University of Rajshahi **Rajshahi-6205** **Bangladesh.**

RUCL Institutional Repository **http://rulrepository.ru.ac.bd**

Institute of Environmental Science (IES) PhD Thesis

2020

Ethnobotanical Study of Some Selected Plants Used in the Treatment of Skin and Diabetic Diseases in Bangladesh

Sadat, A F M Nazmus

University of Rajshahi

http://rulrepository.ru.ac.bd/handle/123456789/1067

Copyright to the University of Rajshahi. All rights reserved. Downloaded from RUCL Institutional Repository.

Ph.D.
THESIS

ETHNOBOTANICAL STUDY OF SOME SELECTED PLANTS USED IN THE TREATMENT OF SKIN AND DIABETIC DISEASES IN BANGLADESH

June, 2020

ETHNOBOTANICAL STUDY OF SOME SELECTED PLANTS USED IN THE TREATMENT OF SKIN AND DIABETIC DISEASES IN BANGLADESH

Ph.D. Thesis

Submitted by

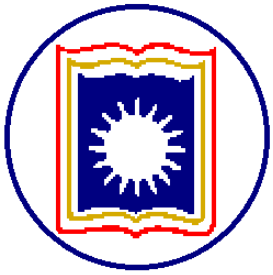
A F M Nazmus Sadat
ID: 14114
Session: 2014-2015

A thesis submitted in fulfillment of the requirements
for the degree of Doctor of Philosophy

Institute of Environmental Science
University of Rajshahi
Rajshahi-6205, Bangladesh

June, 2020.

ETHNOBOTANICAL STUDY OF SOME SELECTED PLANTS USED IN THE TREATMENT OF SKIN AND DIABETIC DISEASES IN BANGLADESH



Ph.D. Thesis

Submitted by

A F M Nazmus Sadat
ID: 14114
Session: 2014-2015

A thesis submitted in fulfillment of the requirements
for the degree of Doctor of Philosophy

Institute of Environmental Science
University of Rajshahi
Rajshahi-6205, Bangladesh

June, 2020.

DEDICATED

TO

MY BELOVED PARENTS,
CHILDREN'S,
SUPERVISORS AND
ALL WELL WISHERS

DECLARATION

I do hereby declare that the Ph.D. thesis entitled **"ETHNOBOTANICAL STUDY OF SOME SELECTED PLANTS USED IN THE TREATMENT OF SKIN AND DIABETIC DISEASES IN BANGLADESH"** submitted to the Institute of Environmental Science, University of Rajshahi, Bangladesh is an independent work carried out by me in Botanical Pesticide and Environmental Microbiology Research Lab of the Institute of Environmental Science of University of Rajshahi under the joint supervision of Dr. Md. Abul Kalam Azad, Professor, Institute of Environmental Science, University of Rajshahi, and Dr. Md. Matiar Rahman, Professor, Department of Biochemistry and Molecular Biology, University of Rajshahi. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Rajshahi
Date: June 30, 2020

(A F M Nazmus Sadat)
Ph.D. Research Fellow
Session: 2014-2015
ID: 14114
Institute of Environmental Science
University of Rajshahi, Bangladesh.

CERTIFICATE

We do hereby certify that the thesis entitled **"ETHNOBOTANICAL STUDY OF SOME SELECTED PLANTS USED IN THE TREATMENT OF SKIN AND DIABETIC DISEASES IN BANGLADESH"** is an original research work conducted by A F M Nazmus Sadat for partial fulfillment of the requirements for the degree of Doctor of Philosophy (Ph. D.) in Environmental Science. To the best of our knowledge, this research work is researcher's own achievement, free from duplication of any previous work. This thesis or its any part has not been submitted to any other university for higher degree.

Principal Supervisor

Dr. Md. Abul Kalam Azad
Professor
Institute of Environmental Science
University of Rajshahi, Bangladesh

Co-Supervisor

Dr. Md. Matiar Rahman
Professor
Department of Biochemistry and Molecular Biology
University of Rajshahi, Bangladesh.

ACKNOWLEDGEMENTS

I have been able to complete this research work with support and active co-operation of concerned authorities and several persons. I would like to express deep feelings of gratitude to my supervisor Dr. Md. Abul Kalam Azad, Professor, Institute of Environmental Science (IES), University of Rajshahi (RU), Bangladesh for his valuable guidance at different stages of this research work. He provided me the solid foundation of this work by his continuous scholastic guidance, constant encouragement, valuable suggestions, timely and solitary instructions and kind help during of this work.

I am intensely indebted to my co-supervisor Dr. Md. Matiar Rahman, Professor, Department of Biochemistry and Molecular Biology, RU, Bangladesh for his very useful guidance and counseling in overcoming various bottlenecks during the study.

I am grateful to Professor Dr. Golam Sabbir Sattar, Director, IES, RU, Professor Dr. Sultan-ul Islam, former Director, IES, RU and Professor Dr. Md. Azizul Islam, former Director, IES, RU who helped me a lot during this research. I am grateful to all faculty members of the IES who conducted theory courses and helped me enriching my knowledge on environmental science. I am ever grateful to the provost and other staffs of Mir Kaium International Dormitory, RU for providing me a wonderful accommodation in RU campus.

I want to give my special thanks to Ms. Afroza Sultana and a group of my students who worked voluntarily with me. My thanks are also to my friends all around the world who conveyed valuable suggestion during this study.

Finally, my heartiest thanks go to my family, my wife Dr. Sumiya Hossain, my son AFM Nafis Anwar Sadat and my daughter Farzana Farisha Sadat Nayaar whose proximity, love and affection provided my joy and relaxation though they sacrificed a lot of my company.

Last but not least, I would like to show my gratitude towards my beloved parents, Md. Abdus Sattar and Mrs. Tahamina Khatun, whose everyday prayers made it possible to complete this thesis. Without their help this work would not be materialized. It would be a smiling ending if it is found to be useful to all concerned.

A F M Nazmus Sadat

ETHNOBOTANICAL STUDY OF SOME SELECTED PLANTS USED IN THE TREATMENT OF SKIN AND DIABETIC DISEASES IN BANGLADESH

Abstract

This study investigated the indigenous people's belief on some medicinal plants those commercially used to cure different types of skin diseases as well as diabetic diseases in Bangladesh. It was observed that around 400 to 1000 medicinal plants available in Bangladesh listed in different database. In the present study ten potential medicinal plants were selected based on their skin diseases curing ability and the anti-diabetic properties through ethnobotanical study. Around 176 peer reviewed publications connected to 24 major tribes' lives in Bangladesh were studied during selection of plants. Ultimately fresh leaves of Neem (*Azadirachta indica*), Babla (*Acacia nilotica*), Pathorkuchi (*Bryophyllum pinnatum*), Sojna (*Moringa oleifera*) and Asham lota (*Mikania cordata*); fresh stems of Shornolota (*Cuscuta reflexa*); dried leaves of Papaya (*Carica papaya*) and Telakochu (*Coccinia grandis*); dried seeds of Ajawin (*Carum copticum*) and finally Kalo Jera (*Nigella sativa*) were enrolled in the present study. The pharmacological actions of these selected plants were assessed scientifically under a controlled laboratory setup.

A comparatively noble and green extraction procedure named "Aqueous Ultrasound Assisted Extraction Method" was used for the preparation of crude extract which provided significantly high yield percentages. More than 30% yield was observed from the dried plant's parts of *N. sativa, C. grandis* and *C. papaya* through this extraction procedure. Around 15% extraction was observed from fresh leaves of *A. nilotica*, *A. indica* and *C. reflexa,* however, still higher than the expected (10%) yield. Promising extraction also found from *M. oleifera* (17.42%), *M. cordata* (18%), *C. copticum* (19.14%) and *B. pinnatum* (21.59%). Most of the crude extracts were found acidic in nature except *C. papaya* and *C. grandis*. It was observed that *M. cordata* and *C. copticum* added more basic compounds during the extraction procedure observed by the pH changes of the solution before and after ultrasound treatment. Drastic pH

changes were observed after ultrasound treatment on the plant material and indicated the nature of compound extracted from the plant parts. Crude extracts were dried by conventional water bath at 55^0C and preserved in a glass vial under freezing condition for further use.

Crude extracts were undergone different phytochemical and pharmacological studies. Gram positive bacteria *S. aureus*, *S. pyogenes* and gram negative bacteria *E. coli* as well as fungi *C. lunata*, *F. chlamydosporum* and *M. furfur* were enrolled in the present study having distinct association on different types of skin diseases. Seven more pathogenic bacteria and 2 fungi were also included in the presents study for comparison of the antimicrobial activities. Antimicrobial activities were conducted by disc diffusion method and found promising antimicrobial activity of *Azadirachta indica* (against *S. aureus*), *Acacia nilotica* (against *S. pyogenes* and *S. aureus*), *Bryophyllum pinnatum* (against *S. pyogenes*, *S. aureus* and *E. coli*), *Cuscuta reflexa* (against *S. aureus* and *S. pyogenes*) and *Moringa oleifera* (against *S. pyogenes*). Comparatively poor sensitivity was observed against three enlisted fungi. These plants may be considered potential antimicrobial agents and may be suitable for curing different types of microorganism infected skin diseases.

Three types of hypoglycemic studies were conducted to observe the anti-diabetic properties of the plants. Most of the enrolled plants were showed distinct properties on lowering blood glucose level of the dexamethasone induced diabetic mice. However extracts of *A. indica, B. pinnatum, C. reflexa, C. papaya* and *C. grandis* were showed very promising anti-diabetic effect almost similar to the standard anti-diabetic drug glibenclamide. From cytotoxic study all crude extracts were proved safer than the standard drug erythromycin and tetracycline.

A purification stages were also performed by using a basic partition theory, and was observed that sufficient purifications were performed which may be considered for large scale purification including industrial setup. The whole study was conducted by minimum impact on the environment. From this study we justified the use of some ethnobotanicals for the treatment of different types of pathogenic skin diseases and diabetic disease.

Table of contents

List of Tables

List of Figures

Abbreviation

ADA	:	American Diabetes Association
ASE	:	Accelerated Solvent Extraction
BMRC	:	Bangladesh Medical Research Council
CBD	:	Convention on Biological Diversity
CHT	:	Chittagong Hill Tracts
DMSO	:	Di-Methyl Sulphur Oxide
FMP	:	Folk Medicinal Practitioner (main stream Kaviraj)
FPG	:	Fasting plasma glucose
GAE	:	Gallic Acid Equivalents
HAE	:	Hot Aqueous Extract
IES	:	Institute of Environmental Science
ILO	:	International Labor Organization
IP	:	Intraperitoneal administration
MAE	:	Microwave Assisted Extraction
MPDB	:	Medicinal Plant Data Base
OGTT	:	Oral Glucose Tolerance Test
OUV	:	Overall Use Values
P	:	Significance level
RU	:	University of Rajshahi
SD	:	Standard Deviation
SFE	:	Supercritical Fluid Extraction
SPME	:	Solid Phase Micro Extraction
TEK	:	Traditional Environmental Knowledge
TFC	:	Total Flavonoid Content
TMP	:	Tribal Medicinal Practitioner (tribal Kaviraj)
TPC	:	Total Phenolic Content
UAE	:	Ultrasound Assisted Extraction
WHO	:	World Health Organization

Chapter 1: Introduction

1.1 Ethnobotany

Ethnobotany is the study of the traditional knowledge of indigenous (ethnic or tribal) groups living in the remote parts of the world. The use of traditional medicines is widespread in Bangladesh. Bangladesh is a subtropical country rich in biodiversity and biological resources, having long history of using medicinal plants for the treatment of different types of diseases. In the present study two major public health problems, skin diseases and diabetics were highlighted. Both types of diseases need long term or even lifelong treatment which is a big burden for the patient. Though effective treatment are available but still treatment are unreachable to a large portion of the patient worldwide due to high cost of drug. However, people living in the remote part or even unconnected to the civilization still fighting against different diseases including the above two types of diseases. These issues are addressed in this research. In the present study, steps were taken to identify natural remedies for the treatment of various types of infectious skin diseases and metabolic disorder like diabetes mellitus through ethnobotanical study among the tribal and folk practitioners in Bangladesh.

1.1.1 History of Ethnobotany

Ethnobotany is the study of a region's plants and their practical uses through the traditional knowledge of a local culture and people (Kochhar 2016). The term ‘ethnobotany’ was first suggested by John Harshberger in 1896 to delimit a specific field of botany and describe plant uses. It was defined as “the use of plants by aboriginal peoples” (Cotton 1996). Richard Evans Schultes, often referred to as the "father of ethnobotany" (Soejarto 2005), explained the discipline in this way: “Ethnobotany simply means, investigating plants used by primitive societies in various parts of the world”. According to Jones (1941) ethnobotany is the discipline concerned with the interactions between people and plants (Hamilton *et al.,* 2003) or simply ‘ethnobotany’ is the part of ethnoecology which concerns plants (Martin 1995). Ethnoecology cover all studies which describe local people’s interaction with the natural environment, including sub-disciplines such as ethnobiology, ethnobotany, ethnoentomology and ethnozoology” (Martin 1995).

Recorded evidence of ethnobotany in the Indian subcontinent may be the earliest in the world and date back to 4000-1500 B.C (Jain & Mudgal, 1999). Pedanius Dioscorides, a Greek physician and historian wrote "De Materia Medica", a catalogue of about 600 plants in the Mediterranean in 77AD (Ponman *et al.,* 2012). The great voyager Christopher Columbus discovered tobacco from *Nicotiana sp.* in Cuba in year of 1492 (Simpson & Ogorzaly, 1986). In 1542, Leonhart Fuchs who was a Renaissance artist listed 400 plants native to Germany and Austria in his "De Historia Stirpium". In the 16th century, one of the French explorers named Jacques Cartier learned a cure for scurvy from a local Iroquoi tribe (Mazal 2016).

For centuries, Bangladesh has been the dwelling place of different ethnic groups. These people are with distinctive social and cultural practices, languages and customs are commonly known as 'Adivasis' by themselves. At least 350 million people worldwide are considered to be indigenous and in Bangladesh about two million indigenous people of 45 different distinct communities are living throughout the country. Latest ethnographic research suggests that the number of tribes within the country approximates 150 (Murmu 2009; Kabir *et al.*, 2014). A vast controversy regarding the number of tribes was observed with Rahman *et al.* (2007) and Uddin (2010) paper as they mentioned the number of major tribes in Bangladesh was only 19 and 35 respectively were living in different hilly areas and as well as in plain lands of Bangladesh (Uddin *et al.,* 2014a). However, Bangladesh is believed by anthropologists to have more than 100 indigenous communities or tribes and these communities are again divided into clans and sub-clans, with every clan and sub-clan possessing their own sets of beliefs and practices (Zahan *et al.,* 2013).

1.1.2 Trends of Ethnobotanical Researches in Bangladesh

The documented ethno-botanical studies in Bangladesh may be started early in the previous century after Hutchinson published a book on Chittagong Hill Tracts (CHTs) in the year of 1909 (Faruque and Uddin, 2011) which is also considered to be the pioneer work on ethnobotany in the Indian subcontinent. The other earliest ethno-botanical reference work may be considered of Lewin (1912) work on local tribe. Rajput (1965) was also worked on the tribes of CHTs and it was also considered another ethno-botanical work in this part. After independence the remarkable works may be included the works of Sirajuddin (1971), Tanchangya (1982) and Shelly

(1992), however their works were less detailed and largely dependent on Hutchinson's (1909) work. The remarkable contribution of Khan and Huq (1975), Hassan and Khan (1986), Mia and Huq (1988) may also be considered as oldest ethnobotanical research in Bangladesh.

Last three decades extensive work has done in the field of ethnobotany in Bangladesh. In this period some remarkable works on ethnobotany was done on the tribal people of Chittagong and CHT, most of which were published after 1990. Extraordinary work was done by Kadir (1990) on medicinal plants of Bangladesh and their conservation strategy. Alam (1992) worked on Marma community and documented the ethno-botanical information and medicinal plant and also conducted a subregional training workshop on applied ethnobotany. Uddin *et al.,* (2008) worked on medicobotanical report on the Chakma people of Bangladesh.

Though last three decades the popularity of ethno-botanical studies in Bangladesh is increases many fold, still studies on ethno-medicinal information of ethnic communities in Bangladesh are at initial stage. However, some recent remarkable ethnomedicinal studies in Bangladesh may be mentioned including the work of Ahmed *et al.* (2017), Akber *et al.* (2011), Anisuzzaman *et al.* (2007), Arumugam *et al.* (2013), Azam *et al.* (2013), Biswas *et al.* (2011), Rahman (2013), Rahmatullah *et al.* (2009). Last two decade's Rahman and Rahmatullah did extensive and series of ethnobotanical survey covering almost all vital places of Bangladesh.

1.1.3 Medicinal Plants in the world

As per the published report by the Royal Botanic Gardens, Kew, in the United Kingdom in their "State of World's Plants", around 391,000 species of vascular plants, of which about 369,000 species (or 94%) are flowering plants are available in the world (Kew 2016). The Food and Agriculture Organization estimated in 2002 that over 50,000 medicinal plants are used across the world (Schippmann *et al.,* 2002) whereas the Royal Botanic Gardens, Kew more conservatively estimated in 2016 that 17,810 plant species have a medicinal use, out of some 30,000 plants for which a use of any kind is documented (Kew 2016).

There is no such scientifically proven data for the estimation of total plants species are available in Bangladesh. From literature survey it was found that, Charles Baron Clarke (1832-1908) wandered two and half years on boat and made more than 7000

botanic collection from Sylhet, Madhupur jungle and Comilla. He also made collection trips to Barisal, Sundarbans, Dhaka, Jessore and Chittagong. So the actual number might be more. According to the Banglapedia, more than 6000 plants species occur in Bangladesh (Islam and Miah, 2012) and Ahmed *et al.* (2009) estimated around 6.5 thousand plant species present in Bangladesh. However, around 500 (Ahmed *et al.,* 2009) to 1000 (Mia 1990) plant species have medicinal values and grow in the country's forests, wetlands, homestead forests, and even roadside as indigenous, naturally occurring, or cultivated plants (Ghani 2003). Yusuf *et al.* (1994), Ghani (1998) and Yusuf *et al.* (2009) documented more than 500 Bangladeshi plants with their medicinal properties. More recent and scientifically proven data was come from Ashraf *et al.* (2014), as they were more concisely estimated 406 medicinal plants with their corresponding scientific, family and local names as well as utilized parts for the treatment from different districts of Bangladesh proven by MPDB1.0 (Medicinal Plant Data Base) software.

1.2 Skin Disease

Skin diseases are the most common illness among people around the world. The World Health Organization (WHO) has found a high prevalence of skin diseases in the general population of developing countries, some diseases being more prevalent in children (Mahbub *et al.,* 2017). There are hundreds of skin conditions that affect humans. However, keratinized surface, low pH, high salt content and presence of lysozyme prevent skin from microorganism's infection (Jyothilakshmi *et al.,* 2016). Most of the skin diseases are caused by bacterial and fungal infection (Azman *et al.,* 2016). Common bacterial skin infections include cellulitis, erysipelas, impetigo, folliculitis, furuncles and carbuncles (Stulberg *et al.,* 2002).

A bacterial infection occurs when bacteria successfully invade the soft tissues through small wounds on the skin surface or through existing condition. Late prevention of bacterial infection can cause to life-threatening situation. Bacterial infection may affect both the skin and the subcutaneous tissues beneath, and can spread to the lymph nodes and bloodstream. *Staphylococcus aureus* a gram-positive, round-shaped bacterium found in the nose, respiratory tract, and on the skin, common cause of skin infections (Lowy 1998). However, *S. aureus* are one the most common bacterial infections in humans and are the causative agents of multiple human infections, including bacteremia, infective endocarditis, skin and soft tissue infections (e.g.,

impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (e.g., pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections (Tong *et al.*, 2015). Most strains of *Staphylococcus aureus* are relatively low virulent and harmless if the infection is restricted to superficial layers of intact skin but they cause infection once they gain entry into damaged skin (Jyothilakshmi *et al.,* 2016). *Streptococcus pyogenes* is also capable of causing infection on damaged skin (Jensen *et al.,* 1997). Besides this bacterium, cellulitis (one of the skin diseases) is caused by large varieties of organisms which are known to colonize chronic wounds, including *Streptococci, Staphylococci, Pseudomonas spp.,* and *Bacteriodes spp.* (Cox and Lawrence, 1998). Impetigo, cellulitis, hair follicle infections, *Staphylococcal* scaled syndrome and necrotizing fascilitis are the major skin diseases caused by *Staphylococcus aureus* whereas impetigo, cellulitis, erysipelas and necrotizing fasciitis are the major skin diseases caused by *Streptococcus pyogenes* (Jyothilakshmi *et al.,* 2016). Common superficial fungal infections of the skin include *Tinea capitis*, *Tinea corporis*, and *Pityriasis versicolor* (Kelly 2012). Eczema is a collective term for some medical conditions that can cause the skin to get irritated or inflamed, the most common form of eczema being known as atopic dermatitis (Mahbub *et al.,* 2017) whereas scabies is caused by a parasite.

1.3 Diabetes

Diabetes, also referred to as diabetes mellitus, is a multiple metabolic disease characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism (Wild *et al.,* 2004; Araki *et al.,* 2017). It is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The distinguishing symptoms of diabetes are polyuria (frequent urination), polydypsia (thirsty), polyphagia (extrem hunger), and unexpected weight loss (Altan 2003). According to WHO report 2016, the total burden of deaths from high blood glucose in 2012 has been estimated to amount to 3.7 million. This number includes 1.5 million diabetes deaths, and an additional 2.2 million deaths from cardiovascular diseases, chronic kidney disease and tuberculosis related to higher-than-optimal blood glucose

(Packirisamy *et al.,* 2018). It has become a major global epidemic over the past few decades (Attanayake *et al.,* 2016). Global estimate of the number of diabetics within the past three decades showed an increase from 153 million in 1980 to 347 million in 2008 (Alamin *et al.,* 2015). Currently the prevalence of diabetes is estimated at 415 million people (6.4% of the adult population worldwide) in 2015 and the number is projected to increase to 642 million in 2040 and it is predicted to become the 7th leading cause of death in the world by the year 2040 (Zimmet *et al.,* 2014). Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macrovascular complications of diabetes, which are the major causes of morbidity and death (ADA 2014). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (ADA 2014). Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Diabetes may be classified into four groups (Diabetes Care 1997, Genuth *et al.,* 2003; ADA 2014),

(I) Type 1 diabetes: *β*-cell destruction, usually leading to absolute insulin deficiency

(II) Type 2 diabetes: may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance

(III) Gestational diabetes: Diabetes developed during pregnancy

(IV) Other specific types.

a. Genetic defects of *β*-cell function (eg. Transient neonatal diabetes, Permanent neonatal diabetes etc.)
b. Genetic defects in insulin action (eg. Type A insulin resistance, Leprechaunism)
c. Diseases of the exocrine pancreas (eg. Pancreatitis, Neoplasia, Cystic fibrosis etc.)
d. Endocrinopathies (eg. Hyperthyroidism, Somatostatinoma etc.)
e. Drug or chemical induced (eg. Nicotinic acid, Glucocorticoids, Thiazides etc.)
f. Infections (eg. Congenital rubella, Cytomegalovirus etc.)

1.4 Ethnobotanicals uses for Curing Skin Diseases and Diabetes

Ethnobotanicals used in skin diseases and diabetes were identified through secondary ethnobotanical survey. Ethnobotanical survey on different TMPs (Tribal Medicinal Practitioners) and FMPs (Folk Medicinal Practitioners) in Bangladesh and published in different peer reviewed journal in the period of 2000-2017 were considered. Total 176 papers (out of around 300) were finally selected for this study. Around 160 medicinal plants were identified from the surveyed paper from where 94 were extensively used for the treatment of different types of skin diseases (Table 2.1 in the chapter 2) and 75 plants were found for the treatment of diabetes (Table 2.2 in the chapter 2). However, interestingly 46 medicinal plants were found having both skin and diabetic disease curing properties and widely used by many TMPs and FMPs in Bangladesh.

1.5 Rational of this Research Work

Bangladesh is a subtropical country rich in biodiversity and biological resources, having long history of using medicinal plants for the treatment of different types of diseases. Due to subtropical climate, infectious diseases are the major public health problems in Bangladesh. On the other hands, diabetes is one of the major public health problems in world ranked it the fourth or fifth leading cause of death (Alamin *et al.,* 2015). Though sufficient numbers of antibiotics are available in the market for treating infectious diseases but indiscriminate uses increases the incidence of multiple antibiotic resistances which has become a global concern (Westh *et al.,* 2004) now a day. Over the century worldwide indigenous people are successfully managing these diseases by using natural resources available in their surroundings. Management of different types of skin diseases and diabetes using traditional remedies are also widespread in Bangladesh. There has been increasing demand for the use of plant products due to low cost, easy availability and lesser side effects. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug because of the unmatched availability of chemical diversity. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections.

The study is significant in a number of ways. Considering the economic importance of the people of Bangladesh, the use of traditional medicines gains its importance (Basak *et al.,* 2016). The study of indigenous local medicinal knowledge may have practical

implications for developing new drug. In the present study, steps were taken to identify natural remedies for the treatment of various types diseases especially infectious skin diseases and metabolic disorder mainly diabetes mellitus through ethnobotanical study among the tribal and folk practitioners in Bangladesh.

1.6 Plant Selection Criteria

The research work was started by selecting some potential plants having capacity for the curing of skin and diabetic diseases. Bangladesh is believed by anthropologists to have more than 100 indigenous communities or tribes and these communities are again divided into clans and sub-clans, with every clan and sub-clan possessing their own sets of beliefs and practices (Zahan *et al.,* 2013). It was very difficult to conduct ethnobotanical survey among all the groups available in Bangladesh. In this regard secondary data was considered. Data was collected from published papers connected to the ethnobotany, ethnomedicine, ethnobiology published on Bangladeshi Traditional Medicinal Practitioners (TMPs) and Folk Medicinal Practitioners (FMPs). Around 176 such publications connected to 24 major tribes (Table 1.1) lives in Bangladesh were studied during selection of plants.

The prime objective of the present study was to justify the use of plants for curing skin diseases and diabetes by the ethnic people in Bangladesh. About 94 plants had identical properties of curing different types of skin diseases (Table 2.1 in Chapter 2) and 75 for controlling diabetes (Table 2.2 in Chapter 2). From the primary identified plants, 10 promising plants (Table 2.2) were selected based on the following criteria;

(i) The plants were widely used (or very rarely used) in different ethnic groups (TMPs and/or FMPs) in Bangladesh as well as abundant in our surroundings

(ii) The plants having capacity to cure one or more types of skin diseases

(iii) The plants having capacity to control or cure diabetes diseases

Table 1.1: Types of papers considered for ethnobotanical survey in the present study

Sl#	Name of Ethnic Tribes	No. of Papers Surveyed
1	Bagdi	1
2	Bede	1
3	Chakma	9
4	Garo	14
5	Goala	1
6	Hajong	2
7	Khasia	1
8	Khumi	3
9	Koch	1
10	Kole tribes	1
11	Lushai	1
12	Manipuri	1
13	Marma	6
14	Murmu	1
15	Oraon	1
16	Pahan	2
17	Pankho	1
18	Rai tribes	2
19	Raibongshi	1
20	Rakhain	2
21	Santal	15
22	Teli tribe	2
23	Tonchongya tribe	2
24	Tripura	10
25	Mixed TMPs	11
26	FMPs	84
	Total Number of papers surveyed	**176**

1.7 Collection and Identification of Medicinal Plants

All plant materials (Table 1.2) were collected from two sources:

1. Botanical Pesticide Garden, University of Rajshahi, Rajshahi, Bangladesh.
2. University campus, University of Rajshahi, Rajshahi, Bangladesh.

Samples were identified by the professional taxonomist of the Department of Botany, University of Rajshahi, Bangladesh. Herbarium specimen for each plant was prepared and placed in the Botanical Pesticide Laboratory, Institute of Environmental Science, University of Rajshahi, Rajshahi, Bangladesh for record.

Table 1.2: List of Plants selected for the present study

Sl#	Scientific Name	Family	Local Name	Plant Parts Used
1.	*Azadirachta indica*	Meliaceae	Neem	Leaf
2.	*Acacia nilotica*	Fabaceae	Babla	Leaf
3.	*Bryophyllum pinnatum*	Crassulaceae	Pathor kuchi	Leaf
4.	*Cuscuta reflexa*	Cuscutaceae	Shorno lota	Stems
5.	*Mikania cordata*	Caricaceae	Asham lota	Leaf
6.	*Moringa oleifera*	Moringaceae	Sojna	Leaf
7.	*Carica papaya*	Caricaceae	Papaya	Leaf
8.	*Coccinia grandis*	Cucurbitaceae	Telakochu	Leaf
9.	*Carum copticum*	Apiaceae	Ajawin	Seeds
10.	*Nigella sativa*	Ranunculaceae	Kalo jera	Seeds

1. Taxonomic classification of Neem

(Girish and Bhat, 2008).

Order	Rutales
Suborder	Rutinae
Family	Meliaceae
Subfamily	Melioideae
Tribe	Melieae
Genus	*Azadirachta*
Species	*A. indica*

Figure 1.1: Neem plant

2. Taxonomical classification of Babla (Raheel *et al.,* 2014)

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	*Acacia*
Species	*A. nilotica*

Synonym: *Acacia arabica* (Lam.) Willd.

Figure 1.2: Babla plant

3. Taxonomical classification of Pathorkuchi (Nagaratna and Hedge, 2015; Prasad *et al.,* 2012)

Kingdom	Plantae
Sub kingdom	Tracheobionta
Division	Spermatophyta
Subdivision	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Rosales
Family	Crassulaceae
Genus	Bryophyllum
Species	*Bryophyllum pinnatum* (lam.)Oken

Figure 1.3: Pathorkuchi plant

4. Taxonomical classification of Shorno lota (Vijikumar *et al.,* 2011)

Kingdom	Plantae
Sub kingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Angiospermes
Class	Eudicots
Subclass	Asterids
Order	Solanales
Family	Cuscutaceae
Alternate	Convolvulaceae
Genus	*Cuscuta*
Species	*C. reflexa*

Figure 1.4: Shornolota plant

5. Taxonomical classification of Asham lota

Kingdom	Plantae
Order	Asterales
Family	Asteraceae
Tribe	Eupatorieae
Genus	*Mikania*
Species	*M. micrantha* *M. cordata*

Figure 1.5: Ashamlota plant

6. Taxonomical classification of Sojna (Raja *et al.,* 2016)

Kingdom	Plantae
Order	Brassicales
Family	Moringaceae
Genus	*Moringa*
Species	*M. oleifera*

Figure 1.6: Sojna plant

7. Taxonomical classification of Papaya

Kingdom	Plantae
Sub kingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Subdivision	Magnoliophyta
Class	Magnoliopsida
Subclass	Dilleniidae
Order	Violales
Family	Caricaceae
Genus	*Carica* L
Species	*Carica papaya* L.

Figure 1.7: Papaya plant

8. Taxonomical classification of Telakochu (Pekamwar *et al.*, **2013)**

Kingdom	Plantae
Order	Cucurbitales
Family	Cucurbitaceae
Sub family	Convolvulaceae
Tribe	Benincaseae
Genus	*Coccinia* Wight & Arn
Species	*Coccinia indica*
	Coccinia grandis

Figure 1.8: Telakochu plant

9. Taxonomical classification of Ajawin (Jeet *et al.,* 2012; Fazeli-nasab and Fooladvand, 2016.)

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Apiales
Family:	Apiaceae
Genus:	*Trachyspermum*
Species:	***T. ammi***

Synonyms

Ammi copticum L.
Carum copticum (L.) Link
Trachyspermum copticum Link
Sison ammi L.

Figure 1.9: Ajawin seeds and plant

10. Taxonomical classification of Kalo Jera (Ansari and Satish, 2013)

Kingdom : Plantae

Division : Magnoliophyta

Order : Ranunculales

Family : Ranunculaceae

Genus : *Nigella*

Species : *sativa*

Figure 1.10: Kalo jera seeds and plants

1.8 Objectives of the Present Research Work

The study was conducted considering the following objectives:

- To study the traditional medicinal plants using in the rural area of Bangladesh.
- To prepare a list of medicinal plants used widely for the treatment of different types of skin diseases and diabetes mellitus by tribal and folk medicinal practitioners in different area of Bangladesh.
- To develop an effective green extraction procedure, purification of the compounds and pharmacological study of botanicals using simple, cost effective and environment friendly approaches.

References

ADA (American Diabetes Association). 2014. Diabetes and driving. *Diabetes Care*, 37(1): 97-103.

Ahmed KMI, Bashar MS, Tanny UT, Akter N, Mandal BK, Islam E and Rahmatullah M. 2017. Ethnomedicinal formulations of a Garo tribal healer in Haluaghat, Mymensingh district, Bangladesh. *Journal of Medicinal Plants Studies*, 5(1): 219-223.

Ahmed ZU, Hassan MA, Begum ZNT, Khondker M, Kabir SMH, Ahmad M, Ahmed ATA, Rahman AKA and Haque EU. 2009. Encyclopedia of Flora and Fauna of Bangladesh: Index Volume-Flora. *Asiatic Society of Bangladesh, Dhaka, Bangladesh,* 6: 1- 408.

Akber M, Seraj S, Islam F, Ferdausi D, Ahmed R, Nasrin D, Nahar N, Ahsan S, Jamal F and Rahmatullah M. 2011. A survey of medicinal plants used by the traditional medicinal practitioners of Khulna City, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, 5: 177-195.

Alam MK. 1992. Medical ethno-botany of the Marma tribe of Bangladesh. *Economic Botany*, 46(3): 330-335.

Alamin MA, Yagi AI and Yagi SM. 2015. Evaluation of antidiabetic activity of plants used in Western Sudan. *Asian Pacific Journal of Tropical Biomedicine,* 5(5): 395-402.

Altan VM. 2003. The Pharmacology of diabetic complications. *Current medicinal chemistry,* 10(15): 1317-1327.

Anisuzzaman M, Rahman AHMM, Rashid MH, Naderuzzaman ATM and Islam AKMR. 2007. An Ethnobotanical Study of Madhupur, Tangail. *Journal of Applied Sciences Research, Pakistan*, 3(7): 519-530.

Ansari Z and Satish T. 2013.Traditional uses of *Nigella sativa*, in Malegaon region of Nashik-A Review, *International Journal of Pure and Applied Bioscience*, 1(2): 19-23.

Araki E, Haneda M, Kasuga M, Nishikawa T, Kondo T, Ueki K and Kadowaki T. 2017. New glycemic targets for patients with diabetes from the Japan Diabetes Society. *Journal of Diabetes Investigation*, 8(1): 123-125.

Arumugam G, Manjula P and Paari N. 2013. A review: Anti diabetic medicinal plants used for diabetes mellitus. *Journal of Acute Disease,* 2(3): 196-200.

Ashraf MA, Khatun A, Sharmin T, Mobin F, Tanue AR, Morshed T, Fakir TA, Begum RA and Nabi AHMN. 2014. MPDB1.0: a medicinal plant database of Bangladesh. *Bioinformation,* 10(6): 384-386.

Attanayake AP, Jayatilaka KAPW, Pathirana C and Mudduwa LKB. 2015. Antihyperglycemic activity of *Coccinia grandis* (L) Voigt in streptozotocin induced diabetic rats. *Indian Journal of Traditional Knowledge,* 14(3): 376-381.

Azam MNK, Ahmed MN, Rahman MM and Rahmatullah M. 2013. Ethnomedicines used by the Oraon and Gor tribes of Sylhet district, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, 7: 391-402.

Azman MA, Sidek HJ, Sharudin MSM, Halim NANA, Sahrom RMAAR. 2016. Phytochemical Screening and Antibacterial Activity of *Azadirachta indica* Leaves Extract on Common Skin Infection Bacteria. *Jurnal Intelek*, 11(1): 18-23.

Basak S, Banerjee A and Mann CK. 2016. Role of some ethno medicines used by the Santal tribal people, of the district Bankura, W.B., India, for abortifacient purposes. *Journal of Medicinal Plants Studies*, 4(2):125-129.

Biswas KR, Ishika T, Rahman M, Swarna A, Khan T, Monalisa MN and Rahmatullah M. 2011. Antidiabetic plants and formulations used by folk medicinal practitioners of two villages in Narail and Chuadanga districts, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, 5: 158-167.

Cotton CM. 1996. Ethnobotany: principles and applications. John Wiley & Sons Ltd. Chichester: 424.

Cox N and Lawrence CM. 1998. Diagnostic Problems in Dermatology. Mosby, London: 146-7.

Diabetes Care. 1997. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care,* 20: 1183-1197.

Faruque O and Uddin SB. 2011. Ethnodiversity of Medicinal Plants Used by Tripura Community of Hazarikhil in Chittagong District of Bangladesh. *Journal of Taxonomy and Biodiversity Research,* 5: 27-32.

Fazeli-nasab B and Fooladvand Z. 2016. A Review on Iranian *Carum copticum* (L.): Composition and Biological Activities. *European Journal of Medicinal Plants,* 12(1): 1-8.

Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, Kitzmiller J, Knowler WC, Lebovitz H, Lernmark A, Nathan D, Palmer J, Rizza R, Saudek C, Shaw J, Steffes M, Stem M, Tuomilehto J, Zimmet P. 2003. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care,* 26: 3160-3167.

Ghani A. 1998. Medicinal plants of Bangladesh, Chemical constituents and uses, *Asiatic Press*, Dhaka : 1-33.

Ghani A. 2003. Medicinal Plants of Bangladesh with Chemical Constituents and Uses. Asiatic Society of Bangladesh, Dhaka, Bangladesh.

Girish K and Bhat SS. 2008. Neem, "A green treasure". *Electronic Journal of Biology*, 4: 102-111.

Hamilton AC, Shengji P, Kessy J, Khan AA, Witte SL and, Shinwari ZK. 2003. The purposes and teaching of Applied Ethnobotany. People and Plants Working Paper 11. WWF, Godalming, UK.

Hassan MA and Khan MS. 1986. Ethnobotanical records in Bangladesh. Plants use for healing fractured bones. *Journal of the Asiatic Society of Bangladesh (Science)*, 12: 33-39.

Hutchinson SRH. 1909. The Chittagong Hill Tracts. Vivek Publishing Company, Delhi, India.

Islam S and Miah S. 2012. Flora. National Encyclopedia of Bangladesh (Banglapedia). 2nd Edition (The first Edition was 2003). Published by Asiatic Society of Bangladesh.

Jain SK and Mudgal V. 1999. A Hand Book of Ethnobotany. *Nordic Journal of Botany,* 20(2): 202-202.

Jeet K, Devi N, Narender, Sunil T, Lalit S and Raneev T. 2012. *Trachyspermum ammi* (Ajwain): a comprehensive review. *International Research Journal of Pharmacy,* 2(5): 133-138.

Jensen MM, Wright DN and Robison RA. 1997. Microbiology for the Health Sciences, 4th edition. Upper Saddle River, New Jersey: Prentice-Hall Inc.

Jones V. 1941. The nature and scope of ethnobotany. *Chronica Botanica*, 6: 219-221.

Jyothilakshmi M, Jyothis M and Latha MS. 2016. *Mikania Micrantha*-a Natural Remedy to Skin Infections. *International Journal of Current Microbiology and Applied Sciences,* 5(2): 742-745.

Kabir MH, Hasan N, Rahman MM, Rahman MA, Khan JA, Hoque NT, Bhuiyan MRQ, Mou SM, Jahan M and Rahmatullah M. 2014. A survey of medicinal plants used by the Deb barma clan of the Tripura tribe of Moulvibazar district, Bangladesh. *Journal of Ethnobiology and Ethnomedicine*, 10: 19-46.

Kadir MH. 1990. Bangladesh flora as a potential source of medicinal plants and its conservation Strategies. In: A. Ghani (ed.), Traditional Medicine. Institute of Life Science, Jahangirnagar University, Savar, Dhaka: 73-77.

Kelly BP. 2012. Superficial fungal infections. *Pediatrics in Review,* 33: 22-37.

Kew 2016. State of the world's plants report-2016. Royal Botanic Gardens, Kew.

Khan MS and Huq AM, 1975. Medicinal Plants of Bangladesh, BARC, Dhaka. 2.

Kochhar SL. 2016. Economic Botany: A Comprehensive Study (5 ed.). Cambridge University: 644.

Lowy FD. 1998. *Staphylococcus aureus* infections. *New England Journal of Medicine*, 339(8): 520-32.

Lewin TH. 1912. A Fly on the Wheel. Blackwell Pub. Ltd Oxford, London: 241.

Mahbub N, Mazumder SH, Morshed MZ, Haq WM, Jahan R, Hossain MS and Rahmatullah M. 2017. Folk Medicinal Use of Plants to Treat Skin Disorders in Chandpur District, Bangladesh. *American Journal of Ethnomedicine,* 4 (2): 1-5.

Martin GJ. 1995. Ethnobotany: A methods manual. Chapman and Hall, London.

Mazal O. 2016. "Dioscorides: De Materia Medica". Uchicago.edu. University of Chicago.

Mia MK and Huq AM. 1988. A preliminary ethnobotanical survey in the Jaintapur, Tamabil and Jaflong area, Sylhet. Bull., Bangladesh National Harbarium, Dhaka. 3:1-10.

Mia MMK. 1990. Traditional medicines of Bangladesh. In: Ghani A. (Ed.), Traditional Medicines. Jahangirnagar University, Dhaka.

