Evaluation of Analgesic and Anthelmintic Activities of Edible Mangrove Fruits in the Sundarbans

Khulna University Library
Thesis Acc. No: 4285
Authorised Sign:
Date: 01.9.2024

A thesis

Submitted to

Khulna University, Khulna

In partial fulfillment of requirement's for the degree of

Master of Science

In

Biotechnology and Genetic Engineering

By

Roll No: MS-170704

Session: 2016-2017

BIOTECHNOLOGY AND GENETIC ENGINEERING DISCIPLINE

LIFE SCIENCE SVHOOL, KHULNA UNIVERSITY

KHULNA-9208, BANGLADESH

DECEMBER, 2019

@Khulna University

Evaluation of Analgesic and Anthelmintic Activities of Edible Mangrove Fruits in the Sundarbans

A Thesis
Submitted to
Khulna University, Khulna

In Partial Fulfillment of the Requirements for the Degree of
Master of Science
In
Biotechnology and Genetic Engineering
By

Anirban Biswas
Student ID: MS-170704
Session: 2016-2017

Approved as to style and contents by:

Dr. Sheikh Julfikar Hossain Supervisor
Professor Biotechnology and Genetic Engineering Discipline Life Science School Khulna University, Khulna

17.12.2020 Dr. Ayesha Ashraf Chairman Examination Committee and Head, Biotechnology and Genetic Engineering discipline

Khulna University
Khulna -9208, Bangladesh
DECEMBER, 2019

@Khulna University

DEDICATED

TO

MY BELOVED PARENTS

@Khulna University

TITLE

Evaluation of Analgesic and Anthelmintic Activities of Edible Mangrove Fruits in the Sundarbans

DECLARATION

This thesis is original research work of the author, except as otherwise stated. The material presented has not submitted either in whole or in part for a degree for this or any other university. The findings of the research has not been/will not be published anywhere without the written concurrence of the concerned supervisor. Legal action will be taken in any case of infringement regarding the declaration.

Anirban.

15.12.2020

Anirban Biswas

Date:

Student ID: MS-170704

@Khulna University

ACKNOWLEDGEMENT

At the onset I would like to pay gratitude to most gracious, most merciful creator of this universe the Almighty God who has kept me healthy, strong and energetic to complete the thesis. Individual efforts alone can never contribute in totality to successful completion of any venture.

I am obliged to offer my deepest sense of gratitude and paramount honor to my supervisor, Dr. Sheikh Julfikar Hossain, Professor, Biotechnology and Genetic Engineering Discipline, Life Science, Khulna University, Khulna for his scholastic guidance, valuable suggestions and providing constant support and cooperation during the research work, without which this piece of work would not be taken its present shape.

My profound thanks also to other honorable teachers and assistant register (lab)(especially Mr. L. C. Roy), of Biotechnology and Genetic Engineering Discipline, Khulna University.

I have the pleasure in expressing my heartfelt thanks and regards to Md. Rabiul Islam, a respected senior of my lab for their skill and active guidance and practical demonstration. I am really grateful to him for their valuable contribution to carry out the thesis work.

I express my heartiest thanks to all my friends especially Md. Zakir Hosen and Nurjahan Islam Smrity and classmates for their effective help and co- operation for continuing my thesis work. I'm also very much thankful to my juniors including Mst. Rima Khatun, Nadia Islam Luna and Sharmin Akhter.

And finally, I express my deepest gratitude to my parents and to my two younger sisters Shila Biswas & Kakoli Biswas for their continuous inspiration, support and encouragement to complete my thesis successfully.

THE AUTHOR

DECEMBER, 2019

@Khulna University

CONTENTS

KHULNA UNIVERSITY Central Library KHULNA.

@Khulna University

@Khulna University

LIST OF FIGURES

@Khulna University

@Khulna University

LIST OF TABLES

@Khulna University

Abstract

In this study, solvent extracts were prepared from edible mangrove fruits in the Sundarbans' and their analgesic and anthelmintic activities were evaluated. The analgesic activity was investigated by acetic acid induced writhing test (chemical method) and hot plate test (thermal method) on Swiss albino mice, whereas anthelmintic activity was tested on the ruminant parasite, *Paramphistomum cervi*. All the extracts (EtOH:MeOH 1:1) inhibited acetic acid induced writhing. *C. decandra* showed the highest activity (44.5%) followed by *H. fomes* (38.1%), *A. corniculatum* (36.4%) and *P. paludosa* (34.8%) at 250 mg/kg body weight whereas diclofenac sodium, a positive control showed 51.3% of inhibition at 25 mg/kg. Similarly in hot plate test, *C. decandra* showed the highest response time (17.4 sec.) at 250 mg/kg followed by *H. fomes* (14.2 sec.) and *A. corniculatum* (13.6 sec.) at 90 min whereas morphine, a positive control showed 18.2 sec. All fruits also significantly ($p<0.05$) showed anthelmintic activity, compared with the control group. The anthelmintic activity of the fruits was evaluated by observing both the paralysis time and the time until death. Among them, the *A. corniculatum* showed the strongest anthelmintic activity (26.3±2.41 and 46.2±3.71 min required for paralysis and death, respectively) at a dose of 10 mg/ml followed by *P. paludosa* (143.6 ± 11.43 and 200.6 ± 23.48 min required for paralysis and death, respectively). Albendazole, a standard drug at 15 mg/ml, showed a paralysis time of 78.4±7.87 min and a time until death of 106.3±12.33 min; corresponding times for the control group were 316.8±17.37 and 371.29 ± 26.75 min, respectively. Furthermore, five different successive fractions (n-hexane, di-ethyl ether, chloroform, ethanol and methanol) of *A. corniculatum* were studied for anthelmintic activity, where methanol fraction showed the strongest activity. Therefore, it can be concluded that the edible fruits in the Sundarbans' possess various types of potential bioactive compounds which might be used as analgesic and anthelmintic medication.

@Khulna University

CHAPTER ONE: INTRODUCTION

Mangroves are a taxonomically various group of plant that grow at the interface between land and sea in tropical and sub-tropical latitudes where they exist in conditions of high salinity, extreme tides, strong winds, high temperatures and muddy, anaerobic soils (Kathiresan and Bingham, 2001). The term mangrove is also used to designate halophytic (salt loving) and salt resistant marine tidal forests comprising of trees, shrubs, palms, epiphytes, ground ferns and grasses, which are located in groves or stands (Bandaranayake, 2002). Mangroves are usually found only in tropical climates, as they need consistently warm conditions for development and survival. Mangroves have long been a source of astonishment for the layman and of interest for scientist, which occur approximately in 112 countries and are largely confined to the regions between 30° north and south of the equator (Bandaranayake, 2002). The distribution, density and species composition of mangroves are determined by the water and air temperatures during the winter, exposure to wave action and tidal currents, the range of the tide, the type of sediment and the chemistry of the seawater. Asia contains most of the world's mangroves with 46%, followed by America with 35% and Africa with 17% (MAP, 1990). The global distribution of mangroves is shown in Figure1.

Natural mangroves are distributed in the Southwest and Southeastern parts of Bangladesh and mangrove plantations are mostly in the central part of the coast viz. in the Meghna estuary. The natural mangrove in the Southwestern part of Bangladesh is the world's largest single tract of mangrove forest - the Sundarbans and lies between the latitudes 21°31' and 22°30' N and longitudes 89° and 90° E is shown in figure 2. The Sundarban is a deltaic mangrove forest, formed about 7000 years ago by the deposition of sediments from the foothills of the Himalayas

@Khulna University

through the Ganges river system, and is situated Southwest of Bangladesh and South of West Bengal, India (Aziz and Paul, 2015). The Sundarbans is the single largest continuous mangrove forest in the world and has been recognized as an international important Ramsar Wetland Site and the UNESCO declared it as a World Heritage Site (WHS) in 1997. The Sundarban forests tract including the Indian part covering an area 10,000 km^2 on which 66% are land, the rest is water (Hussain and Acharya, 1994). About 62% of the Sundarbans forest is in Bangladesh and the residual in India. The Sundarbans is rich in biological diversity. There are total 334 plants, 165 algal, 13 special orchids, 17 fern, 87 monocotyledon and 230 dicotyledon belonging to 245 genera and 75 families have been reported so far from the Sundarbans. Mangroves can grow in such highly saline substrates and even grow better in the presence of some salt (Connor, 1969; Downton, 1982) suggests that they are able to control the intake of salt and maintain a water balance that is physiologically acceptable.

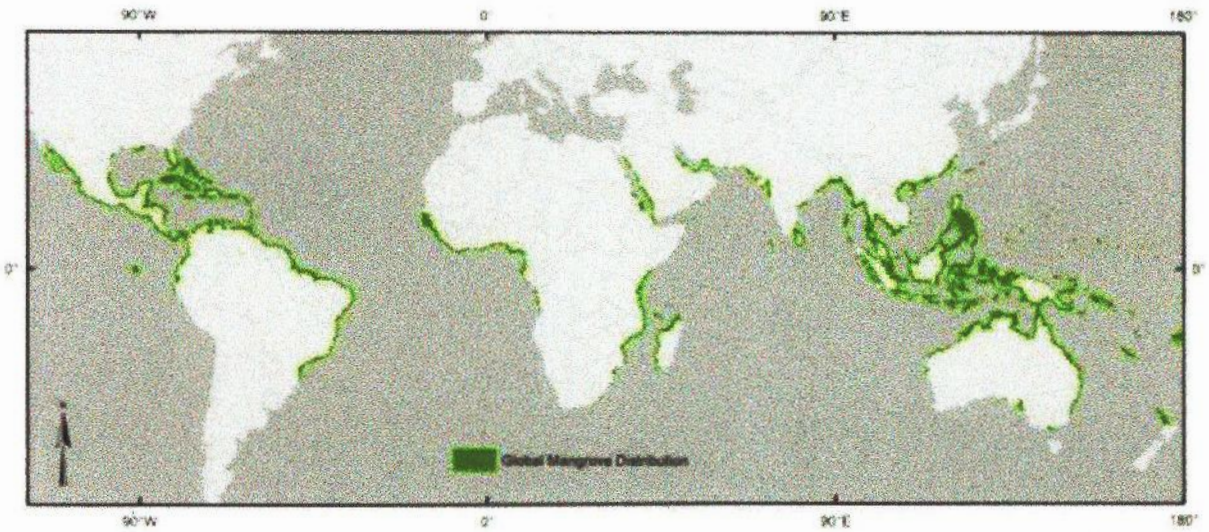


Figure 1.1: World distribution of mangroves (http://www.coastalwiki.org/wiki/Mangroves).

@Khulna University

KHULNA UNIVERSITY
Central Library
KHULNA

Mangroves have a long historical link with human culture and civilization. Mangroves are a great sources of wood, timber, poles, thatch and fuel, and the bark is used for tanning materials; some species have food or medicinal value (Hamilton et al.,1988). Mangrove fruit has been long consumed by society living in coastal area as a food material or ingredient in various processed food products. Salinity, drought, high light intensity and nutrient deficiency etc. are the environmental stress which could result in oxidative burst so that it leads to the production of reactive oxygen species (ROS) that causes physiological and biochemical changes (Xie et al, 2019). Mangrove plants are biochemically unique, producing a wide range of novel natural products which have anti-bacterial, anti-cancer, anti-diadetic, anti-diarrhoeal, anti-viral (Vadlapudi et al, 1998). The mangrove plants are traditionally used for the treatment of asthma, rheumatism, painful arthritis, inflammation and hepatoprotective actions (Roome et al., 2011). Fruit, Stem, root, seed, flower, leavers, twig exudates and modified organs are the different parts of mangroves plants. They are a good source of secondary metabolites such as alkaloids, flavonoids, polyphenols, glycosides, saponins, steroids, tannins, and triterpenes which have interesting bioactive properties and medicinal values (Edeoga et al., 2005).

Skin disorders and sores (leprosy) may be treated with ashes or bark infusions of certain species of mangrove. Reported to be a hemostat, styptic, astringent, emmenagogue, tonic and expectorant, red mangrove is a public remedy for constipation, convulsions, diarrhea, dysentery, dyspepsia, angina, asthma, backache, boils, elephantiasis, eye ailments, fever, fungal infections, headaches, hemorrhage, inflammation, jaundice, sores, sore throat, syphilis, toothache, ulcers, wounds tuberculosis, kidney stones, lesions, malaria, malignancies, rheumatism and snakebites, (Rahman et al., 2011).

@Khulna University

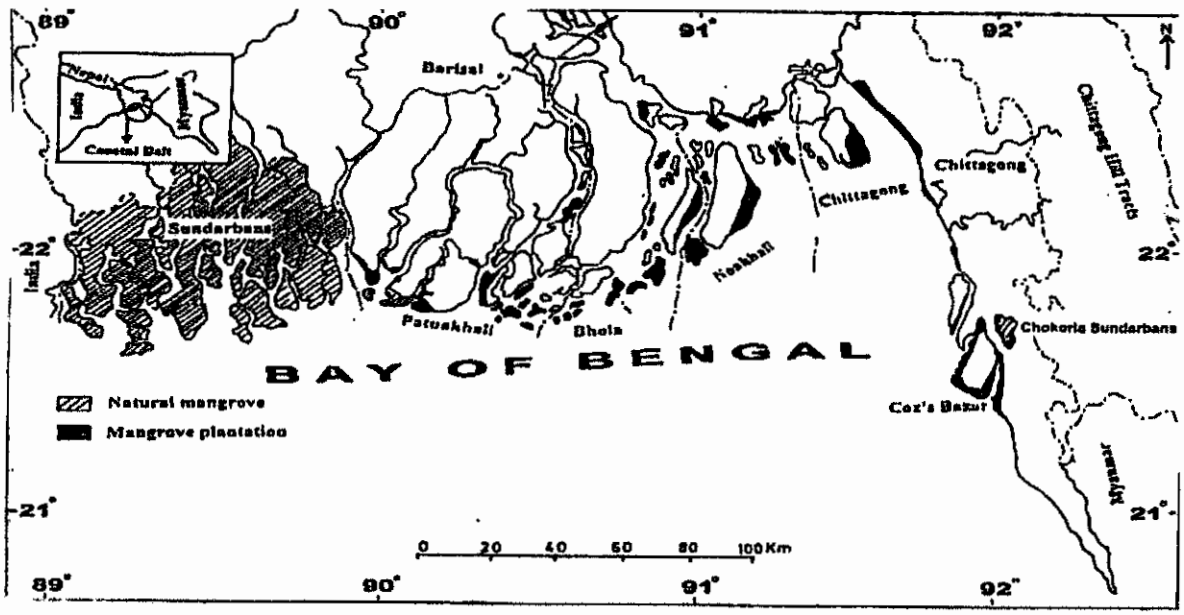


Figure 1.2: Distribution of natural and planted mangroves in Bangladesh (Hoque and Datta, 2005).

Mangrove plants produce a group of secondary metabolites, are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. Secondary metabolites such as phenolic compounds have free radical scavenging activity. They serve as important tool in plant defense system (Meot-Duros and Magne, 2009). These compounds have higher antioxidant activity due to their redox properties along with metal chelation, hydrogen donation and singlet oxygen scavenging (Rice-Evans et al., 1995). Now-a-days, phenolic compounds have a great application in food, cosmetic and pharmaceutical industry (Ksouri et al., 2009). Traditionally, *P. paludosa* is used as antipyretic and antiinflammatory agent in the early days in various regions (Saronika, 2002).

@Khulna University

Figure 1.3: The Sundarbans' (https://www.google.com/search?tbm=isch&sa.....GLiF3cM:).

Phenolic compounds including simple phenols and phenolic acids, hydroxycinnamic acid derivatives and flavonoids are bioactive substances occurring widely in the mangroves plants. Phenolic compounds are closely associated with the sensory and nutritional quality of fresh and processed plant foods. Many phenolic compounds in plants are good sources of natural antioxidants. It is a great interest in recent years that many phenolic compounds in foods have inhibitory effects on mutagenesis and carcinogenesis (Ho et al, 1991). Another property of the polyphenols is their metal-chelating potential which may also play a role in the protection against iron- and copper-induced free radical reactions.

The major families and genus of mangroves: are Fabaceae (*Pongamia pinnata, Derris scandens*); Tamaricaceae (*Tamarix indica*); Acanthaceae (*Acanthus hirsutus, Acanthus ilicifolius*); Rhizophoraceae (*Ceriops decandra*); Clusiaceae (*Calophyllum inophyllum*); Euphorbiaceae

@Khulna University

(*Excoecaria agallocha*); Arecaceae (*Nypa fruticans*); Pandanaceae (*Pandanus foetidus*); Convolvulaceae (*Ipomoea imperati, I. pes-caprae*); Sterculiaceae (*Heritiera littoralis*); Myrsinaceae (*Aegiceras corniculatum*); Avicenniaceae (*Avicennia officinalis*); Lecythidaceae (*Barringtonia racemosa*) and Leguminosae (*Caesalpinia mimosoides*).

There are many species in the Sundarbans that produce edible fruits. These are *Avicennia, Aegiceras, Bruguiera, Bariringtonia, Ceriops, Sonneratia, Lumnitcera, Waru* and *Xylocarpus* (Noor et al., 2006). *Sonneratia caseolaris* is a popular one, which is non-toxic (Chen *et al.*, 2009), soft in texture having specific flavours and nice taste, various food products have been produced from it, including syrup (Abeywickrama and Jayasooriya, 2010), cakes and steamed pudding (Brown et al., 2006). Therefore, plant derived natural compounds such as polyphenols flavonoids, terpenes, alkaloids etc. have received considerable attention in recent years due to their pharmacological properties including antioxidant, anti-microbial and anti-inflammatory activities (Reddy and Grace, 2016).

The fruit of *Sonneratia caseolaris* (L.) is used in poultice in sprains and swelling and *Sonneratia apetala*, locally known as keora which is used for medication by the native people adjacent to the Sundarbans'. The roots of *Avicennia officinalis* are as used to cure small pox and the unripe seeds of this plant are used as poultice to cure suppuration of boils and abscesses. The decoction of the bark of *Ceriops candollena* is used to stop hemorrhage and applied to malignant ulcers and the decoction of the shoot is used as a substitute for quinine in the African coast (Gbindasamy, 2012). The bark of *Rhizophora mucronata* is used as a cure of diabetes and the leaves of *Nypa fruticans* are used to cure ulcers (Mercer et al., 1984 and Mohammed et al., 2010). *Avicenna*

@Khulna University

marinais is a type of mangrove plant belonging to the family of Aviceniaceae which leaves contain phenolic groups like alkaloids, flavonoids, catechin, triterpenoids, tanins and anthroquinones that show different bioactivity. *A. corniculatum* extract demonstrated effectiveness against *Mycobacterium tuberculosis* as well (Janmanchi et al., 2017). Novel anticancer compounds were isolated and characterized from different mangroves species such as *Aegiceras corniculatum, Excoecaria agallocha, Rhizophora stylosa, Sonneratia paracaseolaris, Xylocarpus* genus, *Ceriops tagal*, and *Bruguiera cylindricall* (Dahibhate et al., 2018). *Amorphophallus campanulatus* (Roxb.) (family: Araceae) is an annual herb with large, depressedglobose, much-warted tubers. It contains protease inhibitors, trypsin and chymotrypsin. Corm contains triacontane, lupeol, betulinic acid, stigmasterol, β-sitosterol, glucose, galactose, rhamnose and xylose. Sapogenins are also present in the corms (Ghani, 2003). The tuberous roots of the plant have been used traditionally for the treatment of piles, abdominal pain, tumors, and enlargement of spleen, asthma and rheumatism. (Kirtikar et al., 1998). *Avicennia marina* (Forssk.) produces some phytochemical compounds like phenols, tannins, azadirachtin, ricinine that are toxic to nematodes and parasites (Mian et al., 1982; Rich et al., 1989). *A. marina* has been used traditionally for the management of acute ulcers and skin related inflammatory diseases (Kathiresan et al., 2001; Bandaranayake, 2002; Khafagi et al., 2003).

Some of the mangrove plants have already been scientifically investigated for their chemical composition. For example, *Acanthus illicifolius*, is a herb used traditionaly in the treatment of paralysis, asthma, rheumatic pains and the possessing analgesic and anti-inflammatory activities. Stigma sterol, a common plant steroid, abundantly present in this plant 2-Benoxazoline was isolated from this plant which has a central nervous system (CNS) depressant and anti-pyretic,

@Khulna University

anti-furarium, anti-inflammatory, hypnotic and muscle relaxant activities (Wahidulla et al., 2001).

Pain is a symptom, which is always subjective (Lichtman et al., 1996). Pain is often classified as acute or chronic. Acute pain is frequently associated with anxiety and hyperactivity of the sympathetic nervous system (e.g. tachycardia, increased respiratory rate and BP, diaphoresis, dilated pupils). Chronic pain does not involve sympathetic hyperactivity but may be associated with vegetative signs (e.g., fatigue, loss of libido, and loss of appetite) and depressed mood (Amir et al., 2009; Ayyappa et al., 2009). Widely opioid analgesics and NSAID's are used for the management of pain. However these drugs pose serious side effects like sedation, respiratory depression constipation, serious gastrointestinal tract reactions, bone marrow disturbances, liver disorders, dyspepsia, nausea and vomiting, skin reactions, analgesic associated nephropathy etc (Kiritikar and Basu, 1991). In this regard, search for new, effective and low toxic compounds is desired.

To date, besides the commonly used medicinally important terrestrial plants, several mangrove plants also have ethnopharmacological relevance which were examined and recognized as a potential source of novel natural product for exploitation in medicine which contributes pain management (Adhikari et al., 2016). Secondary metabolites are rich source of the wide range of bioactive compounds and natural products. It includes terpenoids, alkaloids, phenolics, saponins, flavonoids and steroids. The bioactive and natural compounds may serve as therapeutic precursors and industrial raw materials. The pharmacological activities of phenolics, terpenoids, and limonoids from mangrove species indicate that they can serve as health promoting and pharmaceutical agents. Therefore research is focused on natural products and phytotherapy may contribute better therapeutic performance than conventional drugs. *Papaver somniferum*, *Cannabis sativa*, Capsicum and Salix species are some plants which have a great contribution in relevant drug

@Khulna University

development for pain management (Calixto et al., 2001). *Rhizophora mucronata* is a very popular mangrove plant which has been used as ethno-medicine from ancient time. Ethanolic extract of *Rhizophora mucronata* L. leaves showed significant inhibition in acetic acid induced writhing response in mice.

Infections caused by gastrointestinal nematodes are major threat to livestock industry in the developing countries. They cause direct effects in form of loss in production and indirect economic losses due to high cost of anthelmintic drugs (Kassai, 1999). Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, development of resistance in helminthes against conventional anthelmintics is a foremost problem in treatment of helminthes diseases (Coles, 1997, Tagbota et al., 2001). Henceforth it is important to look for alternative strategies against gastrointestinal nematodes, which have led to the proposal of screening safe and newer medicinal product mainly from plant source for their anthelmintic activity. Plant products have potential of curing various ailments including parasitism. The World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs for their primary health care needs (Mahadev et al., 2017). The use of medicinal plant is growing worldwide because of the increasing toxicity and allergic manifestations of the synthetic drugs (Tuse et al., 2011). Hence there is an increasing demand towards natural anthelmintic. A number of plant species have been used and reported in different parts of world against nematode infections in animals and humans (Akhter et al., 2000).

A large proportion of world population, especially in the developing countries depends on the traditional system of medicine for a variety of diseases. Several hundred plant genera are used

@Khulna University

medicinally mainly in the form of herbal preparation in indigenous system of medicine in different countries. Plant products are also a source of very potent and powerful drugs that have stood the test of times and modern chemistry has not been able to replace most of them (Singh et al., 2000).

The uses of mangroves are many and varied and are often quoted in scientific and popular articles (Walsh, 1977; Rollet, 1981; FAO, 1985; Tomlinson, 1986; Vannucci, 1989; Field, 1995, Bandaranayake, 2002; Banerjee et al., 2008; Hossain et al., 2013; Hossain et al., 2016;). Mangroves are of great importance to many people who live along tropical shorelines. The sundarbans is a deltaic mangrove forest which has rich natural resources. Therefore, some edible fruits were selected from the sundarbans to test its biochemical activity and identification of bioactive compouds. These were: Keora (*S. apetala*), Ora (*S. caseolaris),* Passur (*X. mekongensis*), Sundari (*H. fomes*), Khalshi (*A. corniculatum*), Baen (*A. officinalis*), Kankra (*B. gymnorrhiza*), Goran (*C. decandra*), Golpata (*N. fruticans*), Bawali lota (*S. globosus*) and Hental (*P. paludosa*) which are consumed from the ancient time by the local people at raw or processed states.

In Sundarbans, local people have identified the medicinal properties of the mangrove plants. They used different mangrove plants in different forms as medicine to cure some common as well as chronic ailments like fever, malaria, cold and cough, bronchitis, asthma, skin diseases, ulcers, leprosy, small pox, diarrhoea, dysentery, diabetes, antifertility etc (Mondal et al., 2008). *Almarina, Aegicers corniculatum, Bruguiera gymnorhiza,* and other mangrove species can uptake and accumulate the nutrients and heavy metals (Boonsong et al., 2003). A mixed mangrove plantations is responsible for increasing biodiversity, food web connectivity and net ecosystem production (Alongi, 2011). For example, in China, in a small-scale mangrove plantation

@Khulna University

program, the non-native *S. apetala* was planted together with native mangrove species *S. caseolaris* (Zan et al., 2003). Both *Sonneratia* species show high tolerance to environmental stresses and can ameliorate in low inter-tidal zones where other indigenous species survive difficulty (Liao et al., 1996). Thus the present study was designed to evaluate the analgesic and anthelmintic activities of edible mangrove fruits, which may lead to a new possibility in the drug exploration.