Murmu M. 2009. Adivasi Anneshon. Nawroze Kitabistan, Dhaka, Bangladesh: 188-189.

Nagaratna A and Hedge P. 2015. Comprehensive review on Parnabeeja [*Bryophyllum pinnatum* (Lam.) Oken]. *Journal of Medicinal Plants Studies,* 3(5): 166-171.

Packirisamy M, Ayyakkannu P and Sivaprakasam M. 2018. Antidiabetic effect of *Coccinia grandis* (L.) Voigt (Cucurbitales: Cucurbitaceae) on streptozotocin induced diabetic rats and its role in regulating carbohydrate metabolizing enzymes. *Brazilian Journal of Biological Sciences*, 5(11): 683-698.

Pekamwar SS, Kalyankar TM and Kokate SS. 2013. Pharmacological Activities of *Coccinia grandis*: Review. *Journal of Applied Pharmaceutical Science,* 3(5): 114-119.

Ponman, Bussmann, Bruce E and Rainer W. 2012. Medicinal Plants and the Legacy of Richard E. Schultes. Missouri Botanical Garden.

Prasad AK, Kumar S, Iyer SV, Sudani RJ and Vaidya SK. 2012. Pharmacognostical, Phytochemical and Pharmacological Review on *Bryophyllum pinnata*. *International Journal of Pharmaceutical & Biological Archives,* 3(3): 423-433.

Rahman MA, Uddin SB and Wilcock CC. 2007. Medicinal plants used by Chakma tribe in Hill Tracts districts of Bangladesh. *Indian Journal of Traditional Knowledge*, 6(3): 508-517.

Rahman AHMM. 2013. Ethno-medicinal investigation on ethnic community in the northern region of Bangladesh. *American Journal of Life Sciences*, 1(2): 77-81.

Raheel R, Aslam MS, Asghar S and Ashraf M. 2014. Phytochemical, Ethnopharmacological Review of *Acacia nilotica* (Desi Kikar) and Taxo-Pharmacology of Genus Acacia. *Indian Research Journal of Pharmacy and Science,* 1(2): 65-72.

Raja RR, Sreenivasulu M, Vaishnavi S, Navyasri DM, Samatha G, Geethalakshmi S. 2016. *Moringa oleifera*-An Overview. *Research and Analysis Journal of Applied Research*, 2(09): 620-624.

Rajput AB. 1965. The Tribes of Chittagong Hill Tracts. The Inter Services Press Ltd., Karachi (Pakistan): 13-19.

Rahmatullah M, Mukti IJ, Haque AKMF, Mollik MAH, Parvin K, Jahan R, Chowdhury MH and Rahman T. 2009. An Ethnobotanical Survey and Pharmacological Evaluation of Medicinal Plants used by the Garo Tribal Community living in Netrakona district, Bangladesh. *Advances in Natural and Applied Sciences,* 3(3): 402-418.

Schippmann U, Leaman DJ and Cunningham AB. 2002. Impact of cultivation and gathering of medicinal plants on biodiversity: Global Trends and Issues- 2.

Shelly MR. 1992. The Chittagong Hill Tracts of Bangladesh. The Untold Story. Centre for Development Research, Bangladesh (CDRB), Dhaka : 124.

Simpson BB and Conner-Ogorzaly MC. 1986. Economic Botany: Plants in Our World. *Brittonia*, 38(3): 219.

Sirajuddin AM. 1971. The Rajas of Chittagong Hill Tracts and their relation with the Mughals and the East India Company in the Eighteenth Century. *Journal of the Pakistan Historical Society,* 19(1): 1-52.

Soejarto DD, Fong HHS, Tan GT, Zhang HJ, Ma CY, Franzblau SG, Gyllenhaal C, Riley MC, Kadushin MR, Pezzuto JM, Xuan LT, Hiep NT, Hung NV, Vu BM, Loc PK, Dac LX, Binh LT, Chien NQ, Hai NV, Bich TQ, Cuong NM, Southavong B, Sydara K, Bouamanivong S, Thuy TV, Rose WC, Dietzman G. 2005. Ethnobotany/ ethnopharmacology and mass bioprospecting: Issues on intellectual property and benefit-sharing. *Journal of Ethnopharmacology,* 100(1-2): 15-22.

Stulberg DL, Penrod MA and Blatny RA. 2002. Common bacterial skin infections. *American Family Physician*, 66(1): 119-24.

Tong SY, Davis JS, Eichenberger E, Holland TL and Fowler VG. 2015. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews,* 28(3): 603-61.

Tanchangya JC. 1982. Tanchangya Upajatir Sankhipto Parichiti (in Bangla). Tribal Cultural Institute, Rangamati, Bangladesh: 126.

Uddin M, Roy S, Hassan MA and Rahman MM. 2008. Medicobotanical report on the Chakma people of Bangladesh. *Bangladesh Journal of Plant Taxonomy,* 15(1): 67-72.

Uddin N. 2010. Politics of cultural difference: Identity and marginality in the Chittagong hill tracts of Bangladesh. *South Asian Survey*, 17(2): 283-294.

Uddin SB, Faruque MO, Talukder S. 2014a. A Survey of Traditional Health Remedies of the Chakma Indigenous Community of Rangamati District, Bangladesh. *Journal of Plant Science,* 1(2): 106-11.

Vijikumar S, Ramanathan K and Devi BP. 2011. *Cuscuta reflexa* ROXB. – A Wonderful Miracle Plant in Ethnomedicine. *Indian Journal of Natural Sciences*, 1(9): 676-683.

Westh H, Zinn CS and Rosdadahl VT. 2004. An International multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. *Microbial Drug Resistance,* 10: 169-176.

Wild S, Roglic G, Green A, Sicree R and King H. 2004. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care*, 27(5):1047-1053.

Yusuf M, Choudhury JU, Wahab MA and Begum J. 1994. Medicinal Plants of Bangladesh. Bangladesh Council of Scientific and Industrial Research. Dhaka, Bangladesh: 1-340.

Yusuf M, Chowdhury JU, Hoque MN and Begum J. 2009. Medicinal plants of Bangladesh. BCSRI, Chittagong-4220, Bangladesh: 1-692.

Zahan T, Ahmed I, Omi SI, Naher K, Islam S, Mahmud ASMSHB, Sultana S, Shahriar S, Khatun Z and Rahmatullah M. 2013. Ethnobotanical uses of medicinal plants by the Tudu sub-clan of the Santal tribe in Joypurhat district of Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, 7(3): 137-142.

Zimmet PZ, Magliano DJ, Herman WH and Shaw JE. 2014. Diabetes: A 21st century challenge. *Lancet Diabetes Endocrinology,* 2(1): 56-64.

Chapter 2: Review of Literature

The indigenous medicinal history of Bangladesh goes back to centuries of years ago. In Bangladesh both Folk Medicinal Practitioners (FMPs) and Tribal Medicinal Practitioners (TMPs) have their own field of expertise and unique range of medicinal plants, which can be vary greatly from tribe to tribe and between individual Kavirajes of even the same area (Kabir *et al.,* 2014). In most of the situation, the knowledge of medicinal plants usually kept within the family and passed on from generation to generation. Over the years, this successive accumulation of knowledge has led the Kavirajes to develop considerable expertise in the use of medicinal plants (Rahmatullah *et al.,* 2011d). Traditional healers employ methods based on the ecological, socio-cultural and religious background of their people to provide health care. Plant derived medicines are widely used because they are relatively safer than the synthetic alternatives and are easily available, cheaper also (Guha and Chakma, 2015).

If a plant is used for the treatment of similar types of diseases in different places across the world then its pharmacological effect could be accepted and considered as a vital source for new drug development. Therefore, it is suggested that such studies may contribute to indigenous ethno-botanical knowledge as well as studies of the sourcing of raw materials for development of commercial pharmaceuticals (Cakilcioglu and Turkoglu, 2010).

Therefore, the aim of the present study was to document the experiences of both the tribal medicinal practitioners (TMPs) and the folk medicinal practitioners (FMPs) practiced in different tribes and communities in Bangladesh.

In the present study, around 300 published papers on ethnobotanical survey in Bangladesh were studied and screened 94 medicinal plants (Table 2.1) which had proven medicinal uses in different tribes based on their skin disease curing properties. Accordingly 75 plants (Table 2.2) were identified on the basis of their anti-diabetic uses by different TMPs and FMPs.

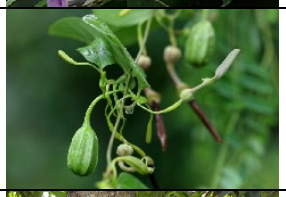
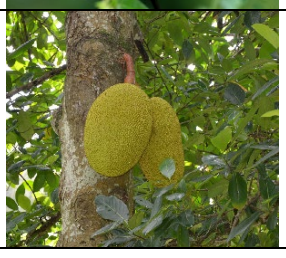
Table 2.1: List of ethnobotanicals used for the treatment of different types of skin diseases

SL#	Scientific name	Family	Local name	Picture	References
1.	*Abelmoschus moschatus* (L.) Medik.	Malvaceae	Musk-dana, Lota-kustori, Kala-kosturi/ Shrub		Chakma, CHT (Rahman *et al.*, 2007); Garo, Haluaghat, Mymensingh (Ahmed *et al.*, 2017); Lushai, Bandarban District (Uddin *et al.*, 2015a)
2.	*Abrus precatorius* L.	Fabaceae	Kuch/ Tree		Santal Tribe in Rajshahi district (Shahidullah *et al.*, 2009); Santal Tribe of Joypurhat District (Rahman 2015a); Tonchongya, Rangamati District (Islam *et al.*, 2015b)
3.	*Abutilon indicum* L. Sweet	Malvaceae	Berela, Kanghi , atibala / Shrub		Santal Tribe, Thakurgaon District (Rahmatullah *et al.*, 2009a); Rakhain, CHT (Hanif *et al.*, 2009)
4.	*Acacia catechu* (L.f.) Willd.	Fabaceae Mimosa-ceae	Khoyer / Tree		Tribal people of Madhupur (Garo, Koch and Hajong communities) (Islam *et al.*, 2014); Garo, Haluaghat, Mymensingh (Ahmed *et al.*, 2017)
5.	*Acacia concinna*	Fabaceae Mimosa-ceae	Vai An Thur, Boba lot/ Shrub		Lushai, Bandarban District (Uddin *et al.*, 2015a);
6.	*Acacia farnesiana* L. Willd.	Mimosa-ceae	Gokul, Belati babul, Torua kadam/ Tree		Chakma, CHT (Rahman *et al.*, 2007); Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015); Tripura tribe of Bangladesh (Hossan *et al.*, 2009)
7.	*Acacia nilotica* L. *Acacia arabica*	Mimosa-ceae Fabaceae	Babla/ Tree		Santal Tribal, Chapai nobabgonj (Jamila and Rahman, 2016); Santal Tribe, Thakurgaon (Rahmatullah *et al.*,2009a); Shantal, Rajshahi (Shahidullah *et al.*, 2009)

SL#	Scientific name	Family	Local name	Picture	References
8.	*Acalypha indica* L.	Euphorbia-ceae	Mukta Jhuri/ Herb		Santal Tribe of Joypurhat District (Rahman 2015a); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)
9.	*Achyranthes aspera* L.	Amarant-haceae	Apang / Herb		Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Tripura tribe of Bangladesh (Hossan *et al.*, 2009); Rakhain, CHT (Hanif *et al.*, 2009)
10.	*Acorus calamus* L.	Araceae Acoraceae	Bach, Ghora bach; Sweet grass/ Herb		Tripura of Hazarikhil In Chittagong District (Faruque and Uddin, 2011); Chakma, Rangamati District (Tasannun *et al.*, 2015); Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015)
11.	*Adhatoda zeylanica* Medic.	Acanthac-eae	Bashok, Sada Bashok/ Herb		Lushai, Bandarban District (Uddin *et al.*, 2015a); Santal Tribe of Joypurhat District (Rahman 2015a); Chakma community of Bangladesh (Roy *et al.*, 2008)
12.	*Adiantum caudatum*	Adiantac-eae	Goyali lota/ Herb		Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015)
13.	*Adina cordifolia*	Rubiaceae	Dakom, keli-Kadam/ Tree		Tripura tribe of Bangladesh (Hossan *et al.*, 2009)
14.	*Aegle marmelos* (L.) Corr.	Rutaceae	Beal/ Tree		Deb barma clan of the Tripura tribe of Moulvibazar District (Kabir *et al.*, 2014); Santal Tribe of Joypurhat District (Rahman 2015a); Folk practitioner, Bogra District (Keya and Rahman, 2017)

SL#	Scientific name	Family	Local name	Picture	References
15.	*Aerva sanguinolenta* L.	Amarant-haceae	Chaya, Mountain knot grass/ Herb		Deb barma clan of the Tripura tribe of Moulvibazar District (Kabir *et al.*, 2014); Santal Tribe in Rajshahi district (Shahidullah *et al.*, 2009)
16.	*Ageratum conyzoides* L.	Asterace-ae	Nackfull, Ati chada, Goat weed / Herb		Sigibe clan of the Khumi tribe of Thanchi sub district in Bandarban District (Sarker *et al.*, 2012); Deb barma clan of the Tripura tribe of Moulvibazar District (Kabir *et al.*, 2014); Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015)
17.	*Alangium lamarckii*	Alangia-ceae	Aakar, Ankara, Bagh Aankra / Shrub		Folk practitioner, Bangladesh (Arumugam *et al.*, 2013)
18.	*Albizia odoratissima*	Mimosa-ceae	Kakur sirish / Tree		Folk practitioner, Bangladesh (Arumugam *et al.*, 2013)
19.	*Allium sativum* L.	Liliaceae	Rosun/ Herb		Chakma, Khagrachori District (Uddin *et al.*, 2015b); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Tonchongya, Rangamati District (Islam *et al.*, 2015b);
20.	*Allophyllus triphyllus* (Burm.f.) Merr.	Sapinda-ceae	Rakhal phul/ Shrub		Chakma, CHT (Rahman *et al.*, 2007);
21.	*Aloe vera* L. Burm f. *Aloe indica* L. *Aloe barbadensis* Mill.	Liliaceae Asphodel-aceae; Aloeace-ae Xanthor-rhoeaceae	Ghrito kumari / Herb		Chakma community of Bangladesh (Roy *et al.*, 2008); Madhupur, Tribal people (Garo, Koch and Hajong communities) (Islam *et al.*, 2014); Santal Tribe of Joypurhat District (Rahman 2015a)

SL#	Scientific name	Family	Local name	Picture	References
22.	*Alpinia conchigera* Griff.	Zingiber-aceae	KolKo m, Galang a/ Tree		Tudu sub clan of the Santal, Joypurhat District (Zahan *et al.*,2013); Chakma community of Bangladesh (Roy *et al.*, 2008); Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015);
23.	*Alternanthera sessilis* (L.) R.Br. ex DC.	Amarant-haceae	Mati kondur ri/ Herb		Hembrom clan of the Santal tribe, Setabganj of Dinajpur District (Moniruzzaman *et al.*, 2015); Folk practitioner, Bogra District (Keya and Rahman, 2017)
24	*Amaranthus spinosus* L. *Amaranthus viridis* L	Amarant-haceae	Kanta notya, Lal notya shate shak/ Herb		Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015); Folk practitioner, Bogra District (Keya and Rahman, 2017) Santal Tribe, Thakurgaon District (Rahmatullah *et al.*,2009a)
25.	*Amomum dealbatum* Roxb.	Zingibera-ceae	Carda mum, Black Carda-mum/ Herb		Chakma, CHT (Khisha *et al.*, 2012); Chakma, CHT (Rahman *et al.*, 2007)
26.	*Amorpho-phallus campanulatus* *Amorpho-phalus paenifolius*	Araceae	Ol, Oal/ Herb		Garo, Netrokona District (Rahmatullah *et al.*, 2009c); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)

SL#	Scientific name	Family	Local name	Picture	References
27.	*Andrographis paniculata* (Burm.f.) Wall. ex Nees.	Acantha-ceae	Chirota, Kalomegh, Green chirayta/ Herb		Chakma, Rangamati District (Tasannun *et al.*, 2015); Deb barma clan of the Tripura tribe of Moulvibazar District (Kabir *et al.*, 2014); Tribal people of Madhupur (Garo, Koch and Hajong communities) (Islam *et al.*, 2014)
28.	*Annona squamosa* L.	Annona-ceae	Ata, Sharifa / Shrub		Folk practitioner, Bogra District (Keya and Rahman, 2017); Santal Tribe of Joypurhat District (Rahman 2015a)
29.	*Anthocephal-us chinensis* (Lam.) A. Rich. ex Walp.	Rubiace-ae	Kadam / Tree		Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Santal Tribe, Thakurgaon District (Rahmatullah *et al.*, 2009a); Folk practitioner, Bogra District (Keya and Rahman, 2017)
30.	*Argemone mexicana* L.	Papaver-aceae	Shialkanta/ Herb		Folk practitioner, Bogra District (Keya and Rahman, 2017); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Santal Tribe of Joypurhat District (Rahman 2015a)
31.	*Argyreia speciosa* (L.f.) Sweet	Convolv-ulaceae	Bichtarak, Sona paruraalata/ Herb		Garo, Netrokona District (Rahmatullah *et al.*, 2009c)
32.	*Aristolachia indica*	Aristola-chiaceae	Isshormul, Ishramul/ Herb		Santal Tribe of Joypurhat District (Rahman 2015a)
33.	*Artocarpus heterophyllus* Lam.	Morace-ae	Kanthal/ Tree		Rakhain, CHT (Hanif *et al.*, 2009); Santal Tribe of Joypurhat District (Rahman 2015a); Chakma, Khagrachori District (Uddin *et al.*, 2015b)

SL#	Scientific name	Family	Local name	Picture	References
34.	*Asparagus racemosus* Willd.	Asparag-aceae	Satamuli / Herb, climber	 	Deb barma clan of the Tripura tribe of Moulvibazar District (Kabir *et al.*, 2014); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Tripura, Rangamati District (Faruque and Uddin, 2011)
35.	*Averrhoa carambola* L.	Oxalida-ceae	Kamranga / Tree		Folk practitioner, Bogra District (Keya and Rahman, 2017); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Santal Tribe of Joypurhat District (Rahman 2015a)
36.	*Axonopus compressus*	Poaceae	Ballagash, Carpetgash, Jhoradan/ Herb		Folk practitioner, Bangladesh (Arumugam *et al.*, 2013)
37.	*Azadirachta indica* A. Juss.	Meliace-ae	Nim, Neem/ Tree		Garo, Netrokona district (Rahmatullah *et al.*, 2009c); Sigibe clan of the Khumi tribe (Sarker *et al.*, 2012); Lushai (Uddin *et al.*, 2015a)
38.	*Bambusa arundinacea*	Poaceae	Bash/Tree		Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)
39.	*Basella alba* L.	Basella-ceae	Pui Shakh/ Herb, climber		Chakma, CHT (Khisha *et al.*, 2012); Hembrom clan of the Santal tribe, Setabganj of Dinajpur District (Moniruzzaman *et al.*, 2015); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)

SL#	Scientific name	Family	Local name	Picture	References
40.	*Berberis vulgaris*	Berberi-daceae	Berberis, Darui/ Shrub		Folk practitioner, Bangladesh (Arumugam *et al.*, 2013)
41.	*Bidens sulphurea*	Asterac-eae	Cosmos/ Herb		Chakma, CHT (Khisha *et al.*, 2012); Chakma, CHT (Rahman *et al.*, 2007)
42.	*Blumea balsamifera*	Asterac-eae	Shial mutra/ Herb		Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015)
43.	*Blumea lacera* *Blumea lancelaria*	Asterac-eae	Kukur muta, Kukur sunga/ Herb		Chakma, CHT (Rahman *et al.*, 2007); Tudu subclan of the Santal, Joypurhat District (Zahan *et al.*, 2013); Chakma, CHT (Khisha *et al.*, 2012)
44.	*Bombax ceiba* L.	Bombac-aceae	Shimul / Tree		Chakma community of Bangladesh (Roy *et al.*, 2008); Garo, Netrokona District (Rahmatullah *et al.*, 2009c); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)
45.	*Bryophyllum pinnatum* *Kalanchoe pinnata*	Crassula-ceae	Pathor kuchi / Herb		Chakma, Rangamati (Tasannun *et al.*, 2015); Folk practitioner of Village Sabgram of Bogra, (Keya and Rahman, 2017); Khumi, Marma and Tripura, Bandarbon (Motaleb *et al.*, 2015)
46.	*Caesalpinia digyna* Rottler	Fabaceae Legumi-nosae	Krishno chura, Radha chura/ Tree		Folk practitioner, Bangladesh (Arumugam *et al.*, 2013); Tripura tribe of Bangladesh (Hossan *et al.*, 2009)

SL#	Scientific name	Family	Local name	Picture	References
47.	*Calotropis gigantea* L.	Asclepi-adaceae	Akando / Herb		Chakma community of Bangladesh (Roy *et al.*, 2008); Tribal people of Madhupur (Garo, Koch and Hajong communities) (Islam *et al.*, 2014); Tripura of Hazarikhil In Chittagong District (Faruque and Uddin, 2011)
48.	*Canna indica* L.	Cannac-eae	Kolaboti / Herb		Garo, Haluaghat, Mymensingh (Ahmed *et al.*, 2017); Santal Tribal, Chapai nobabgonj (Jamila and Rahman, 2016); Santal Tribe, Thakurgaon District (Rahmatullah *et al.*, 2009a)
49.	*Cardiosper-mum halicacabum* L.	Sapind-aceae	Kanfutki, Noafutki, Kopal-futki/ Herb		Chakma community of Bangladesh (Roy *et al.*, 2008), Chakma, CHT (Khisha *et al.*, 2012); Garo, Haluaghat, Mymensingh (Ahmed *et al.*, 2017)
50.	*Carica papaya* L.	Caricac-eae	Pepe/ Shrub		Madhupur, Tribal people (Garo, Koch and Hajong communitie) (Islam *et al.*, 2014); Santal Tribal, Chapai nobabgonj (Jamila and Rahman, 2016); Santal Tribe of Joypurhat (Rahman 2015b)
51.	*Cassia alata* L.	Caesalp-iniaceae	Dad mordon / Shrub		Chak, Chakma, Marma, Murong, Rakhain, and Tonchonga tribes in CHT (Rahmatullah *et al.*, 2010a); Chakma, Khagrachori (Uddin *et al.*, 2015b); Garo, Netrokona (Rahmatullah *et al.*, 2009a)
52.	*Cassia fistula* L.	Fabace-ae Caesalp-iniaceae	Sonali, Sonalu, Bandar lathi / Tree		Chakma, CHT (Uddin *et al.*, 2015b); Tripura of Hazarikhil In Chittagong District (Faruque and Uddin, 2011); Chak, Chakma, Marma, Murong, Rakhain, and Tonchonga tribes in CHT (Rahmatullah *et al.*, 2010a)

SL#	Scientific name	Family	Local name	Picture	References
53.	*Cassia sophera* L.	Caesalp-iniaceae Fabaceae Legumi-nosae	Kalkasu-nde/ Shrub		Chakma, CHT (Rahmatullah *et al.*, 2011b); Rakhain, CHT (Hanif *et al.*, 2009)
54.	*Catharanthus roseus* L. G. Don	Apocyn-aceae	Noyonta-ra/ Herb		Chakma, Khagrachori District (Uddin *et al.*, 2015b); Folk practitioner, Bogra District (Keya and Rahman, 2017); Rakhaing, Cox's Bazar (Uddin *et al.*, 2013)
55.	*Celosia cristata* L.	Amaran-thaceae	Moragphool / Herb		Folk practitioner, Bogra District (Keya and Rahman, 2017); Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)
56.	*Centella asiatica* (L.) Urb.	Umbelli-ferae Apiaceae	Thankuni / Herb		Santal Tribe of Joypurhat District (Rahman 2015a); Tudu subclan of the Santal, Joypurhat District (Zahan *et al.*, 2013); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)
57.	*Cinnamomum tamala* (Buch.Ham.) Nees & Eberm.	Lauraceae	Tejpata / Tree		Santal Tribe, Thakurgaon (Rahmatullah *et al.*,2009a); Madhupur, Tribal people (Garo, Koch and Hajong communities) (Islam *et al.*, 2014); Folk practitioner of Bogra (Keya and Rahman, 2017)
58.	*Cissus quadrangla-ris* L.	Vitaceae	Harajora lota, Haraghunuca/ Herb		Chakma, Rangamati District (Tasannun *et al.*, 2015); Santal Tribe in Rajshahi district (Shahidullah *et al.*, 2009); Santal Tribe, Thakurgaon District (Rahmatullah *et al.*, 2009a)

SL#	Scientific name	Family	Local name	Picture	References
59.	*Citrus grandis* (L.) Osbeck	Rutac-eae	Batabi lebu, Jambur a / Tree		Folk practitioner, Bogra District (Keya and Rahman, 2017); Santal Tribe, Thakurgaon District (Rahmatullah *et al.*, 2009a); Tribal people of Madhupur (Garo, Koch and Hajong communities) (Islam *et al.*, 2014)
60.	*Clerodend-rum indicum* (L.) Kuntze	Verben-aceae	Bamun hati, Kuthap / Herb		Chakma community of Bangladesh (Roy *et al.*, 2008); Folk practitoner, Bangladesh (Rahmatullah *et al.*,2011d); Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015)
61.	*Clerodend-rum viscosum* Vent.	Verben-aceae	Bake pata, viti, Beeg gaas/ Shrub		Folk practitoner, Bangladesh (Rahmatullah *et al.*, 2011c); Rakhain, CHT (Uddin *et al.*, 2015b); Tonchongya, Rangamati District (Islam *et al.*, 2015b)
62.	*Clitoria ternatea* L.	Fabace-ae	Aporaji ta/ Herb, Climbe r		Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Tonchongya, Rangamati District (Islam *et al.*, 2015b); Tribal people of Madhupur (Garo, Koch and Hajong communities) (Islam *et al.*, 2014)
63.	*Coccinia grandis* (L.) Voigt *Coccinia cordifolia* (L.)	Cucurbit-aceae	Telako chu/ Herb, Climbe r		Chakma, Rangamati (Tasannun *et al.*, 2015); Santal Tribal, Chapai nobabgonj (Jamila and Rahman, 2016); Santal Tribe of Joypurhat (Rahman, 2015b)
64.	*Cocos nucifera* L.	Arecac-eae	Narikel / Tree		Folk practitioner, Bangladesh (Arumugam *et al.*, 2013); Hembrom clan of the Santal tribe, Setabganj of Dinajpur District (Moniruzzaman *et al.*, 2015); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)

SL#	Scientific name	Family	Local name	Picture	References
65.	*Colocasia esculenta* (L.) Schott	Araceae	Kochu/ Herb		Deb barma clan of the Tripura tribe of Moulvibazar District (Kabir *et al.*, 2014); Hembrom clan of the Santal tribe, Setabganj of Dinajpur District (Moniruzzaman *et al.*, 2015); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)
66.	*Cordia dichotoma* G.Forst.	Boragin-aceae	Boch, Shelukа, Buhal, Bohubara/ Tree		Chakma, CHT (Khisha *et al.*, 2012); Chakma, CHT (Rahman *et al.*, 2007); Lushai, Bandarban District (Uddin *et al.*, 2015a)
67.	*Costus speciosus* (J. Koenig) Sm.	Costac-eae Zingiber-aceae	Ketoki, Bijuful gach, Rangabishoma, Keo/ Herb		Folk practitioner, Bangladesh (Arumugam *et al.*, 2013); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Tripura, Rangamati District (Faruque and Uddin, 2011)
68.	*Croton bonplandia-num* Baill.	Euphor-biaceae	Bon Tulshi, Chur churi / Herb		Folk practitioner, Bogra District (Keya and Rahman, 2017); Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)
69.	*Curcuma longa* L.	Zingibe-raceae	Holud / Herb		Hembrom clan of the Santal tribe, Setabganj of Dinajpur District (Moniruzzaman *et al.*, 2015); Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)
70.	*Cuscuta reflexa* Roxb.	Convol-vulaceae	Shornolota/ Herb, climber		Rakhain, CHT (Hanif *et al.*, 2009); Garo tribe, Mymensingh (Rahmatullah, *et al.*, 2012); Santal Tribe of Joypurhat District (Rahman 2015a)

SL#	Scientific name	Family	Local name	Picture	References
71.	*Cynodon dactylon* (L.) Pers.	Poaceae	Duglo gach, Durba ghas/ Herb		Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Santal Tribe of Joypurhat District (Rahman 2015a); Santal Tribe, Thakurgaon District (Rahmatullah *et al.*, 2009a)
72.	*Datura metel* L.	Solana-ceae	Dhotur a / Herb		Chakma, Rangamati District (Tasannun *et al.*, 2015); Garo, Netrokona District (Rahmatullah *et al.*, 2009c); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)
73.	*Dillenia indica* L.	Dilleni-aceae	Chalta/ Tree		Chakma, Khagrachori District (Uddin *et al.*, 2015b); Folk practitioner, Bangladesh (Arumugam *et al.*, 2013); Santal Tribe of Joypurhat District (Rahman 2015a)
74.	*Eclipta alba* (L.) Hassk	Asterac-eae	Kalo-keshor, Bringo raj, Kaloke shi/ Herb		Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Santal Tribe of Joypurhat District (Rahman 2015a); Santal Tribe, Thakurgaon District (Rahmatullah *et al.*, 2009a)
75.	*Embelia ribes* Burm.	Myrsina-ceae	Long dhama shak / Shrub		Chakma, CHT (Khisha *et al.*, 2012); Folk practitioner, Bangladesh (Arumugam *et al.*, 2013)
76.	*Euphorbia hirta* L.	Euphorb-iaceae	Bara dudhia, Dudh ghas, Asthm a plant/ Herb		Garo, Haluaghat, Mymensingh (Ahmed *et al.*, 2017); Santal Tribe, Thakurgaon District (Rahmatullah *et al.*,2009a); Sigibe clan of the Khumi tribe of Thanchi subdistrict in Bandarban District (Sarker *et al.*, 2012)

SL#	Scientific name	Family	Local name	Picture	References
77.	*Ficus hispida* L. f.	Morace-ae	Dumur, Joiggi dumur / Tree		Chakma, Khagrachori District (Uddin *et al.*, 2015b); Deb barma clan of the Tripura tribe of Moulvibazar District (Kabir *et al.*, 2014); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)
78.	*Foeniculum vulgare* Mill.	Apiaceae	Mouri , Deihnak / Herb		Lushai, Bandarban (Uddin *et al.*, 2015a)
79.	*Gmelina arborea* L.	Verbena-ceae	Gamhar, Rang / Tree		Lushai, Bandarban District (Uddin *et al.*, 2015a); Chakma, Khagrachori District (Uddin *et al.*, 2015b); Folk practitioner, Bogra District (Keya and Rahman, 2017)
80.	*Heliotropium indicum* L.	Boragin-aceae	Hatisur / Herb		Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Santal Tribe of Joypurhat District (Rahman 2015a); Santal Tribe, Thakurgaon District (Rahmatullah *et al.*, 2009a)
81.	*Hibiscus rosa-sinensis* L.	Malvace-ae	Joba/ Shrub		Rakhain, CHT (Hanif *et al.*, 2009); Tudu subclan of the Santal, Joypurhat District (Zahan *et al.*, 2013); Chakma, Khagrachori District (Uddin *et al.*, 2015a)
82.	*Jatropha gossypifolia* L.	Euphorb-iaceae	Laal-vendar, Lalkundu/ Shrub		Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015);; Santal Tribe, Thakurgaon District (Rahmatullah *et al.*, 2009a); Sigibe clan of the Khumi tribe of Thanchi subdistrict in Bandarban District (Sarker *et al.*, 2012)

SL#	Scientific name	Family	Local name	Picture	References
83.	*Lawsonia inermis* L.	Lythrac-eae	Mehedi / Shrub		Tribal people of Madhupur (Garo, Koch and Hajong communities) (Islam *et al.*, 2014); Santal Tribe of Joypurhat District (Rahman 2015a); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)
84.	*Mikania cordata* *Mikania micrantha*, *Eupatorium odoratum* L.	Compos-itae Asterac-eae	Assam lata, Jarman lota/ Shrub		Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015);; Santal Tribe of Joypurhat District (Rahman 2015a); Sigibe clan of the Khumi tribe of Thanchi subdistrict in Bandarban District (Sarker *et al.*, 2012)
85.	*Moringa oleifera* Lam.	Moringa-ceae	Sojna/ Tree		Tripura (Debbarma *et al.*,2017); Chakma, Khagrachori (Uddin *et al.*, 2015b); Garo, Netrokona (Rahmatullah *et al.*, 2009a);
86.	*Nigella sativa*	Apiaceae	Kali jeera / Herb		Tripura, Chittagong (Faruque and Uddin, 2011)
87.	*Ocimum sanctum* L.	Lamiace-ae	Tulsi/ Herb		Tribal people of Madhupur (Garo, Koch and Hajong communities) (Islam *et al.*, 2014); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Chakma, Khagrachori District (Uddin *et al.*, 2015a)
88.	*Psidium guajava* L.	Myrtace-ae	Peyara/ Tree		Chakma, Khagrachori District (Uddin *et al.*, 2015a); Folk practitioner, Bangladesh (Arumugam *et al.*, 2013); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016)

SL#	Scientific name	Family	Local name	Picture	References
89.	*Scoparia dulcis* L.	Scrophu-lariaceae Plantagi-naceae	Josti modhu/ Herb		Garo, Haluaghat, Mymensingh (Ahmed *et al.*, 2017); Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015);; Tripura, Rangamati District (Faruque and Uddin, 2011)
90.	*Semecarpus anacardium* L.	Anacard-iaceae	Vhallata, Bhalla-taka, Nut / Tree		Folk practitioner, Bangladesh (Arumugam *et al.*, 2013)
91.	*Solanum torvum* Swartz.	Solanac-eae	Tit Begun/ Shrub		Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Folk practitioner, Bogra District (Keya and Rahman, 2017); Khumi, Marma and Tripura, Thanchi Upazila, Bandarban (Motaleb *et al.*, 2015);
92.	*Syzygium aromaticum* L.	Myrtace-ae	Long, Clove / Tree		Garo, Haluaghat, Mymensingh (Ahmed *et al.*, 2017)
93.	*Syzygium cumini* L. *Skuls.*	Myrtace-ae	Jaam / tree		Garo, Netrokona District (Rahmatullah *et al.*, 2009c); Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Santal Tribe, Thakurgaon District (Rahmatullah *et al.*,2009a)
94.	*Vitex negundo* L.	Verbena-ceae	Nishin da, Nirgun di, Samalu / Herb		Santal Tribal, Chapai Nobabgonj District (Jamila and Rahman, 2016); Folk practitioner, Bangladesh (Arumugam *et al.*, 2013); Santal Tribe of Joypurhat District (Rahman 2015a)

Table 2.2: List of medicinal plants used for diabetic treatment in Bangladesh identified through secondary ethnobotanical survey

SL#	Scientific name	Family	Local name	Picture	References
1.	*Abelmoschus esculentus*	Malvacea	Vendi/ Herb		Haque *et al.* 2017
2.	*Abroma augusta*	Sterculiac-eae	Ulotkombol/ Shrub		Haque *et al.* 2017; Uddin *et al.*, 2019
3.	*Abutilon indicum*	Malvaceae	Berela, Kanghi, atibala/ Shrub		Rahmatullah *et al.*, 2010b
4.	*Acacia arabica* *Acacia nilotica*	Mimosaceae Fabaceae	Babla/ Tree		Esha *et al.*, 2012
5.	*Acacia concinna*	Fabaceae Mimosaceae	Bon ritha , Boba lot/ Shrub		Debbarma *et al.*, 2017
6.	*Achyranthes aspera*	Amaranth-aceae	Apang / Herb		Biswas *et al.*, 2014
7.	*Aegle marmelos*	Rutaceae	Bel/ Tree		Mawla *et al.*, 2012; Haque *et al.* 2017
8.	*Agaricus campestris*	Agaricaceae	Mashroom/ Herb		Haque *et al.* 2017

SL#	Scientific name	Family	Local name	Picture	References
9.	*Allium sativum*	Amaryllida-ceae	Rosun/ Herb		Ocvirk *et al.*, 2013; Haque *et al.* 2017
10.	*Andrographis paniculata*	Acanthaceae	Kalomegh, Chirota, Green chirayta,/ Herb		Tasannun *et al.*, 2015; Debbarma *et al.*, 2017; Haque *et al.* 2017
11.	*Argemone mexicana*	Papaverace-ae	Shialkanta/ Herb		Rahmatullah *et al.*, 2011a
12.	*Asparagus racemosus*	Asparagace-ae, Liliaceae	Satamuli / Herb, climber		Rahmatullah *et al.*, 2011a
13	*Averrhoa bilimbi*	Averrhoace-ae	Bilimb/ Tree		Haque *et al.* 2017
14.	*Azadirachta indica*	Meliaceae	Nim, Neem/ Tree		Ocvirk *et al.*, 2013; Haque *et al.* 2017
15.	*Bambusa tulda*	Poaceae	Midinga-bash/ Tree		Roy *et al.*, 2008
16.	*Bacopa monneiri*	Scrophular-iaceae	Noinna shakh/ Herb		Haque *et al.* 2017
17.	*Bombax ceiba*	Bombacac-eae	Shimul/ Tree		Rahmatullah *et al.*, 2011a; Haque *et al.* 2017

SL#	Scientific name	Family	Local name	Picture	References
18.	*Bryophyllum pinnatum* *Kalanchoe pinnata*	Crassulace-ae	Pathorkuchi / Herb		Rahmatullah *et al.*, 2011a
19.	*Carissa carandas*	Apocyana-ceae	Koromcha/ Shrub		Haque *et al.* 2017
20.	*Catharanthus roseus*	Apocyana-cea	Noyontara/ Herb		Haque *et al.* 2017 Islam *et al.*, 2014; Khatun *et al.*, 2013
21.	*Carum copticum*	Apiaceae	Ajwain/ Herb		Mukti *et al.*, 2012
22.	*Caesalpinia digyna*	Fabaceae Leguminos-ae	Mukhoi-chechai/ Tree		Rahmatullah *et al.*, 2009b
23.	*Canna indica*	Cannaceae	Kolaboti / Herb		Esha *et al.*, 2012
24.	*Carica papaya*	Caricaceae	Pepe/ Tree		Haque *et al.* 2017
25.	*Cassia sophera*	Caesalpini-aceae Fabaceae	Kalkasunde/ Shrub		Rahmatullah *et al.*, 2010b
26.	*Centella asiatica*	Apiaceae	Thankuni/ Herb		Ocvirk *et al.*, 2013

SL#	Scientific name	Family	Local name	Picture	References
27.	*Clerodendrum viscosum*	Verbenac-eae	Bake pata, viti, Beeg gaas/ Herb		Rahmatullah *et al.*, 2011a
28.	*Coccinia grandis* *Coccinia cordifolia* *Coccinia indica*	Cucurbita-ceae	Telakochu/ Herb, Climber		Rahmatullah *et al.*, 2010c; Pandey and Mavinkurve, 2014; Roy *et al.*, 2008
29.	*Cocos nucifera*	Arecaceae	Narikel/Tree		Rahmatullah *et al.*, 2009d
30.	*Costus speciosus*	Costaceae Zingibera-ceae	Ketoki, Bijuful gach, Ranga bishoma, Keo/ Herb		Rahmatullah *et al.*, 2009b; Rahmatullah *et al.*, 2011a
31.	*Cuscuta reflexa*	Convolvul-aceae	Shornolota/ Herb, Parasitic weed		Esha *et al.*, 2012; Rahmatullah *et al.*, 2012
32	*Cynodon dactylon*	Poaceae	Duglo gach, Durba ghas/ Herb		Rahmatullah *et al.*, 2009b
33.	*Datura stramonium*	Solanaceae	Dhotura/ Herb		Ocvirk *et al.*, 2013
34.	*Dillenia indica*	Dilleniaceae	Chalta/ Tree		Rahmatullah *et al.*, 2009b; Haque *et al.* 2017
35.	*Eclipta alba*	Asteraceae	Kalo-keshor, Bringoraj, Kalokeshi/ Herb		Esha *et al.*, 2012

SL#	Scientific name	Family	Local name	Picture	References
36.	*Embelia ribes*	Myrsinaceae	Long dhama shak/ Shrub		Rahmatullah *et al.*, 2009b
37.	*Ficus benghalensis*	Moraceae	Bot, Kathali Pata Bot/ Tree		Ocvirk *et al.*, 2013
38.	*Ficus hispida* *Ficus racemosa*	Moraceae	Dumur, Joiggidumur / Tree		Kabir et al., 2014; Haque *et al.* 2017 Ocvirk *et al.*, 2013;
39.	*Foeniculum vulgare*	Apiaceae	Deihnak; Mouri/ Herb		Ocvirk *et al.*, 2013
40.	*Gynura nepalensis*	Asteraceae	Diabetes pata/ Herb		Haque *et al.* 2017
41.	*Heliotropium indicum*	Boraginac-eae	Hatisur/ Herb		Ocvirk *et al.*, 2013
42.	*Hemidesmus indicus*	Apocynace-ae	Anantomul/ Herb		Ocvirk *et al.*, 2013
43.	*Hibiscus rosa-sinensis*	Malvaceae	Jaba/ Shrub		Rahmatullah *et al.*, 2010b

SL#	Scientific name	Family	Local name	Picture	References
44.	*Jatropha gossypifolia*	Euphorbiac-eae	Laal-vendar, Lalkundu/ Shrub		Keya and Rahman, 2017; Rahmatullah *et al.*, 2010c
45.	*Lawsonia inermis*	Lythraceae	Mehedi/ Shrub		Kabir et al., 2014
46.	*Lagerstroemia speciosa*	Lythraceae	Jarul / Tree		Ocvirk *et al.*, 2013
47.	*Mangifera indica*	Anacardiac-eae	Aam / Tree		Ocvirk *et al.*, 2013; Haque *et al.* 2017
48.	*Mikania cordata* *Mikania micrantha* *Eupatorium odoratum*	Compositae; Asteraceae, Compositae	Assam lata/ Herb, climber		Uddin *et al.*, 2019
49.	*Mimosa pudica*	Fabaceae	Lojjaboti, Sada lojjaboti/ Herb		Ocvirk *et al.*, 2013
50.	*Momordica charantia*	Cucurbitac-eae	Korola/ Herb, climber		Ocvirk *et al.*, 2013
51.	*Moringa oleifera*	Moringace-ae	Sojna/ Tree		Islam *et al.*, 2014; Rahmatullah *et al.*, 2009b; Rahman 2015a

SL#	Scientific name	Family	Local name	Picture	References
52.	*Musa paradisiaca*	Musacea	Attya kola/ Tree		Haque *et al.* 2017
53.	*Nigella sativa* *Bunium persicum*	Apiaceae	Kalojera, Kailla jera/ Herb		Ocvirk *et al.*, 2013; Haque *et al.* 2017
54.	*Ocimum sanctum*	Lamiaceae	Tulsi/ Herb		Rahmatullah *et al.*, 2009b; Haque *et al.* 2017
55.	*Psidium guajava*	Myrtaceae	Piyara/ Tree		Rahmatullah *et al.*, 2009b; Biswas *et al.*, 2014
56.	*Phyllanthus acidus*	Euphorbiac-eae	Arboro / Tree		Haque *et al.* 2017
57.	*Phyllanthus emblica*	Phyllantha-ceae	Amloki/ Tree		Ocvirk *et al.*, 2013; Haque *et al.* 2017
58.	*Scoparia dulcis*	Scrophulari-aceae Plantaginac-eae	Josti modhu/ Herb		Esha *et al.*, 2012
59.	*Semecarpus anacardium*	Anacardiac-eae	Bhallata, Bhallataka, Nut/ Tree		Rahmatullah *et al.*, 2009b

SL#	Scientific name	Family	Local name	Picture	References
60.	*Solanum torvum*	Solanaceae	Tit begun/ Shrub		Biswas *et al.*, 2014
61.	*Spondias pinnata*	Anacardiaceae	Amra / Tree		Haque *et al.* 2017
62.	*Syzygium aromaticum*	Myrtaceae	Long, Clove / Tree		Skalli *et al.*, 2019
63.	*Syzygium aqueum* *Syzygium samarangense*	Myrtaceae	Jamrul/ Tree		Rahmatullah *et al.*, 2012; Haque *et al.* 2017
64.	*Swertia chirata*	Gentianaceae	Chirota/ Harb		Ocvirk *et al.*, 2013
65.	*Swietenia mahagoni*	Meliaceae	Mahogany/ Tree		Ocvirk *et al.*, 2013; Haque *et al.* 2017
66.	*Syzygium cumini*	Myrtaceae	Jaam / Tree		Biswas *et al.*, 2014; Esha *et al.*, 2012; Haque *et al.* 2017
67.	*Tamarindus indica*	Fabaceae	Tetul/ Tree		Ocvirk *et al.*, 2013; Haque *et al.* 2017

SL#	Scientific name	Family	Local name	Picture	References
68.	*Terminalia arjuna*	Combretac-eae	Arjun / Tree		Ocvirk *et al.*, 2013; Haque *et al.* 2017
69.	*Terminalia bellirica*	Combretac-eae	Bohera, Jonglee Bohera/ Tree		Ocvirk *et al.*, 2013; Haque *et al.* 2017
70.	*Terminalia chebula* .	Combretac-eae	Horituki/ Tree		Ocvirk *et al.*, 2013; Haque *et al.* 2017
71.	*Tinospora cordifolia*	Menisperm-aceae	Gulancha lota / Shrub, climber		Ocvirk *et al.*, 2013; Haque *et al.* 2017
72.	*Trigonella foenum-graecum*	Fabaceae	Methi/ Herb		Ocvirk *et al.*, 2013; Haque *et al.* 2017
73.	*Vitex negundo*	Verbenace-ae, Lamiaceae	Nirgundi, Nishinda, Samalu/ Shrub		Rahmatullah *et al.*, 2009b
74.	*Withania somnifera*	Solanaceae	Aswagand-ha/ Shrub		Ocvirk *et al.*, 2013
75.	*Zizipus mauritiana*	Rhamnaceae	Boroi/ Tree		Haque *et al.* 2017

References

Ahmed KMI, Bashar MS, Tanny UT, Akter N, Mandal BK, Islam E and Rahmatullah M. 2017. Ethnomedicinal formulations of a Garo tribal healer in Haluaghat, Mymensingh district, Bangladesh. *Journal of Medicinal Plants Studies*, 5(1): 219-223.