Objectives:

Considering the above prospects, this study was undertaken to find out the following objectives:

- whether the edible mangrove fruits show strong analgesic activity and
- whether the edible mangrove fruits display potential anthelmintic activity.

@Khulna University

CHAPTER TWO: PLANT PREVIEW

2.1 *Aegiceras corniculatum* (L.) Blanco

A. corniculatum, commonly known as Black Mangrove, River Mangrove or Khalsi, is a species of shrub or tree mangrove with a distribution in coastal and estuarine areas ranging from India through South East Asia to southern China, New Guinea and Australia. *A. corniculatum* grows as a shrub or small tree up to 7 m high, though often considerably less. Its leaves are alternate, obovate, 30–100 mm long and 15–50 mm wide, entire, leathery and minutely dotted. Its fragrant, small, white flowers are produced as umbellate clusters of 10–30, with a peduncle up to 10 mm long and with pedicels 10–18 mm long. The calyx is 2–4 mm long and corolla 4–6 mm long. The fruit is curved and cylindrical or horn-shaped, light green to pink in colour and 20–75 mm long. It grows in mud in estuaries and tidal creeks, often at the seaward edge of the mangrove zone.

(**Source**: WWW.Wikipedia .com, Ellison et al., 2010; Roome et al., 2011, Hossain, 2015)

Figure 2.1: Fruits of *A. corniculatum*

@Khulna University

Local name: Khalsi, Khalisha

English name: Black mangrove, River mangrove

Systemic Classification of *A. corniculatum*

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Ericales

Family: Primulaceae

Genus : *Aegiceras*

Species: *A. corniculatum*

Traditional Medicinal Uses of *A. corniculatum*

A. corniculatum extract has analgesic properties and it is also used in various traditional medicinal system(s) for the treatment of rheumatism, painful arthritis and inflammation (Ellison et al., 2013). This bark of this species is used as a fish poison and as a dye. It is also used as a medicine. The leaves are also eaten. Therefore, the pharmacological studies of its antinociceptive effect was undertaken to validate its traditional use (Roome et al., 2011).

2.2 *Avicennia officinalis* L.

A. officinalis is a species of mangrove also known as Indian Mangrove. The young tree forms a low, dense bushy crown. When it matures, it forms a columnar tree up to 15 m and grows up to

@Khulna University

30 m. The shiny green leaves, 10 cm long by 5 cm wide, have rounded apexes and golden-brown under leaf and grow in opposites. The flower, the largest among the *Avicennia* species has a diameter of 6 to 10 mm when expanded. It is orange yellow to lemon yellow in color. The bark is smooth, dirty green to dark gray in color. It is slightly fissured and does not flake. The fruit is green or brown, heart-shaped abruptly narrowed to a short beak is 2.5 cm long or more.

(**Source**: WWW.Wikipedia.com, Duke et al., 2006; Hossain, 2015)

Figure 2.2: Fruits of *A. officinalis*

Common name: Bina, Baen, Bani

English name: Indian Mangrove, White mangrove

@Khulna University

Systemic Classification *of A. officinalis*

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Lamiales

Family: Acanthaceae

Genus: *Avicennia*

Species: *A. officinalis*

Traditional Medicinal Uses of *A. officinalis*

The bitter fruits and seeds are sometimes used for food after rather an elaborate processing. The fruits are eaten after baking or steaming. Indian mangrove is a folk remedy for boils and tumours -the fruits are plastered onto tumours (Hartwel, 1967–1971) .The roots are said to be aphrodisiac. Unripe seeds are poulticed onto abscesses, boils, and smallpox sores. The bark is used for treating skin afflictions, especially scabies. A green, bitter, resinous substance that exudes from the bark is said to act as a contraceptive, and apparently can be taken all year long without ill effects (Perry, 1980). The resin is also used to treat snakebite. The bark is used for dying cloth, the ash for washing it in India (Watt and Breyer-Brandwijk, 1962).

@Khulna University

2.3 *Bruguiera gymnorrhiza* (L.) Lam.

B. gymnorrhiza is one of the most important and widespread mangrove species in the Pacific. *B. gymnorrhiza* is a small tree up to 10 m high that belongs to the family Rhizophoraceae. It is found on the seaward side of mangrove swamps, often in the company of Rhizophora. Its bark is rough and reddish brown. The tree develops short prop-roots rather than long stilt-roots. Flowers are creamy white soon turning brown. The sepals are persistent, narrow and slightly tapered. When mature, the spindle-shaped fruits drop and become embedded in the mud in an upright position, where they rapidly develop roots. The flowers are red and remain attached to the propagule when it falls. The propagules are green and cigar-shaped, between 10 and 20 cm long and can be found throughout the year.

(**Source**: WWW.Wikipedia.com, Peter and Sivasothi, 1999 Duke et al., 2006, Hossain, 2015)

Local name: Kankra, Kaṇḍu

English name: Large-leafed orange mangrove

Figure 2.3: Fruits of *B. gymnorrhiza*

@Khulna University

Systemic Classification *of B. gymnorrhiza*

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Malpighiales

Family: Rhizophoraceae

Genus: *Bruguiera*

Species: *B. gymnorrhiza*

Traditional Medicinal Uses of *B. gymnorrhiza*

The propagules or green pods are eaten as a cooked vegetable. They are peeled first and then boiled, the water being discarded and renewed at least four times. The propagules of this species are more appreciated than those of *B. cylindrica*. The fruit of this tree needs some special preparation due to high tannin content and should not be used unprepared. *B. gymnorrhiza* have also been used in treatment of diarrhoea, malaria and burns whereas its fruits have been used in the treatment of shingles and eye diseases (Seepana et al., 2016).

2.4 *Ceriops decandra* (Griff.) Ding Hou

Ceriops decandra is a mangrove plant of tropical Asia in the family Rhizophoraceae. The specific epithet decandra is from the Greek meaning "ten male", referring to the flower having ten stamens. *Ceriops decandra* grows as a shrub or small tree up to 15 metres (50 ft) tall with a trunk diameter of up to 30 cm (12 in). Flowering and fruiting occurs throughout the year in *C.*

@Khulna University

decandra. Its bark is pale brown. The flowers are white. The ovoid to conical fruits measure up to 1.8cm (0.7 in) long. *Ceriops decandra* grows naturally in India and Bangladesh (including the Sundarbans), Burma, Thailand and Peninsular Malaysia. Its habitat is mangrove swamps and tidal creeks. (**Source**: WWW.Wikipedia .com, Duke et al., 2010, Hossain, 2015)

Figure 2.4: Fruits of *Ceriops decandra*

KHULNA UNIVERSITY Central Library KHULNA.

Local name: Goran

English name: Goran,Cuttia, Tengar, Palun

Systemic Classification *of C. decandra*

Kingdom: Plantae

Phylum: Tracheophyta

@Khulna University

Class: Magnoliopsida

Order: Malpighiales

Family: Rhizophoraceae

Genus: *Ceriops*

Species: *C. decandra*

Traditional Medicinal Uses of *C. decandra*

The bark is astringent. A decoction is used to treat haemorrhages. The bark yields around 25 - 37% of a high quality tannin. Both bark and leaves are used for tanning in South-East Asia and India. The bark of older trees has higher contents of tannin. The sap of the bark yields a black dye used in the 'batik' industry. The wood is a pale whitish-yellow when freshly cut, turning orange-brown on exposure to air. It is usually somewhat less heavy than the wood of the related *Ceriops taga.* The branches are used for tool handles, and bent ones for boat ribs. Some wood of this species has been chipped for pulp. The wood is used for fuel. When dry, the wood burns with a hotter flame than that of most other mangrove species. It is used in traditional medicine to cure hepatitis and ulcers (Umamaheswara and Nagababu, 2015).

2.5 *Heriteria fomes* (Buch.-Ham.)

Heritiera fomes is a species of mangrove tree in the family Malvaceae. Its common names include sunder, sundri, jekanazo and pinlekanazo. It is the dominant mangrove tree species of the Sundarbans of Bangladesh and India, and comprises about 70% of the trees in the area. Heritiera fomes is a medium-sized evergreen tree growing to a height of 15 to 25 metres (49 to 82 ft). The roots are shallow and spreading and send up pneumatophores. The trunk develops buttresses and

@Khulna University

is grey with vertically fissured bark. Trees with girths of 2 metres (6 ft 7 in) used to be found but these large trees have mostly been harvested for their timber. The trunk has few large branches and the canopy is open. The leathery leaves are elliptical and tend to be clustered at the ends of the twigs. The pink or orange bell-shaped flowers are each about 5 mm (0.2 in) across. They form in panicles, each flower being either male or female. The fruit carpels are up to 5 cm (2.0 in) long and 3.8 cm (1.5 in) wide. They ripen between June and August and the seed germinate readily.

(**Source**: WWW.Wikipedia.com, Ghosh et al., 2004; Kotowski et al., 2007; Hossain, 2015)

Figure 2.5: Fruits of *Heritiera fomes*

Local name: Sundor, Sundri, Jekanazo and Pinlekanazo

English name: Sundari

@Khulna University

Systemic Classification *of H. fomes*

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Malvales

Family: Malvaceae

Genus: *Heritiera*

Species: *H. fomes*

Traditional Medicinal Uses of *H. fomes*

Timber produced from *H. fomes* is hard, fine-grained, tough and elastic. The heartwood is dark red or reddish brown and the sapwood is a paler reddish brown. The timber has many uses; in bridge building, house construction, boat building and joinery, as utility poles and tool handles, making hardboard and as firewood. The tree is grown commercially in plantations. The bark of *H. fomes* is rich in procyanidins (Kathiresan et al., 2010). The ethanol extract has been shown to have antioxidant properties. It also shows antimicrobial activities against *Kocuria rhizophila*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* and is non-toxic in brine shrimp toxicity tests (Wangensteen et al., 2009).

2.6 Nypa fruticans (Wurmb)

N. fruticans is a large, creeping, unarmed, pleonanthic, monoecious palm. The nipa palm's trunk grows beneath the ground and only the leaves and flower stalk grow upwards above the surface.

@Khulna University

Thus, it is an unusual palm tree, and the leaves can extend up to 9 m (30 ft) in height. The flowers are a globular inflorescence of female flowers at the tip with catkin-like red or yellow male flowers on the lower branches. The flower produces woody nuts arranged in a globular cluster up to 25 cm (10 in) across on a single stalk. The ripe nuts separate from the ball and are floated away on the tide, occasionally germinating while still water-borne. Fruits develop from carpel, compressed and irregularly angled, pyramidal, 10-15 x 6-8 cm, brown to blackish, exocarp smooth, mesocarp fibrous, endocarp thick and composed of interwoven fibrous strands.

(**Source**: WWW.Wikipedia .com, Prashad et al, 2013, Hossain, 2015 GRIN and ARS 2017)

Local name: Golpata

English name: Mangrove palm

Figure 2.6: Fruits of *N. fruticans*

@Khulna University

Systemic Classification *of Nypa fruticans*

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Monocotyledones

Order: Arecales

Family: Arecaceae

Genus: *Nypa*

Species: *N. fruticans*

Traditional Medicinal Uses of *Nypa fruticans*

In many zones, the flower cluster can be tapped before it blooms to yield a sweet, edible sap collected to produce a local alcoholic beverage called *tuba*, *bahal*, or *tuak*. The sap is used to make palm sugar, honey substitute, a fresh drink (Dennett, 1927 and Davis, 1986). The inner endosperm of the fruits is consumed by local people. The endosperm of the fruits could be used as a potential source of natural antioxidant which can lower the incidence of certain types of cancer, cardiovascular diseases, and diabetes (Proasad et al., 2013). Various parts of nipa palm are a source of traditional medicines (e.g. juice from young shoots is used against herpes, ash of

@Khulna University

burned nipa material against toothache and headaches) and material for salt extraction (Koji tsuji et al., 2011).

2.7 *Phoenix paludosa* (Roxb.)