Arumugam G, Manjula P and Paari N. 2013. A review: Anti diabetic medicinal plants used for diabetes mellitus. *Journal of Acute Disease,* 2(3): 196-200.

Biswas B, Arafat MY, Hossain MS, Zarnan MS, Khatun Z, Rahmatullah M. 2014. Ethnomedicinal practices Oia village folk medicinal practitioner in Earidpur district, Bangladesh. *American Eurasian Journal of Sustainable Agriculture*, 8: 20-27.

Cakilcioglu U and Turkoglu I. 2010. An ethnobotanical survey of medicinal plants in Sivrice (Elazig-Turkey). *Journal of ethnopharmacology*, 132(1): 165-75.

Debbarma M, Pala NA, Kumar M, Rainer W and Bussmann RW. 2017. Traditional knowledge of medicinal plants in tribes of Tripura in northeast, India. African *Journal of Traditional, Complementary and Alternative Medicines*, 14(4): 156-168.

Esha RT, Chowdhury MR, Adhikary S, Haque KMA, Achariee M, Nurunnabi Ml et al. 2012. Medicinal plants used by tribal medicinal practitioners of three clans of the Chakma tribe residing in Rangamati district, Bangladesh. *American Eurasian Journal of Sustainable Agriculture,* 6: 74-84.

Faruque O and Uddin SB. 2011. Ethnodiversity of Medicinal Plants Used by Tripura Community of Hazarikhil In Chittagong District of Bangladesh, *Journal of Taxonomy and Biodiversity Research*, 5: 27-32.

Guha A and Chakma D. 2015. Traditional Usage of Ethno-Medicinal Plants among the Chakma Community of Tripura, India. *Global Journal of Pharmacology*, 9(4): 377-384.

Hanif A, Hossan MH, Mia MMK, Islam MJ, Jahan R and Rahmatullah M. 2009. Ethnobotanical Survey of the Rakhain Tribe Inhabiting the Chittagong Hill Tracts Region of Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, 3(2): 172-180.

Haque T, Uddin MZ , Hassan MA and Saha ML. 2017. Plants used for the treatment of diabetes in Brahmanbaria, Bangladesh. *Clinical and Experimental Homeopathy,* 4(3): 8-18.

Hossan MS, Hanif A, Khan M, Bari S, Jahan R and Rahmatullah M. 2009. Ethnobotanical survey of the Tripura tribe of Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, 3(2): 253-261.

Islam I, Siddik AB, Hanee U, Guha A, Zaman F, Mokarroma U, Zahan H, Jabber S, Naurin S, Kabir H, Jahan S and Rahmatullah M. 2015b. Medicinal plants of a Tonchongya tribal healer in Rangamati district, Bangladesh. *Journal of Chemical and Pharmaceutical Research,* 7(2): 357-361.

Islam MK, Saha S, Mahmud I, Mohamad K, Awanc K, Uddin SJ, Rahman MM and Shilpi JA. 2014. An ethnobotanical study of medicinal plants used by tribal and native people of Madhupur forest area, Bangladesh. *Journal of Ethnopharmacology*, 151: 921-930.

Jamila M and Rahman AHM. 2016. Ethnobotanical Study of Traditional Medicinal Plants Used by the Santal Tribal Practitioners at the Village Jamtala of Chapai Nawabganj District, Bangladesh. *Journal of Progressive Research in Biology*, 3(1): 142-159.

Kabir MH, Hasan N, Rahman MM, Rahman MA, Khan JA, Hoque NT, Bhuiyan MRQ, Mou SM, Jahan M and Rahmatullah M. 2014. A survey of medicinal plants used by the Deb barma clan of the Tripura tribe of Moulvibazar district, Bangladesh, *Journal of Ethnobiology and Ethnomedicine,* 10(19): 1-28.

Keya MA and Rahman AHMM. 2017. Angiosperm Diversity at the Village Sabgram of Bogra, Bangladesh with Emphasis on Medicinal Plants. *American Journal of Plant Biology,* 2(1): 25-34.

Khatun A, Khan MAA, Rahman MA, Akter MS, Hasan A, Parvin W, Ripa RJ, Moniruzzaman M, Mahal MJ, Rahmatullah M. 2013. Ethnomedicinal usage of plants and animals by folk medicinal practitioners of three villages in Chuadanga and Jhenaidah districts, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture,* 7: 319-339.

Khisha T, Karim R, Chowdhury SR and Banoo R. 2012. Ethnomedical Studies of Chakma Communities of Chittagong Hill Tracts, Bangladesh. *Bangladesh Pharmaceutical Journal*, 15(1): 59-67.

Mawla F, Khatoon S, Rehana F, Jahan S, Shelley MMR, Hossain S, Haq W, Rahman S, Debnath K and Rahmatullah M. 2012. Ethnomedicinal plants of folk medicinal practitioners in four villages of Natore and Raishahi districts, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture,* 6: 406-16.

Moniruzzaman BJJ, Kamal Z, Shoma JF, Tonmoy AJ, Islam MT, Das PR and Rahmatullah M. 2015. Ethnomedicinal practices among the Hembrom clan of the Santal tribe in Setabganj of Dinajpur District, Bangladesh. *Journal of Chemical and Pharmaceutical Research,* 7(6): 76-79.

Motaleb MA, Mamun MMAA, Hossain MK, Alam MK and Sultana M. 2015. Herbal Healing: An old Practice for Healthy Living among Khumi, Marma and Tripura Communities of Thanchi Upazila, Bangladesh. *European Journal of Medicinal Plants*, 5(1): 23-52.

Mukti M, Ahmed A, Chowdhury S, Khatun Z, Bhuiyan P, Debnath K and Rahmatullah M. 2012. Medicinal plant formulations of Kavirajes in several areas of Faridpur and Rajbari districts, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture,* 6(4): 234-247.

Ocvirk S, Kistler M, Khan S, Talukder SH, Hauner H. 2013. Traditional medicinal plants used for the treatment of diabetes in rural and urban areas of Dhaka, Bangladesh-an ethnobotanical survey. *Journal of Ethnobiology and Ethnomedicine,* 9(43): 1-8.

Pandey A and Mavinkurve RG. 2014. Ethno-Botanical usage of Plants by the Chakma Community of Tripura. *Northeast India, Bulletin of Environment, Pharmacology and Life Sciences,* 3(6): 11-14.

Rahman MA, Uddin SB and Wilcock CC. 2007. Medicinal plants used by Chakma tribe in Hill Tracts districts of Bangladesh. *Indian Journal of Traditional Knowledge*, 6(3): 508-517.

Rahman AHMM. 2015a. Ethno-medicinal Survey of Angiosperm Plants Used by Santal Tribe of Joypurhat District, Bangladesh. *International Journal of Advanced Research,* 3(5): 990-1001.

Rahman AHMM. 2015b. Ethno-botanical Survey of Anti-Diabetic Medicinal Plants Used by the Santal Tribe of Joypurhat District, Bangladesh. *International Journal of Research in Pharmacy and Biosciences,* 2(5): 19-26.

Rahmatullah M, Mollik MAH, Azam ATMA, Islam MR, Chowdhury MAM, Jahan R, Chowdhury MH and Rahman T. 2009a. Ethnobotanical Survey of the Santal Tribe Residing in Thakurgaon District, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, 3(4): 889-898.

Rahmatullah M, Mukti IJ, Haque AKMF, Mollik MAH, Parvin K, Jahan R, Chowdhury MH and Rahman T. 2009b. An Ethnobotanical Survey and Pharmacological Evaluation of Medicinal Plants used by the Garo Tribal Community living in Netrakona district, Bangladesh. *Advances in Natural and Applied Sciences,* 3(3): 402-418.

Rahmatullah M, Mukti IJ, Haque AKMF, Mollik MAH, Parvin K, Jahan R, Chowdhury MH and Rahman T. 2009c. An Ethnobotanical Survey and Pharmacological Evaluation of Medicinal Plants used by the Garo Tribal Community living in Netrakona district, Bangladesh. *Advances in Natural and Applied Sciences,* 3(3): 402-418.

Rahmatullah M, Mollik MAH, Paul AK, Jahan R, Khatun MA, Seraj S, Chowdhury AR, Bashar ABMA, Wahab SMR and Rahman MT. 2010a. A comparative analysis of medicinal plants used to treat gastrointestinal disorders in two sub-districts of Greater Khulna Division, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, 4: 22-28.

Rahmatullah M, lslam MR, Kabir MZ, Harun-or-Rashid M, Jahan R, Begum R, et al. 2010b. Folk medicinal practices in Vasu Bihar village, Bogra district, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, 4: 86-93.

Rahmatullah M, Hasan MR, Hossan MS, Jahan R, Chowdhury MH, Serai S, et al. 2010c. A survey of medicinal plants used by folk medicinal practitioners of six villages in Greater Naogaon district, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, 4: 309-325.

Rahmatullah M, Mollik AH, Ali M, Abbas FB, Jahan R, Khatun A, Seraj S and Ahsan S. 2011a. An Ethnomedicinal Survey of Vitbilia Village in Sujanagar Sub-District of Pabna District, Bangladesh. *American-Eurasian Journal of Agricultural & Environmental Sciences*, 10: 106-111.

Rahmatullah M, Chakma P, Paul A, Nasrin D, Ahmed R, Jamal F, Ferdausi D, Akber M, Nahar N, Ahsan S and Jahan R. 2011b. A survey of preventive medicinal plants used by the Chakma residents of Hatimara (south) village of Rangamati district, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture,* 5(1): 92-96.

Rahmatullah M, Jahan M, Azam FMS, Hossan S, Mollik MAH and Rahman T. 2011c. Folk Medicinal Uses of Verbenaceae Family Plants in Bangladesh. *African Journal of Traditional, Complementary and Alternative Medicines,* 8(5):53-65.

Rahmatullah M, Al-Mahmud A, Rahman MA, Uddin MF, Hasan M, Khatun MA, Bashar ABMA, Ahsan S, Mou SM, Begum R and Jahan R. 2011d. An Ethnomedicinal survey Conducted Amongst Folk medicinal practitioners in the two Southern districts of Noakhali and Feni, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture,* 5, 115- 131.

Rahmatullah M, Azam MNK, Khatun Z, Seraj S, Islam F, Rahman MA, Jahan S and Aziz MS. 2012. Medicinal Plants Used For Treatment of Diabetes by the Marakh Sect of

The Garo Tribe Living in Mymensingh District, Bangladesh. *African Journal of Traditional, Complementary and Alternative Medicines,* 9(3): 380-385.

Roy S, Uddin MZ, Hassan MA and M. Matiur Rahman MM. 2008. Medico-botanical report on the Chakma community of Bangladesh. *Bangladesh Journal of Plant Taxonomy,* 15(1): 67-72.

Sarker B, Akther F, Ayman U, Sifa R, Jahan I, Sarker M, Chakma SK, Podder PK, Khatun Z, Rahmatullah M. 2012. Ethnomedicinal investigations among the Sigibe clan of the Khumi tribe of Thanchi sub-district in Bandarban district of Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, 6(4): 378-386.

Shahidullah M, Al-Mujahidee M, Uddin SMN, Hossan MS, Hanif A, Bari S and Rahmatullah M. 2009. Medicinal Plants of the Santal Tribe Residing in Rajshahi District, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, 3(2): 220-226.

Skalli S, Hassikou R and Arahou M. 2019. An ethnobotanical survey of medicinal plants used for diabetes treatment in Rabat, Morocco. *Heliyon*, 5(3): 14-21.

Tasannun T, Ruba FA, Bhuiyan BU, Hossain KM, Khondokar J, Malek I, Bashar ABMA, Rahamatullah M. 2015. Indigenous medicinal Practices: medicinal plants of Chakma tribal medicinal practitioners in Rangamati district. *American-Eurasian Journal of Sustainable Agriculture,* 9(5): 28-35.

Uddin SB, Sultana R, Faruque MO and Ratna R. 2013. Ethnobotanical Study on Medicinal Plants of Rakhaing Indigenous Community of Cox's Bazar District of Bangladesh. *Journal of Pharmacognosy and Phytochemistry,* 2(4): 164-174.

Uddin MS, Chowdhury V, Uddin SB, Mazumder AAM and Howlader MSA. 2015a. Ethnobotanical Survey of Medicinal Plants Used by the Lushai Community in Bandarban District, Bangladesh. *Journal of Advanced Botany and Zoology*, 2(4): 1-9.

Uddin MS, Chakma JJ, Alam KMM and Uddin SB. 2015b. Ethno-medico studies on the uses of plant in the Chakma community of Khagrachari district, Bangladesh. *Journal of Medicinal Plants Studies,* 3(1): 10-15.

Uddin MZ, Mitu FY, Rifat AB and Kaium AA. 2019. Ethnomedicinal study focusing on anti-diabetic plants used by the community living in and around Dhaka. *Bangladesh Journal of Plant Taxonomy,* 26(2): 231-247.

Zahan T, Ahmed I, Omi SI, Naher K, Islam S, Mahmud ASMSHB, Sultana S, Shahriar SMS, Khatun Z and Rahmatullah M. 2013. Ethnobotanical uses of medicinal plants by the Tudu sub-clan of the Santal tribe in Joypurhat district of Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, 7(3): 137-142.

Chapter 3: Phytochemical Study of Aqueous Ultrasound Assisted Extract of Some Plants

Abstract

Phytochemical screening tests are the qualitative study of the extract for identifying the presence of different types of pharmacologically active compounds in the crude extract. In the present study ten medicinal plants were selected on the basis of Tribal and Folk Medicinal Practitioner's behavior in Bangladesh for treating different types of skin diseases and diabetes. A comparatively noble and green extraction procedure named "Aqueous Ultrasound Assisted Extraction Method" was used for the preparation of crude drugs which provided significantly high yield percentages. More than 30% yield was observed from the dried plant's parts of *N. sativa, C. grandis* and *C. papaya* through this extraction procedure. Around 15% extraction was observed from fresh leaves of *A. nilotica*, *A. indica* and *C. reflexa,* however, still higher than the expected (10%) yield. Promising extraction also found from *M. oleifera* (17.42%), *M. cordata* (18%), *C. copticum* (19.14%) and *B. pinnatum* (21.59%). Most of the crude extracts were found acidic in nature except *C. papaya* and *C. grandis*. It was observed that *M. cordata* and *C. copticum* added more basic compounds during the extraction procedure observed by the pH changes of the solution before and after ultrasound treatment. Crude extracts from the listed plants were observed instantly soluble in water whereas freely soluble in methanol, ethanol or even chloroform. From phytochemical study it was observed that aqueous UAE crude extract of *Carica papaya* from dried leaves contain all the tested compounds including tannin, saponin, flavonoid, steroid, terpenoid, glycoside, alkaloid, anthraquinones and vitamin C. It was also observed that presence of 8 phytochemical in *A. nilotica* and *M. oleifera* and 7 phytochemical in *A. indica*, *B. pinnatum*, *C. reflexa* and *N. sativa*. Lowest number of phytochemical 5 and 3 were present in *C. grandis* and *C. copticum* respectively. The above results indicated that the aqueous UAE method was satisfactory and suitable for this present study.

Key Words: Ultrasound, green extraction, tannin, saponin, flavonoid, steroid, terpenoid, glycoside, alkaloid, anthraquinones, vitamin C, yield.

3.1 Introduction

Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs (Ramesh *et al.,* 2013 and Nidavani *et al.,* 2014). Natural products have been an integral part of the ancient traditional medicine systems like Ayurveda, Chinese and Egyptian (Mittal *et al.,* 2014). Since ancient times, plants have been an exemplary source of medicine (Aboelsoud 2010). Plants are a reservoir of diverse kinds of bioactive chemical agents and have often been utilized either in the form of traditional preparations or as pure active principles (Borkataky *et al.,* 2013). The phytochemical constituents of the plants are important because these decide the pharmacological and biological activities (Barua *et al.,* 2014). Studies on the natural product are aimed to determine medicinal values of plants by exploration of existing scientific knowledge, traditional uses and discovery of potential therapeutic agents (Raka *et al.,* 2019). Phytochemical screening is the extraction, screening, and identification of medicinally active compounds found in plant parts e.g., leaves, stems, barks, roots, etc. or whole plant. Flavonoids, alkaloids, tannins, antioxidants, glycosides, etc. are different major compounds having medicinal properties in a plant (Raka *et al.,* 2019).

Ten medicinal plants were selected for the present study named *Azadirachta indica*, *Acacia nilotica*, *Bryophyllum pinnatum*, *Cuscuta reflexa*, *Mikania cordata*, *Carica papaya*, *Coccinia grandis*, *Carum copticum*, *Moringa oleifera* and *Nigella sativa* with strong ethnobotanical evidence of curing skin and diabetic diseases commonly prescribed by the Tribal Medicinal Practitioners (TMPs) and Folk Medicinal Practitioners (FMPs) in Bangladesh. An optimized green extraction method "Aqueous Ultrasound Assisted Extraction (UAE)" (Sadat *et al.,* 2019) was introduced in the present study for preparing crude extract. As per Chemat *et al.* (2012) "Green Extraction is based on the discovery and design of extraction processes which will reduce energy consumption, allows use of alternative solvents and renewable natural products and ensure a safe and high quality extract or product". Generally, the classical extraction methods are fairly simple, standard and continue to have widespread use, but these methods can also be insufficient and slow, consume large quantities of organic solvents, quite laborious, time- or energy-consuming and ultimately, may cause some target molecule degradation. Recent trends in extraction techniques have largely focused on assuring environment and health safety as well as to get high efficiency (yield of extraction) and efficacy (potency i.e., magnitude of

bioactivity or the capacity to produce an effect) of the extract (Gupta *et al.,* 2012). On the basis of recent trends and environment safety UAE method was considered for this study.

Ultrasound Assisted Extraction (UAE) is a comparatively noble extraction method introduced end of the last century. Ultrasonic extraction is very useful for the isolation and purification of bioactive principles (Ishtiaq *et al.,* 2009). The ultrasonic extraction is not only more efficient but also convenient for the recovery and purification of the active ingredients. The sonication-assisted extraction can be carried out at lower temperatures which are favorable for the thermally unstable compounds (Wu *et al.,* 2001). Ultrasound Assisted Extraction (UAE) is comparatively noble approach in the field of bioactive compound extraction from plant materials introduced in the beginning of the current century. UAE involves application of high-intensity, high-frequency sound waves and their interaction with materials. UAE is a potentially useful technology as it does not require complex instruments and is relatively low-cost. It may be used both for small and large scale extraction (Dai and Mumper, 2010). Under ultrasonic action solid and liquid particles are vibrated and accelerated and because of that solute quickly diffuses out from solid phase to solvent (Cares *et al.,* 2009). Several probable mechanisms for ultrasonic enhancement of extraction, such as cell disruption, improved penetration and enhanced swelling, capillary effect, and hydration process have been proposed (Huaneng *et al.,* 2007). If the intensity of ultrasound is increased in a liquid, then it reaches at a point at which the intra-molecular forces are not able to hold the molecular structure intact, so it breaks down and bubbles are created, this process is called cavitation (Baig *et al.,* 2010). Collapse of bubbles can produce physical, chemical and mechanical effects which result in the disruption of biological membranes to facilitate the release of extractable compounds and enhance penetration of solvent into cellular materials and improve mass transfer (Cares *et al.,* 2009; Metherel *et al.,* 2009). Previous studies observed that UAE provides better extraction of the vanillin in shorter time period for different solvents as compared to the soxhlet method (Jadhav *et al.,* 2009). Extraction variables, particularly extraction time and temperature, strongly influence the UAE of total phenolics, antioxidants, and anthocyanins from grape seeds (Ghafoor *et al.,* 2009). Sonication-assisted extraction of ginseng saponins was about three times faster than the traditional extraction method (Wu *et al.,* 2001). UAE gives the highest extraction

yield of some flavonoids in lesser time in comparison to maceration and Soxhlet extraction (Sun *et al.,* 2011).

Phytochemical screening tests are the qualitative study of the extract for identifying the presence of different types of pharmacologically active compounds in the crude extract. In the present study, crude extracts of ten selected plants were prepared by using aqueous UAE method (Toma *et al.,* 2001; Sadat *et al.,* 2019) and the extraction efficiency as well as the phytochemical constituents were measured (Edeoga *et al.,* 2005; Marinova *et al.,* 2005; Harbone 1999; Tiwari *et al.,* 2011).

The objective of the present study was to introduce and justify a green extraction procedure without sacrificing extract quantity and quality.

3.2 Materials and Methods

3.2.1 Plants Name and Parts Used

Ten plants were selected by ethnobotanical survey in the present study are,

(i) Fresh leaves of *Azadirachta indica*
(ii) Fresh leaves of *Acacia nilotica*
(iii) Fresh leaves of *Bryophyllum pinnatum*
(iv) Fresh leaves of *Mikania cordata*
(v) Fresh leaves of *Moringa oleifera*
(vi) Fresh steams of *Cuscuta reflexa*
(vii) Dried leaves of *Carica papaya*
(viii) Dried leaves of *Coccinia grandis*
(ix) Dried seeds of *Carum copticum*
(x) Dried seeds of *Nigella sativa*

3.2.2 Collection of Plant

Leaves of *Azadirachta indica*, *Acacia nilotica*, *Bryophyllum pinnatum*, *Mikania cordata*, *Moringa oleifera, Carica papaya*, *Coccinia grandis* and steam of *Cuscuta reflexa* were collected from the Botanical Pesticide Garden of the Institute of Environmental Science (IES) of University of Rajshahi (RU). Dried seeds of *Carum copticum* and *Nigella sativa* were collected from the grocery shop of the Station Market adjunct to the campus of RU.

3.2.3 Identification of the Plants

All plant's parts were identified by the Taxonomist of the Department of Botany, Rajshahi University. Herbarium sheets of all plants were duly preserved in the Botanical Pesticide and Environmental Microbiology Lab, IES, RU for further reference.

3.2.4 Extraction Procedure

Two methods were used for preparing crude extracts of selected plants parts;

i. Preparation of Crude Extract from Fresh Plant's Parts by UAE Method (Sadat *et al.,* 2019)
ii. Preparation of Crude Extract from dried Plant's Parts by UAE Method (Toma *et al.,* 2001)

Solvent system used in both procedures was distilled water

3.2.4.1 Preparation of Crude Extract from Fresh Plant's Parts by UAE Method

Fresh leaves of *Azadirachta indica*, *Acacia nilotica*, *Bryophyllum pinnatum*, *Mikania cordata*, *Moringa oleifera* and *Cuscuta reflexa* were washed properly by running tap water followed by distilled water for removing debris, and placed in a shade for drying out the surface water. Within 6 hours 50 gm of each plant's was taken in a conventional juice blender machine by addition of 250 ml distilled water i.e., material: water ratio was 1:5 (Sadat *et al.,* 2019). 10-15 minutes blending were sufficient to get fine particle in the leave juice which was easily passed through a 20 mesh size net. A half portion of the juice was filtered by three layer cloth and measured the pH. Rest part of juice was transferred to a 500 ml conical flask and placed in an ultrasonic bath for ultrasound treatment. Total 30 minutes ultrasound treatments were done in the following manner 15 minutes treatment + 15 minutes rest + 15 minutes treatment at 40^0C bath temperature. The mixture was then filtered by three layer of cloth. pH of the filtrate was measured and compare with the pH before ultrasound treatment. Filtrate was dried at 55^0C temperature in a water bath. After drying the amount (efficiency) of the crude extracts were measured. The dried extract was collected in a vial and preserved in the refrigerator (for further use). The process was previously validated in the Botanical Pesticide and Environmental Microbiology Lab, University of Rajshahi.

3.2.4.2 Preparation of Extract from Dried Plant's Parts by UAE Method

Leaves of *Carica papaya* and *Coccinia grandis* and seeds of *Carum copticum* and *Nigella sativa* washed properly by running tap water followed by distilled water to remove debris and placed in a shade for drying. Dried leaves and seeds were further dried at 40^0C in the oven before grinding. Heavy duty grinding machine (Model: Small Scale Rice Grinding Machine) was used for getting fine particles of the dried plant's parts. Larger particles were separated out by using 20 mesh size sieves i.e., particle size less than 841 µm were used in the study. 50 gm powder was taken in a 500 ml conical flask and adds 250 ml distilled water i.e., material: solvent ratio was 1:5 (Toma *et al.,* 2001) and mixed properly by vortex mixture. A half portion of the mixture was filtered by three layer cloth and measured pH of the solution. Rest part of the mixture was transferred to a 500 ml conical flask and placed in an ultrasonic bath for ultrasound treatment. Total 30 minutes ultrasound treatments were done in the following manner 15 minutes treatment + 15 minutes rest + 15 minutes treatment at 40^0C bath temperature. The mixture was then filtered by three layer of cloth. pH of the filtrate was measured and compare with the pH before ultrasound treatment. Filtrate was dried at 55^0C temperature in a water bath. After drying the amount (efficiency) of crude extract was measured. Efficacy was compared by phytochemical screening test and antimicrobial study by disk diffusion method. The dried extract was collected in a vial and preserved in the refrigerator for further use. The process was previously validated in the Botanical Pesticide and Environmental Microbiology Lab, University of Rajshahi.

3.2.5 Calculation of Yield of Crude Extracts

Percent (%) Yield is one of the simplest methods of measuring the extraction efficiency. In the presents study extraction yields were calculated as per slight modified Terblanche *et al.,* (2017) method.

$$\%\, Yield = \left(\frac{Crude\ extract\ g\ x\ 100}{\text{Weight of plant material}}\right) = \frac{(W1\ x\ 100)}{W2}$$

Here, W1= weight of the dried crude extract (after evaporation of solvent);

W2= weight of the starting material (fresh or dried) loaded for extraction.

Expected % yield: Minimum 10% of the Yield was expected in the present study.

3.2.6 Dissolution Status Study of Crude Extract

Dissolution status provides an idea about the polarity characteristics of the crude extracts. Approximately 20 mg crude extract was dissolved in 2 ml distilled water, methanol, ethanol, chloroform, ethylacetate, dichloromethane, dimethyl sulphur oxide (DMSO) for examining the solubility pattern of the extract.

Dissolution pattern were classified as per following scale;

(i)	Instantly soluble	(+++)	Dissolved within 1 minute
(ii)	Freely soluble	(+++)	Dissolved within 1-3 minutes
(iii)	Sparingly soluble	(+++)	Dissolved within 5-10 minutes
(iv)	Insoluble	(+++)	Not dissolved within 10 minutes

3.2.7 Measurement of in-process Extraction Efficiency

All extraction was done through aqueous solvent system. Variation of the pH in aqueous solutions before and after ultrasound treatment was used for measuring the nature (acidic or basic) of the major component extracted in the crude extract.

3.2.8 Phytochemical Screening of Crude Extracts

In the present study phytochemical tests for plant secondary metabolites were performed for alkaloids, anthraquinones, flavonoids, glycosides, saponins, steroids, tannins, terpenoids, and vitamin C as per procedure of Allen (1973) and Harborne (1980) with slight modification (Edeoga *et al.,* 2005; Marinova *et al.,* 2005; Harbone 1999, Tiwari *et al.,* 2011).

3.2.9 Purification of Bioactive Compounds from the Crude Extracts

In the present study successive extraction (Hussain and Hussain, 2012) by non-polar to polar solvents (i.e., Diethylether, Dichloromethane, Chloroform and Ethylacetate) were considered for separation of some desirable bioactive compounds such as Alkaloids, Terpenoids, Flavonoids, Glycosides etc. Four fractions were separated and yield values were calculated after air drying. Each fraction was provided an identical code number and preserved in cold chamber for further uses. Qualitative phytochemical screening of the isolated fraction from each plant was conducted for measuring the purification status of the isolated compounds.

3.3 Results and Discussion

Phytochemical screening is the extraction and identification of medicinally active compounds found in plant parts e.g., leaves, stems, barks, roots, etc. or whole plant. In the present study fresh leaves of *Azadirachta indica*, *Acacia nilotica*, *Bryophyllum pinnatum*, *Mikania cordata*, *Moringa oleifera*, fresh stems of *Cuscuta reflexa,* dried leaves of *Carica papaya*, *Coccinia grandis*, seeds of *Carum copticum* and *Nigella sativa* were extracted by a comparatively noble and green extraction procedure name "Aqueous Ultrasound Assisted Extraction" method. Extraction efficiency of this method was measured on the basis of (i) % yield value and physical observation, (ii) types of compounds in the crude extracts on the basis of pH and (iii) the presence of phytochemical constituents.

3.3.1 Extraction Yield

In the present study plants materials were extracted by an optimized aqueous UAE (Ultrasound Assisted Extraction) method. The percentage yield values of crude extracts were calculated. It was observed that all the selected plants showed significantly high extraction percentage through the aqueous UAE method (Table 3.1). Maximum extraction was observed from dried plants than the fresh plant parts. This may be due to the presence of water content in the fresh plant's parts. It was observed that dried seeds of *N. sativa* (32.50±1.48%), dried leaves of *C. papaya* (31.50±1.10%) and *C. grandis* (30.22±0.46%) showed higher extraction than the fresh leaves of *A. nilotica* (14.3±0.061)*, A. indica* (15.42±0.51%)*, C. reflexa* (16.32±0.84%)*, M. oleifera* (17.42±0.49%) and *M. cordata* (18.00±0.66%). However all the extraction yield found in the present study was significantly higher than the expected 10% yield ($P<0.05$).

Table 3.1: Percent (%) Yield of Plants Extract by Aqueous UAE Method

List of Selected Plants	% Yield (Mean±SEM) (3 extraction)	*P value (H_0: %Yield Expected=10%)
Fresh leaves of *A. indica*	15.42 ±0.51	0.009
Fresh leaves of *A. nilotica*	14.3±0.061	0.019
Fresh leaves of *B. pinnatum*	21.59±0.38	0.001
Fresh stems of *C. reflexa*	16.32±0.84	0.017
Fresh leaves of *M. cordata*	18.00±0.66	0.007
Fresh leaves of *M. oleifera*	17.42±0.49	0.004
Dried leaves of *C. papaya*	31.50±1.10	0.003
Dried leaves of *C. grandis*	30.22±0.46	0.001
Dried seeds of *C. copticum*	19.14±1.40	0.010
Dried seeds of *N. sativa*	32.50±1.48	0.004

*P <0.05 indicate significant difference (here higher than the expected value)

In present study, extraction yield of aqueous UAE crude extracts from fresh leaves of *Azadirachta indica* was found 15.42±0.51% (Table 3.1) which was significantly (p=0.009) higher than the expected yield value (10%). So far this type of extraction was followed first time with this plant material. In that reason comparison was made with the conventional extraction procedure. From literature survey it was observed that Francine *et al.* (2015) found 2.2 gm crude extract from 25 gm fresh leaves (i.e., % yield was 8.8%) and 1.7 gm crude extract from 20 gm dried leaves (i.e., yield was 8.5%) by using conventional aqueous extraction method which was much less than the newly applied method in our present study. They also found 2.1 gm crude extract from 20 gm dried leaves (i.e., % yield was 10.5%) of *A. indica* after conventional ethanol extraction procedure which was also less than the above mentioned extraction method. Latha *et al.* (2015) found 6.5% yield from methanol, 3.3% yield from hexane and 3% yield from ethyl acetate extract of dried leaves. Comparison with Francine *et al.* (2015) and Latha *et al.* (2015) it was observed that aqueous UAE method provided much better extraction than the conventional aqueous, methanol or even ethanol extraction procedure.

Similarly % yield of aqueous UAE crude extracts from fresh leaves of *Acacia nilotica* was found 14.3±0.061% (Table 3.1), which was significantly (p=0.019) higher than the expected yield value (10%). So far this type of extraction was applied first time with this plant material. From literature survey it was observed that Jangade *et al.* (2014) found 9.48% yield from methanol extraction and Howlader *et al.* (2012) found approximately 13.26% yield from ethanol extraction of dried leaves. Comparison with the above two findings, it was observed that aqueous UAE method from fresh leaves provided much better extraction than the conventional methanol (Jangade *et al.,* 2014) and ethanol (Howlader *et al.,* 2012) extraction procedure.

Extraction yield of aqueous UAE crude extracts from fresh leaves of *Bryophyllum pinnatum* was found 21.59±0.38% (Table 3.1) which was significantly (p=0.001) higher than the expected yield value (10%). So far this type of extraction was followed first time for this plant material. Ganju and Ganju (2016) reported yield value 41.1% by soxhlet extraction continued for 8 cycles (6 hrs) using methanol as a solvent. It seems to be better than the present studies. However, Aprioku and Igbe (2017) was found percentage yield (7%) from aqueous cold maceration of leaves of *B. pinnatum*. So it was observed that aqueous UAE method provided a promising extraction than the conventional method.

Extraction yield from aqueous UAE crude extracts from fresh stems of *Cuscuta reflexa* was found 16.32±0.84% (Table 3.1) which was significantly (p=0.017) higher than the expected yield value (10%). So far this type of extraction was followed first time with this plant material. In that reason comparison was made with the conventional extraction procedure. Perveen *et al.* (2013) observed that % yield of *C. reflexa* mainly depends on host plant. Maximum % yield was found in methanol extract (13±0.83%) from *C. reflexa* host plant *Zizyphs jojoba*. From literature survey it was observed that the extraction yield may also depends on the host plant (Perveen *et al.,* 2013), however, comparison with the overall conventional extraction procedure, the present extraction procedure was proved better.

Extraction yield from aqueous UAE crude extracts from fresh leaves of *Mikania cordata* was found 18.00±0.66% (Table 3.1) which was significantly (p=0.007) higher than the expected yield value (10%). So far this type of extraction was followed first time with this plant material. In that reason comparison was made with the conventional extraction procedure. Nayeem *et al.* (2011) found 2.13% yield from the ethanolic extract. Jyothilakshmi *et al.* (2015) observed the percentage yield of

Petroleum ether extract 1.2%, ethyl acetate extract 3.4% and methanolic extract 9.8%. It was observed that, comparatively higher extraction was found in the present extraction procedure.

Extraction yield from aqueous UAE crude extracts from fresh leaves of *Moringa oleifera* was found 17.76±0.59% (Table 3.1) which was significantly (p=0.004) higher than the expected yield value (10%). So far this type of extraction was followed first time with this plant material. In that reason comparison was made with the conventional extraction procedure. From previous studies, it was observed that Mahdi *et al.* (2016) found the percent yields for the 95% ethanol, 50% ethanol and water extracts which extracted at 45^0C for 48 hrs and 1:10 dried powdered leaves to extraction solvent ratio were 25.022, 38.196 and 37.838%, respectively. From the above results it was observed that extraction from fresh leaves was comparatively smaller than the dried leaves. Okumu *et al.* (2017) found 14.23% yield from water and 17.51% yield from methanol (80%) extract. On the basis of previous findings, the present extraction method was proved almost similar to the conventional organic solvent extraction.

Extraction yield from aqueous UAE crude extracts from dried leaves of *Carica papaya* was found 31.50±1.10% (Table 3.1) which was significantly (p=0.003) higher than the expected yield value (10%). Mojulat and Surugau (2018) did similar study and found that the optimal extraction conditions for aqueous extraction were determined to be at 70^0C for 20 minutes where its TPC (total phenolic content) was 9.97±0.47 mg GAE/ml (mg of gallic acid equivalents per ml of sample) and TFC (total flavonoid content) was 2.63±0.52 mg QUE/ml (mg of quercetin equivalents per gram of sample). Fauziah and Wakidah (2019) did similar study using methanol as solvent and found through GC-MS study that the presence of neophytadiene (1.59%), palmitic acid (1.35%) and methyl linolenate (3.33%). Whereas the average percentage yield of alcohol extract of *C. papaya* leaves was found to be 2.6 % w/w (Sinha *et al.,* 2018) and methanol extract was 8.78% (Hossain *et al.,* 2019). Yusha'u *et al.* (2009) found 6.25% yield from ethanol extraction. It was observed that comparatively higher extraction was found in the present extraction procedure.

Extraction yield from aqueous UAE crude extracts from dried leaves of *Coccinia grandis* was found 30.20±0.46% (Table 3.1) which was significantly (p=0.001) higher than the expected yield value (10%). So far this type of extraction was followed first time with this plant material. From literature survey it was found that Packirisamy *et*

al. (2018) reported the percent yields of ethanol extract was 6.87% ± 0.47% which was much smaller than the present extraction procedure. Though Baha and Iwo (2018) found 14.65% yield from the maceration method of ethanol extract but comparatively higher extraction was found in the present extraction method.

Extraction yield from aqueous UAE crude extracts from dried seeds of *Carum copticum* was found 19.14±1.40 % (Table 3.1) which was significantly (p=0.010) higher than the expected yield value (10%). So far this type of extraction was followed first time with this plant material. Khajeh *et al.* (2004) found that the extraction yield, based on hydrodistillation was 2.8% (v/w) and extraction yield based on the SFE (supercritical fluid extraction) varied in the range of 1.0-5.8% (w/w) under different conditions. Herzi *et al.* (2013) observed that the yield obtained using hydrodistillation, SFE, hexane and ethanol Soxhlet extractions were found to be 0.6, 1.6, 40.4 and 21.2–27.4 gm/kg i.e., 0.06%, 0.14%, 4.04% and 2.12-2.74%, respectively. On the basis of the previous conventional study, the present extraction was proved better.

Extraction yield from aqueous UAE crude extracts from dried seeds of *Nigella sativa* was found 32.50±1.48% (Table 3.1) which was significantly (p=0.004) higher than the expected yield value (10%). So far this type of extraction was followed first time with plant material. Bornare *et al.* (2015) found the petroleum ether extract yield 33.0% oil and acetone extract yield 31.4% from *N. sativa* seeds. Shimeles (2017) investigates and compares the effect of solvents, ethanol and hexane in the extraction process and optimization was done in the two factors named as, extraction time and seed to solvent ratio. The maximum yield of extraction for hexane solvent was found to be 35.73% with seed solvent ratio 1:8 and 4 hour and for ethanol 35.51% with the factor of the 1:6 of seed solvent ratio and with extraction time 6 hour. Topcagic *et al.* (2017) observed yield 4.73% in water and 5.28% in methanol by reflux method, 18.73% in hexane, 18.32% in methanol by soxhlet method and 2.22% in hexane and 2.42% in methanol by using ultrasonic method. Compare to the previous study, the present extraction procedure was proved almost similar to the soxhlet method and much better than other method. The aqueous UAE method may be applicable for further laboratory and industrial study.

3.3.2 Physical Observation and Dissolution Pattern of the Crude Extracts

Aqueous UAE (Ultrasound Assisted Extraction) method is a comparatively noble method in the phytochemical extraction field. Ultrasound was used to breakdown the cell wall of the plants. So basically all the compounds inside the cell forcefully mixed with the surrounding solvents. In the present study deionized and slightly worm water was used as solvent. Water is a universal solvent and has capacity to hold both polar and non-polar substances. After filtration water was evaporated by constant heat at 55 ± 5^0C in a conventional water bath. After drying all the crude extracts were found hard and very much sticky to the beaker. No granules or powder from was found. It was very difficult for withdrawing the dried crude extracts from the beaker surface, even metal spatula failed to create any scratch on the dried crude extracts. Small amount of methanol was added for softening and withdrawing the dried extracts. Crude extracts were observed dark-greenish (*A. indica* and *A. nilotica*), yellow-greenish (*B. pinnatum*), brown (*C. reflexa*), dark green (*M. cordata*), greenish (*M. oleifera*), Green yellowish (*C. papaya*), blackish (*C. grandis*), yellow-blackish (*C. copticum*) and blackish color (*N. sativa*) presented in Table 3.2. All crude extracts were instantly soluble in water whereas most of the crude extracts were freely soluble in methanol, ethanol and chloroform but sparingly soluble in DMSO, ethylacetate, dichloromethane (Table 3.3). Solubility profile indicated that the crude extracts contain both polar (hydrophilic) and non-polar (lipophilic) compounds. On the basis of polarity profile and using partition coefficient theory expected compounds may be separated.

Table 3.2: Physical observation after drying of the Crude Extract

Crude Extracts	Optical Observation	Texture
Fresh leaves of *A. indica*	Dark-greenish color	Hard and sticky to the beaker
Fresh leaves of *A. nilotica*	Dark-greenish color	Hard and sticky to the beaker
Fresh leaves of *B. pinnatum*	Yellow-greenish color	Hard and sticky to the beaker
Fresh stems of *C. reflexa*	Brown color	Hard and sticky to the beaker
Fresh leaves of *M. cordata*	Dark green	Hard and sticky to the beaker
Fresh leaves of *M. oleifera*	Greenish color	Hard and sticky to the beaker
Dried leaves of *C. papaya*	Green-yellowish color	Hard and sticky to the beaker
Dried leaves of *C. grandis*	Blackish color	Hard and sticky to the beaker
Dried seeds of *C. copticum*	Yellow-blackish	Hard and sticky to the beaker
Dried seeds of *N. sativa*	Blackish color	Hard and sticky to the beaker

Table 3.3: Dissolution pattern of the dried crude extract

Solvent	Crude Extracts									
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
Distilled water	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Methanol	++	++	++	++	++	++	++	++	++	++
Ethanol	++	++	++	++	++	++	++	++	++	++
Chloroform	++	++	++	+	+	+	++	+	++	++
DMSO	+	+	+	+	+	+	+	+	+	++
Ethylacetate	+	+	+	+	+	+	+	+	+	+
Dichloromethane	+	+	+	+	+	+	+	+	+	+

Here, (I) *A. indica* (II) *A. nilotica* (III) *B. pinnatum*, (IV) *C. reflexa*, (V) *M. cordata*, (VI) *M. oleifera*, (VII) *C. papaya*, (VIII) *C. grandis*, (IX) *C. copticum* and (X) *N. sativa*
+++ Instantly soluble;
++ Freely soluble (1-3 minutes);
+ Sparingly soluble (> 5 minutes and vigorous shaking to dissolve)

3.3.3 Characterization of Crude Extracts based on pH

In the present study aqueous UAE method was used for extraction purpose. The pH of the solution was measured before and after ultrasound treatment on the plant particle and significant changes were observed (Table 3.4). Most of the crude extracts were observed acidic in nature except *C. papaya* and *C. grandis*. Though the crude extracts of *C. papaya* and *C. grandis* were basic in nature, but it was observed that more acidic compounds were added during extraction process. After ultrasound treatment the pH of most plants was reduced. Reduction of pH indicated the addition of more acidic compounds than the basic compounds in the solution during ultrasound treatment. Only *M. cordata* and *C. copticum* were added more basic compounds than the acidic compounds during the extraction process. Highest and lowest changes of pH observed in case of *N. sativa* and *B. pinnatum* respectively.

Table 3.4: Characterization of the Extract

Crude extract	pH before ultrasound treatment	pH after ultrasound treatment	pH difference	P value	Extract type
A. indica	5.79±0.008	5.55±0.006	(-) 0.24±0.009[a]	0.001	Acidic
A. nilotica	5.42±0.006	5.03±0.006	(-) 0.39±0.01[a]	0.001	Acidic
B. pinnatum	4.43±0.007	4.39±0.006	(-) 0.037± 0.003[a]	0.008	Acidic
C. reflexa	5.34±0.017	5.23±0.009	(-) 0.113±0.009[a]	0.006	Acidic
M. cordata	5.78±0.009	6.04±0.015	(+) 0.27±0.023[b]	0.008	Acidic
M. oleifera	5.48±0.006	5.42±0.006	(-) 0.06±0.01[a]	0.027	Acidic
C. papaya	8.14±0.009	7.77±0.009	(-) 0.37 ± 0.015[a]	0.002	Basic
C. grandis	9.11±0.009	8.45±0.009	(-) 0.66±0.006[a]	0.000	Basic
C. copticum	5.34±0.007	5.44±0.006	(+) 0.097±0.009[b]	0.008	Acidic
N. sativa	5.68±0.009	4.27±0.009	(-) 1.41±0.012[a]	0.000	Acidic

[a] Addition of acidic compounds in the solution after ultrasound treatment
[b] Addition of basic compounds in the solution after ultrasound treatment
$p<0.05$ indicated the significance difference

This type of parameter was so far first time used in the extraction process to know the type (acidic or basic) of compounds in the crude extract. This parameter may be used to quantify the amount of crude extract by preparing standard curve by using serial amount of plant material and pH changes during extraction. Further studies required to standardize the method. This parameter may be used for all types of aqueous extraction method.

3.3.4 Presence of Phytochemical in the Selected Plants

Phytochemical screening tests are the qualitative study of the extract for identification of different kinds of pharmacologically active compounds in crude extract. In the present study qualitative study of alkaloid, anthraquinones, flavonoid, glycoside, saponin, steroid, tannin, terpenoid and vitamin C were performed. Results presented in Table 3.5. Aqueous UAE crude extracts of dried *C. papaya* leaves showed the presence of all the tested phytochemicals (Figure 3.1). Aqueous UAE crude extracts of fresh leaves of *A. nilotica*, *B. pinnatum* and *M. oleifera* showed all the tested phytochemicals except saponin, anthraquinone and steroid, respectively. Two kinds of phytochemicals were absent in *A. indica* (anthraquinones and steroid), *C. reflexa* (saponin and steroid) and *N. sativa* (alkaloid and vitamin C). *M. cordata* crude extracts showed all the compounds except tannins, saponin and steroid. Similarly *C. grandis* was observed the absence of tannin, saponin, steroid and anthraquinones. Only three compounds including flavonoid, glycoside and alkaloid were found in the crude extract of *C. copticum*.

Table 3.5: Phytochemical screening of aqueous UAE crude extract

Phytochemical Tests	Crude extract									
	I	II	III	IV	V	VI	VII	VIII	IX	X
1.Alkaloid, (i) Dragendroffs' test	+	+	+	+	+	+	+	+	+	-
(ii) Mayer's test	+	+	+	+	+	+	+	+	+	-
2. Anthraquinones	-	+	-	+	+	+	+	-	-	+
3. Flavonoid (i): by H_2SO_4	+	+	+	+	+	+	+	+	+	+
(ii): by aluminum	+	+	-	+	+	+	+	+	+	+
4. Glycoside	+	+	+	+	+	+	+	+	+	+
5. Saponin	+	-	+	-	-	+	+	-	-	+
6. Steroid	-	+	+	-	-	-	+	-	-	+
7. Tannin	+	+	+	+	-	+	+	-	-	+
8. Terpenoid	+	+	+	+	+	+	+	+	-	+
9. Vitamin C	+	+	+	+	+	+	+	+	-	-

Here, (+) indicated presence of compound, and (–) indicated absence of compound

(I) *Azadirachta indica* (II) *Acacia nilotica* (III) *Bryophyllum pinnatum*, (IV) *Cuscuta reflexa*, (V) *Mikania cordata*, (VI) *Moringa oleifera*, (VII) *Carica papaya*, (VIII) *Coccinia grandis*, (IX) *Carum copticum* and (X) *Nigella sativa*

Figure 3.1: Pictorial representation of phytochemical tests of *Carica papaya*

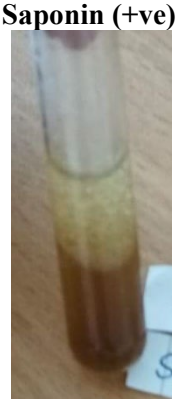
Mother solution

The color of the mother solution was slightly yellowish

Tannin (+ve)

2 ml mother solution + few drop 0.1% ferric chloride. observed blue-black coloration

Saponin (+ve)



After shaken vigorously a stable persistent froth was formed

Flavonoid (i) (+ve)

Mother solution + Conc. H_2SO_4.
The yellow coloration observed which was disappeared on standing

Flavonoid (II) (-ve)

Mother solution + Few drops of 1% aluminium solution.
A yellow coloration was not observed

Steroid (+ve)

Mother solution + 2 ml of acetic anhydride + 2 ml concentrated H_2SO_4. Color change from violet to blue was observed

Terpinoid (+ve)

Mother solution + 2 ml of $CHCl_3$ + 3 ml Conc. H_2SO_4 was added to from a layer. A reddish brown coloration of the inter face was formed

Glycoside (+ve)

Mother solution + 2 ml of glacial acetic acid + one drop of ferric chloride solution. This was underlayed with 1 ml Conc. H_2SO_4. Brown ring of the interface was observed

Alkaloid /Dragendroff test (+ve)

0.5ml of mother solution + 2ml of HCl + 1ml of Dragendroffs reagent.
Orange precipitate found

Alkaloid: Mayer test (+ve)

1.2 ml mother solution + 0.2 ml dilute HCl + 0.1 ml of Mayer's reagent. A yellowish Puff colored precipitate was developed

Anthraquinone (+ve)

5 ml of $CHCl_3$ + 2ml mother solution + 10% ammonia solution. A bright pink color in the aqueous layer was not observed

Vitamin C (+ve)

Mother solution + 1 drop 5% w/v sodium nitroprusside + 1 ml of diluted NaOH + 0.4 ml HCl. The yellow color turns blue

Qualitative phytochemical screen tests showed that aqueous UAE crude extract from fresh leaves of *Azadirachta indica* contain most of the compound including alkaloid, flavonoid, glycoside, saponin, tannin, terpenoid and vitamin-C whereas steroid and anthraquinone were not found in the crude extract. From literature survey it was observed that Galeane *et al.* (2017) did not find anthraquinone, glycosides and alkaloids in *A. indica* aqueous leaves extracts. Similarly Ramadass and Subramanian (2018) did not find tannins, saponins and terpenoids in their aqueous leaves. They also observed that terpenoids was absent in ethanol and chloroform extract of *A. indica* leaves extract. Biu *et al.* (2009) did not found anthraquinones in their aqueous leaf extract. Comparison of the above results, the present extraction procedure was proved quite satisfactory and may be applicable for further use.

Similarly aqueous UAE crude extract from fresh leaves of *Acacia nilotica* contain most of the compound including alkaloid, anthraquinones, flavonoid, glycoside, steroid, tannin, terpenoid and vitamin-C but saponin was absent. Das *et al.* (2016) also did not find saponin in their ethanol extract whereas Howlader *et al.* (2012) did no found flavonoids in their ethanol extract. Jangade *et al.* (2014) reported the absence of saponin and anthraquinones in their methanol extract whereas Ukwuani-Kwaja *et al.* (2016) reported the absence of glycoside and anthraquinones in their hydromethanolic (70% methanol) extract. Comparison with the above result, it may be concluded that the aqueous UAE crude extraction method was more effective than the conventional extraction method.

Qualitative phytochemical tests showed that the aqueous UAE crude extract from fresh leaves of *Bryophyllum pinnatum* contain most of the compound including tannin, saponin, flavonoid, steroid, terpinoid, glycoside, alkaloid and vitamin C but anthraquinone was not found in the crude extract. Biswas *et al.* (2011) reported the presence of alkaloids, glycosides, steroids, gums, flavonoids, saponins, reducing sugars and tannins in ethanolic extract of leaves. Ganju and Ganju (2016) also found alkaloids, flavonoids, saponins, tannins, phenolic compounds in their methanolic extract. Phytochemical analysis of aqueous *B. pinnatum* leaf extract (distilled water for 72 hours by cold maceration) showed the presence of alkaloids, carbohydrates, flavonoids, saponins, terpenoid, tannins/phenols, anthraquinone and steroids (Aprioku and Igbe, 2017). Abhijit (2015) reported absence of flavonoids in the aqueous extract and steroid and flavonoids in petroleum ether extract.

From phytochemical tests it was observed that aqueous UAE crude extract from fresh stems of *Cuscuta reflexa* contain most of the compound including Tannin, flavonoid, terpinoid, glycoside, alkaloid, anthraquinone and vitamin C but saponin and steroid were not found in the crude extract. Vikasa *et al.* (2010) did not found alkaloids in water, methanol, ethylacetate, chloroform and petroleum ether extract of *C. reflexa* steam but saponin and steroid were present most of the extract. Whereas Adhikary *et al.* (2011) reported the presence of alkaloids in methanolic extracts similar to the present study.

From phytochemical tests it was observed that aqueous UAE crude extract from fresh leaves of *Mikania cordata* contain most of the compound including flavonoid, terpinoid, glycoside, alkaloid, anthraquinone and vitamin C, whereas tannin, saponin and steroid were not found in the crude extract. Barua *et al.* (2014) reported the presence of saponins, tannins and steroids in the ethanolic extract. Nayeem *et al.* (2011) reported that the absence of saponins and flavonoid in ethanol extract.

Aqueous UAE crude extract from fresh leaves of *M. oleifera* contained most of the compound including tannin, saponin, flavonoid, terpinoid, glycoside, alkaloid, anthraquinone and vitamin C, only steroid was absent in the crude extract. Ajayi and Fadeyi (2015) reported the absence of steroid in their aqueous leaf extract. Castillo *et al.* (2013) reported absence of tannin and saponin in aqueous and ethanolic leaves extract. Fahal *et al.* (2018) observed that absence of saponin in aqueous extract, tannin in ethanolic extract, saponin in methanolic extract and flavonoids and tannins in chloroform extract pod.

From phytochemical tests it was observed that aqueous UAE crude extract from dried leaves of *Carica papaya* contain most of the compound including tannin, saponin, flavonoid, steroid, terpinoid, glycoside, alkaloid, anthraquinone and vitamin C. Sinha *et al.* (2018) found the presence of alkaloids, glycosides, saponins, phytosterols, tannins and amino acids from the preliminary phytochemical screenings of the aqueous extract of leaves of *C. papaya*. Fresh, green leaves as specified in the works of Ayoola and Adeyeye (2010) and Nguyen *et al.* (2014) reported that ethanolic and chloroform extract contain alkaloids, flavonoids, tannins and saponins, while water extract contains tannins and saponins only. From the above result, it may be concluded that the aqueous UAE crude extraction method was more effective than the conventional extraction method.

Qualitative phytochemical screen tests found that aqueous UAE crude extract from dried leaves of *Coccinia grandis* contain most of the compound including flavonoid, terpinoid, glycoside, alkaloid and vitamin C, whereas tannin, saponin, steroid and anthraquinone were absent. Hossain *et al.* (2014) also reported the absence of tannin and flavonoids in their ethanol leaves extract. However, Packirisamy *et al.* (2018) reported the presence of saponin in ethanol leaf extract. Ashish *et al.* (2011) reported that saponin was absent in methanolic extract of *Coccinia grandis* L. leaves. Tamilselvan *et al.* (2011) did not find saponin and tannin in their aqueous extract which was much better than the ethanol, chloroform and petroleumether extract.

From qualitative phytochemical screen tests it was observed that aqueous UAE crude extract from dried seeds of *Carum copticum* contain very few compounds including flavonoid, glycoside and alkaloid. Most of the tested compound including tannin, saponin, steroid, terpinoid, anthraquinone and vitamin C was absent in the crude extract. Jeet *et al.* (2012) reported absence of saponin in methanol extract, saponin and steroids in acetone, tannin, saponins, alkaloids, glycosides and anthraquinone in chloroform extract and saponin, glycosides, anthraquinone in hexane extract.

Aqueous UAE crude extract from dried seeds of *Nigella sativa* contained most of the compounds including tannin, saponin, flavonoid, steroid, terpinoid, glycoside and anthraquinone. Only alkaloid and vitamin C were absent in the crude extract. Ishtiaq *et al.* (2013) observed that absence of terpenoids and anthraquinone in methanol and ethanol extract whereas tannins, anthraquinone, saponins were absent in chloroform extract and steroids, tannins, anthraquinone, glycoside and saponin were absent in aqueous extract.

Aqueous UAE method was applied on the above ten plants and found satisfactory extraction yield containing maximum number of phytochemical in the crude extracts. This method complied with the green extraction principles proposed by Chemat *et al.* (2012). This extraction method may be suitable for both laboratory and industrial extraction purpose instead of expensive and hazardous extraction method.

3.3.5 Purification of the Bioactive Compounds from the Crude Extracts

In the present study partitioning hypothesis was applied for separating bioactive compounds likely alkaloids, terpenoids, flavonoids and glycosides. Successive fractional separation by using diethylether, dichloromethane, chloroform and ethylacetate were used to separate the crude extracts in the sequence of alkaloid, terpenoid, flavonoid and glycoside presented in the Table 3.6. The qualitative phytochemical studies showed that most of the purified crude extract fractions were almost pure (Table 3.7). Though 100% purity was not found, but sufficient amount was separated by using minimum cost and short time.

It was observed that flavonoid reached compounds (chloroform fraction) and terpenoid reached compounds (dichloromethane) were found 8.4% and 6.4% respectively considered maximum in *A. indica*. Similarly terpenoid compounds (dichloromethane) and flavonoid compounds (chloroform fraction) were found 9% and 7.2%, respectively considered maximum in *A. nilotica*. It was also observed that terpenoid and flavonoid compounds were also found in the crude extracts of *B. pinnatum*, *C. reflexa*, *M. cordata, M. oleifera*, *C. papaya*, *C. grandis* and *N. sativa,* respectively. Diethylether fraction was found negligible in *C. copticum* and *N. sativa* crude extract.

Figure 3.2: Dichloromethane (DM) fraction of *B. pinnatum*

Figure 3.3: Drying stage of DM fraction of *B. pinnatum*

Figure 3.3: Presence of Terpenoid in DM fraction of *B. pinnatum*

Table 3.6: Purification of the crude extracts

Crude Extracts	Fraction*	Volume of Solvent Used (ml)	Weight of Extract after Drying (gm)	% Yield**	Identification Code of the Fraction
Azadirachta indica	Diethylether	20	0.08	1.6	S-AI-DE-1
	Dichloromethane	20	0.32	6.4	S-AI-DM-2
	Chloroform	20	0.42	8.4	S-AI-CL-3
	Ethylacetate	20	0.12	2.4	S-AI-EA-4
Acacia nilotica	Diethylether	20	0.09	1.8	S-AN-DE-5
	Dichloromethane	20	0.45	9	S-AN-DM-6
	Chloroform	20	0.36	7.2	S-AN-CL-7
	Ethylacetate	20	0.14	2.8	S-AN-EA-8
Bryophyllum pinnatum	Diethylether	20	0.06	1.2	S-BP-DE-9
	Dichloromethane	20	0.45	9	S-BP-DM-10
	Chloroform	20	0.23	4.6	S-BP-CL-11
	Ethylacetate	20	0.12	2.4	S-BP-EA-12
Cuscuta reflexa	Diethylether	20	0.03	0.6	S-CR-DE-13
	Dichloromethane	20	0.11	2.2	S-CR-DM-14
	Chloroform	20	0.23	4.6	S-CR-CL-15
	Ethylacetate	20	0.08	1.6	S-CR-EA-16
Mikania cordata	Diethylether	20	0.08	1.6	S-MC-DE-17
	Dichloromethane	20	0.44	8.8	S-MC-DM-18
	Chloroform	20	0.28	5.6	S-MC-CL-19
	Ethylacetate	20	0.12	2.4	S-MC-EA-20
Moringa oleifera	Diethylether	20	0.03	0.6	S-MO-DE-21
	Dichloromethane	20	0.15	3	S-MO-DM-22
	Chloroform	20	0.45	9	S-MO-CL-23
	Ethylacetate	20	0.04	0.8	S-MO-EA-24
Carica papaya	Diethylether	20	0.04	0.8	S-CP-EA-25
	Dichloromethane	20	0.6	12	S-CP-DM-26
	Chloroform	20	0.48	9.6	S-CP-CL-27
	Ethylacetate	20	0.11	2.2	S-CP-EA-28
Coccinia grandis	Diethylether	20	0.015	0.3	S-CC-DE-29
	Dichloromethane	20	0.78	15.6	S-CC-DM-30
	Chloroform	20	0.46	9.2	S-CC-CL-31
	Ethylacetate	20	0.04	0.8	S-CC-EA-32
Carum copticum	Diethylether	20	-	-	S-CG-DE-33
	Dichloromethane	20	0.04	0.8	S-CG-DM-34
	Chloroform	20	0.34	6.8	S-CG-CL-35
	Ethylacetate	20	0.16	3.2	S-CG-EA-36
Nigella sativa	Diethylether	20	-	-	S-NS-DE-37
	Dichloromethane	20	0.47	9.4	S-NS-DM-38
	Chloroform	20	0.5	10	S-NS-CL-39
	Ethylacetate	20	0.1	2	S-NS-EA-40

*Successive fractions were taken in the sequence of non-polar to polar solvent. The objectives of this fraction was to purify the alkaloid reached compounds through diethylether fraction, terpenoid reached compounds through dichloromethane fraction, flavonoid reached compounds through chloroform fraction and glycoside reached compounds through ethylacetate fraction.

** Yield was calculate considering the amount of crude drugs used for separation (here, 5 gm sample was used)

Table 3.7: Qualitative phytochemical identification test of pure fractions

Crude Extracts	Fraction	Phytochemical Tests										
		I	II	III (A)	III (B)	IV	V	VI	VII (A)	VII (B)	VIII	IX
A. indica	S-AI-DE-1	-	-	-	-	-	+	-	+++	+++	-	-
	S-AI-DM-2	-	-	+	+	-	+++	-	+	+	-	-
	S-AI-CL-3	-	-	+++	+++	-	+	+	+	-	-	-
	S-AI-EA-4	-	-	-	-	-	-	+++	-	-	-	-
A. nilotica	S-AN-DE-5	-	-	-	-	-	+	-	+++	+++	-	-
	S-AN-DM-6	-	-	+	+	-	++	-	++	+	-	-
	S-AN-CL-7	-	-	+++	++	-	+	+	+	-	-	-
	S-AN-EA-8	-	-	-	-	-	-	++	-	-	-	-
B. pinnatum	S-BP-DE-9	-	-	-	-	-	+	-	++	++	-	-
	S-BP-DM-10	-	-	+	-	-	+++	-	+	+	-	-
	S-BP-CL-11	-	-	+++	++	-	+	+	+	-	-	-
	S-BP-EA-12	-	-	-	-	-	-	++	-	-	-	-
C. reflexa	S-CR-DE-13	-	-	-	-	-	-	-	++	+++	-	-
	S-CR-DM-14	-	-	+	+	-	++	-	+	+	-	-
	S-CR-CL-15	-	-	+++	++	-	+	+	+	-	-	-
	S-CR-EA-16	-	-	-	-	-	-	++	-	-	-	-
M. cordata	S-MC-DE-17	-	-	-	-	-	+	-	+++	++	-	-
	S-MC-DM-18	-	-	+	+	-	+++	-	+	-	-	-
	S-MC-CL-19	-	-	+++	+++	-	+	-	+	+	-	-
	S-MC-EA-20	-	-	-	-	-	-	+++	-	-	-	-
M. oleifera	S-MO-DE-21	-	-	-	-	-	+	-	+++	++	-	-
	S-MO-DM-22	-	-	+	+	-	++	-	+	+	-	-
	S-MO-CL-23	-	-	+++	++	-	+	+	+	-	-	-
	S-MO-EA-24	-	-	-	-	-	-	++	-	-	-	-
C. papaya	S-CP-EA-25	-	-	-	-	-	-	-	+++	++	-	-
	S-CP-DM-26	-	-	+	+	-	++	-	+	+	-	-
	S-CP-CL-27	-	-	+++	++	-	-	+	+	-	-	-
	S-CP-EA-28	-	-	-	-	-	-	++	-	-	-	-
C. grandis	S-CC-DE-29	-	-	-	-	-	+	-	++	++	-	-
	S-CC-DM-30	-	-	+	+	-	++	-	+	-	-	-
	S-CC-CL-31	-	-	++	++	-	+	+	+	-	-	-
	S-CC-EA-32	-	-	-	-	-	-	++	-	-	-	-
C. copticum	S-CG-DE-33	-	-	-	-	-	-	-	-	-	-	-
	S-CG-DM-34	-	-	+	+	-	-	-	++	++	-	-
	S-CG-CL-35	-	-	++	++	-	-	+	+	-	-	-
	S-CG-EA-36	-	-	-	-	-	-	++	-	-	-	-
N. sativa	S-NS-DE-37	-	-	-	-	-	+	-	-	-	-	-
	S-NS-DM-38	-	-	+	+	-	++	-	-	-	-	-
	S-NS-CL-39	-	-	++	++	-	+	+	-	-	-	-
	S-NS-EA-40	-	-	-	-	-	-	++	-	-	-	-

Here, (I) Tannin (II) Saponin (III:A) Flavonoid test by H_2SO_4 (III:B) Flavonoid test by aluminum solution (IV) Steroid (V) Terpenoid (VI) Glycoside (VII:A) Alkaloid, Dragendroffs' test (VII:B) Alkaloid, Mayer's test (VIII) Anthraquinones (IX) Vitamin C

And,

+++ strong presence of phytochemicals; ++ moderate presence; + trace presence of phytochemicals

3.4 Conclusion

In the present study, ten ethnobotanicals were extracted by an aqueous UAE method which was proved more efficient compare with the conventional extraction procedure. It was observed that the yield of extraction was significantly higher than the expected yield. A remarkable pH changes was observed before and after ultrasound treatment, which established a new parameter to measure the acidic or basic nature of the extracted compounds. It was observed that most of the plants reduces pH after ultrasound treatment, i.e., added more acidic compound in the aqueous solution. Whereas *M. cordata* and *C. copticum* added basic compounds in the aqueous solution during the ultrasound treatments. Dissolution pattern study of the crude extracts indicated that both polar and non-polar compounds were present in crude extracts. *C. papaya* crude extract contained all phytochemicals. Crude extract of the *A. nilotica*, *A. indica*, *B. pinnatum*, *M. oleifera* also contained maximum type of phytochemicals. Only three phytochemicals were found in the crude extract of *C. copticum*. However, from the phytochemical study it may be concluded that listed plants contained a wide variety of medicinally active compounds. Crude extracts were separated in to four fractions by successive separation of non-polar to polar solvents having immiscibility phenomenon to the aqueous solvent. Crude extracts were separated into alkaloid, terpenoid, flavonoid and glycoside. Aqueous UAE method was proved as a suitable green approaches for extraction of important phytochemical from the plant materials. At the same time the fractional separation also proved as a suitable method of purifying desired compounds from the crude drug.

References

Aboelsoud NH. 2010. Herbal medicine in ancient Egypt. *Journal of Medicinal Plants Research,* 4(2): 82-86.

Adhikary P, Roshan KC, Kayastha D, Thapa D, Shrestha R, Shrestha TM and Gyawali R. 2011. Phytochemical Screening and Anti-Microbial Properties of Medicinal Plants of Dhunkharka Community, Kavrepalanchowk, Nepal. *International Journal of Pharmaceutical & Biological Archives,* 2(6): 1663-1667.

Ajayi AO and Fadeyi TE. 2015. Antimicrobial Activities and Phytochemical Analysis of *Moringa oleifera* Leaves on *Staphylococcus aureus* and *Streptococcus species*. *American Journal of Phytomedicine and Clinical Therapeutics,* 3(10): 643-653.

Allen SE, Grinshaw HM, Parkinson JA and Quarmbay C. 1973. Chemical Analysis of Ecological Materials. 1st edition, Blackwell Scientific Publication, London.

Aprioku JS and Igbe I. 2017. Effects of Aqueous *Bryophyllum pinnatum* Leaf Extract on Hematological, Renal and Sperm Indices in Wistar Rats. *Indian Journal of Pharmaceutical Science,* 79(4): 521-526.

Ashish SA, Ujwala RS, Sugandha GC, Deshpande SV, Aniket AG and Sandeep GT. 2011. Analgesic and antipyretic activity of methanolic extract of *Coccinia grandis* L. leaves in experimental animals. *Research Journal of Pharmaceutical Biological and Chemical Sciences,* 2(4): 175-182.

Abhijit R. 2015. Phytochemical screening and in-vitro evaluation of antioxidant, cytotoxicity, antifungal activities of *Kalanchoe pinnata*. *Journal of Science and Arts*, 4(33): 343-350.

Ayoola PE and Adeyeye A. 2010. Phytochemical and nutrient evaluation of *Carica papaya* (Pawpaw) leaves. *International Journal of Recent Research and Applied Studies,* 5: 325-328.

Baha K and Iwo MI. 2018. Immunomodulatory Activity of Ethanolic Extract of Ivy Gourd [*Coccinia grandis* (L.) Voigt] Leaves on Balb/C Mice. *World Journal of Pharmacy and Pharmaceutical Sciences,* 7(8): 60-77.

Baig S, Farooq R and Rehman F. 2010. Sonochemistry and its industrial applications. *World Applied Sciences Journal,* 10: 936-944.

Barua N, Absar N, Paul S, Barua A, Gazi MY, Saha M, Islam MS, Belaly JM. 2014. Invitro phytochemical, cytotoxicity and mineral composition analyses of *Micania cordata* (Bumr.f.) B.L. Robinson leaves. *International Journal of Biosciences,* 5(8): 154-160.

Biswas SK, Chowdhury A, Das J, Karmakar UK and Shill MC. 2011. Assessment of cytotoxicity and antibacterial activities of ethanolic extracts of *Kalanchoe pinnata* Linn. (Family: Crassulaceae) leaves and stems. *International Journal of Pharmaceutical Sciences and Research,* 2(10): 2605-2609.

Biu AA, Yusufu SD and Rabo JS. 2009. Phytochemical screening of *Azadirachta indica* (Neem) (Meliaceae) in Maiduguri, Nigeria. *Bioscience Research Communications*, 21(6): 281-283.

Borkataky M, Kakoty BB and Saikia LR. 2013. Antimicrobial Activity and Phytochemical Screening of Some Common Weeds of Asteraceae Family. *International Journal of Pharmaceutical Sciences Review and Research*, 23(1): 116-120.

Bornare DT, Pathan JY and Ahmed ST. 2015. Extraction and Utilization of *Nigella Sativa* L. Oil in Development of Value Added Cookies. *International Journal of Engineering Research & Technology,* 4(8): 386-389.

Cares MG, Vargas Y, Gaete L, Sainz J, Alarcon J. 2009. Ultrasonically assisted extraction of bioactive principles from Quillaja Saponaria Molina. *Physics Procedia*, 3: 169-178.

Castillo JAT, García SRS, Ávila GCGM, Flores ABL, González EIS, Arzola VEA, Acosta RIT, Sáenz EO, Hernández EO and Díez AG. 2013. *Moringa oleifera*: phytochemical detection, antioxidants, enzymes and antifungal properties. *International Journal of Experimental Botany,* 82: 193-202.

Chemat F, Vian MA and Cravotto G. 2012. Green extraction of natural products: concept and principles. *International Journal of Molecular Sciences,* 13: 8615-8627.

Dai J and Mumper RJ. 2010. Review Plant Phenolics: Extraction, Analysis and their Antioxidant and Anticancer Properties. *Molecules,* 15: 7313-7352.

Das M, Rahman MS, Maniruzzaman M and Uddin MB. 2016. Evaluation of Phytochemical, Invitro Antibacterial and Cytotoxic Properties of Ethanol Extract of *Acacia Nilotica* (L) Leaves. *Indo American Journal of Pharmaceutical Sciences*, 3(12): 1492-1497.

Edeoga HO, Okwu DE and Mbebie BO. 2005. Phytochemical constituents of Nigerian medicinal plants. *African Journal of Biotech,* 46: 685-688.

Fahal EM, Rani AMB, Aklakur MD, Chanu TI and Saharan N. 2018. Qualitative and Quantitative Phytochemical Analysis of *Moringa oleifera* (Lam) Pods. *International Journal of Current Microbiology and Applied Sciences,*7(5): 657-665.

Fauziah L and Wakidah M. 2019. Extraction of Papaya Leaves (*Carica papaya* L.) Using Ultrasonic Cleaner. *Eksakta: Jurnal Ilmu-ilmu MIPA*, 19(1): 35-45.

Francine U, Jeannette U and Pierre RJ. 2015. Assessment of antibacterial activity of Neem plant (*Azadirachta indica*) on *Staphylococcus aureus* and *Escherichia coli*. *Journal of Medicinal Plants Studies,* 3(4): 85-91.

Galeane MC, Martins CHG, Massuco J, Bauab TM and Sacramento LVS. 2017. Phytochemical screening of *Azadirachta indica* A. Juss for antimicrobial activity. *African Journal of Microbiology Research,* 11(4): 117-122.

Ganju K and Ganju E. 2016. Phytochemical analysis of methanolic extract of leaves of *Kalanchoe pinnata* (Lam.). *European Journal of Pharmaceutical and Medical Research,* 3(5): 359-361.

Ghafoor K, Yong-hee C, Jeon J and Jo I. 2009. Optimization of Ultrasound-Assisted Extraction of Phenolic Compounds, Antioxidants, and Anthocyanins from Grape (*Vitis vinifera*) Seeds. *Article in Journal of Agricultural and Food Chemistry,* 57(11): 4988-94.

Gupta A, Naraniwal M and Kothari V. 2012. Modern Extraction Methods for Preparation of Bioactive Plant Extracts. *International Journal of Applied and Natural Sciences*, 1(1): 8-26.

Harbone.1999. Harbone (Ed.), Phytochemical Dictionary, Taylor and Francis, London. 2nd edition.

Harborne JB. 1980. Phytochemical Methods. 2nd edition, Chapman and Hall, London: 288-293.

Herzi N, Bouajila J, Camy S, Romdhane M, Condoret JS. 2013. Comparison of different methods for extraction from *Tetraclinis articulata*: Yield, chemical composition and antioxidant activity. *Food Chemistry*, 141: 3537-3545.

Hossain MA, Hitam S and Ahmed SHI. 2019. Pharmacological and toxicological activities of the extracts of papaya leaves used traditionally for the treatment of diarrhea. *Journal of King Saud University-Science*, 32(2020): 962-969.

Hossain SA, Uddin SN, Salim MA and Haque R. 2014. Phytochemical and Pharmacological screening of *Coccinia grandis* Linn. *Journal of Scientific and Innovative Research*, 3(1): 65-71.

Howlader MSI, Hossain MH, Dey SK, Hira A, Ahmed A. 2012. Evaluation of antinociceptive, antimicrobial and cytotoxic activities of ethanolic extract of *Acacia nilotica* leaves from Bangladesh. *Pharmacology Online,* 2: 73-81.

Huaneng X, Yingxin Z and Chaohong H. 2007. Ultrasonically assisted extraction of isoflavones from stem of *Pueraria lobata* (Willd.) Ohwi and its mathematical model. *Chinese Journal of Chemical Engineering,* 15: 861-867.

Hussain F and Hussain MM. 2012. Cytotoxic effect of crude extracts of *Acacia nilotica*. *International Journal of Pharmaceutical Science and Research*, 3(6): 1652-1655.

Ishtiaq S, Ashraf M, Hayat MQ and Asrar M. 2013. Phytochemical Analysis of *Nigella sativa* and its Antibacterial activity against Clinical Isolates Identified by Ribotyping. *International Journal of Agriculture & Biology,* 15: 1511-1156.

Ishtiaq F, Farooq R, Farooq U, Farooq A, Siddique M, Shah H, Hassan MU and Shaheen MA. 2009. Application of Ultrasound in Pharmaceutics. *World Applied Sciences Journal,* 6(7): 886-893.

Jadhav D, Rekha BN, Gogate PR, Rathod VK. 2009. Extraction of vanillin from vanilla pods: A comparison study of conventional Soxhlet and ultrasound assisted extraction. *Journal of Food Engineering,* 93: 421-426.

Jangade NM, Nagargoje PB and Shirote PJ. 2014. Isolation, Phytochemical and Biological Evaluation of *Acacia nilotica* (L) Willd. Leaf Extract. *International Journal of Pharmacognosy and Phytochemical Research*, 6(2): 179-182.

Jeet K, Devi N, Narender, Sunil T, Lalit S and Raneev T. 2012. *Trachyspermum ammi* (Ajwain): a comprehensive review. *International Research Journal of Pharmacy,* 2(5): 133-138.

Jyothilakshmi M, Jyothis M, and Latha MS. 2015. Antidermatophytic Activity of *Mikania micrantha* Kunth: An Invasive Weed. *Pharmacognosy Research,* 7(1): 20-25.

Khajeh M, Yamini Y, Sefidkon F and Bahramifar N. 2004. Comparison of essential oil composition of *Carum copticum* obtained by supercritical carbon dioxide extraction and hydrodistillation methods. *Food Chemistry,* 86(4): 587-591.

Khatun A, Khan MAA, Rahman MA, Akter MS, Hasan A, Parvin W, Ripa RJ, Moniruzzaman M, Mahal MJ and Rahmatullah M. 2013. Ethnomedicinal usage of plants and animals by folk medicinal practitioners of three villages in Chuadanga and Jhenaidah Districts, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, 7: 319-339.

Ukwuani-Kwaja A, Dabai Y, Samuel R and Odoh J. 2016. Antibacterial Activity of Column Fractions of *Acacia nilotica* Leaves Extract. *The pharmaceutical and chemical journal,* 3(3): 38-42.

Latha RCR, Kavitha S and Sathya S. 2015. Antimicrobial Efficacy of *Azadirachta indica* Leaf Extracts. *International Journal of Science and Nature,* 6 (3): 432-440.

Mahdi HJ, Yousif EM, Khan NAK, Mahmud R, Murugaiyah VAL and Asmawi MZB. 2016. Optimizing Extraction Conditions of *Moringa oleifera* Lam leaf for percent yield, total phenolics content, total flavonoids content and total radical scavenging activity. *International Journal of Advanced Research,* 4(11): 682-695.

Marinova D, Ribarova F and Atanassova M. 2005. Total phenolic and flavonoids in fruits and vegetables. *Journal of Chemical Technology and Metallurgy*, 40(3): 255-260.

Metherel AH, Taha AY, Izadi H and Stark KD. 2009. The application of ultrasound energy to increase lipid extraction throughput of solid matrix samples [flaxseed]. *Prostaglandins Leukot Essent Fatty Acids*, 81: 417-423.