The trees grow in clusters, to 5 m high, usually forming dense thickets. The leaves are 2 to 3 m long and recurved. Leaves like Nypa but smaller and arranged at the crown of the plant. Anthophylls remains at the base of the petiole. The species contains slender, cylindrical and unbranched stem. The stem is covered with dark fibrous sheath. Leaves are petiolate, compound, imparipinnate with many ensiform segments, waxy, strong midrib and ending into strong sharp spine at apex; few pairs of leaflet at the lower segment are modified into sharp spines. Leaves are arching and contain light green leaflets. The inflorescence is a spadix, peduncle branched; spathe about 45 cm long, boat shaped, yellow, spongy glabrous and deciduous. Both the male and female flowers are sessile and erect; but female flowers are shorter that the male. Fruits are drupes, fleshy, one seeded, purple to black when ripe and arranged in cluster. Seeds are woody and very hard on maturity.

(**Source**: WWW.Wikipedia.com, Roxburgh, 1832, Lima, et al., 2010; Ellison et al., 2010, Hossain, 2015)

@Khulna University

Figure 2.7: Fruits of *Phoenix paludosa*

Local name: Hental

English name: Mangrove date palm

Systemic Classification *of Phoenix paludosa*

Kingdom: Plantae

Phylum: Tracheophyta

Class: Liliopsida

Order: Arecales

Family: Arecaceae

Genus: *Phoenix*

Species: *Phoenix peludosa*

@Khulna University

Traditional Medicinal Uses of *Phoenix paludosa*

Leaves are used for making mats, ropes, umbrellas and thatching materials. Stems are used to build cottages and fuel wood. Pith of the stem is edible. The fruits are eaten in curries. The fruits have been used as a coffee substitute. The pith of the stem is edible. *P. paludosa* is used as antipyretic and anti-inflammatory agent in the early days in various regions (Lima et al., 2010).

2.8 *Sarcolobus globosus* (Wall.)

S. globosus is a twining shrub native to tropical regions of Asia with stout glabrous branches, root-stock thick, and fleshy; roots thick. Leaves are simple, opposite, 3-6x2-4.5 cm, ovate or oblong, thick and fleshy, acute or obtuse at apex, rounded at base. Inflorescence cymose. Flowers small, starry, crowded, in axillary corymbose cymes, 2–3 mm across; corolla purplish, lobes pubescent inside. Follicles brown, 4–5 cm across, sub-globose; seeds are many and flattened. Cotyledons are often large, radicleterete. In the mangroves of India it is often found in association with and climbing on *Phoenix paludosa*. Flowering and fruiting occur during June–September, October–January, respectively.

(**Source**: WWW.Wikipedia .com, Wangensteen, 2005; Hossain, 2015)

@Khulna University

Figure 2.8: Fruits of *S. globosus*

Local name: Bawali lota

English name: Protruding, Sarcolobus

Systemic Classification *of S. globosus*

Domain: Eukaryota

Kingdom: Plantae

Phylum: Tracheophyta

Class: Spermatopsida

Order: Gentianales

Family: Apocynaceae

Genus: *Sarcolobus*

@Khulna University

Species: *S. globosus*

Traditional Medicinal Uses of *S. globosus*

S. globosus is listed by the U.S. Food and Drug Administration (FDA) as poisonous plant. The seeds are known to be highly toxic to mammals (Arokiasamy, 1968). Native people of Asia widely use it to kill dogs and wild animals. It was demonstrated that it effectively killed cats. The plants extract causes inhibition of the neuro-muscular system. The symptoms of poisoning in animals include blood urine and nephrosis. The plant has been used as a herbal medicine for treatment of rheumatism, dengue and fever (Mustafa et al., 1990). The plant is known to contain barbigerene which is validated to have significant antioxidant property, highly effective against the malaria parasite and with anti-cancer potential as it causes apoptosis of murine lung cancer cells (Li et al., 2009).

2.9 *Sonneratia apetala* (Bauch.-Ham.)

S. apetala is one of the woody mangrove species with high adaptability and seed production. The height of the species ranges from 15 to 20 m. The species is naturally distributed in India, Bengal and Sri Lanka as a dominant species in local mangrove communities (Jayatissa et al., 2002). The species was introduced to China in 1985 for restoration purposes. *S. apetala* first growing, light demanding and hardy species is found in the upstream estuarine zone in the low to mid-intertidal zone. It requires a sunny position and grows best in a heavy soil. It also prefers a pH in the range 6.8 - 7.2, tolerating 6.5 - 7.5. The flowers are nocturnal, opening in the evening and falling off in the morning.

@Khulna University

Figure 9: Fruits of *S. apetala*

Some others Characteristics of *S. apetala*-

- Dense crown and slender drooping branches, appearance similar to that of a Eucalyptus tree
- Leaves: Narrow, simple, opposite, narrow, leathery.
- Petals absent, hence apetala
- Fruit: very small (2cm diameter)
- Flowers: Bisexual, cream colored, four sepals

(Source: WWW.Wikipedia .com, Jayatissa et al., 2002, Kathiresan et al., 2010; Hossain, 2015)

English name: Mangrove apple,

Local name: Keora, chipi

@Khulna University

Systemic Classification of *S. apetala*

Domain: Eukaryota

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Myrtales

Family: Lythraceae

Genus: *Sonneratia*

Species: *Sonneratia apetala.*

Traditional Medicinal Uses of *S. apetala*

Leaves of this plant have been used traditionally to treat hepatitis (Bandaranayake, 2002) dysentery, sprain & bruises, in treatment of eye troubles and open sores in children in ears and also in heart troubles (Bandaranayake, 1995). Hossain et al. (2013), reported that the mangrove fruit *Sonneratia apetala* Buch.-Ham. is extensively consumed by the people which consist of very high content of polyphenols (300 ± 8.2 mg GAE/g extract), flavonoids (30.6 ± 0.7 CE/g extract), anthocyanins (2.3 ± 0.03 µmol/g extract) and vitamin C (4.0 ± 0.08 mg/g extract) and also reported (2015) that *S. apetala* fruits are attributed to presence of various active compounds. Seeds fractions found to have potential as antibacterial, anti-diarrhoeal, analgesic, and cytotoxic constituents. In many zones, sour sauce is produced by the fruit of *S. apetala*. Fermented juice of this fruit is used for arresting haemorrhage. Barks and fruits of the *S. apetala*

@Khulna University

are used for treatment of febrifuge, asthma, ulcers, swellings, sprains, bleeding, piles and hemorrhages (Bandaranayake et al., 1998). Antioxidant, antibacterial, antidiabetic and anticancer activities are obtained from bark and leaf extracts of *S. apetala* (Patra et al., 2015).

2.10 *Sonneratia caseolaris* (L.) Engl.

Sonneratia caseolaris is a type of mangrove growing up to 20 m in height and with a trunk reaching a maximum diameter of 50 cm. Leaves are simple, opposite, minute recurved point at the apex, blunt or rounded tip; petioles are short and reddish to pink in colour. Flowers are large, reddish or purple. It opens at dusk and last only for one night. The fruit is berry with persistent calyx and pointed style. Previously this species was classified under Sonneratiaceae family. The tree has red flowers. The fruit are large (4 cm wide) green, leathery berries with a star-shaped base.

(**Source**: WWW.Wikipedia .com, Kathiresan et al., 2010; Hossain, 2015)

Figure 2.10: Fruits of *Sonneratia caseolaris*

@Khulna University

English name: Mangrove apple

Local name: Ora, Soila

Systemic Classification of *S. caseolaris*

Domain: Eukaryota

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Myrtales

Family: Lythraceae

Genus: *Sonneratia*

Species: *S. caseolaris.*

Traditional Medicinal Uses of *S. caseolaris*

The fruit are edible and appreciated as food in many areas. In Sri Lanka, where the fruit is known as *kirala gédi* in Sinhala, the pulp of the fruit is mixed with coconut milk extract and made into a milk shake. Leaves are used as forage, and tannins from bark for dyes. In the Philippines the fruits can be eaten raw or as soup, and are used as a comestic. Fruits are major source of dietary functional components such as polyphenols, flavonoids, vitamin C, antioxidant which reduce risk of cardiovascular diseases and cancer (Kris-Etherton et al., 2002). This plant is used for followings treatments: bleeding, hemorrhages, piles, sprain poultices (Simlai and Roy, 2013).

@Khulna University

Malayans use old fruit walls for worms, half-ripe fruits for coughs, and pounded leaves for hematuria and smallpox (Perry, 1980).

2.11 *Xylocarpus mekongensis* (Pierre)

Xylocarpus is a genus of plants in the mahogany family (Meliaceae). *X. mekongensis* is commonly known as Passur. It is a glabrous, medium-sized tree that grows generally on the inter-tidal silty but consolidated clay or on the sandy or rocky bay distributed throughout the tropical and subtropical regions of Southeast Asia including the Sundarbans. The plant has well developed aerial blunt end pneumatophores or root suckers and green coloured fruit of diameter generally not exceeding 15 cm.

(**Source**: WWW.Wikipedia .com, Raju, 2003, Duke, 2006; FAO. 2007, Hossain, 2015)

Figure 2.11: Fruits of *X. mekongensis*

@Khulna University

Local name: Passur

English name: The puzzle fruit tree

Systemic Classification of *X. mekongensis*

Domain: Eukaryota

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Sapnidales

Family: Meliaceae

Genus: *Xylocarpus*

Species: *X. mekongensis*

Traditional Medicinal Uses of *X. mekongensis*

Leaves, barks, pneumatophore and fruits of these plants have been reported for various ethnomedicinal uses such as fever, malaria, inflammation, dysentery, diarrhoea, cholera, abdominal problems, diabetes, elephantiasis, antimicrobials etc and are also reported for their antioxidant, anticancer, antidyslipidemia, antifilarial, antiulcer and cardiotonic activities (Ellison, 2005). The oil extracted from seeds has been used as an illuminant and as hair oil (Raju, 2003). The astringent bark has some medicinal uses.

@Khulna University

CHAPTER THREE: LITERATURE REVIEW

The Sundarbans is the most important natural mangrove forest of Bangladesh and rich in flora diversity compared to other mangrove forests in the world. This forest ecosystem is dynamic and complex in nature. The Sundarbans is the single tract largest mangrove forest in the whole world. The forest occupies the southwest corner of Bangladesh between longitudes 88°02′00″ and 89°00′00″ E and latitudes 21°27′30″ and 22°30′30″ N. The forest cover an area of 6017 km², of which 4143 km² are landmass and remaining 1874 km² are under water bodies (Siddiqui, 2016). Mangroves are economically and ecologically important as a natural renewable resource (Field, 1995). A fundamental function of all forests has been to supply timber for cooking, heating and constructing dwellings, and mangrove forests are no exception (Watson, 1929; FAO, 1982). Traditionally, people have used mangroves for the benefit of the local community, but increasing populations have led to an increasing non-sustainable abuse of the resources. The mangrove wetland is a major type of natural wetland in tropical and subtropical regions, which is important in the removal of nutrients, heavy metals, and organic pollutants from wastewater within estuarine systems due to the presence of oxidized and reduced conditions (Zhang et al., 2010).

In the Sundarban, eight dominant plants were recognized to form forest categories: *Heritiera fomes* (Sundari), *Sonneratia apetala* (Keora), *Excoecaria agalocha* (Gewa), *Xylocarpus mekongensis* (Passur), *X. granatum* (Dhundul), *Bruguiera gymnorhiza* (Kankra), *Ceriops decundra* (Goran) and *Avicennia officinalis* (Baen) (Chaffey et al., 1985). There are many common chemical constituents present in the plants of Sundarbans. They are amino acids and alkaloids, aliphatic alcohols and acids, carbohydrates, carotenoids, hydrocarbons, free fatty acids including polyunsaturated fatty acids (PUFAs), lipids, pheromones, phorbol esters, steroids,

@Khulna University

triterpenes and their glycosides, tannins, other terpenes, phenolics, and related compounds (Ksouri et al., 2007). The additional newer components like gums and glues to alkaloids and saponins and other substances of interest to modern industry and medicine. Chemicals such as amino acids, carbohydrates and proteins, are products of primary metabolism and are vital for the maintenance of life processes, while others like alkaloids, phenolics, steroids, terpenoids, are products of secondary metabolism and have toxicological, pharmacological and ecological importance (Revathi et al., 2013).

Many plant species have medicinal properties and beneficial impact on health, e.g. antioxidant activity, anti-inflammatory activity, antimicrobial activityl, digestive stimulation action, hypolipidemic, anticarcinogenic potential and antimutagenic effects (Aaby et al., 2004).

Global agriculture production and food security is seriously affected by high salinity and scarcity of water (Subudhi and Baisakh, 2011). In fact, only a small group of higher plants, the halophytes, can grow under saline conditions. The potential utilization of halophytic species for the improvement of sustainable agriculture as well as sources of economy was discussed by Khan and Qaiser (2006). Plants have evolved adaptive mechanisms which can be understood and exploited as an important resource for development of crops tolerant to extremities. Halophytic species that live under conditions of high salinity exhibit succulence by altering their energy metabolism (Aghaleh et al., 2011) and salinity tolerance in the form of osmotic adjustment (Flowers and Colmer, 2008).