Mittal M, Gupta M, Parashar P, Mehra P and Khatri M. 2014. Phytochemical evaluation and pharmacological activity of *Syzygium aromaticum*: a comprehensive review. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(8): 67-72.

Mojulat MBC and Surugau N. 2018. Effect of extraction conditions of *Carica papaya* leaves aqueous extracts and its resulting infusion with "kelulut" honey to its antioxidant activity. *ASM Science Journal,* 11(2): 75-86.

Nayeem AA, Khatun A, Rahman MS and Rahman M. 2011. Evaluation of phytochemical and pharmacological properties of *Mikania cordata* (Asteraceae) leaves. *Journal of Pharmacognosy and Phytotherapy*, 3(8): 118-123.

Nguyen T, Parat MO, Hodson MP, Shaw PN and Hewavitharana AK. 2014. Investigation of compounds with cytotoxic activity in *Carica papaya* leaves. In: 6th International Conference on Drug Discovery & Therapy: Abstracts. ICDDT 2014: 6th International Conference on Drug Discovery and Therapy, Dubai, United Arab Emirates: 126-12.

Nidavani RB and Mahalakshmi AM. 2014. Teak (*Tectona grandis* Linn.): a renowned timber plant with potential medicinal values. *International Journal of Pharmacy and Pharmaceutical Sciences,* 6(1): 48-54.

Okumu MO, Mbaria JM, Kanja LW, Gakuya DW, Kiama SG and Ochola FO. 2016. Phytochemical profile and antioxidant capacity of leaves of *Moringa oleifera* (Lam) extracted using different solvent systems. *Journal of Pharmacognosy and Phytochemistry,* 5(4): 302-308.

Packirisamy M, Ayyakkannu P and Sivaprakasam M. 2018. Antidiabetic effect of *Coccinia grandis* (L.) Voigt (Cucurbitales: Cucurbitaceae) on streptozotocin induced diabetic rats and its role in regulating carbohydrate metabolizing enzymes. *Brazilian Journal of Biological Sciences*, 5(11): 683-698.

Perveen S, Bukhari IH, Qurat-Ul-Ain, Kousar S & Rehman J. 2013. Antimicrobial, Antioxidant and Minerals Evaluation of *Cuscuta europea* and *Cuscuta reflexa* collected from different hosts and exploring their role as functional attribute. *International Research Journal of Pharmaceutical and Applied Sciences*, 3(5): 43-49.

Raka SC, Mishu SA, Rahman MM and Rahman A. 2019. Assessment of phytochemical, cytotoxic, anthelmintic and thrombolytic activity of *Mikania micrantha* leaves: a new addition in phytomedicine. *Pharmacologyonline,* 1: 246-255.

Ramadass N and Subramanian N. 2018. Study of phytochemical screening of neem (*Azadirachta indica*). *International Journal of Zoology Studies,* 3(1): 209-212.

Ramesh BN, Mahalakshmi AM, Mallappa SH. 2013. Towards a better understanding of updated ethnopharmacology of *Celosia argentea* L. *International Journal of Pharmacy and Pharmaceutical Sciences,* 5(3): 54-59.

Sadat AFMN, Mizan MRB, Sultana A, Rahman MM and Azad MAK. 2018. Comparative study of the Antimicrobial Activity of Methanol Extract and Ultrasound Assisted Water Extract of the Leaves of *Azadirachta indica*. *Rajshahi University Journal of Environmental Science,* 7: 40-47.

Shimeles M. 2017. Extraction and Evaluation of Fixed Oil from Black Cumin (*Nigella sativa*) using Hexane and Ethanol Solvents. A Thesis Submitted to the School of Chemical and Bio-Engineering submitted in partial Fulfillment of the Requirements for the Degree of Master of Science (Process Engineering), School of Chemical and Bio-Engineering, Addis Abeba University.

Sinha RK, Pratap R and Varma MC. 2018. Hypoglycemic Activity of *Carica papaya* Leaf Aqueous Extract in Normal and Diabetic Mice. *International Journal of Advances in Scientific Research and Engineering*, 4(6): 12-16.

Sun Y, Liu Z and Wang J. 2011. Ultrasound-assisted extraction of five isoflavones from *Iris tectorum* Maxim. *Separation and Purification Technology*, 78(1): 49-54.

Tamilselvan N, Thirumalai T, Elumalai EK, Balaji R and David E. 2011. Pharmacognosy of *Coccinia grandis*: a review. *Asian Pacific Journal of Tropical Biomedicine,* 1(2): 299-302.

Topcagic A, Zeljkovic SC, Karalija E, Galijasevic S, Sofic E. 2017. Evaluation of phenolic profile, enzyme inhibitory and antimicrobial activities of *Nigella sativa* L. seed extracts. *Bosnian Journal of Basic Medical Sciences*, 17(4): 286-294.

Terblanche U, Semakalu CC, Mtunzi F and Pillay M. 2017. Screening of Variables Influencing Extraction Yield of *Cotyledon orbiculata*: 2^3 Full Factorial Design. *International Journal of Pharmacognosy and Phytochemical Research,* 9(3): 303-312.

Tiwari P. 2011. Phyto Chemical Screening and Extraction; A Review. *International Pharmaceutical Science,* 1(1): 98-106.

Toma M, Vinatoru M, Paniwnyk L and Mason TJ. 2001. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. *Ultrason Sonochem,* 8: 137-142.

Vikasa K, Sa YP, Udayaa PS, Raja BH, Amara R, Kamaruzb ZM. 2010. Pharmacognostical evaluation of *Cuscuta reflexa* Roxb. *Pharmacognosy Journal,* 2(6): 74-82.

Wu J, Lin LD and Chau FT. 2001. Ultrasound-assisted extraction of ginseng saponins from ginseng roots and cultured ginseng cells. *Article in Ultrasonics Sonochemistry*, 8(4): 347-52.

Yusha'u M, Onuorah FC and Murtala Y. 2009. In-vitro sensitivity pattern of some urinary tract isolates to *Carica papaya* extracts. *Bayero Journal of Pure and Applied Sciences*, 2(2): 75-78.

Chapter 4: Antimicrobial Activity of Some Ethnobotanicals on Bacteria and Fungus Responsible for Skin Diseases

Abstract

Skin diseases are common in Bangladesh. Medicinal plants have been proved to be effective in the treatment of infectious diseases with little or no side effects. On the basis of ethnobotanical study in Bangladesh, ten medicinal plants were selected for the present study. Three bacteria *S. aureus* (Gram +ve), *S. pyogenes* (Gram +ve), *E. coli* (Gram -ve) and three fungi *C. lunata*, *F. chlamydosporum* and *M. furfur* were enrolled in the present study based on their distinct connection on different types of skin diseases. Seven more bacteria including *B. subtilis*, *A. species*, *S. boydii*, *S. flexniri, S. typhi, S. sonnei*, *S. dysenteriae* and 2 fungi including *F. oxysporum* and *C. gloeosporioide* also enrolled in the present study for reference purpose. It was observed that three botanicals (*A. indica, B. pinnatum* and *C. reflexa*) showed high sensitivity (≥16 mm Zone of Inhibition) against *S. aureus*. Similarly *A. nilotica, B. pinnatum, C. reflexa* and *M. oleifera* showed high sensitivity (≥ 16mm) against *S. pyogenes*. Only *B. pinnatum* showed high sensitivity against *E. coli*. *A. indica, A. nilotica, B. pinnatum, C. reflexa, M. oleifera* showed promising antimicrobial activities. However, moderate sensitivity was observed against all the listed fungi in the study. *C. copticum* and *N. sativa* showed very poor sensitivity against all the listed microorganisms. Minimum Inhibitory Concentrations (MIC) were measured against *S. aureus* and *S. pyogenes* and found 60 µg/ml to 100 µg/ml. From cytotoxicity study LC_{50} of the aqueous UAE crude extracts was found safer than the standard antibiotic tetracycline and erythromycin. From the above study *A. indica*, *A. nilotica*, *B. pinnatum*, *C. reflexa* and *M. oleifera* showed promising antimicrobial activities against skin diseases causing pathogens. Results suggested that the above plants may be useful for treating different types of skin diseases caused by pathogens.

Key words: Skin diseases, Cytotoxicity, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Curvularia lunata*, *Fusarium chlamydosporum* and *Malassezia furfur.*

4.1 Introduction

Skin diseases are the most common illness among people around the world. According to the World Health Organization (WHO), a high prevalence of skin diseases is in the general population of developing countries, some diseases being more prevalent in children (Mahbub *et al.,* 2017). There are hundreds of skin conditions that affect humans. The skin disorder has no total cure in modern medicine, however symptoms can be alleviated with various types of drugs including corticosteroids (Mahbub *et al.,* 2017). The overuse of antibiotics has caused the development of resistant strains of bacteria (Davies and Davies, 2010). Antibiotic resistance has become a global concern (Westh *et al.,* 2004). The widespread use of a limited number of antimicrobial agents concomitantly with the reduced arsenal of drugs with antimicrobial function, has led to the development of resistance to drugs that oppose both fungal and bacterial infections, which has been an increasing problem (Rex and Pfaller, 2002; Zida *et al.,* 2016). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug – resistant pathogens (Bandow *et al.,* 2003). In recent years, due to the constant emergence of microorganisms resistant to conventional antimicrobials, challenges come to develop new antimicrobial drugs. Medicinal plants have been proved to be effective in the treatment of infectious diseases with little or no side effects as experienced with synthetic drugs (Iwu 1999).

In the present study ten medicinal plants were selected on the basis of ethnobotanical survey including *Azadirachta indica* (locally known as Neem), *Acacia nilotica* (locally known as Babla), *Bryophyllum pinnatum* (locally known as Pathorkuchi), *Cuscuta reflexa* (locally known as Shornolota), *Mikania cordata* (locally known as Ashamlota), *Moringa oleifera* (locally known as Sojna), *Carica papaya* (locally known as Papaya), *Coccinia grandis* (locally known as Telakochu), *Carum copticum* (locally known as Ajwain) and *Nigella sativa* (locally known as Kalo-jera). These plants have important role of curing skin diseases and prescribed by the TMPs and FMPs practice different region in Bangladesh. The antimicrobial effect of crude extracts were studied against 15 microorganisms (10 bacteria and 5 fungi) whereas 6 (3 bacteria and 3 fungi) solely responsible for skin diseases on human. Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pyogens* have direct association of developing different types of skin diseases (Azman *et al.,* 2016). *Escherichia coli* also possess some distinct influence of skin disease. Similarly fungi including *Malassezia*

furfur, Curvularia lunata and *Fusarium chlamydosporum* have some association of developing different types of skin disease including dandruff, allergy, etc. *Bacillus subtilis* was used as the reference non-pathogenic gram-positive bacteria. Some gram-negative bacteria were also used in the present study due to their antibacterial resistance tendency of some marked antibiotics. These pathogens helped us to identify the efficacy (broad spectrum nature) of the crude extracts.

Bioactive compounds in high doses are (almost) always toxic (Megawati *et al.,* 2012). In vitro killing power of the compounds against the animal organism can be used to screening plant extracts which has bioactivity, and to monitor bioactive fraction during the fractionation and purification. One of organism suitable for toxicity animal testing is a brine shrimp (crayfish) (Meyer *et al.,* 1982). Brine Shrimp Lethality Assay (BSLA) has been applied as an alternative bioassay technique to screen the toxicity of plant extracts (Meyer *et al.,* 1982; McLaughlin *et al.,* 1998; Moshi *et al.,* 2010; Ogugu *et al.,* 2012; Gadir 2012; Solanki and Selvanayagam, 2013; Sharma *et al.,* 2013, Hamidi *et al.,* 2014). It is a simple and inexpensive bioassay used for testing the efficacy of phytochemical present in the plant extracts. This method mainly reported the cytotoxic and antitumor activities of plant extracts (Krishnarajua *et al.,* 2005; Ahmed *et al.,* 2010; Ramachandran *et al.,* 2011; Olowa and Nunez, 2013 and Singh *et al.,* 2015). Several studies proved that there is a good correlation between the results for the lethal concentration that kills 50% of the exposed population (LC_{50}) obtained with the Brine Shrimp Lethality Assay using *A. salina* and the results of the Acute Oral Toxicity Assay in Mice (Parra *et al.,* 2001; Arlsanyolu and Erdemgil, 2006; Hamidi *et al.,* 2014). The Brine Shrimp Lethality Assay has been developed for toxicity testing of various concentrations of pure compounds and crude plant extracts. The results for the toxicity of tested herbal preparations gained by using crude plant extracts were more accurate than by testing pure compounds isolated from the same plant. This difference in the toxicity results is probably due to the chemical complexity of the crude or partially purified extract, which seemed to be essential for the bioavailability of the active constituents of the examined plant (Hamidi *et al.,* 2014).

The prime objectives of the present study was to conduct antibacterial, antifungal and cytotoxicity study of the selected plants for identifying the pharmacological efficacy among different skin disease causing pathogens and the safety profile on mammal.

4.2 Materials and Methods

4.2.1 Plants Used in the Research

Ten plants were selected through ethnobotanical survey in the present study on the basis of their skin disease curing properties:

i. Fresh leaves of *Azadirachta indica*
ii. Fresh leaves of *Acacia nilotica*
iii. Fresh leaves of *Bryophyllum pinnatum*
iv. Fresh leaves of *Mikania cordata*
v. Fresh leaves of *Moringa oleifera*
vi. Fresh stems of *Cuscuta reflexa*
vii. Dried leaves of *Carica papaya*
viii. Dried leaves of *Coccinia grandis*
ix. Dried seeds of *Carum copticum*
x. Dried seeds of *Nigella sativa*

4.2.2 Plant Collection

Most of the plant's parts were collected from the Botanical Pesticide Garden, University of Rajshahi. The seeds of *C. copticum* and *N. sativa* were collected from the local market of University area.

4.2.3 Identification of Plants

All plant's parts were identified by the Taxonomist, Department of Botany, University of Rajshahi. Herbarium sheets of all plants were duly preserved in the Botanical Pesticide and Environmental Microbiology Lab, IES, RU for further reference.

4.2.4 Preparation of Crude Extracts

Crude extracts from selected plants were prepared by;

i. Aqueous UAE from dried plants parts (Toma *et al.,* 2001). Extraction procedure described in the Section 3.2.4.1 in Chapter 3.
ii. Aqueous UAE from fresh plants parts (Sadat *et al.,* 2019). Extraction procedure described in the Section 3.2.4.2 in Chapter 3.

4.2.5 Microorganisms Tested in the Study

Total 15 microorganisms were tested, 6 microorganisms had direct association for skin diseases and 9 were used for as reference.

Gram positive bacteria

(i) *Staphylococcus aureus**

(ii) *Streptococcus pyogens**

(iii) *Bacillus subtilis*

Gram negative bacteria

(i) *Escherichia coli**

(ii) *Agrobacterium species*

(iii) *Shigella boydii*

(iv) *Shigella dysenteriae*

(v) *Shigella flexniri*

(vi) *Salmonella typhi*

(vii) *Shigella sonnei*

Fungi

(i) *Malassezia furfur**

(ii) *Fusarium chlamydosporum**

(iii) *Curvularia lunata**

(iv) *Fusarium oxysporum*

(v) *Collectotrichum gloeosporioides*

* Responsible for different types of skin diseases (Azman *et al.,* 2016; Coimbatore *et al*., 2015; Hay 2007; Gaitanis *et al*., 2012)

4.2.5.1 Sources of Microorganisms

All bacterial and fungal pure strains were collected form, Microbiology Laboratory, Department of Biochemistry and Molecular Biology, University of Rajshahi.

4.2.6 Method of Sensitivity Study

Disk diffusion method (Baker *et al.,* 1993 and Mukhtar and Tukur, 2000) was used in this study. Nutrient agar and potato dextrose agar was used for sub-culturing bacteria at 37^0C and fungus at 25^0C respectively. The degree of sensitivity of the organisms to the extracts was determined by measuring diameter of visible zones of inhibition to the nearest millimeter with respect to each isolate and extract concentration. Minimum Inhibitory Concentration, (MIC) of the extracts was determined using the tube dilution method (Baker *et al.,* 1993) against selected pathogens.

Sensitivity level:

From literature review it was found that 0 mm zone of inhibition – indicates no effects, less than 8mm zone of inhibition – indicates low sensitivity and more than 8mm zone of inhibition – indicates high sensitivity (Mukhtar and Okafor, 2002). However, in the present antimicrobial activity was evaluated by measuring zone of inhibition by using Hi-media zone scale i.e., inhibition zones with diameter less than 12 mm were considered as having low antimicrobial activity, diameters between 12 mm and 16 mm were considered moderately active and these with 16mm were considered highly active (Latha *et al.,* 2015).

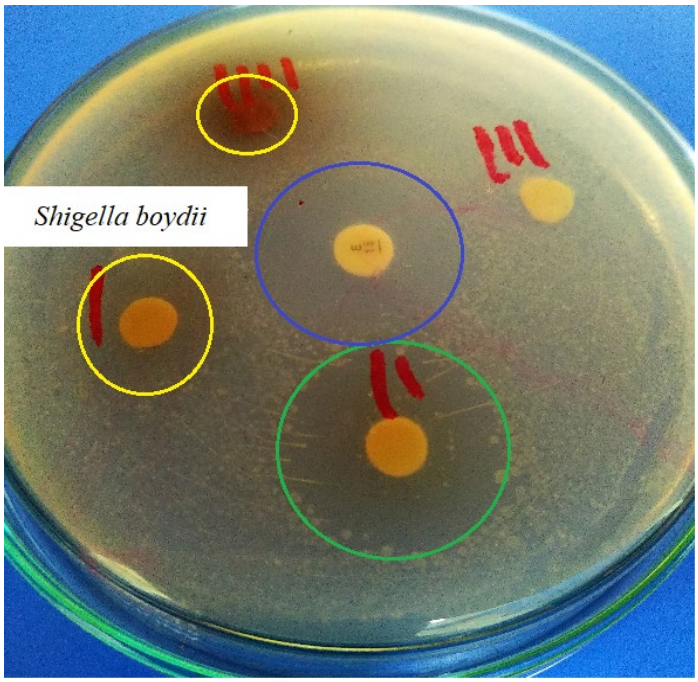


Figure 4.1: Measuring zone of inhibition

4.2.7 Cytotoxicity Study by Brine Shrimp Lethality Bioassay

Eggs of *Artema salina* (about 10 mg) were placed into a conical shaped or beaker shaped vessel (Figure 4.2) hatching chamber containing sea water (instant sea water was prepared by dissolving 38 gm/L NaCl and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 48 hrs (Krishnarajua *et al.,* 2005). After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. Experiments were conducted along with control (vehicle treated) with different concentrations. 80 mg equivalent weight of crude extracts (for sample) and standard drugs erythromycin or tetracycline tablet powder (for standard) was weighed into sterile vials and dissolved in 1.0 ml sterile distilled water. Serial dilution was made for getting 400, 200, 100, 50, 25, 12.50, 6.25, 3.215, 1.56 and 0.78 µg/ml concentrations respectively. Ten nauplii were drawn through a glass capillary (or Pasteur's pipette) and placed in each vial containing 4.5 ml of brine solution. In each experiment, 1 ml of the plant extract was added to 4 ml of brine solution and maintained at room temperature for 24 hr under the light and surviving larvae were counted. The vial used for the control experiment was stained with 1 ml sterilized distilled water. The volume of each vial was adjusted to 10 ml with sea-water. Twenty- four hours after the inoculation, the number of surviving shrimp larvae at each dosage was counted and recorded. LC_{50} values were determined by log concentration method (Saha *et al.*, 2014) and compared with the standard drugs (Figure 4.3) for measuring safety profile.

Figure 4.2: Hatching Eggs of *Artema salina*

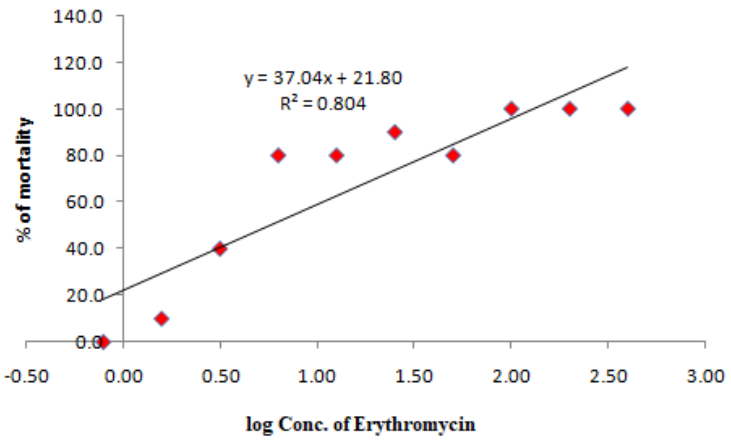


Figure 4.3: LC_{50} of Erythromycin (Here, LC_{50} = 5.74 µg/ml)

4.3 Results and Discussion

Antibacterial, antifungal and cytotoxicity studies were performed by using aqueous UAE crude extracts of the fresh leaves of *Azadirachta indica*, *Acacia nilotica*, *Bryophyllum pinnatum*, *Mikania cordata*, *Moringa oleifera*; fresh stream of *Cuscuta reflexa*; dried leaves of *Carica papaya* and *Coccinia grandis*; dried seeds of *Carum copticum* and *Nigella sativa*. The prime objective of the antimicrobial study was to assess the potency against skin diseases causing pathogen. Brine shrimp lethality (cytotoxicity) studies were also performed for comparing the safety profile of the crude extracts with the standard antibiotic.

4.3.1 Antibacterial Activity of Extracts against Skin Disease Causing Bacteria

Antimicrobial sensitivity studies of crude extracts were performed against 10 bacteria including *Staphylococcus aureus*, *Streptococcus pyogens*, *Escherichia coli*, *Bacillus subtilis*, *Agrobacterium species*, *Shigella boydii*, *Shigella flexniri, Salmonella typhi, Shigella sonnei* and *Shigella dysenteriae.* Among them *S. aureus* and *S. pyogens* were widely known bacteria responsible for creating different types of skin disease. It was also observed that *E. coli* had some association of skin disease. In the present study most of the crude extracts were observed somewhat sensitive against the listed bacteria. Crude extracts of *Azadirachta indica*, *Acacia nilotica, Cuscuta reflexa, Moringa oleifera, Bryophyllum pinnatum, Coccinia grandis* and *Carica papaya* showed better sensitivity over all the listed bacteria in the study (Table 4.1.1 and Table 4.1.2). *Mikania cordata*, *Carum copticum* and *Nigella sativa* showed comparatively poor sensitivity over all bacteria. Poor antimicrobial sensitivity was also observed in case of standard antibiotic tetracycline. Erythromycin showed slightly better antimicrobial activity specially *S. pyogenes* and *S. aureus*. We had three pathogenic bacteria *S. pyogenes, S. aureus* and *E. coli* responsible for different types of skin diseases. *Azadirachta indica*, *Acacia nilotica, Bryophyllum pinnatum* showed excellent sensitivities against all three bacteria whereas *Cuscuta reflexa* and *Moringa oleifera* showed sensitivity against two except *E. coli*. *Mikania cordata, Carica papaya* and *Coccinia grandis* showed moderate sensitivity over all these three bacteria. Standard antibiotic erythromycin (10 µg/disc) showed better activity than the tetracycline (30 µg/disc) against most of the enrolled microorganisms in the study.

Table 4.1.1: Antimicrobial activity of aqueous UAE crude extracts against bacteria

Plant extract (300 µg/ml)	Zone of Inhibition (mm)				
	*Staphylococcus aureus**	*Streptococcus pyogenes**	*Escherichia coli**	*Bacillus subtilis*	*Agrobacterium species*
A. indica	16.00±1.73	14.67±1.45	14.67±0.67	11.33±0.88	12.67±0.58
A. nilotica	15.33±0.33	17.67±0.67	13.00±0.58	15.00±0.58	13.67±0.88
B. pinnatum	17.00±0.58	18.00±0.58	16.67±0.33	16.67±0.33	16.67±0.33
C. reflexa	17.67±0.67	16.33±0.58	14.00±0.58	14.00±0.58	12.33±0.33
M. cordata	14.00±0.58	12.33±0.33	13.67±0.33	8.33±0.88	12.33±0.67
M. oleifera	14.33±0.33	17.67±0.67	15.67±0.67	13.67±1.67	18.67±0.33
C. papaya	12.67±0.33	12.67±0.67	10.33±0.67	13.67±1.67	14.67±0.67
C. grandis	11.33±0.33	10.00±0.58	10.67±0.33	12.67±0.33	11.33±0.33
C. copticum	8.33±0.33	8.33±0.33	8.33±0.33	9.33±0.88	8.33±0.67
N. sativa	8.33±0.67	8.67±0.33	10.67±0.33	9.00±0.58	8.33±0.33
Erythromycin	16.67±0.88	19.67±2.19	12.00±1.00	11.67±0.88	8.33±0.33
Tetracycline	8.33±0.33	8.33±0.33	8.33±0.33	8.33±0.33	8.33±0.33

* Bacteria responsible for different types of skin diseases

Table 4.1.2: Antimicrobial activity of aqueous UAE crude extracts against bacteria

Plant extract (300 µg/ml)	Zone of Inhibition (mm)				
	Shigella flexniri	*Salmonella typhi*	*Shigella boydii*	*Shigella sonnei*	*Shigella dysenteriae*
A. indica	15.33±1.45	12.67±0.88	11.33±0.88	11.00±0.58	11.33±0.88
A. nilotica	12.33±0.67	14.00±0.58	12.33±0.33	16.67±0.33	12.33±0.33
B. pinnatum	13.33±0.33	14.00±0.58	13.67±0.33	12.33±0.67	14.00±0.58
C. reflexa	15.33±0.67	16.67±0.33	14.00±0.58	16.67±1.33	14.00±0.58
M. cordata	9.67±0.58	14.67±0.33	12.33±0.33	10.67±0.33	10.67±0.88
M. oleifera	16.67±0.33	14.67±1.33	19.67±1.33	14.33±1.33	12.33±0.33
C. papaya	10.67±0.33	15.33±0.33	12.67±1.33	13.33±0.33	14.33±0.33
C. grandis	9.33±0.33	13.33±0.33	9.67±0.33	9.33±0.67	13.33±0.67
C. copticum	8.67±0.58	8.67±0.33	8.33±0.33	9.33±0.33	10.33±0.33
N. sativa	8.33±0.33	8.67±0.33	9.00±0.58	9.67±0.33	10.00±0.33
Erythromycin	7.67±0.33	8.00±0.58	18.00±0.58	10.00±1.15	17.67±0.67
Tetracycline	11.33±0.33	8.33±0.33	11.67±0.33	10.33±0.33	10.33±0.33

Aqueous UAE crude extract of *A. indica* from fresh leaves showed moderate sensitivity against common skin disease causing bacteria *Streptococcus pyogenes* (14.67±1.45), *Escherichia coli* (14.67±1.45) and *Staphylococcus aureus* (16.00±1.73). Promising sensitivity was also observed against the listed reference bacteria. From previous study it was observed that dichloromethane leaf extract of *A. indica* exhibited maximum inhibitory activity followed by ethanolic and aqueous extracts against various organisms tested including *S. aureus*, *S. pyogenes*, *E. coli* etc. whereas chloroform extract and petroleum ether extract was not effective against any of the organism tested (Rajasekaran *et al.,* 2008). Cheenickal and Mendez (2017) observed that the methanolic extracts (11 mm) showed a low zone of inhibition when compared to the ethanol extracts (17 mm) in *Staphylococcus aureus* but aqueous extracts did not show any anti microbial activity. Francine *et al.* (2015) found that the ethanol leaf extracts were more effective compared to aqueous leaf extracts in both dry and fresh leaves. From the above study it was observed that the crude extract of *A. indica* fresh leaves may be a suitable herbal medicine for curing different types of pathogenic skin diseases.

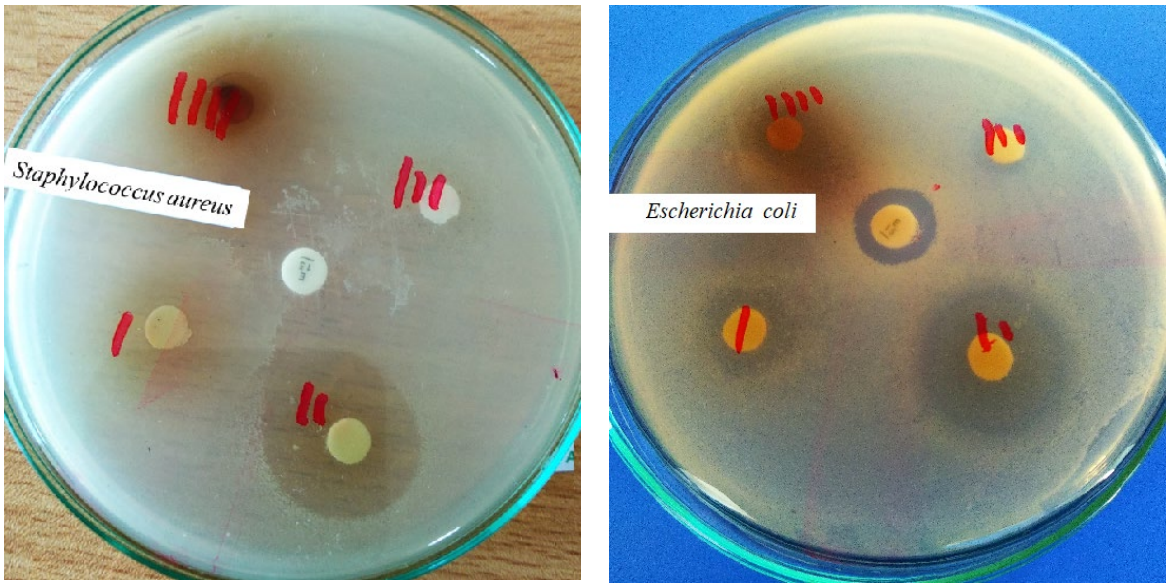


Figure 4.4: Antibacterial study of crude extracts of (I) *A. indica* (300 µg/disc); (II) *B. pinnatum* (300 µg/disc); (III) *C. grandis* (300 µg/disc) and (IIII) *C. papaya* (300 µg/disc) with standard antibiotic erythromycin (15 µg/disc) in the middle against *Staphylococcus aureus* and *Escherichia coli***.**

Aqueous UAE crude extract from fresh leaves of *A. nilotica* showed high sensitivity against common skin disease causing bacteria *Streptococcus pyogenes* (17.67±0.67) and *Staphylococcus aureus* (15.33±0.33) similar to the sensitivity study of erythromycin. Though crude drug showed moderate activity on *Escherichia coli* (13.00±0.58) but higher than the both standard antibiotic included in the present study. Promising sensitivity was also observed against the listed reference bacteria. Howlader *et al.* (2012) and Bashir *et al.* (2014) observed that the ethanol crude extract of *A. nilotica* leaves showed high sensitivity against *S. aureus, S. pyogenes* and *E. coli*. Das *et al.* (2016) found ethanolic extract of *A. nilotica* showed sensitivity against *E. coli, S. dysenteriae, S. boydii, S. sonnei* and *S. flexneri* was showed almost similar antimicrobial action observed in the present study. Hot aqueous extract of *A. nilotica* leaves produced dose dependent zone of inhibition against *Klebsiella pneumoniae, Pseudomonas aeruginosa, E. coli, Bacillus cereus, Staphylococcus aureus*, and *Streptococcus uberis* (Sharma *et al.,* 2014).

Aqueous UAE crude extract of *Bryophyllum pinnatum* from fresh leaves showed moderate sensitivity against skin disease causing bacteria *Streptococcus pyogenes* (18.00±0.58), *Escherichia coli* (16.00±0.33) and *Staphylococcus aureus* (17.00±0.58) which was quite high. Moderate to high sensitivities were also observed against all the listed reference bacteria. Obioma *et al.* (2017) the methanol extracts of *B. pinnatum* plants had a higher antimicrobial activity compared with their ethanol extracts, while the hot water extracts had no antimicrobial activity against *S. aureus* and *E. coli*. Akinsulire *et al.* (2007) observed various antimicrobial activities of this plant extract in different solvents including aqueous extract showed antimicrobial sensitivity against *S. aureus, B. subtilis, E. coli, P. aeruginosa* and *S. flexneri* and confirmed its use traditionally in treating antimicrobial infections like wound infection, sore, ear infection, abscesses and dysentery. Biswas *et al.* (2011) reported the ethanolic extract exhibited significant zone of inhibition against *B. Subtilis, S. aureus* and *E. coli*.

Aqueous UAE crude extract of *Mikania cordata* from fresh leaves showed moderate sensitivity against skin disease causing bacteria *Staphylococcus aureus* (14.00±0.58), *Streptococcus pyogenes* (12.33±0.33) and *Escherichia coli* (13.67±0.33). Moderate to high sensitivities were also observed against all the listed reference bacteria. Jyothilakshmi *et al.* (2016) found 15 mm and 17 mm zone of inhibition against *S. aureus* and *S. pyogenes* respectively by using ethyl acetate extract. Similarly 14 mm and 13 mm zone of inhibition were found against *S. aureus* and *S. pyogenes*

respectively by using ethanol extract (Nayeem *et al.,* 2011). But Baral *et al.,* (2011) did not found any sensitivity of hexane, ethyl acetate, acetone, chloroform, methanol and aqueous extract against *S. aureus* and *E. coli* by using 200 mg/ml whereas moderate to high sensitivities were observed against *F. oxysporum.* Andriani *et al.* (2018) observed that ethanol extract showed strong antimicrobial activity against *S. aureus* and moderate antimicrobial activity against *E. coli*. Matawali *et al.* (2016) did not found any sensitivity against *S. aureus, E. coli, S. typhii* by use in aqueous leaf extract. Borkataky *et al.* (2013) found 19±1 mm against *S. aureus* and 10±1 mm against *E. coli*. The cytotoxic and antimicrobial activities of *M. cordata* leaves have been reported before (Ali *et al.,* 2011).

Aqueous UAE crude extract of *Moringa oleifera* from fresh leaves showed moderate sensitivity against skin disease causing bacteria including, *S. pyogenes* (17.67±0.67), *Escherichia coli* (15.67±0.67) and *S. aureus (*14.33±0.333) which was quite high. Moderate to high sensitivities were also observed against all the listed reference bacteria. Ajayi and Fadeyi (2015) reported that aqueous, petroleum ether and ethanolic extract of *M. oleifera* leaves showed sensitivity activities on *S. aureus* and *Streptococcus* species. However, aqueous extract showed negligible activities compared to the petroleum ether and ethanol extract. Nkya *et al.* (2014) reported leaf extract of *M. oleifera* was comparatively safer than the steam and root extract obtained from brine shrimp cytotoxicity study. Kalpana *et al.* (2013) found satisfactory antimicrobial activities against *E. coli, K. pneumoniae, S. aureus* and *S. pneumonia* by using ethanolic leaves extract. Isitua *et al.* (2016) carried out the susceptibility test on *Staphylococcus aureus, Streptococcus pyogenes, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Shigella sonnei, Shigella dysenteriae, Shigella flexneri, Shigella boydii,* and *Proteus mirabilis* showed susceptibility of these isolates to different concentrations of the plant.

Aqueous UAE crude extract of *Cuscuta reflexa* from fresh stems showed moderate to high sensitivity against skin disease causing bacteria, *Streptococcus pyogenes* (16.33±0.58), *Escherichia coli* (14.00±0.58) and *Staphylococcus aureus* (17.67±0.67). Moderate to high sensitivities were also observed against all the listed reference bacteria. Manirujjaman *et al.* (2016) reported that ethanolic extract provide promising sensitivity against *E. coli* than *B. subtilis* and *S. aureus*. Bais *et al.* (2013) observed remarkable activities on *E. coli, B. subtilis* and *S. aureus*. They also confirmed the host dependent antibacterial activity of *Cuscuta reflexa*. From the above study it was

observed that the crude extract of *C. reflexa* fresh stems may be a suitable herbal medicine for curing different types of pathogenic skin diseases.

Aqueous UAE crude extract of *Carica papaya* from fresh leaves showed moderate sensitivity against skin disease causing bacteria including, *Streptococcus pyogenes* (12.67±0.67), *Escherichia coli* (10.33±0.67) and *Staphylococcus aureus (*12.67±0.33) which was quite high. Moderate to high sensitivities were also observed against all the listed reference bacteria. Suresh *et al.* (2008) studies observed that the antibacterial action of *Carica papaya* leaf extract possesses antibacterial activity against tested gram positive (*Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacteria. Similarly its action against *Salmonella typhi*, paratyphi and typhimurium has also been documented. From the above study it was observed that the crude extract of *C. papaya* dried leaves may be a suitable herbal medicine for curing different types of pathogenic skin diseases.

Aqueous UAE crude extract of *Coccinia grandis* from dried leaves showed low to moderate sensitivity against skin disease causing bacteria *Streptococcus pyogenes* (10.00±0.58), *Escherichia coli* (10.67±0.33) and *Staphylococcus aureus (*11.33±0.33). Dewanjee *et al.* (2007) observed that methanol extract of *Coccinia grandis* leaves had major activity against *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae, Shigella sonnei* and *Pseudomonas aeruginosa*, whilst resistant to *Shigella flexneri* and *Shigella boydii*. Farrukh *et al.* (2008) water extract of leaves showed moderate activity against *Salmonella typhi* and *Shigella boydii* but no activities were observed against *S. pyogenes*, *S. aureus and E. coli* whereas hexane extract of leaves were moderately active against all gram positive and negative bacteria. Sivaraj *et al.* (2011) found ethanol leaf extract of *Coccinia grandis* showed high antibacterial activity against *S. aureus, E. coli* and *S. pyogens*. They also found that acetone, methanol and aqueous extracts showed intermediate activity against *S. aureus, E. coli,* and *S. pyogens*. Ekram *et al.* (2017) found that methanolic crude extract of showed antimicrobial susceptibility against *Pseudomonas sp., Escherichia coli* at a dose of 150 µg/disc. Sivaraj *et al.* (2011) Ethanol leaf extract of *Coccinia grandis* showed high antibacterial activity against *S. aureus, B. cereus, E. coli, K. pneumoniae* and *S. pyogens*. The hexane leaf extracts showed high antibacterial activity against *B. cereus, E. coli, K. pneumoniae* and *S. pyogens*. Whereas, acetone, methanol and aqueous extracts showed intermediate activity against *S. aureus, B. cereus, E. coli, K. pneumoniae* and *S. pyogens*. From the above study it was observed that the crude

extract of *C. grandis* dried leaves may be a suitable herbal medicine for curing different types of pathogenic skin diseases.

Aqueous UAE crude extract of *Carum copticum* from dried seeds showed very poor sensitivity against skin disease causing bacteria including *Streptococcus pyogenes* (8.67±0.45), *Escherichia coli* (8.33±0.335) and *Staphylococcus aureus* (8.33±0.33). Very poor sensitivities were also observed against all the listed reference bacteria. Shrivastava *et al.* (2012) reported aqueous extract did not showed any activity against *B. subtilis, P. auraeuginosa* and *S. aureus*. However ethanol showed moderate to high sensitivity against the above bacteria. Mobaiyen *et al.* (2015) reported essential oil provide significant antimicrobial sensitivity against *B. subtilis, E. coli* and *S. aureus*. Goudarzi *et al.* (2011) reported significant sensitivity against *Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus.* Mahmoudzadeh *et al.* (2016) reported that significant sensitivity against *E. coli*. From the above study it was observed that the crude extract of *C. copticum* fresh leaves may not be a suitable herbal medicine for curing different types of pathogenic skin diseases.

Aqueous UAE crude extract of *Nigella sativa* from dried seeds showed poor sensitivity against skin disease causing bacteria including, *Streptococcus pyogenes* (8.67±0.33), *Escherichia coli* (10.67±0.33) and *Staphylococcus aureus* (8.33±0.67). Very poor sensitivities were also observed against all the listed reference bacteria. Salman *et al.* (2008) reported antimicrobial sensitivity against *Staphylococcus aureus* and *Streptococcus pyogenes*. Hosseinzadeh *et al.* (2007) reported that the aqueous extract did not show any inhibitory effect on either micro-organism including *S. aureus* and *E. coli*. Hannan *et al.* (2008) observed that methicillin resistant *Staphylococcus aureus* was sensitive to *N. sativa* extract at a concentration of 4 mg/disc while the extract had an MIC range of 0.2–0.5 mg/ml. From the above study it was observed that the crude extract of *N. sativa* fresh leaves may be a suitable herbal medicine for curing different types of pathogenic skin diseases.

From the present study it was observed that aqueous UAE crude extracts of *Azadirachta indica*, *Acacia nilotica, Bryophyllum pinnatum*, *Mikania cordata*, *Moringa oleifera* and *Cuscuta reflexa* showed very promising activities against all the enrolled bacteria. Comparatively poor but also promising antimicrobial effects were also observed by *Carica papaya* and *Coccinia grandis*. However, more studies required for *Carum copticum* and *Nigella sativa* by increasing dose and/or changing extraction procedure may be required.