@Khulna University

Generally pain can be defined as a physical suffering or distress, as due to injury and illness or it is a distressing sensation in a particular part of the body (www.dictionary.com). So Pain is a multidimensional experience that is essential for the maintenance and preservation of an individual. It warns of the danger of bodily harm and alerts to trauma and injury. Pain is a specific enteroceptive sensation; it can be perceived as arising from a particular portion of the body and it involves dedicated subsets of peripheral and central neurons. Under normal circumstances, primary afferent pain fibres activate particular central pathways that engage protective mechanisms at several functional levels: autonomic, homeostatic, motoric, behavioral and mnemonic. However, injury or disease can alter the balance of this system and result in persistent, pathological pain. Analgesia is the alleviation or the absence of pain. So analgesic activity means the capacity of a substance that neutralize the pain sensation. Analgesic substances, such as aspirin and morphine, which interact with the transmitters and modulators of the pain system are helpful for many people with pain. However these drugs pose serious side effects like: sedation, respiratory depression constipation, serious gastrointestinal tract reactions, bone marrow disturbances, liver disorders, dyspepsia, nausea and vomiting skin reactions, analgesic associated nephropathy etc (Kiritikar and Basu, 1991). So, there is a great need for the development of better methods for the alleviation and control of both acute (immediate) and chronic (long-term, pathological) pain (Carig and Sorkin, 2001). Excessive pain may be unbearable and causes other effects- sinking sensation, apprehension, sweating, nausa, palpitation, rise or fall in BP (Tripathi, 2001).

Analgesic is an agent that reduces or eliminates pain by acting on the sensory nervous system, either centrally or peripherally without significantly altering consciousness. The function of analgesics is to relieve pain as a symptom, without affecting its cause. They are used when the

KHULNA UNIVERSITY Central Library KHULNA
@Khulna University

noxious stimulus (evoking the pain) cannot be removed or as adjuvant to more etilogical approach to pain (Ghani, 1998, Tripathi, 2001). According to the Arthritis Research, UK Analgesics are divided into two types:

- Opoid/ narcotic/ morphine like analgesics: These are strong analgesics & depress CNS. Examples of this type are codeine, heroine, fentanyl, morphine, ethadone, oxycodone, hydromorphone, buprenorphine, propoxyphene, tapentadol, etc.
- Non-opoid/ non-narcotic/ aspirin like/ antipyretic/ anti-inflammatory analgesics: These are weaker analgesics. They act primarily on peripheral pain mechanisms and also in CNS to raise pain threshold (Tripathi, 2001, Nathan, 2002). Examples of this type are aspirin, acetaminophen, diclofenac, etodolac, flurbiprofen, ibuprofeen, indomethacin, ketorolac, oxaprozin etc.

In modern world all tries to develop economically, less side effect containing but therapeutically potent drugs. Our world is the rich source of many medicinal plants which are the rich raw source of crude drugs. These crude drugs are the first materials for developing a new potent drug. So firstly needs to investigate the plant source which gives analgesic activity by comparing with potent standard and then determine and isolate those active components.

Analgesic activity of drug or any test sample at different steps of pharmacological investigation can be assessed by different methods (www.serlab....). Bently (1983) reported the most of the methods of analgesic activity determination are:

i. Acetic acid induced method,
ii. Hot plate method,
iii. Formaldehyde induced writhing method,

@Khulna University

iv. Radiant heat method,

v. Paw-withdrawal test in rats,

vi. Hot-water tail immersion in mice and

vii. Tail-flick test.

Acetic acid is a pain stimulating agent. Intraperitoneal administration of acetic acid (0.7%) causes the release of free arachidonic acid from tissue phospholipid by the action of phospholipase A_2 and other acyl hydrolases. There are three major pathways in the synthesis of the eicosanoids acid. All the eicosanoids with ring structures that is the prostaglandins, thromboxanes and prostacylines are synthesized via the cyclooxygenase pathway. The leucotrienes, HETE (Hydroxy Eicosatetraenoic Acids) and HPETE (Hydroperoxy Eicosatetraenoic Acids) are hydroxylated derivatives of straight-chain fatty acids and are synthesized via the lipooxygenase pathway (Rang et al., 1993).

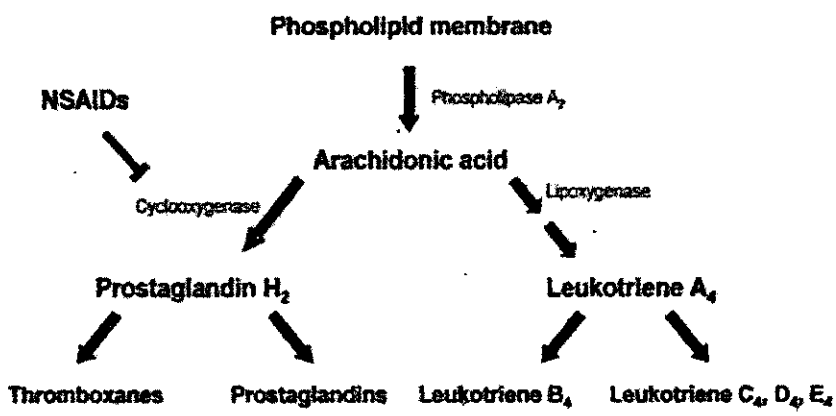


Figure 3.1: Main biochemical pathways of arachidonic acid. (Pardutz and Schoenen, 2010).

Prostaglandins and thromboxane A_2 (TXA_2), collectively termed prostanoids, are formed when arachidonic acid (AA), a 20-carbon unsaturated fatty acid, is released from the plasma membrane by phospholipases (PLAs) and metabolized by the sequential actions of prostaglandin G/H

@Khulna University

synthase, or cyclooxygenase (COX), and respective synthases. There are four principal bioactive prostaglandins generated *in vivo:* prostaglandin (PG) E_2 (PGE_2), prostacyclin (PGI_2), prostaglandin D_2 (PGD_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). They are ubiquitously produced – usually each cell type generates one or two dominant products - and act as autocrine and paracrine lipid mediators to maintain local homeostasis in the body. During an inflammatory response, both the level and the profile of prostaglandin production changes dramatically. Prostaglandin production is generally very low in uninflamed tissues, but increases immediately in acute inflammation prior to the recruitment of leukocytes and the infiltration of immune cells (Dubois et al., 1998).

Diclofenac Na is chemically named 2-[(2, 6-dichlorophenyl)aminophenyl]-acetic acid, used as the positive control. It is a potent analgesic and anti-inflammatory agent (Idris, 2011). Any agent that lowers the number of writhing will demonstrate analgesia by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition.

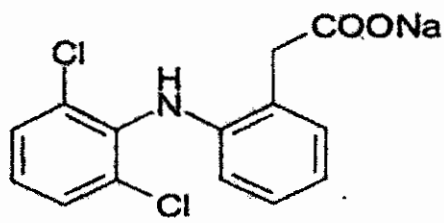


Figure 3.2: **Chemical structure of diclofenac sodium** (Idris, 2011).

The hot plate test is one of the most common and reliable test of the pain response in animals, similar to the tail flick test that is based on a phasic stimulus of high intensity. It is used in basic pain research and in testing the efficacy of analgesics by detecting the reaction to pain caused by heat. This method was first introduced by Eddy and Leimbach in 1953 (Eddy et al., 1953). They used a behavioral model of nociception where behaviors such as jumping and hind paw-licking

@Khulna University

are clicited following a noxious thermal stimulus. Licking is a rapid response to painful thermal stimuli that is a direct indicator of nociceptive threshold. Jumping represents a more elaborated response, with latency, and encompasses an emotional component of escaping (Espejo, 1993). The response of mice on heat stimulus, dose does not depend on mice gender that means the heat stimulus create same effects on both sex of mice (Duman et al., 2006). The paws of mice are very sensitive to heat at temperatures which are not damaging for the skin. The animals are placed on the hot plate and after a certain time the temperature becomes unbearable for them. Actually this stimulus transfer from paw tissue to brain receptor through central nervous system (CNS) and brain produce response (Mishra et al., 2011). So it is narcotic type analgesia. Here the drugs which are strong analgesics and depress CNS are used as positive control like indomethacine (Meena et al., 2009), diclofenac potassium (Mishra et al., 2011), diclofenac Na (Sampth et al., 2012), pentazocine (Hemamalini et al., 2010), morphine sulphate (Yerima et al., 2009).

Morphine is used to treat severe pain. It acts on certain centers in the brain to give you pain relief. This medication is a narcotic pain reliever (opiate-type) (www.healthcentral.com).

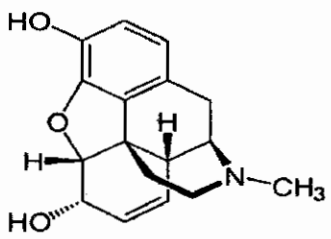


Figure 3.3: Molecular structure of Morphine (www.opiateaddiction...)

Mangrove plants are a great source of novel natural compounds or secondary metabolites, which have significant pharmacological properties and used as ethnomedicine for treating number of

@Khulna University

ailments (Bandaranayake, 2002; Salini, 2015). For example, *Rhizophora mucronata* is one of the medicinally important mangrove plant (family Rhizophoraceae), abundantly found in a number of South Asian countries including India and different parts of this plant are being used traditionally in the treatment of diabetes, diarrhoea, wounds, ulcers and liver disorders (Hoppe-Speer, 2011 ; Manilal, 2015). Gallic acid, syringic, benzoic, trans-cinnamic, p-coumaric and caffeic acids are phenolic acids of plant materials while catechin, kaempferol , naringenin, luteolin and apigenin and chrysin are the flavonoids (Pyrzynska and Biesaga, 2009). The flavonoids and phenolic acids are known sources of antioxidants that act as free radical scavengers (Johnston et al., 2005). Flavonoid also involved in many biological properties such as antibacterial, anti-inflammatory and anti-allergic activities (Gheldof et al., 2002). Recent study revealed that mangrove plants can act as potential analgesic agent which is contributed from its bioactive compounds. Many researchers showed significant analgesic effects of mangrove plant materials.

Adhikari et al. (2016) reported that the ethanolic extract of *Rhizophora mucronata* L. (Sunderban mangrove) leaves is rich in tannin, flavonoids, polyphenols and possess potential peripheral analgesic and mild central analgesic action. The activity was comparable with the standard drug Diclofenac sodium in the dose 10 mg/kg b.w. orally. Diclofenac sodium reduced the writhing response 58.78% in mice compared to the control whereas the test sample in 100 mg/kg dose orally reduced the same by 30.08% and the most effective dose of the extract in a dose of 200 mg/kg orally reduced the same by 44.05%. In the thermal nociception model, the plant extract at a dose of 200 mg/kg body weight (orally) possesses analgesic activity to some extent but the other doses failed to show significant analgesic effect. Standard analgesic drug Diclofenac sodium 10mg/kg orally was used for comparison. The plant extract in a dose of 200 mg/kg orally

@Khulna University

reduced the pain response and increased the latency period
end of the study compared to the control whereas the stand
the pain sensation by 49.01%.

According to Hossain et al. (2013) seeds of *S. apetala* h
activity. Five different solvent extracts of seed are given orally (500 mg/kg b.w.) and n-hexane solvent extract inhibited ($P<0.05$) the abdominal constrictions in mice more than the positive control, diclofenac sodium (25 mg/kg b.w). Besides, at that concentration, diethyl ether extract, chloroform extract, ethanol extract, and methanol extract showed similar inhibitory effects as that of positive control. Control group showed the average writhing numbers of 60±6. In the hot plate method, the five different solvent extracts of the seed increased reaction time with the highest effect at 120 min. At that time, n-hexane showed the highest reaction time (15.1 s), whereas morphine (10 mg/kg b.w.) used as a positive control, showed 16.9 s.

Lima et al. (2010) reported that leaf of *Phoenix paludosa* has a great analgesic activity. In the acetic acid-induced writhing test, the ethanolic extract of leaves of *P. paludosa* showed dose dependent decrease in the total number of writhings after 15 min of administration of acetic acid intraperitoneally. The analgesic effect at the dose of 500 mg/kg body weight was significant ($p=0.009$) and the effect increased with the increase of dose. Diclofenac sodium reduced the writhing response 75% in mice compared to the control whereas the test sample in 250 mg/kg dose orally reduced the same by 8.82% and the most effective dose of the extract in a dose of 200 mg/kg orally reduced the same by 31.61%.