4.3.2 Antifungal Activity of Extracts against Skin Disease Causing Fungi

Antifungal sensitivity studies of crude extracts were performed against 5 fungi including *Curvularia lunata*, *Fusarium chlamydosporum, Malassezia furfur, Fusarium oxysporum* and *Collectotrichum gloeosporioide*. From literature survey it was observed that *Curvularia lunata*, *Fusarium chlamydosporum* and *Malassezia furfur* had some association for causing skin diseases. From Table 4.2, it was observed that *Azadirachta indica*, *Acacia nilotica, Cuscuta reflexa, Bryophyllum pinnatum, Moringa oleifera, Carica papaya* and *Coccinia grandis* showed moderate sensitivity over all the listed fungi whereas *Mikania cordata*, *Carum copticum* and *Nigella sativa* showed very poor sensitivity. Poor antifungal activities were also observed in case of standard antibiotic tetracycline (30 µg/disc) and erythromycin (15 µg/disc). *Azadirachta indica*, *Acacia nilotica, Cuscuta reflexa, Bryophyllum pinnatum, Moringa oleifera, Carica papaya* and *Coccinia grandis* showed moderate sensitivity against all those three fungi.

Table 4.2: Antifungal activity of aqueous UAE crude extracts

Plant Extract (300 µg/ml)	Zone of Inhibition (mm)				
	*Curvularia lunata**	*Fusarium chlamydosporum**	*Malassezia furfur**	*Fusarium oxysporum*	*Collectotrichum gloeosporioides*
A. indica	14.00±0.66	13.00±0.33	12.00±0.67	11.00±0.73	9.00±0.58
A. nilotica	12.00±0.58	9.67±0.33	10.67±0.88	12.33±0.33	12.33±0.67
B. pinnatum	13.33±1.33	12.33±0.67	14.33±0.67	15.33±1.33	13.67±0.33
C. reflexa	13.33±1.67	14.67±0.67	13.00±0.67	15.33±0.33	14.33±1.33
M. cordata	7.67±0.67	7.67±0.67	7.33±0.33	10.33±1.33	8.67±0.33
M. oleifera	15.33±0.67	15.33±0.33	14.67±0.33	14.33±0.33	15.67±1.33
C. papaya	11.67±0.67	12.33±0.33	10.67±0.67	12.33±0.67	11.67±1.33
C. grandis	13.00±0.58	15.33±0.67	11.33±0.67	10.67±0.33	13.67±0.33
C. copticum	8.33±0.33	8.67±0.67	7.33±0.33	7.33±0.33	8.67±0.33
N. sativa	8.67±0.33	9.67±0.67	7.33±0.67	7.33±0.58	8.33±1.33
Erythromycin	11.00±0.58	12.67±1.45	7.67±0.33	8.00±0.58	15.33±1.45
Tetracycline	8.33±0.33	8.33±0.33	10.67±0.33	7.33±0.33	10.33±0.33

* Fungi responsible for different types of skin diseases

Crude extracts of *A. indica* fresh leaves showed moderate sensitivity against the tested fungi in this study. From previous studies it was observed that aqueous, ethanolic and ethyl acetate extracts showed sensitivity against some human pathogenic fungi including *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger,*

Aspergillus terreus, Candida albicans and *Microsporum gypseum* which was gradually increased with concentration (Mahmoud *et al.,* 2011). Khatun and Shamsi (2016) also studied on ethanol extract of *A. inidica* seeds extracts against 9 fungi including *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *A. nidulans*, *Curvularia lunata*, *Penicillium*, *Rhizopus stolonifer* and *Trichoderma viride* and found moderate sensitivity.

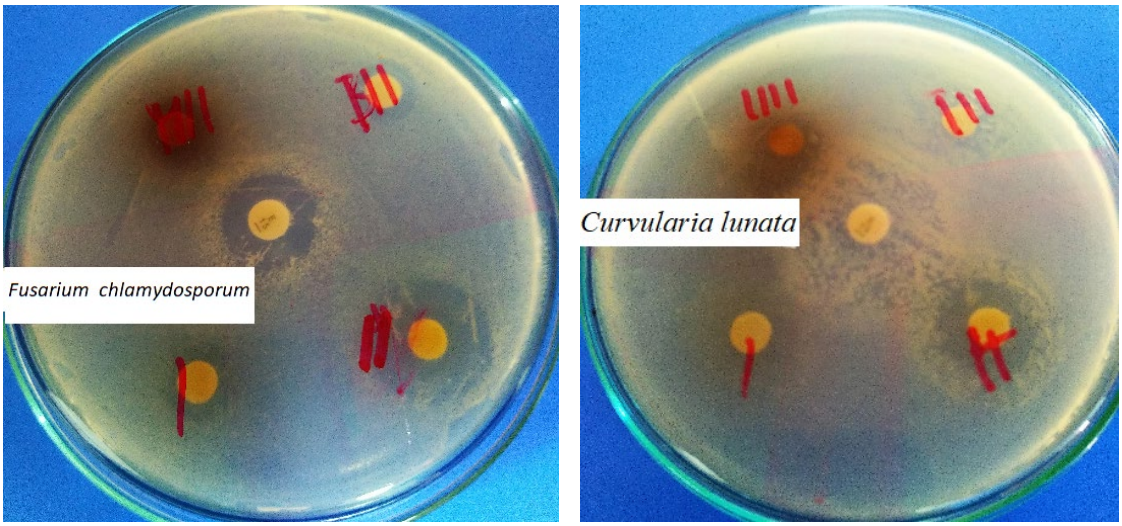


Figure 4.5: Antifungal study of crude extracts of (I) *A. indica* (300 µg/disc); (II) *B. pinnatum* (300 µg/disc); (III) *C. grandis* (300 µg/disc) and (IIII) *C. papaya* (300 µg/disc) with standard antibiotic erythromycin (15 µg/disc) in the middle against *Fusarium chlomydosporum* and *Curvularia lunata***.**

Crude extract of *A. nilotica* fresh leaves showed moderate sensitivity against the listed fungi in this study. From previous studies it was observed that Sharma *et al.* (2014) found dose dependent zone of inhibition against *Aspergillus niger* and *Aspergillus fumigates* by using hot aqueous extract (HAE) of *Acacia nilotica* leaves. Acetone-water extracts of *Acacia nilotica* leaves also showed fungicidal activity against the *Pythium aphanidermatum* (Khan *et al.,* 1996).

Crude extracts of *B. pinnatum* showed promising antifungal activities against the listed fungi in this study. From previous study it was observed that ethanolic extracts of *Bryophyllum pinnatum* plants showed inhibition zones against *Candida albicans* (Umebese and Falana, 2013). Kamal *et al.* (2014) found non-significant anti-fungal activities by dichloromethane and methanol extracts of leaves. However, Pattewar *et*

al. (2013) found significant antifungal activity and they suggested for preparation of antifungal cream.

Crude extracts of *Cuscuta reflexa* showed very promising antifungal activity against the enrolled fungi. From previous studies antifungal potential of *C. reflexa* aqueous extract was observed against five different pathogenic fungi namely, *Alternaria alternate, Aspergillus niger, Fusarium solani, Fusarium oxysporium* and *Macrophomina phaseolina* (Mukhtar *et al.,* 2012). It was also observed that butanol, ethanol and aqueous extracts of *C. reflexa* Roxb. showed effective antifungal activity against the *Fusarium sp*., *Aspergillus sp*., and *Penicillium sp*. whereas acetone and methanol extracts did not show any antifungal activity (Jagtap *et al.,* 2014). Rai and Pal (2015) studied antifungal potential of *C. reflexa* extract against two different pathogenic fungi namely, *Candida albicans, Aspergillus fumigates*. From the above results the crude extracts may be consider for the treatment of skin disease caused by pathogenic fungi.

Crude extracts of *M. cordata* showed insignificant sensitivity against the listed fungi. From previous study ethyl acetate extract exhibited excellent antidermatophytic activity, followed by petroleum ether and methanolic extracts against *Epidermophyton floccosum* var. *nigricans*, *Microsporum canis, Microsporum gypseum* and *Trichophyton rubrum* (Jyothilakshmi *et al.,* 2015).

Crude extracts of *M. oleifera* fresh leaves showed very promising antifungal activities against the enlisted fungi in the present study. Zaffer *et al.* (2015) also found potential antifungal activity particularly against dermatophytic fungi, *M. gypseum*. Dwivedi and Sangeeta (2015) showed promising antifungal potentiality against *F. oxysporum* with maximum inhibition. This plant may be included for the treatment of skin disease caused by fungal infection.

Crude extracts of *Carica papaya* leaves showed moderate sensitivities against the listed fungi in the present study. Chávez-Quintal *et al.* (2011) also observed that the leaf extract of *Carica papaya* exhibited the broadest action spectrum against some pathogenic fungi, *Rhizopus stolonifer*, *Fusarium* spp. and *Colletotrichum gloeosporioides*. Sherwani *et al.* (2013) found antifungal activity of crushed and boiled water extract of *Carica papaya* leaf against 6 saprophytic fungi *Penicillium sp, Aspergilus flavus, Aspergillus niger, Fusarium sp,* Rhizopus and Helminthosporum, 5 dermatophytic fungi *Microsporum canis, Microsporum gypseum, Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton tonsurans* and found activity

against majority of fungi. Based on the above results the plant may be included for the treatment of skin disease caused by pathogenic fungi.

Crude extracts of *C. grandis* leaves showed moderate sensitivity against the enrolled fungi in the present study. Thanabalasingama *et al.* (2015) showed strong antifungal activity of *C. grandis* against the plant pathogen *Cladosporium cladosporioides* and *Nigrospora oryzae*. Gupta *et al.* (2015) found antifungal activity by "food poisoning method" against *Fusarium oxysporum.* Shaheen *et al.* (2018) observed that the antifungal activity of petroleum ether and methanol extracts of *C. grandis* showed maximum activity tested using dermatophytes like *Candida albicans, Microsporum gypsum* and *Microsporum canis* and other fungi on Asthana and Hawker's and Sabourad's dextrose agar media. From the above study this plants may be included in the treatment of skin disease caused by pathogenic fungi.

Crude extracts of *C. copticum* seeds showed insignificant activities against the enrolled pathogens in the present study. Previous study observed that essential oil of *C. copticum* possess in vitro antifungal activity against *Alternaria alternate* (Khosravi *et al.,* 2015). Siripornvisal (2010) observed antifungal activity against three strains of *Fusarium oxysporum* and results suggested that ajowan oil has potential use as a biofungicide against the wilt pathogens. However, further study required to enroll the extracts in the treatment of skin disease caused by pathogenic fungi.

Crude extracts of *N. sativa* seeds showed insignificant activities against the enrolled pathogens in the present study. Previous study observed that nigella seed oil has a strong antifungal activity compared to the conventional fungicides (Asdadi *et al.,* 2014). However further study is required to conform the activity against the pathogenic fungi.

4.3.3 Minimum Inhibitory Concentration (MIC)

From the antibacterial and antifungal study, *A. indica, A. nilotica, M. cordata, C. reflexa, B. pinnatum, M. oleifera, C. papaya and C. grandis* showed moderate to high sensitivities against skin disease causing bacteria *Staphylococcus aureus* and *Streptococcus pyogenes.* From MIC study, it was observed that *A. indica* showed 60 µg/ml against both microorganisms. *C. reflexa* showed MIC 100 µg/ml against the both microorganisms. *C. grandis* failed to show MIC within 100 µg/ml.

Table 4.3: Determination of Minimum Inhibitory Concentration (MIC)

Micro-organi-sms	Crude Extracts	Turbidity					
		Aqueous UAE crude extract					
		20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	Solvent control
Staphylococcus aureus	*Azadirachta indica*	++	++	MIC	-	-	-
	Acacia nilotica	++	++		MIC	-	-
	Mikania cordata	++	++	++	++	++	-
	Cuscuta reflexa	+++	++	++	+	MIC	-
	Bryophyllum pinnatum	++	++	+	MIC	-	-
	Moringa oleifera	++	++	+	MIC	-	-
	Carica papaya	++	++	+	MIC	-	-
	Coccinia grandis	++	++	++	++	++	-
Streptococcus pyogenes	*Azadirachta indica*	++	++	MIC	-	-	-
	Acacia nilotica	++	++	++	++	MIC	-
	Mikania cordata	+++	+++	++	++	++	-
	Cuscuta reflexa	+++	++	++	+	MIC	-
	Bryophyllum pinnatum	++	++	++	+	MIC	-
	Moringa oleifera	++	++	MIC	-	-	-
	Carica papaya	++	++	MIC	-	-	-
	Coccinia grandis	++	++	++	++	++	-

MIC- Minimum Inhibitory Concentration,
- No Growth, + Mild Turbidity, ++ Moderately turbidity, +++ More turbidity

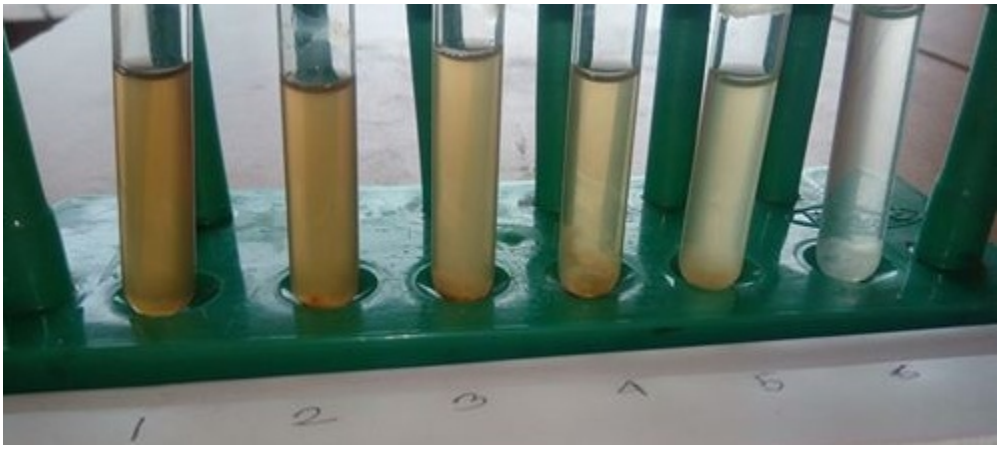


Figure 4.4: MIC of *M. cordata* crude extracts against *S. aureus*

4.3.4 Antimicrobial Study of the Purified Crude Extracts

Crude extracts were separated into four fractions based on the polarity. All fractions were undergone antimicrobial study against *S. aureus* and *S. pyogenes* for measuring their pharmacological efficiency of curing skin diseases (Table 4.4). Chloroform fraction of *A. indica*, dichloromethane fraction of *C. reflexa*, *M. oleifera*, ethylacetate fraction of *C. papaya* showed promising antibacterial activity against the two prominent skin disease causing bacteria.

Table 4.4: Antimicrobial sensitivity study of purified fraction

Name of Pure Fraction	Zone of Inhibition (mm) [considered 3 tests]	
	Staphylococcus aureus	*Streptococcus pyogenes*
S-AI-DE-1	10.33±0.67	10.33±0.33
S-AI-DM-2	11.33±0.33	9.67±0.67
S-AI-CL-3	16.33±0.33	15.67±0.67
S-AI-EA-4	-	-
S-AN-DE-5	-	-
S-AN-DM-6	13.33±0.33	11.67±1.33
S-AN-CL-7	-	-
S-AN-EA-8	-	-
S-BP-DE-9	12.67±0.67	14.33±0.33
S-BP-DM-10	-	-
S-BP-CL-11	10.33±0.67	11.67±0.67
S-BP-EA-12	-	-
S-CR-DE-13	-	-
S-CR-DM-14	15.67±1.33	12.33±0.67
S-CR-CL-15	11.33±0.67	10.67±0.67
S-CR-EA-16	-	-
S-MC-DE-17	-	-
S-MC-DM-18	12.67±0.67	13±1.67
S-MC-CL-19	13.33±0.33	11±0.67
S-MC-EA-20	-	-
S-MO-DE-21	-	-
S-MO-DM-22	17.33±0.67	13.67±0.67
S-MO-CL-23	14.33±0.33	11.33±0.33
S-MO-EA-24	-	-
S-CP-EA-25	16.33±1.33	15±0.67
S-CP-DM-26	-	-
S-CP-CL-27	-	-
S-CP-EA-28	10.67±0.67	11.33±0.33
S-CG-DE-29	9.67±0.67	8.00±1.67
S-CG-DM-30	-	-
S-CG-CL-31	10.33±0.33	11.33±0.33
S-CG-EA-32	-	-
S-CC-DE-33	-	-
S-CC-DM-34	-	-
S-CC-CL-35	12.33±0.33	9.33±0.67
S-CC-EA-36	-	-
S-NS-DE-37	-	-
S-NS-DM-38	-	-
S-NS-CL-39	9.67±0.33	10±0.67
S-NS-EA-40	-	-

4.3.5 Cytotoxicity Study of Crude Extracts

Brine shrimp lethality study was performed by log concentration method and compared with the standard antibiotic tetracycline and erythromycin (Table 4.5). Crude extracts of *A. indica* and *A. nilotica* were observed highly cytotoxic among all crude extracts which was almost similar to tetracycline ($p>0.05$) but safer than the erythromycin ($p<0.05$). Except these two extract rest of the extract showed significantly less toxic than the standard antibiotic tetracycline and erythromycin. Crude extract of *C. copticum* and *N. sativa* showed comparatively safer among all tested plants.

Table 4.5: Cytotoxicity study of aqueous UAE crude extracts

Tested Drugs	LC_{50} in µg/ml (Mean±S.D)	P value	
		Tetracycline=Extract	Erythromycin=Extract
Tetracycline	23.43±4.02	-	-
Erythromycin	6.84±1.52	-	-
A. indica	22.14±4.12	0.805	0.015
A. nilotica	36.58±8.68	0.157	0.018
B. pinnatum	61.79±11.82	0.023	0.011
M. cordata	60.06±16.98	0.029	0.015
M. oleifera	56.44±16.12	0.037	0.028
C. reflexa	48.86±7.10	0.045	0.004
C. papaya	55.96±6.08	0.045	0.004
C. grandis	66.32±15.74	0.044	0.015
C. copticum	71.29±12.16	0.011	0.008
N. sativa	71.87±12.46	0.024	0.008

* Cytotoxicity study was performed by log concentration method considering successive three studies (Standard curve presented in Appendix)
**P<0.05 indicates significant difference & p>0.05 indicates significant similarities

The Brine Shrimp Lethality Assay has been developed for toxicity testing of various concentrations of pure compounds and crude plant extracts. The results for the toxicity of tested herbal preparations gained by using crude plant extracts were more accurate than by testing pure compounds isolated from the same plant. This difference in the toxicity results is probably due to the chemical complexity of the crude or partially purified extract, which seemed to be essential for the bioavailability of the active constituents of the examined plant (Hamidi *et al.,* 2014).

Aqueous UAE crude extract from fresh leaves of *A. indica* showed LC_{50} 22.14±4.12 µg/ml (consider three different tests) which was quite high and almost similar to the standard antibiotic tetracycline. However, the recommended cut off point for detecting cytotoxic activity using brine shrimp lethality test was 20 µg/ml (Geran *et al*, 1972; Massele and Nshimo, 1995; Latha *et al*., 2007). It therefore concludes that *A. nilotica* extracts may not be toxic to humans. In previous study, reported that the leaves ethanolic extract of *A. indica* showed LC_{50} value of 37.15 mg/ml (Emran *et al.,* 2011) and 28 µg/mL (Elhardallou 2011) when tested against *A. salina*. Another cytotoxicity study on the ethanolic extracts shows LC_{50} value of 36.813 mg/ml when evaluated after 24 h of exposure (Chowdhury *et al.,* 2008). Researcher also suggested that the crude ethanol extracts of *A. indica* that were tested at range of 10-1000 ppm had $LC_{50}$of 23 ppm after 24 hrs (Rahmani *et al.,* 1992). From previous study of *A. salina* screening with leaf crude extracts of *A. indica*, the results showed LC_{50} values that were varied whereby the leaf crude extract of hexane, butanol, and ethylacetate extracts had LC_{50} of 1.3, 10.2, and 0.61 µg/ml respectively (Islam *et al.,* 2012).

In the present study aqueous UAE crude extract from fresh leaves of *A. nilotica* was observed 36.58±8.68 µg/ml (considering 3 tests). Howlader *et al.* (2012) performed cytotoxicity (LC_{50} = 32 µg/ml) study by ethanolic leaf extract of *A. nilotica* which was almost similar to the present study. Hussain and Hussain (2012) also found similar cytotoxic activity observed in the present study. Toxicity studies of the ethanol extracts revealed that they exhibited no significant toxicity (LC_{50} of 253.27 µg/ml) against *Artemia salina* (Okoro *et al.,* 2014).

Aqueous UAE crude extract from fresh leaves of *Bryophyllum pinnatum* showed LC_{50} 61.79±11.82 µg/ml (consider three different tests) which was quite safe. However, Abhijit (2015) was found LC_{50} 25.12 µg/ml. Another study reported that the ethanol extract has LC_{50} value higher than methanol extract with LC_{50} 176.19 µg/ml and 481.98 µg/mL, respectively whereas water extract were non active (Megawati *et al.,* 2011). Based on the recommended cut off point for detecting cytotoxic activity using brine shrimp lethality test was 20 µg/ml (Geran *et al*, 1972; Massele and Nshimo, 1995; Latha *et al*., 2007), the extracts may not be toxic to humans.

Aqueous UAE crude extract from fresh leaves of *M. cordata* showed LC_{50} 60.06±16.98 µg/mL (consider three different tests) which was quite safe. In previous study, Nayeem *et al.* (2011) found LC_{50} 90 µg/ml from ethanol extract which was proved much safer than Raka *et al.* (2009) findings 7.51 µg/ml. However, Barua *et al.*

(2014) reported that the ethanolic extract did not show any noticeable toxicity in brine shrimp lethality bioassay. Based on the recommended cut off point for detecting cytotoxic activity using brine shrimp lethality test was 20 µg/ml (Geran *et al*, 1972; Massele and Nshimo, 1995; Latha *et al*., 2007), the extracts may not be toxic to humans.

Aqueous UAE crude extract from fresh leaves of *Moringa oleifera* showed LC_{50} 56.44±16.12 µg/ml (consider three different tests) which was quite safe. From previous study it was observed that the bioassay of the extracts of *Moringa oleifera* of the various fractions showed that all the fractions; petroleum ether, crude extract and ethyl acetate with LC_{50} values of 145.684 µg/ml, 468.070 µg/ml and 203.1522 µg/ml, respectively (Usman *et al.,* 2018). Rafshanjani *et al.* (2014) observed the ethanolic extract showed LC_{50} value of 8.12 µg/ml. Methanolic *Moringa oleifera* stem bark extract were LC_{50} value 0.33 µg/ml (Tonny *et al.,* 2018). Whereas, Nkya *et al.* (2014) observed that petroleum ether, ethyl acetate and ethanol extract of leaves showed 103.796 µg/ml, 697.18 µg/ml and 117.14 µg/ml respectively. Dried leaf of *Moringa oleifera* were subjected to brine shrimp lethality bioassay and the LC_{50} values of methanol, ethanol, petroleum ether, n-hexane and chloroform were found to be 0.747 µg/ml, 0.712 µg/ml, 1.632 µg/ml, 2.163 µg/ml and 0.633 µg/ml respectively (Shahriar *et al.,* 2012).

Aqueous UAE crude extract from fresh leaves of *Cuscuta reflexa* showed LC_{50} 48.86±7.10 µg/mL (consider three different tests) which was quite safe. From previous study it was observed that the bioassay of the extracts of ethanol extract of *C. reflexa* flower showed LC_{50} value 36.72 µg/ml (Sakib *et al.,* 2015). Udavant *et al.* (2012) observed that the methanolic extract and Ethyl acetate fraction of methanolic extract of *Cuscuta reflexa* showed LC_{50} 257.73 µg/ml 184.86 µg/ml respectively. Sayeed *et al.* (2013) found methanolic extract of *C. reflexa* showed LC_{50} 35.75 µg/ml. Based on the recommended cut off point for detecting cytotoxic activity using brine shrimp lethality test was 20µg/ml (Geran *et al.*, 1972; Massele and Nshimo, 1995; Latha *et al*., 2007), the extracts may not be toxic to humans.

Aqueous UAE crude extract from fresh leaves of *C. papaya* showed LC_{50} 55.96±6.08 µg/mL (consider three different tests) which was quite safe. From previous study it was observed that Sinha *et al.* (2018) found the LD_{50} of the aqueous extract of *C. papaya* was 2495 mg/kg. Madjos and Luceño (2019) suggested that the extract may possess some cytotoxic activities at higher dose (1000 µg/ml). Nugrahaningsih *et al.*

(2019) aqueous leaf extract showed the LC_{50} value 88.5 mg/ml. Sahgal *et al.* 2010 studied on seed and found the LC_{50} for the extract was 0.68 mg/ml. Haque *et al.* 2015 observed LC_{50} values for chloroform, methanol and aqueous extracts were 17.142 µg/ml, 15.404 µg/ml and 18.126 µg/ml respectively. Based on the recommended cut off point for detecting cytotoxic activity using brine shrimp lethality test was 20 µg/ml (Geran *et al*, 1972; Massele and Nshimo, 1995; Latha *et al*., 2007), the extracts may not be toxic to humans.

Aqueous UAE crude extract from fresh leaves of *C. grandis* showed LC_{50} 66.32±15.74 µg/mL (consider three different tests) which was quite safe. From previous study Hasan and Sikdar (2016) observed that the extract showed LC_{50} against brine shrimp nauplii was 15.00 µg/ml. The plant extract also showed moderate pesticidal activities towards *S. oryzae* adults. Alamgir and Rahman (2014) observed the ethanolic leaf extract showed strong inhibitory effect on brine shrimp lethality with LC_{50} at 24.20 µg/ml. Orech *et al.* (2005) found LC_{50} 100.6 µg/ml by using the extract of the ethanol and chloroform mixture.

Aqueous UAE crude extract from dried seeds of *C. copticum* and *N. sativa* showed LC_{50} 71.29±12.16 µg/mL and 71.29±12.16 µg/mL respectively which was quite safe. Sufficient work on cytotoxicity study was not done on these plants. However, Bajracharya and Tuladhar (2011) observed LC_{50} 26.09 µg/ml by using seeds oil of *C. copticum*. Sharififar *et al.,* (2017) observed Petroleum ether and chloroform extract of *N. sativa* showed the most cytotoxicity with LC_{50} values 7 µg/ml and 21 µg/ml respectively. Further study required for drawing conclusion.

4.4 Conclusion

From the present study it was observed that aqueous UAE crude extracts of *Azadirachta indica*, *Acacia nilotica, Bryophyllum pinnatum*, *Moringa oleifera* and *Cuscuta reflexa* showed very promising activities against all the enrolled bacteria and fungi. Comparatively moderate antimicrobial effects were observed for the extracts of *Carica papaya, Coccinia grandis* and *Mikania cordata*. However, *Carum copticum* and *Nigella sativa* showed negligible antimicrobial activities. As like crude extracts, purified fractions were also showed promising effects against skin disease causing bacteria including *Staphylococcus aureus* and *Streptococcus pyogenes*. The results indicated the effectiveness of the studied plants for the treatment of pathogenic skin diseases.

Most of the tested plants showed MIC bellow 100 µg/ml which was also promising for implement those plants for the treatment purpose. Through cytotoxicity (brine shrimp lethality) study, it was also observed that most of the crude extracts were safer than the standard drug (antibiotic) widely used for the treatment of different type of skin diseases. Based on the above study it may be concluded that the aqueous UAE crude extracts of *Azadirachta indica* (fresh leaves), *Acacia nilotica* (fresh leaves), *Bryophyllum pinnatum* (fresh leaves), *Mikania cordata* (fresh leaves), *Moringa oleifera* (fresh leaves), *Cuscuta reflexa* (fresh stems), *Carica papaya* (dried leaves) and *Coccinia grandis* (dried leaves) and their purified fractions are suitable for the treatment of different types of pathogenic skin diseases.

References

Abhijit R. 2015. Original Paper Phytochemical Screening and In-Vitro Evaluation of Antioxidant, Cytotoxicity, Antifungal Activities of *Kalanchoe pinnata* (L). *Journal of Science and Arts,* 4(33): 343-350.

Ahmed Y, Sohrab H, Al-Reza SM, Tareq FS, Hasan CM and Sattar MA. 2010. Antimicrobial and cytotoxic constituents from leaves of *Sapium baccatum*. *Food and Chemical Toxicology*, 48: 549-552.

Ajayi AO and Fadeyi TE. 2015. Antimicrobial Activities and Phytochemical Analysis of *Moringa oleifera* Leaves on *Staphylococcus aureus* and *Streptococcus species*. *American Journal of Phytomedicine and Clinical Therapeutics*, 3(10): 643-653.

Akinsulire OR, Aibinu IE, Adenipekun T, Adelowotan T and Odugbemi T. 2007. In vitro Antimicrobial Activity of Crude Extracts from Plants *Bryophyllum pinnatum* and *Kalanchoe crenata*. *African Journal of Traditional, Complementary and Alternative Medicines*, 4(3): 338-344.

Alamgir ANM and Rahman M. 2014. Phytochemical Characteristics, Antimitotic, Cytotoxic and Antiinflamatory Activities of *Coccinia grandis* (L.) J. Voigt. *Journal of Pharmacognosy and Phytochemistry,* 3(1): 218-225.

Ali MS, Islam MS, Rahman MR, Sayeed MA and Islam MR. 2011. Antibacterial and cytotoxic activity of Ethanol Extract of *Mikania cordata* (Burm.F) B.L Robinson leaves. *Journal of Basic and Clinical Pharmacy,* 2(2): 103-107.

Andriani L, Perawati S, Pratiwi P, Sagita D and Yulianis. 2018. Isolation of Antibacterial Compound from the Leaves of *Mikania micrantha* Kunth. *Journal of Pharmaceutical Sciences and Research,* 2018: 125-130.

Arslanyolu M and Erdemgil FZ, 2006. Evaluation of the antibacterial activity and toxicity of isolated arctiin from the seeds of *Centaurea sclerolepis*. *Journal of Faculty of Pharmacy of Ankara University*, 35: 103-109.

Asdadi A, Harhar H, Gharby S, Bouzoubaâ Z, Yadini AE, Moutaj R, Hadek ME, Chebli B and Hassani LMI. 2014. Chemical Composition and Antifungal Activity of *Nigella Sativa* L. Oil Seed Cultivated in Morocco. *International Journal of Pharmaceutical Science Invention,* 3(11): 9-15.

Azman MA, Sidek HJ, Sharudin MSM, Halim NANA, Sahrom RMAAR. 2016. Phytochemical Screening and Antibacterial Activity of *Azadirachta indica* Leaves Extract on Common Skin Infection Bacteria. *Jurnal Intelek*, 11(1): 18-23.

Bais N, Kakkar A, Mishra VK, Singh R and Khare P. 2013. Comparative study on antibacterial activity of ethyl acetate extract of *Cuscuta reflexa* grown on *Cassia fistula* and *Ficus benghalensis*. *International Journal of Pharmaceutical Sciences and Research,* 5(1): 137-41.

Bajracharya GB and Tuladhar SM. 2011. Brine-shrimp Bioassay for Assessment of Anticancer Property of Essential Oils from Spices. *Nepal Journal of Science and Technology,* 12:163-170.

Baker J, Liu JP, Robertson EJ and Efstratiadis A. 1993. Role of Insulin-like Growth Factors in Embryonic and Postnatal Growth. *Cell*, 75: 73-82.

Bandow JE, Brotz H and Leichert LIO. 2003. Proteomic approach to understanding antibiotic action. *Antimicrobe chemother*, 47: 948-955.

Baral B, Bhattarai N and Vaidya GS. 2011. Pharmacological and Antagonistic Potentials of *Mikania micrantha*. *Nepal Journal of Science and Technology,* 12: 75-84.

Barua N, Absar N, Paul S, Barua A, Gazi MY, Saha M, Islam MS, Belaly JM. 2014.In vitro phytochemical, cytotoxicity and mineral composition analysis of *Micania cordata* (Bumr. f.) B.L. Robinson leaves. *International Journal of Biosciences,* 5: 154-60.

Bashir HS; Mohammed AM, Magsoud AS, Shaoub AM. 2014. Isolation and Identification of Two Flavonoids from *Acacia Nilotica* (Leguminosae) Leaves. *Journal of Forest Products & Industries,* 3(5): 211-215.

Biswas SK, Chowdhury A, Das J, Karmakar UK and Shill MC. 2011. Assessment of cytotoxicity and antibacterial activities of ethanolic extracts of *Kalanchoe pinnata* Linn. (Family: Crassulaceae) leaves and stems. *International Journal of Pharmaceutical Sciences and Research,* 2(10): 2605-2609.

Borkataky M, Kakoty BB and Saikia LR. 2013. Antimicrobial Activity and Phytochemical Screening of Some Common Weeds of Asteraceae Family. *International Journal of Pharmaceutical Sciences Review and Research,* 23(1): 116-120.

Chávez-Quintal P, González-Flores T, Rodríguez-Buenfil I and Gallegos-Tintoré S. 2011. Antifungal Activity in Ethanolic Extracts of *Carica papaya* L. cv. Maradol Leaves and Seeds. *Indian Journal of Microbiology,* 51(1): 54-60.

Cheenickal M and Mendez RM. 2017. Phytochemical screening and the antimicrobial activity of the leaves of *Azadirachta indica*, A. Juss. *International Journal of Scientific & Engineering Research,* 8(5): 721-724.

Chowdhury MK, Ahsan N and Akhand AA. 2008. *Azadirachta indica* and *Terminalia arjuna* Leaf Extracts Induce Bacteria Cells Involving Aggregation of Proteins. *Bangladesh Journal of Microbiology,* 25(2): 115-121.

Clarkson C, Vineshm JM, Neil RC, Olwen MG, Pamisha P, Motlalepula GM, Niresh B, Peters JS and Peter IF. 2004. In vitro antiplasmodial activity of medicinal plants native to or naturalized in South Africa. *Journal of Ethnopharmacology,* 92: 177-191.

Coimbatore S, Chandrasekaran M, Csaba V and Laszlo K. 2015. Clinical Importance of the Genus Curvularia. Medical Mycology: Current Trends and Future Prospects, Publisher: Boca Raton FL: CRC Press, Editors: Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Rai M:147-204

Das M, Rahman MS, Maniruzzaman M and Uddin MB. 2016. Evaluation of Phytochemical, Invitro Antibacterial and Cytotoxic Properties of Ethanol Extract of *Acacia Nilotica* (L) Leaves. *Indo American Journal of Pharmaceutical Sciences*, 3(12), 1492-1497.

Davies J and Davies D. 2010. Origins and Evolution of Antibiotic Resistance. *Microbiology and Molecular Biology Reviews*, 74(3): 417-433.

Dewanjee S, Kundu M, Maiti A, Majumdar R, Majumdar A and Mandal SC. 2007. In Vitro Evaluation of Antimicrobial Activity of Crude Extract from Plants

Diospyros peregrina, *Coccinia grandis* and *Swietenia macrophylla*. *Tropical Journal of Pharmaceutical Research,* 6(3): 773-778.

Dwivedi SK and Sangeeta. 2015. Efficacy of some Medicinal Plant Extract Against *Fusarium oxysporum* f. sp. *ciceri* Causing Chickpea Wilt. *Asian Journal of Crop Science,* 7: *138-146.*

Ekram MAE, Sarker MEH, Talukder AP, Sarker B, Uddin MS and Zaman S. 2017. Biological Activities of the Methanolic Extracts of *Coccinia indica* and *Mikania scandens* Leaves Available in Bangladesh. *Dhaka University Journal of Pharmaceutical Sciences,* 16(1): 87-93.

Elhardallou SB. 2011. Cytotoxicity and Biological Activity of Selected Sudanese Medicinal Plants. *Research Journal of Medicinal Plant.,* 5: 201-229.

Emran AA, Shahed SM, Ahmed F, Saha SK , Das SC and Bachar SC. 2011. Evaluation of Brine shrimp lethality and Antimicrobial activity of *Azadirachta indica* leaf extract on some drug resistance bacteria in Bangladesh. *Pharmacognosy Journal,* 20(3): 66-71.

Farrukh U, Shareef H, Mahmud S, Ali SA and Rizwani GH. 2008. Antibacterial Activities of *Coccinia grandis* L. *Pakistan Journal of Botany*, 40(3): 1259-1262.

Francine U, Jeannette U and Pierre RJ. 2015. Assessment of antibacterial activity of Neem plant (*Azadirachta indica*) on *Staphylococcus aureus* and *Escherichia coli*. *Journal of Medicinal Plants Studies,* 3(4): 85-91.

Gadir SA. 2012. Assessment of bioactivity of some Sudanese medicinal plants using Brine Shrimp (*Artemia salina*) Lethality Assay. *Journal of Chemical and Pharmaceutical Research,* 4: 5145-5148.

Gaitanis G, Magiatis P, Hantschke M, Bassukas ID, and Velegrakid A. 2012. The Malassezia Genus in Skin and Systemic Diseases. *Clinical Microbiology Reviews*, 25(1): 106 –141.

Geran RI, Greenberg H M, McDonald M and Abbott BJ. 1972. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer chemotherapy reports,* 33: 1-17.

Goudarzi GR, Saharkhiz MJ, Sattari M and Zomorodian K. 2011. Antibacterial Activity and Chemical Composition of Ajowan (*Carum copticum* Benth. & Hook) Essential Oil. *Journal of Agricultural Science and Technology,* 13: 203-208.

Gupta AK, Chaudhary S, Samue CO and Upadhyaya PP. 2015. Antifungal Activity of Some Locally Available Plants Against *Fusarium oxysporum* F. Sp. Udum. *International Journal of Current Microbiology and Applied Sciences*, 4(11): 695-699.

Hamidi MR, Jovanova B and Panovska TK. 2014.Toxicological evaluation of the plant products using Brine Shrimp (*Artemia salina* L.) model. *Macedonian Pharmaceutical Bulletin*, 60 (1): 9-18.

Hannan A, Saleem S, Chaudhary S, Barkaat M and Arshad MU. 2008. Anti bacterial activity of *Nigella sativa* against clinical isolates of methicillin resistant *Staphylococcus aureus*. *Journal of Ayub Medical College Abbottabad,* 20(3): 72-74.

Haque N, Faysal AA, Ahmed I and Chowdhury NS. 2015. Phytochemical screening and In vitro bioactivities of the various extracts of *Carica papaya* leaves available in Bangladesh. *International Journal of Research in Pharmacology and Pharmacotherapeutics,* 4(3): 341-348.

Hasan MF and Sikdar B. 2016. Screening of antimicrobial, cytotoxic and pesticidal activities of *Coccinia grandis* (L.) Voigt. *Journal of Microbiology Biotechnology and Food Science,* 5(6): 584-588.

Hay RJ. 2007. *Fusarium* infections of the skin. Current Opeinion in Infectious Diseases, 20: 115-117.

Hosseinzadeh H, Bazzaz BSF and Haghi MM. 2007. Antibacterial Activity of Total Extracts and Essential oil of *Nigella sativa* L. Seeds in Mice. *Pharmacolgyonline,* 2: 429-435.

Howlader MSI, Hossain MH, Dey SK, Hira A, Ahmed A. 2012. Evaluation of antinociceptive, antimicrobial and cytotoxic activities of ethanolic extract of *Acacia nilotica* leaves from Bangladesh. *Pharmacology online,* 2: 73-81.

Hussain F and Hussain MM. 2012. Cytotoxic effect of crude extracts of *Acacia nilotica. International Journal of Pharmaceutical Sciences and Research*, 3(6): 1652-1655.

Isitua CC, Ibeh IN and Olayinka JN. 2016. Antibacterial Activity of Moringa Oleifera Lam Leaves on Enteric Human Pathogens. *Indian Journal of Applied Research,* 6(8): 553-557.

Islam M, Al-Amin M, Siddiqi MMA, Akter S, Haque MM, Sultana N and Chowdhury AMS.2012. Isolation of Quercetin-3-o-beta-d-glucopyranoside from the Leaves of *Azadirachta indica* and Antimicrobial and Cytotoxic screening of the Crude Extracts. *Dhaka University Journal of Science,* 60(1): 11-14.

Iwu MW, Duncan AR and Okunji OO. 1999. "New antimicrobials of plant origin," in Perspective on New Crops & New Uses, J. Janick, Ed., ASHS Press, Alexandria: 457-462.

Jagtap MD, Asabe AS, Telave AB, Mali BS, Chavan SJ and Kanade MB. 2014. Antifungal potential of *Cuscuta reflexa* Roxb. *Central European Journal of Experimental Biology*, 3(3): 30-32.

Jyothilakshmi M, Jyothis M and Latha MS. 2015. Antidermatophytic Activity of *Mikania micrantha* Kunth: An Invasive Weed. *Pharmacognosy Research,* 7(5): 20-25.

Jyothilakshmi M, Jyothis M and Latha MS. 2016. *Mikania micrantha* – a Natural Remedy to Skin Infections. *International Journal of Current Microbiology and Applied Sciences,* 5(2): 742-745.

Kalpana S, Moorthi S and Kumari S. 2013. Antimicrobial activity of different extracts of leaf of *Moringa oleifera* (Lam) against gram positive and gram negative bacteria. *International Journal of Current Microbiology and Applied*, 2(12): 514-518.

Kamal Y, Ahmad BC, Uzair M, Irshad N, Yaseen M and Hussain I. 2014. In vitro evaluation of antibacterial, antifungal and phytotoxicity activity of different extracts of leaves of *Kalanchoe pinnata*. *Journal of Applied Pharmacy,* 6(4): 446-450.

Khan AJ, Zouba A and Seapy DG. 1996. Antifungal activity from leaves of *Acacia nilotica* against Pythium aphanidermatum. *Journal for Scientific Resarch*, 1(1): 7-11.