@Khulna University

Helminthes are recognized as a major health problem throughout the tropics (Adewunmi et al., 2001). Helminth infection is one of the most prevalent diseases in developing and developed countries (Krogstad et al., 1998). Globally, an estimated 2 billion people are infected by intestinal nematodes (Wen et al., 2008). Most diseases caused by helminthes are of a chronic and debilitating in nature, they probably cause more morbidity and greater economic and social deprivation among humans and animals than any other single group of parasites. Helminths consume nutrients from their host, thereby causing or aggravating malnutrition which results in retarded growth and physical development. Consequently, symptoms like retarded cognitive development, iron-deficiency, anemia, abdominal pains and related health problems are characteristic features of most heavy helminth infections (Crompton et al., 2002; Kirwan et al., 2009). Due to the asymptomatic nature of these diseases, the helminths remain undetected and children born in an endemic region may harbor the worms for the most part of their lives (WHO, 1987).

Anthelmintics are drugs that are used for the treatment of infections caused by the worms, flukes, nematodes, round worms, tapeworms etc. These are the tropical and veterinary types of medicines which are of huge importance. According to Dye and Walker (2007), drugs which are used as anthelmintics include piperazine, mebendazole, albendazole, heterocyclics, ivermectin, vinyl pyrimidines, niclosamide etc.

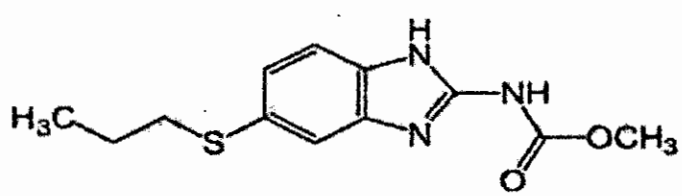


Figure 3.4: Molecular structure of Albendazole (Silvana et al., 2015)

@Khulna University

Although synthetic molecules are effective in the treatment/management of parasitic infections, they are reported for side effects or toxicity. Till the time reported side effects/adverse effects of synthetic anthelmintics include: Epigastric pain, diarrhea, nausea, vomiting, dizziness and headache. Allergic phenomena such as edema, rashes and urticaria, asymptomatic trichuriasis, impaired growth in childrens, increase in serum aminotransferase activity (Bagheri et al., 2004). Abdominal pain, distention, alopecia, reversible neutropenia, agranulocytosis and hypospermia (Rivas et al, 1981). Retinal hemorrhages, severe encephalopathy, tachycardia, arthralgias, intense itching, enlargement and tenderness of the lymph nodes, and sometimes a popular (Horton, 2000) rash, fever, Ocular complications include limbitis, punctate keratitis, uveitis, and atrophy of the retinal pigment epithelium (Dominguez et al., 1983,). It is advised that mebendazole not be taken by pregnant women or to children less than 2 years of age (Adam et al., 2004). In animals, signs of CNS toxicity, including lethargy, ataxia, mydriasis, tremors, and eventually death, occur at very high doses (Campbell, 1993).

The synthetic anthelmintics which are randomly used are not very safe as they cause various side effects and toxicity and many of them are not recommended for young children and pregnant ladies. Consequently, the importance of herbal drugs in medicine has tremendously increased because of their fewer side effects. The phytochemical constituents and their standardization are accelerated with the development of instrumental analysis and this field is becoming important and new for investigation. Hence the development and discovery of new substances acting as anthelmintics are being derived through plants which are considered to be the best source of bioactive substances (Piyush et al., 2013). The natural anthelmintics Known as vermifuge or vermicides natural anthelmintic includes the following components: Tobacco, Walnut, Valerian Aconite, Jalap, quassia wood, podophyllin, stavesacre seeds, turpentines, wormwood, Kamala,

@Khulna University

kuosso and kosin (Dun 1892), American wormseed, oil of chenopodium and santonica (Hall,1924), Clove, Calomel, Kalonji seeds, Garlic, Male-fern, Pineapple, Diatomaceous earth, Soya and other legumes, Honey, water and vinegar are mixed with warm water (Ludow, 1860), mucuna beans, cowhage pink root (Ludow, 1860).

The plant derivatives have the anthelmintic activity mainly due to their phyto-constituents especially due to secondary metabolites. It has not been understand clearly the mechanism of action of herbs for their anthelmintic activity. Phyto-constituents, jointly or separately may act by inhibition of tubulin polymerization and blocking glucose uptake (Jain et al., 2011). Any damage to the mucopolysaccharide membrane of worms will expose the outer layer restricting their movement which finally may cause paralysis and ultimately death of parasite (Chandrashekhar et al., 2008). The anthelmintic effects of tannins may be attributed to its capacity to bind free protein available for larval nutrition and thus reducing the nutrient availability resulting in larval starvation or decrease in gastrointestinal metabolism directly through inhibition of oxidative phosphorylation causing larval death (Scalbert, 1991 and Athanasiadou et al., 2001). According to Roy et al. (2010) alkaloids may act on central nervous system and caused paralysis of the earthworm. The effect can be due to presence of the steroidal alkaloids and oligoglycosides which may suppress the transfer of sucrose from the stomach to the small intestine together with their antioxidant effect which is capable of reducing the nitrate generation which can interfere in local homeostasis that is essential for the development of helminths (Borba et al., 2010).

According to Raju and Patro (2017) *Aegiceras corniculatum* (Myrsinaceae) stem extract has acted as an anthelmintic agent. The study design were investigation of the traditional anthelmintic medicinal plant *Aegiceras corniculatum* using in vitro anthelmintic properties of

@Khulna University

four extracts by using earthworms. The four crude stem extracts of *Aegiceras corniculatum* were petroleum ether extract (50 and 100 mg/ml), chloroform extract (50 and 100 mg/ml), methanol extract (50 and 100 mg/ml) and aqueous extract (50 and 100 mg/ml). The paralysis time of petroleum ether extract (96 ± 6.33 and 76 ± 2.31), chloroform extract (116 ± 4.36 and 92 ± 7.50), ethanol extract (62 ± 3.20 and 34 ± 3.52) and aqueous extract (200 ± 2.22 and 180 ± 3.19) were compared to the standard drug Albendazole suspension 100 mg/5ml (5 ± 1.00). From these findings the four extracts of *Aegiceras corniculatum* are rich source of naturally occurring anthelmintic activity.

Shettar and Vedamurthy (2017) reported that *Kandelia candel* and *Rhizophora apiculata* plants methanol extract showed higher anthelmintic activity when compared to other solvent extracts. In the study the concentrated and dried extracts of *Kandelia candel* and *Rhizophora apiculata* were evaluated for in vitro anthelmintic activity by varying the concentration (50- 250 mg/20ml) with using Indian earth worm (*Pheretima posthuma*) as animal model. In case of both *Kandelia candel* and *Rhizophora apiculata* among different solvent extracts methanol extract exhibited highest anthelmintic activity at 250 mg/20ml concentration with paralysis time 46.66±1.20 (min) and 60.66±0.88 (min) respectively and in case of death time it was observed to be 67.33±1.76 (min) and 84.33±1.45 respectively. Results were compared with standard drug Piperazine citrate which shows paralysis time 31.33±1.76 (min) and death time 36.00±1.15 (min) at 100 mg/20ml concentration. The results indicate that methanol extract of *Kandelia candel* and *Rhizophora apiculata* shown appreciable anthelmintic activity but in performed in-vitro assay methanol extract of *Kandelia candel* exhibited promising significant activity where as other tested extracts of both plants showed the least anthelmintic activity.

@Khulna University

According to Moghal et al. (2016) methanolic extract of *Avicennia marina* (Forssk.) has displayed potential anthelmintic activity. Anthelmintic activity was assessed by recording paralysis and death time with different concentrations of the plant extracts. Anthelmintic test showed that methanol extract of *Avicennia marina* at 40 mg/ml significantly caused paralysis and death of worms and the required time were found to be 19.0 ± 1.46 min and 32.67 ± 0.95 min, respectively which gradually increased with the decrease of concentration. while standard albendazole caused paralysis and death of worms after 35.33 ± 58 min and 71.33 ± 1.15 min of administration at a concentration of 10 mg/ml.

Sardar et al. (2018) reported that aerial parts of *Acanthus ilicifolius* L. have shown potential anthelmintic activity. Anthelmintic activity of the extract was assessed on live parasites P. cervi and H. contortus of cattle. In a concentration-dependent manner, the aerial part extract exhibited significant anthelmintic activity which was comparable with standard drug albendazole. In P. cervi, both paralysis and death occurred faster at higher concentrations of the extract (i.e. 5.50 ± 2.42 min and 10.17 ± 2.71 at 200 mg/mL while 9.83 ± 1.47min and 15.50 ± 2.66 at albendazole 15 mg/mL). In H. contortus, a similar tendency was found (i.e. 9.33 ± 3.82 min and 18.83 ± 3.54 min at 200 mg/mL while 19.17 ± 4.02 min and 31.33 ± 6.62 min at albendazole 15 mg/mL)

Islam et al. (2019) pointed out that Sundarbans' honeys showed potential anthelmintic acivity when compared to control group. At a dose of 100, 200, 300, and 400 mg/mL, the paralysis times were 15.8, 8.6, 5.6, and 4.7 min, respectively, and the times until death were 34.0, 15.8, 9.2, and 8.0 min, respectively. Albendazole, a standard drug, showed a paralysis time of 8.3 min and a time until death of 17.2 min; corresponding times for the control group were 83.5 min and 161.8

@Khulna University

min, respectively. All doses of honey used in this study significantly ($P<0.05$) reduced both the paralysis time and the time until death, compared with the control group.

With respect to use of anthelmintic plants, a perusal of literature reveals that in the beginning quite a few studies on anthelmintic activity of traditional anthelmintic plants, their extracts frequently employed earthworm, *Pheritima posthuma* and Paramphistimum species as a test worm. The test parasites which have more frequently been used to evaluate the anthelmintic efficacy of plants are the ones which are readily available from locally slaughtered domestic animals. As the gastrointestinal helminthes become resistant to currently available synthetic anthelmintic drugs, hence the need to investigate and identify natural substances with anthelmintic activity is huge. There are some well-known edible mangrove fruits: keora (*S. apetala*) Ora (*S. caseolaris*); Passur (*X. mekongensis*); Sundari (*H. fomes*) Khalshi (*A. corniculatum*); Baen (*A. officinalis*); Kankra (*B. gymnorrhiza*); Goran (*C. decandra*); Golpata (*N. fruticans*); Bawali lota (*S. globosus*), and Hental (*P. paludosa*) which are selected for investigation of those noble natural compounds that's are active against GI nematodes and pain management.

Nowadays, bovine are affected of intestinal problems by different parasites, if better anthelmintic activity will be found in any single fruit extract, then it will lead for development of new drugs that interfere with the disease due to internal parasites. Hopefully, the new findings in this investigation will offer a scientific support to the traditional medicinal and therapeutic use of mangrove plant resources. Besides, the present study will demonstrate significant central and peripheral analgesic activity of fruits extract in the Sundarbans.

@Khulna University

Literature survey indicates that there are only few pharmacological studies are reported on these plant part. However these above mentioned plant fruits will be subjected for investigation for their analgesic and anthelmintic activities.

@Khulna University

CHAPTER FOUR: MATERIALS AND METHODS

4.1 Materials

4.1.1 Collection of the Fruit Samples

The eleven type fruits were collected from the different zones of Sundarban's forest division, Bangladesh in August and September, 2018. After collection, the fruits were washed with tap water to remove the undesirable materials and the excess of water was drained off.

4.1.2 Drying

The fruits were then cut into smaller pieces and shade dried for about 15 days to remove water portion from fruits.

4.1.3 Grinding

The dried fruits were ground into fine powder with the help of grinder machine. The powder was stored in an airtight plastic bottle and kept in cool and dry place.

4.1.4 Extraction Procedure-1

Ten grams (10g) of powder of each sample was taken in separate airtight bottle. Then, 200 ml of methanol and ethanol mixture (1:1) was added to each bottle. The mixtures were shaked vigorously and kept for overnight. After 20 hours, the mixtures were filtered through Whatman filter paper no.1. After evaporation, the filtrates were then taken into two dram vials from conical flask followed by drying and stored in a refrigerator at 4°C.