Khan MSA, Ahmad I, Cameotra SS. 2014. *Carum copticum* and *Thymus vulgaris* oils inhibit virulence in *Trichophyton rubrum* and *Aspergillus* spp. *Brazilian Journal of Microbiology,* 45(2): 523-531.

Khatun A and Shamsi S. 2016. In vitro Evaluation of Fungicides and Plant Extracts Against the Fungi Associated With Seeds of Nine Chickpea Varieties. *Dhaka University Journal of Biological Sciences,* 25(1): 83-90.

Khosravi AR, Salehi RM, Yahyaraeyat R, Mokhtari AR and Panahi P. 2015. Chemical Composition and Antifungal Activity of *Trachyspermum copticum* Essential Oil Against *Alternaria alternata* (In-Vitro Study). *Journal of Medicinal Plants,* 14 (53): 82-88.

Krishnarajua AV, Raoa TVN, Sundararajua D, Vanisreeb M, Tsayb HS and Subbarajua GV. 2005. Assessment of Bioactivity of Indian Medicinal Plants using brine shrimp (*Artemia salina*) lethality Assay. *International Journal of Applied Sciences and Engineering,* 3(2): 125-13.

Laboni FR, Sultana T, Kamal S, Karim S, Das S, Rashid MH and Shahriar M. 2017. Biological investigations of the ethanol extract of the aerial part (leaf) of *Coccinia grandis* L. *Journal of Pharmacognosy and Phytochemistry*, 6(2): 134-138.

Latha LY, Sasidharan S, Zuraini Z, Suryani S, Shirley L, Sangetha S, and Davaselvia M. 2007. Antimicrobial Activities and Toxicity of Crude Extract of the *Psophocarpus Tetragonolobus* Pods. *African Journal of Traditional, Complementary and Alternative Medicines,* 4(1): 59-63.

Latha CR, Kavitha S and Sathya S. 2015. Antimicrobial Efficacy of *Azadirachta indica* Leaf Extracts. *International Journal of Science and Nature*, 6(3): 432-440.

Madjos MG and Luceño AM. 2019. Comparative Cytotoxic Properties of Two Varieties of *Carica papaya* leaf extracts from Mindanao, Philippines using Brine Shrimp Lethality Assay. *Bulletin of Environment, Pharmacology and Life Sciences,* 8(2):113-118.

Mahbub N, Mazumder SH, Morshed MZ, Haq WM, Jahan R, *et al.* 2017. Folk Medicinal Use of Plants to Treat Skin Disorders in Chandpur District, Bangladesh. *American Journal of Ethnomedicine,* 4 (2):1-5.

Mahmoud DA, Hassanein NM, Youssef KA, Abou Zeid MA. 2011. Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens. *Brazilian Journal of Microbiology*, 42(3): 1007-1016.

Mahmoudzadeh M, Hosseini H, Nasrollahzadeh J, Khaneghah AM, Rismanchi M, Chaves R, Shahraz F, Azizkhani M, Mahmoudzadeh L, Haslberger A. 2016. Antibacterial Activity of *Carum copticum* Essential Oil Against *Escherichia Coli* O157:H7 in Meat: Stx Genes Expression. *Current Microbiology*, 73(2): 265-272.

Manirujjaman, Suchana S, Collet T, Nawshin L N, Chowdhury MAR. 2016. Antimicrobial Effects of Ethanolic Extracts from *Cuscuta reflexa* Roxb. (Convolvulaceae). *International Journal of Pharmacognosy and Phytochemical Research*, 8(6): 930-932.

Massele AY and Nshimo CM. 1995. Brine shrimp bioassay for biological activity of medicinal plants used in traditional medicines in Tanzania. *East African Medical Journal*, 72(10): 661-663.

Matawali A, Chin LP, Eng HS & Gansau JA. 2016. Antibacterial and Phytochemical Investigations of *Mikania micrantha* H.B.K. (Asteraceae) From Sabah, Malaysia. *Transactions on Science and Technology*, 3(1-2): 244-250.

McLaughlin JL, Rogers LL and Anderson JE. 1998. The use of biological assays to evaluate botanicals. *Drug Information Journal,* 32: 513-524.

Megawati, Darmawan A and Dewijanti ID. 2012. Bioactivity Brine Shrimp Lethality Test Extract and Fractionation from *Kalanchoe pinnata* Lam Pers. Proceeding of International Conference on Drug Development of Natural Resources. June 30th: 147-151.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE and McLaughlin JL. 1982. Brine Shrimp: A convenient general bioassay for active plant constituents. *Planta Medica,* 45: 31-34.

Mobaiyen H, Pour MN and Elmi F. 2015. Phytochemical Composition and Antibacterial Activity of *Trachyspermum copticum* L. essential oil, East Azerbaijan, Iran. *Journal of Medical Microbiology and Infectious Diseases*, 3(3-4): 71-74

Moshi MJ, Innocent E, Magadula J.J, Otieno DF, Weisheit A, Mbabazi PK and Nondo RSO. 2010. Brine shrimp toxicity of some plants used as traditional medicines in Kagera Region, north western Tanzania. *Tanzania Journal of Health Research*, 12: 63-67.

Mukhtar I, Atiq M, Hanan A and Iqbal Z. 2012. Antifungal activity of C*uscuta reflexa* roxb. *Pakistan Journal of Phytopathology* , 24(2): 163-166.

Mukthar MD and Okafor T. 2002. Antibacterial Activity of Ethanolic Extract of *Guiera senegalensis*. *International Journal of Pharmacology*, 56: 213-216.

Mukhtar MD and Tukur A. 2000. Antibacterial Activity of Aqueous and Ethanolic Extracts of *P. stratiotes*. *Journal of the Nigerian Society for Experimental Biology*, 1: 51-59

Nayeem AA, Khatun A, Rahman MS and Rahman M. 2011. Evaluation of phytochemical and pharmacological properties of *Mikania cordata* (Asteraceae) leaves. *Journal of Pharmacognosy and Phytotherapy*, 3(8): 118-123.

Nkya JW, Erasto P and Chacha M. 2014. Antimycobacterial and cytotoxicity activities of *Moringa oleifera* Lam extracts. *American Journal of Research Communication,* 2(9): 108-120.

Nugrahaningsih WH, Titi A and Dewi NK. 2019. Acute toxicity of papaya leaf extract on *Artemia salina* leach larvae. *Journal of Physics: Conference Series,* 1321(032033): 1-5.

Obioma A, Chikanka AT and Dumo I. 2017. Antimirobial activity of Leave Extracts of *Bryophyllum pinnatum* and *Aspilia africana* on Pathogenic Wound Isolates Recovered from Patients Admitted in University of Port Harcourt Teaching Hospital, Nigeria. *Annals of Clinical and Laboratory Research,* 5(3): 185-191.

Ogugu SE, Kehinde AJ, James BI and Paul DK. 2012. Assessment of cytotoxic effects of methanol extract of *Calliandra portoricensis* using Brine Shrimp (*Artemia salina*) Lethality Bioassay. *Global Journal of Bio-Science and BioTechnology,* 2: 257-260.

Oketch-rabah HA, Dossaji JF and Mberu EK. 1999. Antimalarial activity of some Kenyan medicinal plants. *Pharmaceutical Biology*, 37: 329-334.

Okoro SO, Kawo AH and Arzai AH. 2014. Phytochemical screening, antibacterial and toxicological activities of *Acacia nilotica* extracts. *Bayero Journal of Pure and Applied Sciences,* 7(1): 105-115.

Olowa LF and Nunez OM. 2013. Brine Shrimp Lethality Assay of the Ethanolic Extract of Three Selected Species of Medicinal Plants from Iligan city, Philippines. *International Research Journal of Biological Sciences,* 2(11): 74-77.

Orech FO, Akenga T, Ochora J, Friis H and Hansen JA. 2005. Potential toxicity of some traditional leafy vegetables consumed in Nyang'oma Division, western Kenya. *African Journal of Food and Nutritional Sciences,* 5(1): 1-13.

Parra LA, Yhebra RS, Sardiñas IG and Buela LI. 2001. Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD_{50} value) in mice, to determine oral acute toxicity of plant extracts. *Phytomed,* 8: 395-400.

Pattewar SV, Patil DN and Dahikar SB. 2013. Antimicrobial potential of extract from leaves of *Kalanchoe pinnata*. *International Journal of Pharmaceutical Sciences and Research,* 4(12): 4577-80.

Rafshanjani MAS, Parvin S and Kader MA. 2014. Invitro antibacterial activities and brine shrimp lethality bioassay of ethanolic extract from *Moringa oleifera* Lam. Leaves. *International Research Journal of Pharmacy,* 5(11): 856-860.

Rahmani M, Ismail HM, Ahmad F, Manas AR and Sukari MA. 1992. Screening of Tropical Plants for the Presence of Bioactive Compounds. *Pertarnka,* 15(2): 131-135.

Rai DK and Pal A. 2015. Comparative evaluation of antifungal activity of *Cuscuta reflexa* (morning glory) convolvulaceae. *World Journal of Pharmaceutical Research*, 4 (11) 1341-1347.

Rajasekaran C, Meignanam E, Vijayakumar V, Kalaivani T, Ramya S, Premkumar N, Siva R and Jayakumararaj R. 2008. Investigations on Antibacterial Activity of Leaf Extracts of *Azadirachta indica* A. Juss (Meliaceae): A Traditional Medicinal Plant of India. *Ethnobotanical Leaflets,* 12: 1213-17.

Raka SC, Mishu SA, Rahman MM and Rahman A. 2019. Assessment of phytochemical, cytotoxic, anthelmintic and thrombolytic activity of *Mikania micrantha* leaves: a new addition in phytomedicine. *Pharmacologyonline,* 1: 246-255.

Ramachandran S, Vamsikrishna M, Gowthami KV, Heera B and Dhanaraju MD. 2011. Assessment of cytotoxic activity of *Agave cantula* using Brine Shrimp (*Artemia salina*) Lethality Assay. *Asian Journal of Scientific Research,* 4: 90-94.

Rex JH and Pfaller MA. 2002. Has antifungal susceptibility testing come of age? *Clinical Infectious Diseases,* 35: 982-989.

Sadat AFMN, Mizan MRB, Sultana A, Rahman MM and Azad MAK. 2018. Comparative study of the Antimicrobial Activity of Methanol Extract and Ultrasound Assisted Water Extract of the Leaves of *Azadirachta indica*. *Rajshahi University Journal of Environmental Science,* 7: 40-47.

Saha SK, Das S, Chowdhury P and Saha SK. 2014. Biocompatibility of a sonochemically synthesized poly (*Nisopropyl acrylamide*) / silica nanocomposite. *RSC Advances,* 4: 14457-14467.

Sahgal G, Ramanathan S, Sasidharan S, Mordi MN, Ismail S and Mansor SM. 2010. Brine shrimp lethality and acute oral toxicity studies on *Swietenia mahagoni* (Linn.) Jacq. seed methanolic extract. *Pharmacognosy Research*, 2(4): 215-220.

Sakib MH, Hossain MS, Hossain MS, Mahmood AA, Sarkar MY, Rahman S and Shill LK. 2015. In-vitro cytotoxicity and antioxidant property evaluation from methanolic extract of *Cuscuta Reflexa* flowers. *Asian Journal of Medical and Biological Research,* 1(2): 285-291.

Salman MT, Khan RA and Shukla T. 2008. Antimicrobial activity of *Nigella sativa* Linn. seed oil against multi-drug resistant bacteria from clinical isolates. *Natural Product Radiance,* 7(1):10-14.

Sayeed MA, Rashid MM and Taiseer MRA. 2013. Investigation of Cytotoxic Potential of Ethanolic Extract of *Citrus limetta* fruit peel, *Paederia foetida* Leaves and Methanolic Extract of *Cuscuta reflexa*. *Journal of Medicinal Plants Studies*, 1(1): 34-37.

Shaheen SZ, Krishna G and Charya MAS. 2018. Antifungal Activity of *Coccinia indica* Extracts Against Fungal Pathogens". *International Journal of Pharmaceutical Science Invention*, 7(4): 6-10.

Shahriar M, Hossain MI, Bahar ANM, Akhter S, Haque MA, Bhuiyan MA. 2012. Preliminary Phytochemical Screening, In-Vitro Antioxidant and Cytotoxic Activity of Five Different Extracts of *Moringa oleifera* Leaf. *Journal of Applied Pharmaceutical Science,* 2(5): 65-68.

Sharififar F, Assadipour A, Moshafi MH, Alishahi F and Mahmoudvand H. 2017. Bioassay Screening of the Essential Oil and Various Extracts of *Nigella sativa* L. Seeds Using Brine Shrimp Toxicity Assay. *Herbal Medicines Journal*, 2(1): 26-31.

Sharma AK, Kumar A, Yadav SK and Rahal A. 2014. Studies on Antimicrobial and Immunomodulatory Effects of Hot Aqueous Extract of *Acacia nilotica* L. Leaves against Common Veterinary Pathogens. *Veterinary Medicine International,* 2014: 1-9.

Sharma N, Gupta PC, Singh A and Rao CV. 2013. Brine shrimp Bioassay of *Pentapetes phoenicea* Linn. and *Ipomoea carnea* jacq. leaves. *Der Pharmacia Lettre,* 5: 162-167.

Sherwani SK, Bokhari TZ, Nazim K, Gilani SA and Kazmi SU. 2013. Qualitative phytochemical screening and antifungal activity of *Carica papaya* leaf extract against human and plant pathogenic fungi. *International Research Journal of Pharmacy,* 4(7): 83-86.

Shrivastava V, Shrivastava G, Sharma R, Mahajan N, Sharma V and Bhardwaj U. 2012. Anti microbial potential of Ajwain (*Trachyspermum copticum*): An Immense Medical Spice. *Journal of Pharmacy Research,* 5(7): 3837-3840.

Singh MP, Sing J and Rajesh R. 2015. Brine shrimp cytotoxic assay for polyherbal formulation of *Moringa oleifera*, *Viola odorata*, *Allium sativum*. *International Journal of Innovative Pharmaceutical Sciences and Research,* 3(12): 1639-1643.

Sinha RK, Pratap R and Varma MC. 2018. Hypoglycemic Activity of Carica papaya Leaf Aqueous Extract in Normal and Diabetic Mice. *International Journal of Advances in Scientific Research and Engineering*, 4(6): 12-16.

Siripornvisal S. 2010. Antifungal Activity of Ajowan Oil against *Fusarium oxysporum*. *KMITL Science and Technology Journal,* 10(2): 45-51.

Sivaraj A, Jenifa BP, Kavitha M, Inbasekar P, Senthilkumar B and Panneerselvam A. 2011. Antibacterial activity of *Coccinia grandis* leaf extract on selective bacterial strains. *Journal of Applied Pharmaceutical Science*, 1(7): 120-123.

Solanki SS and Selvanayagam M. 2013. Phytochemical screening and study of predictive toxicity of certain medicinal plants and extracts using brine shrimp. *Journal of Herbal Science and Technology,* 10: 1-4.

Suresh K, Deepa P, Harisaranraj R and Achudhan V. 2008. Antimicrobial and Phytochemical Investigation of the Leaves of *Carica papaya* L, *Cynodon dactylon* (L.) Pers, *Euphorbia hirta* L, *Melia azedarach* L. and *Psidium guajava* L. *Ethnobotanical Leaflets*, 12: 1184-91.

Tabatabai MB, Mirjalili M, Yazdiyan F and Hekmatimoghaddam S. 2019. Antibacterial Activity and Cytotoxicity of Nanoliposomic and Nanoniosomic Essential Oil of *Trachyspermum copticum. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 89: 1109-1116.

Thanabalasingama D, Kumara NS, Jayasinghea L and Fujimotoa Y. 2015. Endophytic Fungus *Nigrospora oryzae* from a Medicinal plant *Coccinia grandis*, a High Yielding New Source of Phenazine-1-carboxamide. *Natural Product Communications*, 10(10): 1659-1660.

Toma M, Vinatoru M, Paniwnyk L and Mason TJ. 2001. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. *Ultrason Sonochem,* 8: 137-142.

Tonny TS, Rana M, Akter S and Ria SA. 2018. An Experimental Study of Analgesic Activity and Cytotoxic Potential of Methanolic *Moringa oleifera* Stem Bark Extracts in Animal Models. *EC Pharmacology and Toxicology,* 6(11): 912-921.

Udavant PB, Satyanarayana SV and Upasani CD. 2012. Preliminary screening of *Cuscuta reflexa* stems for Anti inflammatory and cytotoxic activity. *Asian Pacific Journal of Tropical Biomedicine,* 2(3): 1303-1307.

Umebese CE and Falana FD. 2013. Growth, phytochemicals and antifungal activity of *Bryophyllum pinnatum* L. subjected to water deficit stress. *African Journal of Biotechnology,* 12(47): 6599-6604.

Usman AG, Ammani HM and Bala SS. 2018. Comparative Antioxidative and Cytotoxic Activity of Extracts of *Moringa oleifera* and its Mistletoe. *IOSR Journal of Applied Chemistry*, 11(1): 13-18.

Westh H, Zinn CS and Rosdadahl VT. 2004. An International multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. *Microbial Drug Resistance,* 10: 169-176.

Zaffer M, Ganie SA , Gulia SS , Yadav SS , Singh R and Ganguly S. 2015. Antifungal Efficacy of *Moringa oleifera* Lam. *American Journal of Phytomedicine and Clinical Therapeutics,* 3(1): 28-33.

Zida A, Bamba S, Yacouba A, Ouedraogo-Traore R and Guiguemdé RT. 2017. Anti-Candida albicans natural products, sources of new antifungal drugs: A review. *Journal de Mycologie Médicale,* 27(1): 1-19.

Chapter 5: Efficacy of Some Ethnobotanicals on the Experimental Diabetic Mice

Abstract

Diabetes mellitus is a metabolic disorder associated with chronic hyperglycemia and imbalance of carbohydrate, protein and fat metabolism. Ethnobotanical survey reported that medicinal plant may have capacity to combat against diabetes without marked side effects. In the present study 10 medicinal plants were selected on the basis of traditional behavior of local people for the treatment of diabetes in Bangladesh. Crude extracts prepared by aqueous UAE method was used in this study on hormone induced diabetic mice. Three different types of anti-diabetic study including OGTT, FPG and long term effects on FPG were performed for measuring the hypoglycaemic efficacy. Most of the selected plants showed hypoglycaemic properties on diabetic mice. However, *A. indica*, *B. pinnatum*, *M. oleifera, C. papaya* and *C. grandis* showed significantly high anti-diabetic activities. Moderate anti-diabetic activities were observed for the botanicals of *A. nilotica, C. reflexa, M. cordata* and *N. sativa* whereas crude extracts of *C. copticum* was showed very negligible response. Most of the plants successfully prevented unwanted weight loss happening in the diabetic animal. Selected plants also provide marked analgesic properties. Based on the present study crude extracts of *A. indica*, *B. pinnatum*, *M. oleifera, C. papaya* and *C. grandis* may be recommended for the treatment of diabetes.

Key Words: Diabetes, Analgesic, Glucocorticoid, Dexamethasone, OGTT, FPG, Ultrasound.

5.1 Introduction

Diabetes mellitus is a metabolic disorder associated with chronic hyperglycemia and imbalance of carbohydrate, protein and fat metabolism (Adenowo *et al.,* 2014). It is an age-long disease (Ogundele *et al.,* 2007). This disease caused by inherited or acquired deficiency of insulin production or resistance to action of the produced insulin (Setter *et al.,* 2000; Fakeye *et al.,* 2007). The distinguishing symptoms of diabetes are polyuria, polydypsia, polyphagia, and unexpected weight loss (Altan 2003; Sinha *et al.,* 2018). Diabetes and its complications remain a major public health problem worldwide (Sreenivasan *et al.,* 2011; Akhere *et al.,* 2013). Approximately 1.3 % of the population suffers from this disease throughout the world (ADA 2017; Sinha *et al.,* 2018). Global prevalence of diabetes has dramatically continued to increase (Juárez-Rojopa *et al.,* 2014). Long term complications arising from diabetes are major causes of diabetes morbidity and mortality (Akhere *et al.,* 2013). According to American Diabetic Association fasting (no calorie intake for the last eight hours) plasma glucose (FPG) levels ≥7 mmol/l (126mg/dl) and random plasma glucose ≥11.1 mmol/l (200mg/dl) considered as diabetic (hyperglycemia or a hyperglycemic crisis) symptoms (ADA 2014 and TUEPG 2015).

The drugs currently used to treat diabetes mostly target the lowering of blood glucose concentrations to normal levels (Gy *et al.,* 2005; Akhere *et al.,* 2013). Widely used anti-diabetic drugs includes insulin and oral hypoglycaemic agent such as sulfonylureas, metformin, α-glucosidase inhibitors, troglitazone (Holman and Turner, 1991; Sinha *et al.,* 2018). Many synthetic oral anti-diabetic drugs are associated with drawbacks such as resistance and side affects ranging from liver toxicity, increased cardiovascular risk, abdominal discomfort, flatulence and diarrhea (Cheng and Fantus, 2005; Adenowo *et al.,* 2014). Many researchers believe that medicinal plant preparations, which contain different phytochemicals, may combat diabetes at multiple points producing faster and perhaps better resolution of diabetes symptoms (Tiwari and Rao, 2002; Akhere *et al.,* 2013). There are some plants which possess bioactive compounds which have been reported to be used in combating diabetes worldwide and have been used in treating diabetes (Ogundele *et al.,* 2007). Several studies have reported the existence of 306 to 400 plants or fruits used as herbal remedies for diabetes (Andrade-Cetto and Heinrich, 2005; Juarez-Rojop *et al.,* 2012; Rao *et al.,* 1997; Sinha *et al.,* 2018). This has led to the usage of medicinal plants for

the treatment of diabetes, but most of them lack scientific evidence to validate their usage and efficacy (Morelli and Zoorob, 2000; Adenowo *et al.,* 2014).

In the present study *Azadirachta indica* (locally known as Neem), *Acacia nilotica* (locally known as Babla), *Bryophyllum pinnatum* (locally known as Pathorkuchi), *Cuscuta reflexa* (locally known as Shornolota), *Mikania cordata* (locally known as Ashamlota), *Moringa oleifera* (locally known as Sojna), *Carica papaya* (locally known as Papaya), *Coccinia grandis* (locally known as Telakochu), *Carum copticum* (locally known as Ajwain) and *Nigella sativa* (locally known as Kalo-jera) were selected on the basis of their anti-diabetic uses through ethnobotanical survey among the TMPs and FMPs in Bangladesh.

This study was therefore intended to evaluate the anti-diabetic effect of aqueous UAE extract of the above mentioned plants in dexamethasone-induced diabetic mice by oral glucose tolerance test, FPG test by single dose and effect of multiple doses on FPG study. Analgesic potency of the crude extracts was also measured as diabetic patients frequently suffer long term pain. Analgesic potency of the crude extracts was measured by Eddy's hot plate method (Eddy and Leimbach, 1953). The objective of the study was to measure the anti-diabetic efficacy of the crude extracts isolated from the selected plants which are widely used for the treatment of diabetic diseases by the TMPs and FMPs in Bangladesh.

5.2 Materials and Methods

5.2.1 Plant Materials

Leaves of *Azadirachta indica*, *Acacia nilotica*, *Bryophyllum pinnatum*, *Mikania cordata*, *Moringa oleifera, Carica papaya*, *Coccinia grandis* and steam of *Cuscuta reflexa* were collected from the Botanical Pesticide Garden of Institute of Environmental Science of Rajshahi University, Bangladesh. Dried seeds of *Carum copticum* and *Nigella sativa* were collected from the grocery shop of the Station Market adjacent to the campus of University of Rajshahi, Bangladesh. All plant's parts were identified by the Taxonomist of the Department of Botany, University of Rajshahi. Herbarium specimen of all plants were duly preserved in the Botanical Pesticide and Environmental Microbiology Lab, IES, RU for further reference.

5.2.2 Preparation of Crude Extracts

Crude extracts from selected plants were prepared by;

i. Aqueous UAE from dried plants parts (Toma *et al.,* 2001). Extraction procedure described in the Section 3.2.4.1 in Chapter 3.
ii. Aqueous UAE from fresh plants parts (Sadat *et al.,* 2019). Extraction procedure described in the Section 3.2.4.2 in Chapter 3.

5.2.3 Study of Anti-diabetic Properties of Crude Extracts on Diabetic Mice

The following anti-diabetic study was considered in the present study.

a) Effects of crude extracts on oral glucose tolerance test (OGTT)

b) Effect of Crude Extracts on Fasting Plasma Glucose (FPG) Level

c) Effects of Long Term Uses of Crude Extracts on FPG Level and Body Weight

5.2.3.1 Enrolment of the Animal in the Study

Hormone (glucocorticoid created by dexamethasone) induced diabetes mice were enrolled in the present study (Ogawa *et al.,* 1992). All experimental procedures were performed in compliance with institutional and international policies governing the humane and ethical treatment of experimental animals as contained in the United States National Institutes of Health (NIH) guidelines (NIH 1985) after ethical approval by the Ethical Committee of University of Rajshahi.

5.2.3.2 Grouping of Animal for the Treatment

The animals were segregated into twelve groups including four mice of either sex in each (Table 5.1). Group I to Group XII mice were selected from the dexamethasone induced diabetic mice which was created by intraperitoneal injections of 10 mg/kg/day of dexamethasone on normal *Swiss albino* mice for around 7 days. Group I mice were considered diabetic control. Group II mice were served as diabetic treatment by standard anti-diabetic drug glibenclamide (600 µg/kg-bw/dose) and Group III to XII were served as diabetic treatment by extract (300 mg/kg-bw/dose).

Table 5.1: Grouping of animals for anti-diabetic study

Group of Animal	Treatment procedure
Group I	Diabetic control received distilled water
Group II	Glibenclamide
Group III	*Azadirachta indica*
Group IV	*Acacia nilotica*
Group V	*Bryophyllum pinnatum*
Group VI	*Cuscuta reflexa*
Group VII	*Mikania cordata*
Group VIII	*Moringa oleifera*
Group IX	*Carica papaya*
Group X	*Coccinia grandis*
Group XI	*Carum copticum*
Group XII	*Nigella sativa*

5.2.3.3 Effects of Crude Extracts on Oral Glucose Tolerance Test (OGTT)

Oral Glucose Tolerance Test (OGTT) was performed according to the WHO instructions with an equivalent of 75 gm anhydrous glucose dissolved in water. A 2 hours plasma glucose value during an OGTT of ≥11.1 mmol/l (200 mg/dl) considered as diabetic condition (ADA 2014; TUEPG 2015). After overnight fasting, a 0-min blood samples were taken from the tail tip under mild ether anesthesia. Fasting plasma glucose (FPG) levels ≥7 mmol/l (126 mg/dl) was considered as diabetic symptoms (ADA 2014; TUEPG 2015). The test drugs were administered orally as per the Table 5.1. After 60 minutes of administration of extracts or drug to the particular group, glucose solution (2 gm/kg-body weight) was administered by gavaging. Blood

samples were drawn 2-hour after glucose administration by punching the tail-tip of the mice. Determination of the blood glucose level was done by the glucose-oxidase principle using the ONE TOUCH basic instrument and results were reported as mmol/L (which was manually converted to mg/dl). Anti-diabetic properties of the crude extracts were compared with standard anti-diabetic drug glibenclamide by using the value of 2nd hour plasma glucose level. The objective of this study was to measure the hypoglycaemic effect of tested drugs on animal at elevated glucose level in the blood.

5.2.3.4 Effects of Crude Extracts on Fasting Plasma Glucose (FPG) Level

Fasting is defined as no calorie intake for the last eight hours and fasting plasma glucose (FPG) levels ≥7 mmol/l (126 mg/dl) is considered as diabetic condition (ADA 2014; TUEPG 2015). After performing OGTT the same groups were treated for single dose experiment. The duration of resting period was one week. After overnight fasting, a 0-min blood samples were taken from the tail tip of all mice under mild ether anesthesia. The test drugs were administered orally as per the Table 5.1. Blood samples were taken after 2 hours of administration of test drugs. The objective of this study was to measure the hypoglycaemic effect of tested drugs on overnight fasted animals.

5.2.3.5 Effects of Long Term Uses of Crude Extracts on FPG Level and Body Weight

The objective of this study was to observe the long term effects of the crude extract on animal fasting plasma glucose (FPG) level as well as the body weight. It was the extension of the previous study and continued up to 15 days. Crude extracts were administered to the respective groups once in a day through oral route. Diabetic control and diabetic treatment groups were served regular food. Blood samples were collected from the tail-tip after overnight fasting condition and the estimation of blood glucose was carried out as above on 15th day of the drug administration. Base level glucose was considered the same as we found in the above study. All blood samples were taken before scheduled administration of drug or crude extract on animal. Body weights of all the animals were recorded just prior to and on the 15th day of the study to determine the effect of the crude extracts on the body weight, if any.

5.2.4 Analgesic Potency of the Crude Extracts

Eddy's hot plate method (Eddy and Leimbach, 1953; Buchineni *et al.,* 2014) was used for conducting analgesic study of the crude extracts described. Four *Swiss albino* mice of either sex with weight of about 20-30 gm was used in each group. The hot plate consists of electrically heated surface with controlled temperature 45±2^{0}C. Animals were divided into twelve groups, Group I, healthy animals which received only distilled water 2 hours before the experiment. Group II, healthy animals which received aspirin (2 mg/kg-bw) solution 2 hours before the experiment. Group III to XII, healthy animals which received crude extracts 2 hours before the experiment (Table 5.2).

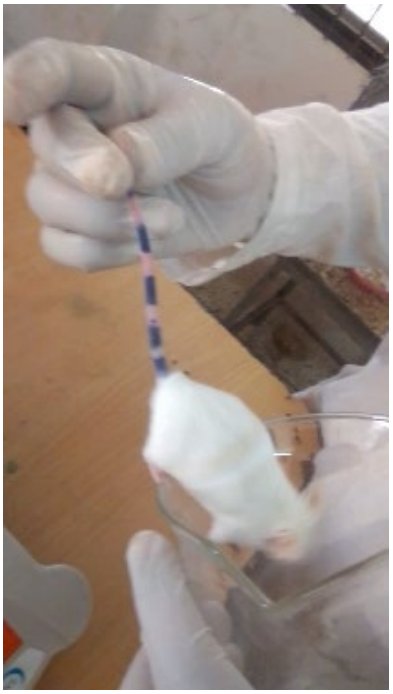
Table 5.2: Grouping of animals for analgesic study

Group	**Treatment for analgesic study**
Group I	Control received distilled water
Group II	Aspirin
Group III	*Azadirachta indica*
Group IV	*Acacia nilotica*
Group V	*Bryophyllum pinnatum*
Group VI	*Cuscuta reflexa*
Group VII	*Mikania cordata*
Group VIII	*Moringa oleifera*
Group IX	*Carica papaya*
Group X	*Coccinia grandis*
Group XI	*Carum copticum*
Group XII	*Nigella sativa*

5.2.5 Statistical Analysis

Statistical analysis was performed using SPSS 16.0 software. Data was described as Mean ± SD. Negative sign (-) indicates decrease and positive (+) sign indicates increase respectively. ANOVA followed by Tukeys multiple comparison tests was used for analysis multiple group comparisons. For all inferential statistical tests a two tailed P value of 0.05 was considered significant

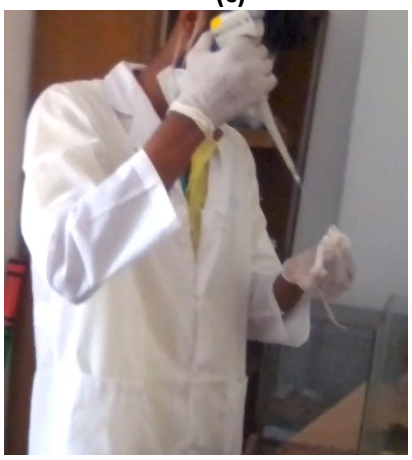
(a)



(b)

(c)

(d)



(e)

(f)

Figure 5.1: Different stages of anti-diabetic study on mice (a) Animal in a cage before enrolment in the study; (b) Identification code (differentiated individual mice by color variation and number of spot, etc.); (c) Weighing of the animal; (d) Dosing adjustment of dexamethasone injection for creating diabetes on mice; (e) Gavaging of phytochemicals and sugar before measuring blood glucose level; (f) Intraperitoneal administration of dexamethasone.

5.3 Results

In the present study anti-diabetic (hypoglycaemic) and analgesic properties was measured on artificially developed diabetic animal (mice) model by using the aqueous UAE crude extracts of *Azadirachta indica*, *Acacia nilotica*, *Bryophyllum pinnatum*, *Cuscuta reflexa*, *Mikania cordata*, *Moringa oleifera*, *Carica papaya*, *Coccinia grandis*, *Carum copticum* and *Nigella sativa*. Dexamethasone (induce glucocorticoid hormone) was used for creating diabetes condition on mice. Efficacy of crude extracts for curing diabetes was measured in comparison with the standard anti-diabetic drug glibenclamide.

5.3.1 Effect of Crude Extracts on OGTT of Experimental Mice

Oral glucose tolerance test (OGTT) was performed on dexamethasone induced diabetic mice and the data presented in Table 5.3. From the tabulated value, it was observed that fasting blood glucose levels of all the enrolled animals were >200 mg/dl indicated that dexamethasone was successfully induced diabetes in the experimented animal. According to the ADA (2014) a 2-hour plasma glucose value during OGTT of ≥11.1 mmol/l (>200 mg/dl) considered as diabetic condition. Crude extracts of *A. indica, B. pinnatum, M. oleifera, C. papaya* and *C. grandis* successfully reduced the plasma glucose level bellow 200 mg/dl and showed significant ($p<0.05$) glucose lowering effect compared with the diabetic control. The effect was observed almost similar to the standard anti-diabetic drug glibenclamide ($p>0.05$). Though crude extracts of *A. nilotica* failed to reduce blood glucose level bellow 200 mg/dl, but statistically it was significant ($p=0.045$) than the diabetic control and similar to the standard drug glibenclamide ($p=0.328$). Though marked reduction of glucose level observed by *C. reflexa*, *M. cordata* and *N. sativa* but that was not statistically significant ($p>0.05$) compared to the diabetic control. However, these three extracts were statistically significant ($p>0.05$) to the standard drug. Only *C. copticum* failed to overcome the OGTT parameter to show the hypoglycaemic effect.

Table 5.3: Effect of Single Dose on Oral Glucose Tolerance Test (OGTT)

Treatment group	Plasma Glucose Level (mg/dl) (Values are mean ± SD for n=4)		P value (After treatment)	
	0 Hour* (Before treatment)	2 Hour** (After treatment)	Control Vs. Treatment	Treatment Std. drug Vs. Extract
Group I: Diabetic control	230.85±15.16	244.80±12.82	-	-
Group II: Glibenclamide	227.70±13.66	188.55±12.06	0.001	-
Group III: *A. indica*	225.00±12.30	193.50±23.26	0.027	0.604
Group IV: *A. nilotica*	241.55±13.72	204.75±27.94	0.045	0.328
Group V: *B. pinnatum*	228.15±17.54	187.20±16.94	0.027	0.931
Group VI: *C. reflexa*	237.60±11.48	202.95±22.16	0.059	0.227
Group VII: *M. cordata*	228.15±17.1	205.65±17.54	0.064	0.329
Group VIII: *M. oleifera*	242.45±22.54	192.60±18.88	0.009	0.635
Group IX: *C. papaya*	234.00±18.60	197.10±20.18	0.007	0.520
Group X: *C. grandis*	223.65±14.28	190.80±16.44	0.012	0.777
Group XI: *C. copticum*	229.05±15.08	219.60±31.62	0.164	0.078
Group XII: *N. sativa*	233.10±11.90	211.95±27.62	0.072	0.249

*At fasting condition, ≥7 mmol/l (>126mg/dl) considered diabetic situation (ADA 2014).

** 2-hr plasma glucose value during OGTT, ≥11.1 mmol/l (200mg/dl) considered diabetic condition (ADA 2014)

$P<0.05$, indicate significant difference

5.3.2 Effect of Crude Extracts on FPG level of Experimental Mice

It was observed that *A. indica, B. pinnatum, M. oleifera, C. papaya* and *C. grandis* successfully reduced the plasma glucose level bellow 126 mg/dl and showed significant ($p<0.05$) glucose lowering effect compared with the diabetic control (Table 5.4). The effect was observed almost similar to the standard anti-diabetic drug glibenclamide ($p>0.05$). Though crude extracts of *M. cordata, C. copticum* and *N. sativa* failed to reduce plasma glucose level below 126 mg/dl, but significant ($p<0.05$) reduction was observed than the diabetic control, statistically similar to the standard drug glibenclamide ($p>0.05$). Results indicated the distinguished anti-diabetic properties of those crude extracts. Plasma glucose lowering effect of crude extracts of *A. nilotica* and *C. reflexa* was insignificant compared to the diabetic control and the standard drug glibenclamide.

Table 5.4: Effect of Crude Extracts on FPG Level of Experimented Mice

Treatment Group	Plasma Glucose Level (mg/dl) (Values are mean ± SEM for n=4)		P value (After treatment)	
	0 Hour (Before treatment)	2 Hour (After treatment)	Control Vs. Treatment	Treatment Std. drug Vs. Extract
Group I: Diabetic control	233.10±7.28	229.95±7.54	-	-
Group II: Glibenclamide	227.70±13.663	100.35±12.32	0.000	-
Group III: *A. indica*	229.95±28.46	119.70±14.28	0.000	0.089
Group IV: *A. nilotica*	232.65±22.54	151.65±10.00	0.001	0.004
Group V: *B. pinnatum*	234.00±18.30	120.15±22.12	0.001	0.288
Group VI: *C. reflexa*	236.25±20.22	153.45±29.96	0.007	0.013
Group VII: *M. cordata*	226.35±13.32	147.60±24.10	0.013	0.078
Group VIII: *M. oleifera*	220.95±16.84	126.00±11.28	0.001	0.067
Group IX: *C. papaya*	239.85±15.38	113.40±12.02	0.000	0.130
Group X: *C. grandis*	219.15±12.50	115.65±20.84	0.003	0.387
Group XI: *C. copticum*	221.85±9.90	134.55±18.44	0.004	0.085
Group XII: *N. sativa*	235.35±17.28	139.50±15.16	0.002	0.059

*At fasting condition, ≥7 mmol/l (>126mg/dl) considered diabetic situation (ADA 2014).

$P<0.05$, indicate significant difference

5.3.3 Effect of Long Term Use of Crude Extracts on FPG level

Long term effect of drug was measured by administrating multiple doses i.e., one dose 300 mg/kg-bw/day up to 15 days results presented in the Table 5.5. The previous study was extended up to 15 days in this purpose. From the tabulated value, it was observed that only *B. pinnatum* successfully maintained the plasma glucose level 126 mg/dl and showed significant ($p<0.05$) glucose lowering effect compared with the diabetic control. Though crude extracts of *A. indica, A. nilotica, M. oleifera, C. papaya* and *C. grandis* failed to maintain plasma glucose level below 126 mg/dl, but significantly ($p<0.05$) lower than the diabetic control and similar to the standard drug glibenclamide ($p>0.05$), indicated the distinguished anti-diabetic properties of those crude extracts. Plasma glucose lowering effect of crude extracts of *C. reflexa, C. copticum* and *N. sativa* were found insignificant ($p< 0.05$).

Table 5.5: Effect of long term uses of crude extracts on FPG Level

Treatment Group	Plasma Glucose Level (mg/dl) (Values are mean ± SEM for n=4)		P value (After treatment)	
	Base level	Day 15	Control Vs. treatment	Treatment Std. drug Vs. Extract
Group I: Diabetic control	233.10±7.28	249.75±8.98	-	-
Group II: Glibenclamide	227.70±13.663	108.00±9.86	0.000	-
Group III: *A. indica*	229.95±28.46	128.70±26.30	0.002	0.145
Group IV: *A. nilotica*	232.65±22.54	130.95±15.16	0.001	0.109
Group V: *B. pinnatum*	234.00±18.30	123.75±8.86	0.000	0.125
Group VI: *C. reflexa*	236.25±20.22	182.25±8.10	0.002	0.003
Group VII: *M. cordata*	226.35±13.32	147.15±23.12	0.001	0.068
Group VIII: *M. oleifera*	220.95±16.84	130.95±21.92	0.002	0.008
Group IX: *C. papaya*	239.85±15.38	128.7±11.62	0.000	0.077
Group X: *C. grandis*	219.15±12.50	129.60±12.20	0.001	0.136
Group XI: *C. copticum*	221.85±9.90	163.35±24.48	0.011	0.010
Group XII: *N. sativa*	235.35±17.28	161.10±4.76	0.002	0.017

*At fasting condition, ≥7 mmol/l (>126mg/dl) considered diabetic situation (ADA 2014).

P<0.05, indicate significant difference

5.3.3.1 Effect of Long Term Use of Crude Extracts on Body Weight

Unwanted weight loss is one of the common symptoms of diabetes disease. The impact of crude extract on body weight of the experimented diabetic mice was observed in the present study. From the tabulated value it was observed that weight loosing effect was only happened in diabetic control mice (Table 5.6). Diabetic group treated by standard anti-diabetic drug glibenclamide was gain significant weight during the study. Crude extracts included in the present study either gain or protect body weight during the experiment.