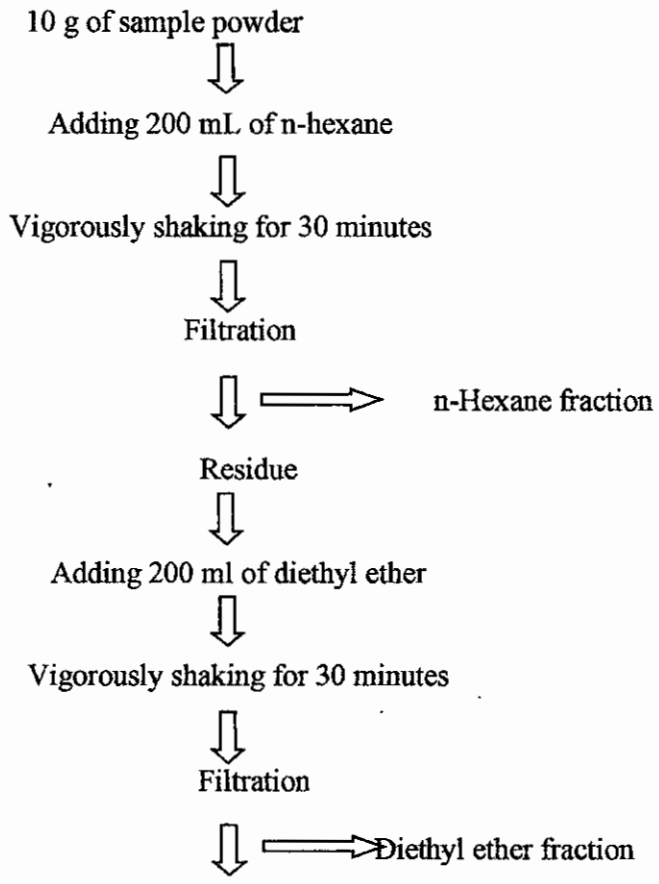
4.1.5 Extraction Procedure-2

Ten grams (10g) of powder of *A. corniculatum* was taken with 200 mL of 100% n-hexane and kept in air tight bottle and vigorously shaked for 30 minutes then it was filtered by Whatman No.1 filter paper to remove any fruits part or powder particle. After that 200 ml of diethyl ether

@Khulna University

was added to the residue of the filtration and vigorously shaked for 30 minutes then it was filtered by the same process. Then, 200 mL of chloroform was added to the remaining part and shaked for 30 minutes then it was filtered by Whatman No.1 filter paper. Next 200 ml of ethanol was added to the residue of the filtration and shaked vigorously for 30 minutes and then filtered by same process. Finally, 200 mL of methanol was added to the residue of the fruits powder and vigorously shaked for 30 minutes and filtered properly. After filtration the filtrates were air dried and stored in the refrigerator (4° C). Finally the extracts were weighted and named as n-hexane, diethyl ether, chloroform, ethanol and methanol extract of *A. corniculatum*, respectively.

The flow chart of extraction of the *A. corniculatum* fruit:



@Khulna University

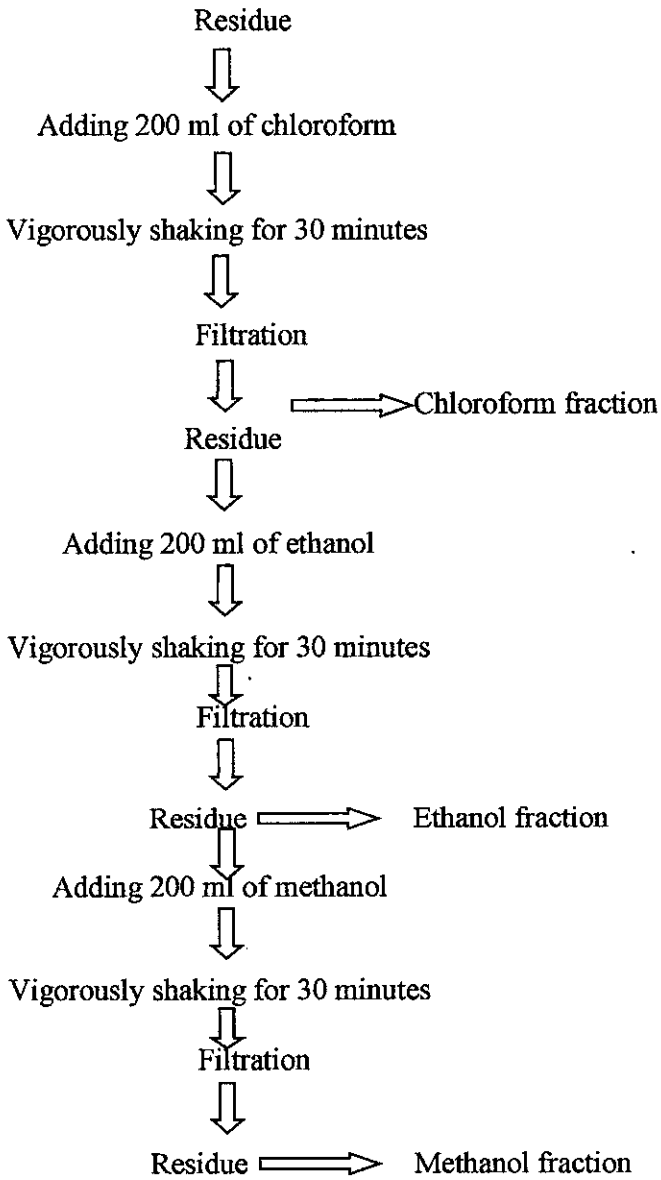


Fig 4.1: A flow chart of extraction method of *A. corniculatum* fruit powder.

@Khulna University

4.1.6 Chemicals

1. n-Hexane
2. Diethyl ether
3. Chloroform
4. Ethanol
5. Methanol
6. Distilled water
7. Tween-80
8. Sodium chloride
9. Potassium chloride
10. di-Sodium bi-phosphate
11. Potassium bi-phosphate

4.1.7 Instruments

1. Electric balance
2. Measuring cylinder
3. Shaking incubator
4. Beaker
5. Conical flask
6. Vortex machine
7. Hot plate
8. Thermo meter
9. Micropipette
10. One dram vial

@Khulna University

11. Two dram vial
12. Funnel
13. Filter paper
14. Extraction chamber
15. Transparent plastic cage
16. 1 mL syringe
17. 3 mL syringe
18. Insulin syringe
19. Feeding needle
20. Stop watch
21. Plastic Box
22. Hand gloves
23. Mask
24. Magnetic stirrer
25. Micropipettes
26. pH meter

4.1.8 Drugs

1. Diclofenac Na (clofenac, Square Pharmaceutical)
2. Morphine (Gonosastho Pharmaceutical).
3. Albendazole (Alben DS, SK^{+}F Pharmaceutical)

@Khulna University

4.1.9 Experimental animals

Young Swiss-Albino mice aged 6-8 weeks, average weight 15-25 g were used for the experiment. The mice were purchased from the Animal Resources Branch of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDRB). They were kept in standard environmental condition up to finishing the experiment. The animals were provided with standard laboratory food and tap water and maintained at natural day night cycle. All the experiments were conducted on an isolated and noiseless condition.

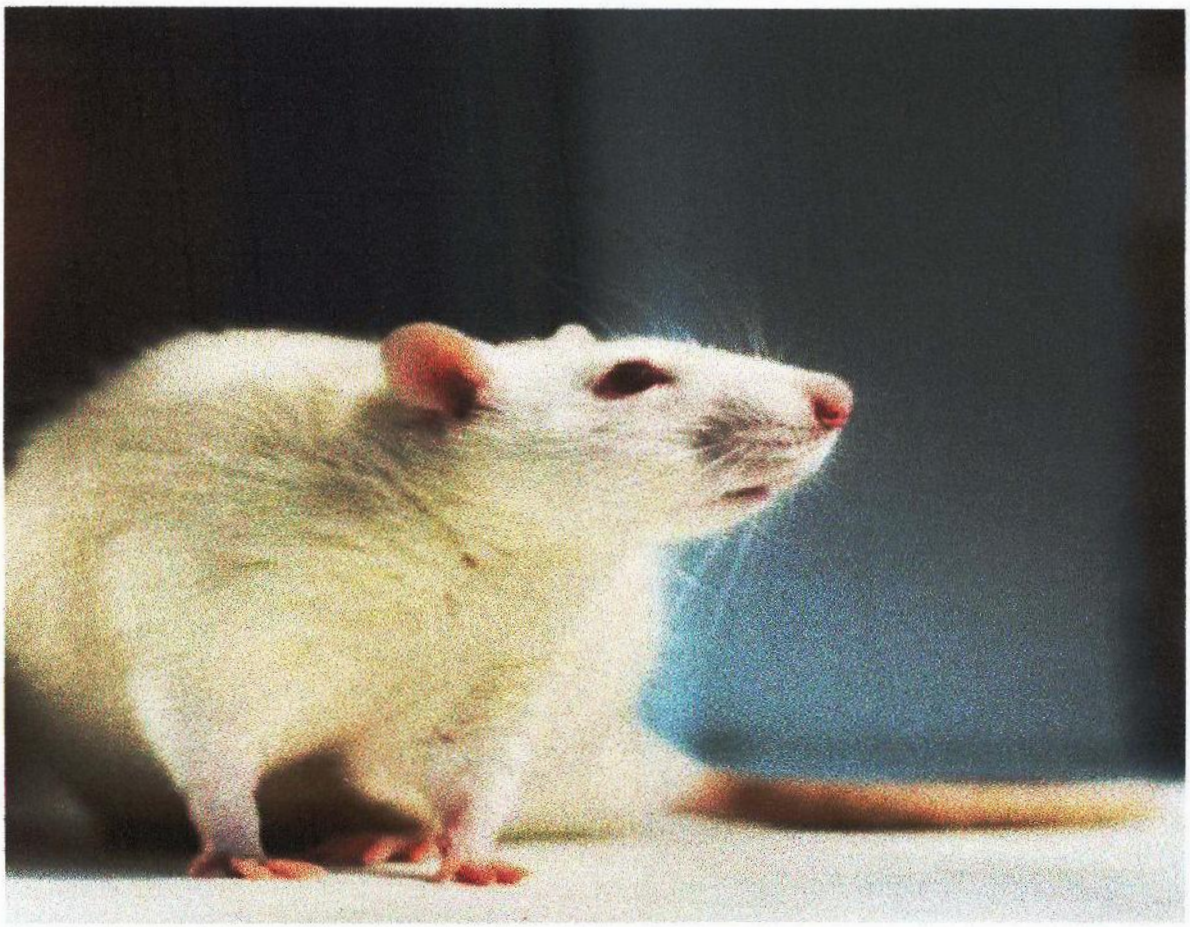


Figure 4.2: A Swiss-Albino mouse collected from ICDDRB

@Khulna University

4.2 Methods

4.2.1 Screening for Analgesic Activity

4.2.1.1 Evaluation of Analgesic Activity by Acetic Acid Induced Writhing Method

Analgesic activity of the different edible mangrove fruits extracts was tested using the model of acetic acid induced writhing in mice described by Whittle (1964).

4.2.1.1.1 Principle

The acetic acid induced writhing method is an analgesic behavioral observation and evaluation method that demonstrates a noxious stimulation in mice. The test consists of injecting 0.7% acetic acid solution intraperitoneally and then observing the animal for specific contraction of body referred as writhing. A comparison of writhing is made between positive control (diclofenac Na), control and test sample given orally 30 minutes prior to acetic acid injection. If the sample possesses analgesic activity, the animal that received the sample will give lower number of writhing than the control group, i.e. the sample having analgesic activity will inhibit writhing. Diclofenac Na is used as reference standard drug. It has analgesic, antipyretic and anti-inflammatory actions at different steps of pharmacological investigation with mild adverse effects. So the drug is used widely.

4.2.1.1.2 Study Design

Experimental animals were randomly selected and divided into three groups denoted as group-I, group-II and group –III. Group-I received normal water with 1% Tween-80 as control. Group-II received diclofenac Na as positive control. Group-III received test extracts at dose of 250 mg/kg

@Khulna University

body weight (bw.). Dose dependent experiment was conducted for *C.decandra* extract and *H. fomes* extracts at 50, 100, 250 and 500mg/kg body weight (bw.) (Ahmed et al., 2004).

Table 4.2.1: Experiment profile to assess the effect of different edible mangrove fruits extract on acetic acid induced writhing of mice

Animal group	Treatment	Dose (mg/kg-body wt)	Route of Administration
I (Control)	1% tween-80 Solution in water	10 mg/kg	Oral
II (Positive control)	Diclofenac Na	25 mg/kg	Intraperitoneal
III (Test group-I)	*A. corniculatum Extract*	250 mg/kg	Oral
III (Test group-II)	*A.officinalis Extract*	250 mg/kg	Oral
III (Test group- III)	*B. gymnorrhiza Extract*	250 mg/kg	Oral
III (Test group-IV)	*C. decandra Extract*	500 mg/kg	Oral
III (Test group-V)	*C. decandra Extract*	250 mg/kg	Oral
III (Test group-VI)	*C. decandra Extract*	100 mg/kg	Oral
III (Test group-VII)	*C. decandra Extract*	50 mg/kg	Oral
III (Test group-VIII)	*H. fomes Extract*	500 mg/kg	Oral
III (Test group- IX)	*H. fomes Extract*	250 mg/kg	Oral
III (Test group-X)	*H. fomes Extract*	100 mg/kg	Oral
III (Test group-XI)	*H. fomes Extract*	50 mg/kg	Oral
III (Test group-XII)	*N. fruticans Extract*	250 mg/kg	Oral
III (Test group-X III)	*P. peludosa Extract*	250 mg/kg	Oral
III (Test group-XIV)	*S. caseolaris Extract*	250 mg/kg	Oral

@Khulna University

III (Test group-XV)	*S. globosus Extract*	250 mg/kg	Oral
III (Test group-XVI)	*X. mekongenesuis Extract*	250 mg/kg	Oral

4.2.1.1.3 Preparation of sample suspension

4.2.1.1.3.1 Dose of extract -500 mg/kg

To prepare suspension of the test extracts at the dose of 500 mg/kg body weight, 105 mg of extract was measured in a vial. 5-6 drops of tween-80 was added and 2.1 mL of distilled water was taken in that vital to make concentration 50 mg/mL. Then it was properly mixed by shaking in vortex. As the dose was 500 mg/kg each mouse was administered $(0.01\times body\ weight)$ mL solution.