Table 5.6: Effects of Crude Extracts on body weight of the diabetic mice

Group	Impacts of extract on body weight after multiple dose administration (Values are mean ± SD for n=4)			p-value
	Body weight before treatment	Body weight after treatment	Weight difference	
Diabetic control	30.58±2.48	25.48±1.68	(-) 5.10±2.72	0.033
Glibenclamide	31.4±2.90	36.45±1.26	5.05±2.20	0.019
A. indica	27.57±3.52	27.8±3.26	0.225±1.16	0.027
A. nilotica	29.07±3.28	29.23±3.26	0.15±0.96	0.775
B. pinnatum	27.6±1.72	30.38±2.72	2.78±2.18	0.85
C. reflexa	27.93±2.52	29.47±8.84	1.55±2.58	0.361
M. cordata	28.05±1.20	30.63±2.94	2.57±2.10	0.09
M. oleifera	28.25±1.14	30.48±3.34	2.23±2.42	0.164
C. papaya	29.67±1.76	34.00±1.16	4.33±2.40	0.069
C. grandis	29.53±2.72	34.47±3.62	4.93±1.12	0.013
C. copticum	28.53±1.48	33.67±2.40	5.13±1.18	0.013
N. sativa	30.33±3.76	35.07±4.06	4.73±0.36	0.001

P<0.05, indicate significant difference

5.3.4 Analgesic Effect of Crude Extracts on Healthy Mice

Diabetic patients generally suffer long term pain associated with different types of diseases. An analgesic property of the crude extract (presented in Table 5.7) was also measured in the present study. Comparison was made with the standard drug aspirin. From tabulated data it was observed that most of the crude extracts showed significant analgesic effect on the mice.

Table 5.7: Analgesic effect of crude extracts

Study Group	Mean ± SEM	P value		
		Control & Standard	Control &. Extract	Standard & Extract
Healthy control (Control)	51±2.08	-	-	-
Aspirin (Standard)	88±4.24	0.008	-	-
A. indica	75.75±7.28	-	0.048	0.234
A. nilotica	78.00±7.93	-	0.047	0.412
B. pinnatum	74.25±9.97	-	0.032	0.372
C. reflexa	74.25±5.72	-	0.014	0.253
M. cordata	74.00±3.49	-	0.006	0.129
M. oleifera	78.25±4.61	-	0.003	0.344
C. papaya	74.00±4.49	-	0.017	0.147
C. grandis	77.25±5.72	-	0.013	0.129
C. copticum	72.00±6.36	-	0.017	0.193
N. sativa	80.16±4.23	-	0.018	0.112

P<0.05, indicate significant difference

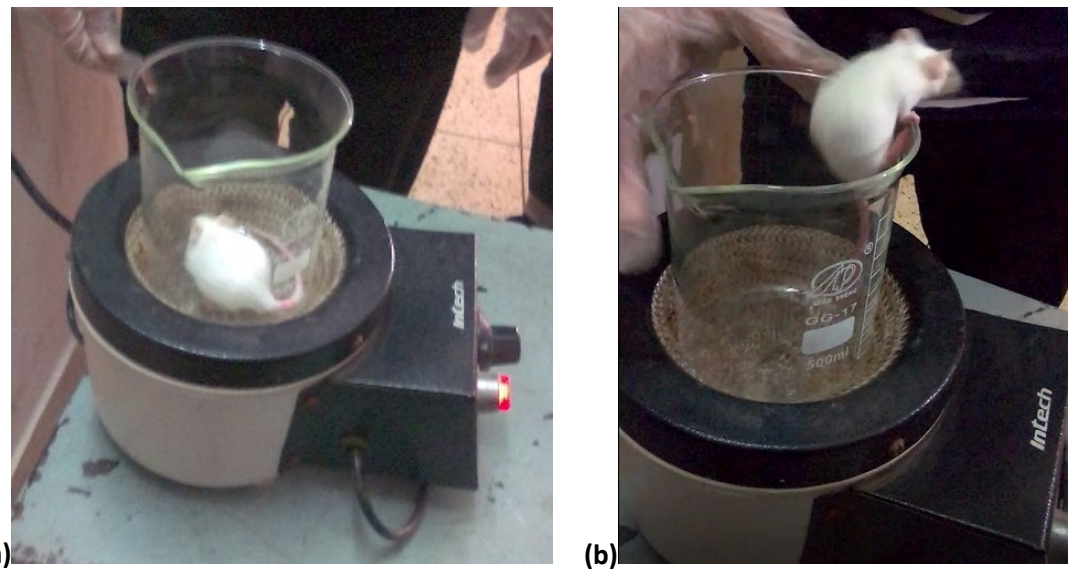


Figure 5.2: Analgesic potency study by Eddy's Hot Plate Method **(a)** Tolerable pain **(b)** Intolerable pain

5.4 Discussion

Form this study, it was observed that the aqueous UAE crude extracts of *Azadirachta indica* fresh leaves had significant anti-diabetic effect equivalent to the standard anti-diabetic drug glibenclamide. *A. indica* crude extracts showed distinct effect of preventing unwanted lost of body weight of the diabetic animal and provided significant analgesic effect similar to the standard analgesic drug aspirin. Similar activities was also observed from Bhat *et al.* (2011) study and they observed that aqueous and methanolic extracts of *A. indica* showed a good oral glucose tolerance than chloroform performed by using *invivo* diabetic murine model. Similarly Nagashayana *et al.* (2014) study concluded that aqueous extract of leaf and some oily extract of *A. indica* produced a marked decrease in blood glucose levels in alloxan induced diabetic animals. Morshed *et al.* (2011) efficacy of *A. indica* leaf (powder, aqueous extract and 80% ethanol) extract as hypoglycaemic on streptozotocin induced diabetic animals. They also found body weight of the animal tends to increase throughout the study period. Buchineni *et al.* (2014) study indicated that the aqueous extract of *A. indica* (leaf) extract revealed significant analgesic activity in thermal and chemical induced pain models. On the basis of above findings *A. indica* leaves may be recommended for the treatment of diabetes.

Crude extract of *Acacia nilotica* fresh leaves showed distinct anti-diabetic effect on the tested mice during the anti-diabetic studies (OGTT, FPG and long term study). However hypoglycaemic effect was not equivalent to the standard anti-diabetic drug glibenclamide. It was observed that *A. nilotica* crude extracts had distinct effect of preventing unwanted loss of body weight of the diabetic animal and provided significant analgesic effect similar to the standard analgesic drug aspirin. From previous study anti-diabetic potential of aqueous leaf extracts of *A. nilotica* on alloxan induced diabetic mice was found (Karau 2013). Kumari *et al.* (2014) concluded that *A. nilotica* powder was effective in management of blood glucose levels even the simple sugars also and can be considered as suitable for diabetics. Tanko *et al.* (2014) studied methanol crude leaves extract and aqueous fraction on blood glucose levels of Alloxan-induced diabetic Wistar rats and concluded that the extract had both significant hypoglycaemic and anti-hyperglycemic effects. Asad *et al.* (2015) observed that *A. nilotica* leaves extract resulted in hypoglycaemic and hypolipidemic effect in alloxan-induced diabetic rats similar to glibenclamide. Rashid *et al.* (2017) studied analgesic activity of the plant *Acacia nilotica* by acetic acid induced writhing

method and moderate analgesic activity. That the aqueous extracts of *A. nilotica* produced non-dose dependent analgesic activity was related to studies by Zarei *et al.* (2015). Verma *et al.* (2014) observed ethanolic extract of *A. nilotica* showed no significant change in body weight of Wistar rats. On the basis of above findings *A. nilotica* leaves may be recommended for the treatment of diabetes.

Crude extracts of *Bryophyllum pinnatum* fresh leaves had significant anti-diabetic effect which was equivalent to the standard anti-diabetic drug glibenclamide used in the present study. The *B. pinnatum* crude extracts had distinct effect of preventing unwanted lost of body weight of the diabetic animal and also provided significant analgesic effect equivalent to the standard analgesic drug aspirin. Patil *et al.* (2013) observed that dichloromethane fraction showed better hypoglycaemic activities than chloroform fraction whereas petroleum ether and aqueous fraction were not able to reduce blood glucose levels as compared to glibenclamide control male albino Wistar rats after OGTT experiment. Kpomah and Arhoghro (2012) reported significant reduction of serum glucose and lipid profile of the alloxan induced diabetic albino rats. Aransiolav *et al.* (2014) also reported that aqueous extract of *B. pinnatum* leaves have significant anti-diabetic properties, and that the performance of the existing drugs (glibenclamide) could be enhanced with the use of the aqueous extract. Igwe and Akunyili (2005) observed increased the pain threshold in rats using the hot plate or thermal methods, inhibited or reduced phenylbenzoquinone-induced writhing or abdominal stretches in mice in a dose-dependent manner and produced a weak or an inferior anti-inflammatory activity than aspirin. Afzal *et al.* (2012) observed that the anti-inflammatory and analgesic activity of aqueous extract of *B. pinnatum was* mainly due to the presence of steroidal compound in the extract. On the basis of above findings *B. pinnatum* leaves may be recommended for the treatment of diabetes.

Crude extracts of *Cuscuta reflexa* fresh stems was observed distinct anti-diabetic effect from the above anti-diabetic studies. However the hypoglycaemic effect was not equivalent to the standard anti-diabetic drug glibenclamide. It was observed that *C. reflexa* crude extracts had distinct effect of preventing unwanted lost of body weight of the diabetic animal and provided significant analgesic effect similar to the standard analgesic drug aspirin. Previous study reported that methanol and aqueous extract provide significant reduction of blood glucose during OGTT in diabetic rats (Rath *et al.,* 2016). Improvement of body weight was also observed from this study. Sandeep and Mittal (2017) reported that the ethanolic extract produced significant

decrease in the blood glucose level when compared with the controls in alloxan induced hyperglycemic rats in the single dose experiment at the tested dose level and are comparable with the standard drug glibenclamide. They also observed that the ethanolic extract reversed the weight loss of the diabetic rats and they returned to near normal. On the basis of above findings *C. reflexa* leaves may be recommended for the treatment of diabetes.

Crude extracts of *Mikania cordata* fresh leaves had marked anti-diabetic effect found in the present study but comparatively very less than the standard anti-diabetic drug glibenclamide. The *M. cordata* crude extract was also provided significant analgesic effect similar to the standard analgesic drug aspirin but significant weight gaining effect was observed during the treatment of diabetic mice. Previous study also found a beneficial effect in the treatment of diabetes mellitus on alloxan-induced diabetic rats and proposed for the therapy of diabetes (Nurhayati *et al.,* 2013). Ahmed *et al.* (2002) observed that the crude extract of *M. cordata* significantly inhibited acetic acid-induced writhing in mice indicated analgesic properties. On the basis of above findings *M. cordata* leaves may be recommended for the treatment of diabetes.

Crude extracts of *Moringa oleifera* fresh leaves showed high anti-diabetic activity which was almost equivalent to the standard anti-diabetic drug glibenclamide. The *M. oleifera* crude extract was also provided significant analgesic effect similar to the standard analgesic drug aspirin. The extract also showed marked weight gaining effect during the long term treatment of diabetic mice. Previous study also observed blood glucose levels became normal after long (6 weeks) term administration of aqueous extract of *M. oleifera* leaves on streptozotocin induced diabetic rats (Adeeyo *et al.,* 2013). They also observed increased body weight after administration of aqueous extract. Paula *et al.* (2017) found that 56.2% reduction in the blood glucose level on the 7^{th} day after I.P. administration of extracted protein from leaf extract of *M. oleifera*. Ndong *et al.* (2007) observed that glucose tolerance test, significantly decreased the blood glucose for Goto-Kakizaki rats and Wistar rats. Chinedu *et al.* (2014) observed that the extract of *Moringa oleifera* significantly lowered the fasting blood glucose at days 7 and 14 compared to controls. Patnaik *et al.* (2018) observed that ethanolic extract had better analgesic effect than aqueous extract of *M. oleifera* leaves compared to the aspirin. Bhattacharya *et al.,* (2014) observed that ethanolic leaf extract of *M. oleifera* exhibited analgesic activity in both models (using acetic acid induced writhing test and Eddy's hot plate test) showing its both central and

peripheral analgesic actions. On the basis of above findings *M. oleifera* leaves may be recommended for the treatment of diabetes.

Crude extracts of *Carica papaya* dried leaves had significantly high anti-diabetic effect which was equivalent to the standard anti-diabetic drug glibenclamide. The *C. papaya* crude extract was provided significant analgesic effect similar to the standard analgesic drug aspirin. The extract also showed marked weight gaining effect during the long term treatment of diabetic mice. Previous study found significant activity of *C. papaya* leaves extracts on alloxan induced diabetic mice of lowering blood glucose level after 6h of oral administration of the extract (Sinha *et al.,* 2018). Juárez-Rojop *et al.* (2012) observed that the aqueous extract of *C. papaya* exert shown hypoglycaemic and antioxidant effect and improvement in the lipid profile on streptozotocin induced diabetes rats. Adenowo *et al.* (2014) observed significant hypoglycaemic effect of *C. papaya* leaf exert. Hasimuna *et al.* (2014) observed ethanol extract of *C. papaya* dose of showed the best analgesic activity over hexane and ethylacetate extract that was comparable to aspirin. Danborno *et al.* (2018) studied analgesic properties of aqueous leaves extract of *C. papaya* using formalin induced hind paw edema in rats, and acetic acid induced abdominal writhing in mice and found strong anti-inflammatory and analgesic property and its analgesic property is dose dependent. On the basis of above findings *C. papaya* leaves may be recommended for the treatment of diabetes.

This study observed that crude extracts of *Coccinia grandis* dried leaves had significantly high anti-diabetic activity which was equivalent to the standard anti-diabetic drug glibenclamide. The *C. grandis* crude extract was also provided significant analgesic effect similar to the standard analgesic drug aspirin. The extract also showed significant weight gaining effect during the long term treatment of diabetic mice. Packirisamy *et al.* (2018) study found that *C. grandis* represent as a good candidate for alternative or complementary medicine in the management of diabetes mellitus. Attanayake *et al.* (2015) reported that hot water crude leaf extract of *C. grandis* had significant effect on serum/blood glycemic parameters, serum lipid parameters and regenerative potential of islet cells on streptozotocin induced diabetic rats. Hossain *et al.* (2014) observed significant analgesic action of ethanol leaves extract compared to the standard analgesic drug diclofenac sodium. However, from Ashish *et al.* (2011) methanolic extract failed to show central analgesic action. On the basis of above findings *C. grandis* leaves may be recommended for the treatment of diabetes.

Crude extracts of *Carum copticum* dried seeds showed anti-diabetic effect but very less compared to the standard anti-diabetic drug glibenclamide. The *C. copticum* crude extract was also provided significant analgesic effect similar to the standard analgesic drug aspirin. The extract also showed significant weight gaining effect during the long term treatment of diabetic mice. Shete and Sathaye (2013) observed significant hypoglycaemic effect by OGTT and FPG test on streptozotocin induced diabetic rats. Rahmatabadi *et al.* (2007) observed that *C. copticum* extract possessed a clear-cut analgesic effect tail-flick latency study on mice. On the basis of above findings *C. copticum* seeds need further study to recommend for the treatment of diabetes. However, the extract may be recommended associate with other anti-diabetic drug as it showed better effect of preventing weight lost during diabetic condition.

Crude extracts of *N. sativa* dried seeds had anti-diabetic effect found in the above study but slightly lower than the standard anti-diabetic drug glibenclamide. The *C. copticum* crude extract was also provided significant analgesic effect similar to the standard analgesic drug aspirin. The extract also showed significant weight gaining effect during the long term treatment of diabetic mice. Previous study reported no effect on glucose levels in diabetic rats (Karimlar *et al.,* 2019). However, Rabey *et al.* (2016) observed that methanolic extract of *N. sativa* significantly reduced the fasting blood sugar after 4 weeks administration on streptozotocin induced diabetic rats. Shete and Sathaye (2013) observed significant hypoglycaemic effect by OGTT and FPG test on streptozotocin induced diabetic rats. Andaloussi *et al.* (2011) observed insignificant effect of *N. sativa* on body weight of *Meriones shawi.* They also observed significance glucose lowering effect by OGTT test on *Meriones shawi*. Ghannadi *et al.* (2005) suggested that *N. sativa* had analgesic and anti-inflammatory effects were studied on mice and rats using the acetic acid-induced writhing, formalin, light tail flick, carrageenan-induced paw edema, and croton oil-induced ear edema tests. On the basis of above findings *N. sativa* seeds required further study to recommend for the treatment of diabetes. However, the extract may be recommended in association with other anti-diabetic drug as it showed better effect of preventing unwanted weight lost during diabetic condition and analgesic properties.

5.5 Conclusion

Most of the selected plants showed somewhat anti-diabetic as well as analgesic activities on diabetic mice. However, *Azadirachta indica*, *Bryophyllum pinnatum*, *Moringa oleifera*, *Carica papaya* and *Coccinia grandis* showed significantly high anti-diabetic effect almost similar to the anti-diabetic drug glibenclamide. Unwanted weight losses of diabetic suffering animal were also overcome by using the above mentioned extracts. From the previous chapter it was observed that the cytotoxic properties of all the crude extracts were comparatively less than the widely used drug. From the study herbal oral preparation of the above mentioned crude extracts may be recommended for curing of diabetic disease.

References

ADA (American Diabetes Association). 2014. Diabetes and driving. *Diabetes Care*, 37(1): 97-103.

Adeeyo AO, Adefule AK, Ofusori DA, Aderinola AA and Caxton-Martins EA. 2013. Antihyperglycemic Effects of Aqueous Leaf Extracts of Mistletoe and *Moringa oleifera* in Streptozotocin-Induced Diabetes Wistar Rats. *Diabetologia Croatica,* 42(3):81-88.

Adenowo AF, Ilori MF, Balogun FO and Kazeem MI. 2014. Protective Effect of Ethanol Leaf Extract of *Carica papaya* Linn (Caricaceae) in Alloxan-induced Diabetic Rats. *Tropical Journal of Pharmaceutical Research November*, 13 (11): 1877-1882.

Adenowo AF, Ilori MF, Balogun FO and Kazeem MI. 2014. Protective Effect of Ethanol Leaf Extract of *Carica papaya* Linn (Caricaceae) in Alloxan-induced Diabetic Rats. *Tropical Journal of Pharmaceutical Research November*, 13 (11): 1877-1882.

Afzal M, Gupta G, Kazmi I, Rahman M, Afzal O, Alam J, Hakeem KR, Pravez M, Gupta R and Anwar F. 2012. Anti-inflammatory and analgesic potential of a novel steroidal derivative from *Bryophyllum pinnatum*. *Fitoterapia,* 83(5): 853-858.

Ahmed MFG, Rahman TM, Alimuzzaman M and Shilpi JA. 2001. Analgesic sesquiterpene dilactone from *Mikania cordata*. *Fitoterapia*, 72(8): 919-921.

Akhere AO, Iyere OO, Afolabi FA, Lekan OA and Ayodeji RA. 2013. Long Term Anti-Diabetic, Anti-Hyperlipidaemic and Anti-Atherogenic Effects of *Carica papaya* Leaves in Streptozotocin Diabetic Rats. *European Journal of Medicinal Plants*, 3(4): 508-519.

Altan VM. 2003. The pharmacology of diabetic complications. *Current Medicinal Chemistry*, 10: 1317-1327.

Andaloussi AB, Martineau L, Vuong T, Meddah B, Madiraju P, Settaf A, and Haddad PS. 2011. The In Vivo Antidiabetic Activity of *Nigella sativa* is Mediated through Activation of the AMPK Pathway and Increased Muscle Glut4 Content. *Evidence-Based Complementary and Alternative Medicine,* 2011: 1-9.

Andrade-Cetto A and Heinrich M. 2005. Mexican Plants with hypoglycaemic effect used in the treatment of diabetes. *Journal of Ethnopharmacology,* 99: 325-348.

Aransiolav EF, Daramola MO, Iwalewa EO, Seluwa AM and Olufowobi OO. 2014. Anti-Diabetic Effect of *Bryophyllum pinnatum* Leaves. *International Journal of Biotechnology and Bioengineering*, 8(1): 89-93.

Asad M, Munir TA, Farid S, Aslam M, Shah SS. 2015. Duration effect of *Acacia nilotica* leaves extract and glibenclamide as hypolipidaemic and hypoglycaemic activity in alloxan induced diabetic rats. *Journal of the Pakistan Medical Association,* 65(12): 1266-1270.

Ashish SA, Ujwala RS, Sugandha GC, Deshpande SV, Aniket AG and Sandeep GT. 2011. Analgesic and antipyretic activity of methanolic extract of *Coccinia grandis* L. leaves in experimental animals. *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* 2(4): 175-182.

Attanayake AP, Jayatilaka KAPW, Pathirana C and Mudduwa LKB. 2015. Antihyperglycemic activity of *Coccinia grandis* (L) Voigt in streptozotocin induced diabetic rats. *Indian Journal of Traditional Knowledge,* 14(3): 376-381.

Bhat M, Kothiwale SK, Tirmale AR, Bhargava SY and Joshi BN. 2011. Antidiabetic Properties of *Azardiracta indica* and *Bougainvillea spectabilis*: In Vivo Studies in Murine Diabetes Model. *Evidence-Based Complementary and Alternative Medicine*, 2011: 1-9.

Bhattacharya A, Agrawal D, Sahu PK, Kumar S, Mishra SS and Patnaik S. 2014. Analgesic effect of ethanolic leaf extract of *Moringa oleifera* on albino mice. *Indian Journal of Pain,* 28: 89-94.

Buchineni M, Kumar MR, Kudagi BL, Pathapati RM, Salmakamal, Haritha M and Chandra VB. 2014. Evaluation of Anti-Inflammatory and Analgesic Activity of *Azadirachta Indica* (leaf) Extract in Chemical and Thermal Induced Pain Models in Rats. *International Journal of Toxicological and Pharmacological Research,* 6(4): 144-147.

Cheng AYY and Fantus IG. 2005. Oral antihyperglycemic therapy for type 2 diabetes Mellitus. *Canadian Medical Association Journal,*172(2): 213-226.

Chinedu AA, Alani SO and Olaide AO. 2014. Effect of the Ethanolic Leaf Extract of *Moringa oleifera* on Insulin Resistance in Streptozotocin Induced Diabetic Rats. *Journal of Plant Sciences,* 2(6-1): 5-12.

Danborno AM , Ibrahim SH and Mallo MJ. 2018. The Anti-Inflammatory and Analgesic Effects of the Aqueous Leaves Extract of *Carica Papaya*. *IOSR Journal of Pharmacy and Biological Sciences,* 13(3): 60-63.

Eddy NB and Leimbach DJ. 1953. *Journal of Pharmacology and Experimental Therapeutics*, 107: 385-393.

Fakeye TO, Oladipupo T, Showande O and Ogunremi Y. 2007. Effects of Coadministration of Extract of *Carica papaya* Linn (family Cariaceae) on Activity of Two Oral Hypoglycaemic Agents. *Tropical Journal of Pharmaceutical Research*, 6(1): 671-678.

Ghannadi A, Hajhashemi V and Jafarabadi H. 2005. An Investigation of the Analgesic and Anti-Inflammatory Effects of *Nigella sativa* Seed Polyphenols. *Journal of Medicinal Food*, 8(4): 488-493.

Gy SY, Cisse A, Nongonierma RB, Sarr M, Mbodj NA and Faye B. 2005. Hypoglycaemic and antidiabetic activity of acetonic extract of *Vernonia colorata* leaves in normoglycaemic and alloxan induced diabetic rats. *Journal of Ethnopharmacology.,* 98: 171-175.

Hasimuna P, Suwendara P and G.I. Ernasaria GI. 2014. International Seminar on Natural Product Medicines, ISNPM 2012 Analgetic Activity of Papaya (*Carica papaya* L.) Leaves Extract. *Procedia Chemistry,* 13: 147-149.

Holman RR and Turner RC. 1991. Oral Agents and Insulin in the Treatment of NIDDM. In: Pickup, J. and Williams, G., Eds., Text Book of Diabetes, Blackwell, Oxford: 467-469.

Hossain SA, Uddin SN, Salim MA and Haque R. 2014. Phytochemical and Pharmacological screening of *Coccinia grandis* Linn. *Journal of Scientific and Innovative Research*, 3(1): 65-71.

Igwe SA & Akunyili DN. 2005. Analgesic Effects of Aqueous Extracts of the Leaves of *Bryophyllum pinnatum. Pharmaceutical Biology*, 43(8): 658-661.

Juárez-Rojopa IE, Tovilla-Zárateb CA, Aguilar-Domíngueza DE, Roa-de la Fuentec LF, Lobato-Garcíac CE, Blé-Castilloa JL, López-Merazd L, Díaz-Zagoyae JC, Bermúdez-Ocañab DY. 2014. Phytochemical screening and hypoglycaemic activity of *Carica papaya* leaf in streptozotocin-induced diabetic rats. *Revista Brasileira de Farmacognosia,* 24: 341-347.

Karau GM. 2013. Biosprospecting of antidiabetic compounds from selected medicinal plants for the management of Diabetes Mellitus in Mbeere and Meru, Kenya. *PhD Thesis*: 57.

Karimlar S, Naderi A, Mohammadi SF, Moslehishad M, Delrish E, Aghajanpour L, Khoshzaban A and Lashay A. 2019. Hypoglycaemic and Hypolipidemic Effects of *Myrtus communis*, *Trachyspermum copticum* and *Ferula gummosa* Essential Oils on Streptozotocin Induced Diabetic Rats. *Nutrition and Food Sciences Research,* 6(1): 1-8.

Kpomah ED and Arhoghro EM. 2012. Effects of doses of *Bryophylum pinnatum* and glibenclamide on serum glucose and lipid profile in alloxan-induced diabetic rats. *Indian Journal of Drugs and Diseases,* 1(5): 124-128.

Kumari M, Jain S and Dave R. 2014, "Babul (*Acacia nilotica*): A potential source of tannin and its suitability in management of type II diabetes". *Nutrition & Food Science*, 44(2): 119-126.

Morelli V and Zoorob RJ.2000. Alternative Therapies: Part 1. Depression, Diabetes, Obesity. *American Family Physician,* 62(5): 1051-1060.

Morshed MA, Fayzunnessa N , Haque MA, Uddin A , Bhoumik A , Ali L and Rokeya B. 2011. Comparative study of *Azadirachta indica* leaf powder and its different extracts on glycemic, insulinemic and lipidemic status of type 2 diabetic model rats. *International Journal of Pharmaceutical Sciences and Research,* 2(4): 954-960.

Nagashayana, Jagadeesh and Revankar SP. 2014. Evaluation of Hypoglycaemic Activity of Neem (*Azadirachta indica*) In Albino Rats. *IOSR Journal of Dental and Medical Sciences,* 13(9): 4-11.

Ndong M, Uehara M, Katsumata S, and Suzuki K. 2007. Effects of Oral Administration of *Moringa oleifera* Lam on Glucose Tolerance in Goto-Kakizaki and Wistar Rats. *Journal of Clinical Biochemistry and Nutrition*, 40: 229-233.

NIH 1985. National Institute of Health. Guide for the care and use of laboratory animals. NIH publication no. 85-23, Bethesda, USA, 1985.

Nurhayati WHW, Arlizan TN and Nurdiana S. 2013. Effect of *Mikania micrantha* leaf extract on the level of blood glucose and hepatic glycogen in the normal and alloxan-induced diabetic rats. *Natural Products an Indian Journal,* 9(10): 398-402.

Ogawa A, Johnson JH, Ohneda M, Mcallister CT, Inman LR, Alam T, Unger RH. 1992. Roles of insulin resistance and beta-cell dysfunction in dexamethasone-induced diabetes. *Journal of Clinical Investigation,* 90: 497-504.

Ogundele AV, Otun KO, Ajiboye A, Olanipekun BE and Ibrahim RB. 2017. Anti-Diabetic Efficacy and Phytochemical Screening of Methanolic Leaf Extract of Pawpaw (*Carica papaya*) Grown in North Central Nigeria. *Journal of the Turkish Chemical Society Section A: Chemistry,* 4(1): 99-114.

Packirisamy M, Ayyakkannu P and Sivaprakasam M. 2018. Antidiabetic effect of *Coccinia grandis* (L.) Voigt (Cucurbitales: Cucurbitaceae) on streptozotocin induced diabetic rats and its role in regulating carbohydrate metabolizing enzymes. *Brazilian Journal of Biological Sciences*, 5(11): 683-698.

Patil T, Patil S, Patil A and Patil S. 2014. *Carica Papaya* Leaf Extracts-An Ethnomedicinal Boon. *International Journal of Pharmacognosy and Phytochemical Research*, 6(2): 260-265.

sPatnaik PK, Majeed MA, Bhuvaneshwari, Kameswari L and Niharika MB. 2018. Comparative study of analgesic effect of *Moringa oleifera* leaf extracts with aspirin on experimental animals. *International Journal of Basic & Clinical Pharmacology,* 7(11): 2096-2100.

Paula PC, Sousa DOB, Oliveira JTA, Carvalho AFU, Alves BGT, Pereira ML, Farias DF, Viana MP, Santos FA, Morais TC and Vasconcelos IM. 2017. A Protein Isolate from *Moringa oleifera* Leaves Has Hypoglycaemic and Antioxidant Effects in Alloxan-Induced Diabetic Mice. *Molecules,* 22(271): 1-15.

Rabey HAE, Seeni MNA and Bakhashwain AS. 2017. The Antidiabetic Activity of *Nigella sativa* and Propolis on Streptozotocin-Induced Diabetes and Diabetic Nephropathy in Male Rats. *Evidence-Based Complementary and Alternative Medicine,* 2017: 1-14.

Rahmatabadi MHD, Hejazian SH, Morshedi A and Rafati A. 2007. The analgesic effect of *Carum copticum* extract and morphine on phasic pain in mice. *Journal of Ethnopharmacology,* 109(2): 226-8.

Rao BK, Giri R, Kesavalu MM and Apparao CH. 1997. Herbal medicine in the management of diabetes mellitus. *Manphar Vaidhya Patrika,* 1: 33-35.

Rashid MM, Islam A , Amran MS and Hossain MA. 2017. Evaluation of Analgesic Activity by Acetic Acid Induced Writhing Method of Crude Extracts of *Acacia nilotica*. *Scholars Academic Journal of Pharmacy,* 6(4): 126-138.

Rath D, Kar DM, Panlgrahi SK and Maharana L. 2016. Antidiabetic effects of *Cuscuta reflexa* Roxb. In streptozotocin induce diabetic rats. *Journal of Ethnopharmacology,* 192: 442-449.

Sadat AFMN, Mizan MRB, Sultana A, Rahman MM and Azad MAK. 2018. Comparative study of the Antimicrobial Activity of Methanol Extract and Ultrasound Assisted Water Extract of the Leaves of *Azadirachta indica*. *Rajshahi University Journal of Environmental Science,* 7: 40-47.

Sandeep and Mittal A. 2017. Antidiabetic activity of *Cuscuta reflexa. International Journal of Pharma and Chemical Research,* 3(3): 572-576.

Setter SM, White JR and Campbell RK. 2000. Diabetes: Textbook of therapeutics, drugs and diseases management, Ed Herfindal E.T and Crowley D.R; Lippincott; Williams and Wilkins Publishing Company, United States of America: 45-50.

Shete H and Sathaye S. 2013. Anti-diabetic activity of polyherbal therapy in Streptozotocin induced diabetes mellitus. *International Journal of Research in Pharmaceutical Sciences,* 4(1): 88-95.

Sinha RK, Pratap R and Varma MC. 2018. Hypoglycaemic Activity of *Carica papaya* Leaf Aqueous Extract in Normal and Diabetic Mice. *International Journal of Advances in Scientific Research and Engineering*, 4(6): 12-16.

Sreenivasan S, Vello S, Naidu RJ and Lachimanan YL. 2011. Antihyperglycaemic effects of ethanol extracts of *Carica papaya* and *Pandanus amaryfollius* leaf in streptozotocin induced diabetic mice. *Natural Product Research,* 25(20): 1982-1987.

Tanko Y, Garkuwa UA, Jimoh A, Sada NM, Mohammed KA, Muhammad A and Makarfi TM. 2014. Hypoglycaemic effects of methanol crude leaves extract and aqueous fraction of *Acacia nilotica* on blood glucose levels on experimental animals. *Annals of Biological Sciences*, 2(1):23-27.

Tiwari AK and Rao JM. 2002. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current Science,* 83(1): 30-38.

Toma M, Vinatoru M, Paniwnyk L and Mason TJ. 2001. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. *Ultrason Sonochem*, 8: 137-142.

TUEPG. 2015. TUE Physician Guidelines. Medical Information to Support the Decisions of TUE Committees Diabetes Mellitus, WADA- World Anti-Doping Program Version 3.0.

Verma P, Singh SP, Vind SK, Kumar GR, Rao CV. 2014. Protective effect of *Acacia nilotica* (bark) against anti tubercular drug induced hepatic damage an experimental study. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(9): 75-79.

Zarei A, Changizi-Ashtiyani S, Taheri S, Hosseini N. 2015. A brief overview of the effects of *Melissa officinalis* L. extract on the function of various body organs. *Zahedan Journal of Research in Medical Sciences*, 15: 29-34.

Conclusion

The present study was designed on the basis of ethnobotanical knowledge of curing skin and diabetic diseases. Ten medicinal plants were selected through literature survey of peer reviewed paper published in Journals on different tribal and folk medicinal practitioners in Bangladesh. Active phytochemicals were extracted from fresh leaves of *Azadirachta indica*, *Acacia nilotica*, *Bryophyllum pinnatum*, *Moringa oleifera* and *Mikania cordata*; fresh stems of *Cuscuta reflexa*; dried leaves of *Carica papaya* and *Coccinia grandis*; dried seeds of *Carum copticum* and *Nigella sativa* through Aqueous Ultrasound Assisted Extraction (UAE) Method.

Aqueous UAE method is comparatively noble and green extraction procedure introduced in the end of the 20th century. Aqueous UAE method was proved as an effective extraction procedure in the present study as it's provide high percentages of yield of crude extracts both from the dried and fresh plant's parts. More than 30% yield was observed from the dried plant's parts of *N. sativa, C. grandis* and *C. papaya*, whereas about 15% extraction was observed from fresh leaves of *A. nilotica*, *A. indica* and *C. reflexa.* Promising extraction was also found from *M. oleifera* (17.42%), *M. cordata* (18%), *C. copticum* (19.14%) and *B. pinnatum* (21.59%) indicated that aqueous UAE method may be a suitable alternative than the conventional cold and hot extraction procedure. Replacing organic solvent with water is made this procedure is more environment friendly (free of health hazards) and cost effective. Introducing simple equipment for ultrasound treatment and avoiding drying stage (apply fresh plant's parts) may also reduce the overall cost and time of extraction.

Differences of the pH of plant materials before and after ultrasound treatment is an indicator for getting idea about the type (acidic or basic) of extracted compound. Generally both acidic and basic compounds were extracted from the plant's material during extraction, however, pH may shifts lower or higher after addition of more acidic or basic compounds, respectively during extraction. In the present study, most of the crude extracts were found acidic in nature except *C. papaya* and *C. grandis*. Crude extracts of *C. papaya* (pH 7.77±0.009) and *C. grandis* (pH 8.45±0.009) were found basic in nature but lower than the untreated solution, indicated that ultrasound treatment successfully extracts more acidic compounds from the cell of the plant's parts. Similarly, *M. cordata* (pH 6.04±0.015) and *C. copticum* (pH 5.44±0.006) were

found acidic in nature which was higher than the untreated solution. Crude extracts from rest of the plants was observed acidic in nature.

Dissolution study of crude extracts with water, methanol, ethanol, chloroform, DMSO, ethylacetate and dichloromethane indicated the presence of both polar and nonpolar compounds in the crude extracts. Dissolution study indicated the ability of the aqueous UAE method to extract wide range of compounds from the plant's material. Conceptually all compounds present in the cell may be available in the crude extracts due to rupturing the cell wall during ultrasound treatment. Aqueous UAE method should assure all possible compounds available in the plant's parts in the crude extract which is difficult by the conventional extraction procedure.

Crude extracts of most of the plants showed the presence of maximum number of phytochemicals compared with conventional extraction. Crude extracts of *C. papaya* contained all the phytochemicals tested in the present study including tannin, saponin, flavonoid, steroid, terpinoid, glycoside, alkaloid, anthraquinone and vitamin C, which showed better performance than the conventional ethanolic and chloroform extract. Similarly, aqueous UAE crude extracts of *A. nilotica*, *B. pinnatum* and *M. oleifera* contained all phytochemicals except saponin, anthraquinone and steroid respectively, proved the extraction efficiency of the aqueous UAE over the conventional extraction procedure. Partitioning hypothesis was applied in the present study for separating the bioactive compounds likely alkaloids, terpenoids, flavonoids and glycosides from crude extracts by using successive fractional separation of diethylether, dichloromethane, chloroform and ethylacetate, respectively. Though 100% purity was not found, but sufficient amount was separated by using minimum organic solvent which was suitable for further purification by column chromatography. This separation technique used in the present study reduced the ultimate cost for purification.

From the above phytochemical study it was observed that "Aqueous UAE Method for dried and fresh plant's parts" was environment friendly (free of health hazards), cost effective, time efficient (i.e., assure faster rate of extraction), user friendly, and produce a high yield crude extract containing a verity of polar & non polar compounds of the plants. On the basis of present study "Aqueous UAE method" may be termed as a green extraction method and suitable alternative of traditional (organic solvent dependent) extraction method both for laboratory and industrial setup.

Pharmacological efficacy of the crude extracts for curing skin diseases were measured in-vitro antimicrobial study on common skin disease causing pathogenic microorganisms. Three bacteria named *S. aureus* (Gram +ve), *S. pyogenes* (Gram +ve), *E. coli* (Gram -ve) and three fungi *C. lunata*, *F. chlamydosporum* and *M. furfur* were enrolled on the basis of their proven association of different types of skin diseases. However, some reference microorganisms were also included in the present study for validation purpose. Promising antimicrobial activity of *Azadirachta indica* (against *S. aureus*), *Acacia nilotica* (against *S. pyogenes* and *S. aureus*), *Bryophyllum pinnatum* (against *S. pyogenes*, *S. aureus* and *E. coli*), *Cuscuta reflexa* (against *S. aureus* and *S. pyogenes*) and *Moringa oleifera* (against *S. pyogenes*) were observed. Those plant extracts also showed promising sensitivity against the enlisted fungi. Comparatively moderate antimicrobial activity was observed for the extracts of *Carica papaya, Coccinia grandis* and *Mikania cordata* whereas *Carum copticum* and *Nigella sativa* showed negligible antimicrobial activities. As like crude extracts, purified fractions were also showed promising antimicrobial effects against skin disease causing bacteria including *Staphylococcus aureus* and *Streptococcus pyogenes*. Most of the tested plants showed MIC bellow 100 µg/ml against *Staphylococcus aureus* and *Streptococcus pyogenes* indicated the effectiveness of the crude extract for treating skin disease. Based on the above study it may be concluded that the aqueous UAE crude extracts of *Azadirachta indica*, *Acacia nilotica, Bryophyllum pinnatum*, *Mikania cordata*, *Moringa oleifera*, *Cuscuta reflexa*, *Carica papaya* and *Coccinia grandis* and their purified fractions are suitable for the treatment of different types of pathogenic skin diseases. From the above study herbal topical preparations including lotion, paste, cream, shop, shampoo, etc. using crude extracts of the above plants may be prepared for different types of infectious skin disease on the basis of relevant clinical trial.

Anti-diabetic properties of plant extracts were measured on laboratory animal (dexamethasone induced diabetic mice) and found significant blood glucose lowering effect in most of the cases. Three different types of anti-diabetic study including OGTT, FPG and long term effects on FPG were performed for measuring the hypoglycaemic efficacy of the selected plants. Most of the selected plants showed hypoglycaemic effects on diabetic mice. However extracts of *A. indica, B. pinnatum, C. reflexa, C. papaya* and *C. grandis* were showed very promising Anti-diabetic effect almost similar to the standard anti-diabetic drug glibenclamide. All the selected plants

successfully prevented unwanted weight loss normally happening in the diabetic animal. From the above study herbal oral preparation of the crude extracts of *A. indica*, *B. pinnatum*, *M. oleifera, C. papaya* and *C. grandis* may be suggested for the treatment of diabetic patients on the basis of relevant clinical trial.

Cytotoxicity study was done through brine shrimp lethality study and observed that most of the crude extracts were safer than the standard antibiotic. Additionally all selected plants provide marked analgesic properties which will be helpful for diabetic patients who are suffering different types of pain. Similarly pain connected to the skin disease will also be cure during the treatment of skin disease. Present study observed that most of the selected plants were somehow used by the tribal and folk medicinal practitioners in Bangladesh for curing diabetes and skin disease was justified.

From the present study it may be concluded that nature have huge wealth to explore for the betterment of the mankind. Health expenditure is increasing day by day due to dependency on conventional medicine whereas drug resistance, toxicity, side effects, contraindication, even drug addiction are the common phenomenon of the allopathic medicine. Therefore, traditional medicine is getting more attention in different countries of the world. Traditional knowledge is disappearing due to rapid modernization of the world as well as migration and accumulation of tribal practitioners in the main stream population. Ethnobotanical study and subsequent laboratory study may have opportunity to accumulate scientific evidence and justification for including the traditional medicine in the main stream of treatment system. In the present study, skin and diabetic diseases were considered, and evaluated scientific evidences of some Bangladeshi plants considering the natural drugs for the treatment purpose of those two diseases. From the present study, a number of promising findings will inspire scientists for further study in this field without polluting the environment.