KHULNA UNIVERSITY Central Library

4.2.1.1.3.2 Dose of extract -250 mg/kg

To prepare suspension of text extracts at the dose of 250 mg/kg body weight, 52.5mg (0.0525g) of extract was measured in a vial. 5-6 drops of tween-80 was added and then 2.1 mL of distilled water was taken in that vital to make concentration 25 mg/mL. Then it was properly mixed by shaking in vortex. As the dose was 250 mg/kg each mouse was administered (0.01× body weight) mL in solution.

4.2.1.1.3.3 Dose of extract -100 mg/kg

To prepare suspension of test extracts at the dose of 100 mg/kg body weight, 21 mg (0.021g) of extract was measured in a vial. Then 5-6 drops of tween-80 was added and finally 2.1 mL of distilled water was taken in that vital to make concentration 10 mg/mL. Then it was properly mixed by shaking in vortex. As the dose was 100 mg/kg each mouse was administered (0.01× body weight) mL in solution.

4.2.1.1.3.4 Dose of extract -50 mg/kg

@Khulna University

To prepare suspension of test extracts at the dose of 50 mg/kg body weight, 21 mg (0.021g) of extract was measured in a vial. Then 5-6 drops of tween-80 were added and Finally 8.4 mL of distilled water was taken in the vital to make concentration 5 mg/mL. Then it was properly mixed by shaking in vortex. As the dose was 50 mg/kg each mouse was administered (0.01× body weight) mL in solution.

4.2.1.1.3.5 Preparation of standard solution: Diclofenac Na injection (75 mg in 2 mL of a single ampoule) was brought and used to prepare positive control. This 2 mL of diclofenac Na was made 30 mL volume by adding 28 mL distilled water to get the desired concentration of 2.5 mg/mL. As the dose of positive control is 25 mg/kg, the mice of positive control group were administered (0.01× body weight)mL in solution.

4.2.1.1.3.6 Preparation of 0.7% acetic acid: For preparation of 0.7% acetic acid solution, 0.35 mL glacial acetic acid was mixed with 49.65 mL of distilled water. Each mouse was injected (0.01× body weight) mL of 0.7% acetic acid solution.

4.2.1.1.4 Methodologies

Test samples, positive and negative control solution were given orally by means of a feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing was induced by intraperitoneally administration of 0.7% acetic acid solution to each of the animals of a group. After an interval of 5 minutes, which was given for adsorption of acetic acid, number of squirms of each mouse of all groups was observed carefully to count the number of writhing that they had made in 15 minutes. The animal do not always perform full writhing, because sometimes the animals begin to produce writhing but they do not complete it. This incomplete writhing was taken as half writhing, so two half-writhing were taken as one full writhing (Prabhu et al., 2011 and Emran et al., 2012).

@Khulna University

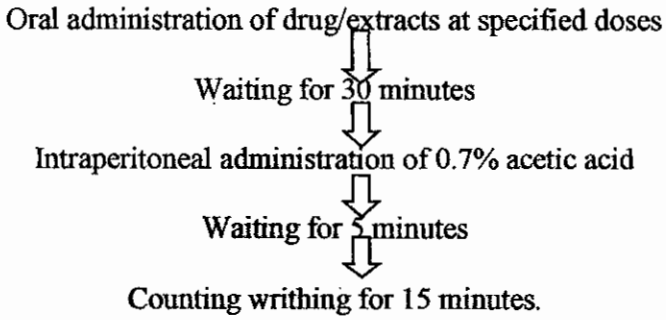


Fig 4.2.2: Schematic representation of acetic acid induced writhing of mice for investigation of analgesic activity..

4.2.1.2 Study of analgesic activity by Hot plate method

Analgesic activity of the different edible mangrove fruits extract was tested using the model described by Eddy and Leimbach (1953) with slide modification.

4.2.1.2.1 Principle

The hot plate in one of the most common tests of nociception that is based on a phasic stimulus of high intensity. Pain induced by thermal stimulus in hot plate is specific for centrally mediated nonciception. The paws of mice are very sensitive to heat at temperatures which are not damaging for the skin. The animals are placed on the hot plate and after a certain times the temperature becomes unbearable for them. Then they start to show physical responses like jumping from the plate, withdrawal of the paws from the plate, licking of the paws etc. The hot plate, which is commercially available, consists of an electrically heated surface is used. This can be a copper plate or a heated glass surface. The temperature is controlled for 55^0 to 56^0C. the animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stopwatch (Venkaraman et al,. 2005).

@Khulna University

4.2.1.2.2 Study design

Experimental animals were randomly selected having either sex and divided into three groups denoted as group-I, group-II and group-III. Group-I received normal water with tween-80 as blank or control and group-II received morphine as positive control at 10 mg/kg bd. (Mulla et al., 2010 and Tammu et al., 2011). Group-III received test extracts at 250 mg/kg dose where 5 mice were selected for each extract. Dose dependent experiment was conducted for *C.decandra* and *H. fomes* extracts at 50, 100, 250 and 500mg/kg body weight (bw.).

Table 4.2.2: Experiment profile to assess the effect of different edible mangrove fruits extracts in the Sundarbans on Hot Plate Method.

Animal group	Treatment	Dose (mg/kg-body wt)	Route of Administration
I (Control)	1% tween-80 Solution in water		Oral
II (Positive control)	Morphine	10 mg/kg	Intraperitoneal
III (Test group-I)	*A. corniculatum Extract*	250 mg/kg	Oral
III (Test group-II)	*A. officinalis Extract*	250 mg/kg	Oral
III (Test group- III)	*B. gymnorrhiza Extract*	250 mg/kg	Oral
III (Test group-IV)	*C. decandra Extract*	500 mg/kg	Oral
III (Test group-V)	*C. decandra Extract*	250 mg/kg	Oral
III (Test group-VI)	*C. decandra Extract*	100 mg/kg	Oral
III (Test group-VII)	*C. decandra Extract*	50 mg/kg	Oral
III (Test group-VIII)	*H. fomes Extract*	500 mg/kg	Oral

@Khulna University

III (Test group- IX)	*H. fomes Extract*	250 mg/kg	Oral
III (Test group-X)	*H. fomes Extract*	100 mg/kg	Oral
III (Test group-XI)	*H. fomes Extract*	50 mg/kg	Oral
III (Test group-XII)	*N. fruticans Extract*	250 mg/kg	Oral
III (Test group-X III)	*P. peludosa Extract*	250 mg/kg	Oral
III (Test group-XIV)	*S. caseolaris Extract*	250 mg/kg	Oral
III (Test group-XV)	*S. globosus Extract*	250 mg/kg	Oral
III (Test group-XVI)	*X. mekongenesuis Extract*	250 mg/kg	Oral

4.2.1.2.3 Inhibition percentage calculation

The result of acetic acid induced writhing test is shown in table 5.1 and 5.2. The % inhibition of writhing was calculated by using the following formula-

$$\% \text{ inhibition} = \frac{\text{Mean writhing of control group} - \text{Mean writhing of treated group}}{\text{Mean writhing of control group}} \times 100$$

4.2.1.2.4 Preparation of sample suspension

4.2.1.2.4.1 Dose of extract -500 mg/kg

To prepare suspension of the extracts at the dose of 500mg/kg body weight, 105 mg of extract was measured by electric weight machine and taken in a vial. 5-6 drops of tween-80 and 2.1 mL of distilled water were taken in the vial to make concentration 50 mg/ml. Then it was properly mixed by shaking in vortex as the dose was 500mg/kg each mouse was administered (0.01× body weight) mL solution.

4.2.1.2.4.2 Dose of extract- 250 mg/kg

To prepare suspension of the test extracts at the dose of 250 mg/kg body weight, 52.5 mg (0.0525 g) of extract was measured by electric weight machine and taken in a vial. Then 5-6

@Khulna University

drops of tween-80 and 2.1 mL of distilled water were taken in the vial to make concentration 25 mg/mL. Then it was properly mixed by shaking in vortex. As the dose was 250 mg/kg each mouse was administered (0.01 × body weight) mL solution.

3.2.1.2.4.3 Dose of extract- 100 mg/kg

To prepare suspension of the test extracts at the dose of 100 mg/kg body weight, 21 mg of extract was measured by electric weight machine and taken in a vial. 5-6 drops of tween-80 and 2.1 mL of distilled water were taken in the vial to make concentration 25 mg/mL. Then it was properly mixed by shaking in vortex. As the dose was 100 mg/kg each mouse was administered (0.01 × body weight) mL solution.

3.2.1.2.4.4 Dose of extract-50 mg/kg

To prepare suspension of test extracts at the dose of 50 mg/kg body weight, 21 mg (0.021g) of extract was measured in a vial. Then 5-6 drops of tween-80 were added and Finally 8.4 mL of distilled water was taken in the vital to make concentration 5 mg/mL. Then it was properly mixed by shaking in vortex. As the dose was 50 mg/kg each mouse was administered (0.01× body weight) mL in solution.

4.2.1.2.4.5 Preparation of standard solution

Morphine injection (15 mg in 1 mL of a single ampoule) was bought and used to prepare positive control. From this 0.5 mL morphine solution was taken in a vial and 7 mL distilled water was added to the vial to make volume 7.5 mL to get the desire concentration of 10 mg/kg. As the dose of positive control was 10 mg/kg, the mice of positive control group (group-2) were administered (0.01 × body weight) mL solution.

@Khulna University

4.2.1.2.4.5 Preparation of control solution

Few drops of tween-80 were mixed with 10 mL of distilled water which was used as negative control. As the dose of negative control is 10 mg/kg, the mice of negative control group were administered (0.01 × body weight) mL solution.

4.2.1.2.5 Methodologies

In hot plate method, prior to the experiment, the hot plate was set for a temperature 55 ± 1 ° C. Foods and water were removed 12 hours before of the experiment. The animals were grouped randomly having either sex and kept in individual cage. The body weight of each animal was recorded and treated with respective treatment. The animal was placed on the hot plate and the time for licking paws or jumping in hot plate whichever appeared first (Kumar et al., 2012) was recorded by using stop watch as a response at 0, 30, 60, 90, 120, 180 and 240 min (Biswas et al., 2011) after the administration of the respective drugs. The cut off time for the reaction was 20 seconds (Sudarwo, 2005, Nikajoo et al., 2009 and Kulkarni et al., 1999). The cut of time is an important factor. If the animal that did not response within 20 second, it would be withdrawn from the hot plate because the paw tissue is very sensitive to heat and excess heat may cause tissue damage.

4.2.3 Anthelmintic Test

4.2.3.1 Study of Anthelmintic Activity by *in-vitro* method

Anthelmintic activity of different mangrove fruits extract and different concentrations was performed using the Ajaiyeoba et al. (2001) with minor modification.

@Khulna University

4.2.3.2 Principle

In-vitro anthelmintic test is a common test to evaluate anthelmintics where all procedures are carried out in petridishes. The parasites were washed and placed in petridishes with sample and standard drug separately in different concentrations. After a period of time the parasites will not show any movement without any external stimulation which indicate that the worm has been paralyzed. When the parasites will not move despite of external stimulation is indicated as death. Comparing the paralysis and death time of test sample with standard drug the efficacy of the sample is determined.

4.2.3.3 Experimental parasites:

Live parasites *Paramphistomum cervi* (Trematoda) were collected from freshly slaughtered cattle at local abattoirs. After cleaning the parasites, they were stored in 0.9% phosphate-buffered saline (PBS) of p^H 7.4, prepared with 8.01g NaCl, 0.20 KCl, 1.78 g Na_2HPO_4 and 0.27 g KH_2PO_4 in 1 liter of distilled water at 37 ± 1°C (Ajaiyeoba et al., 2001).

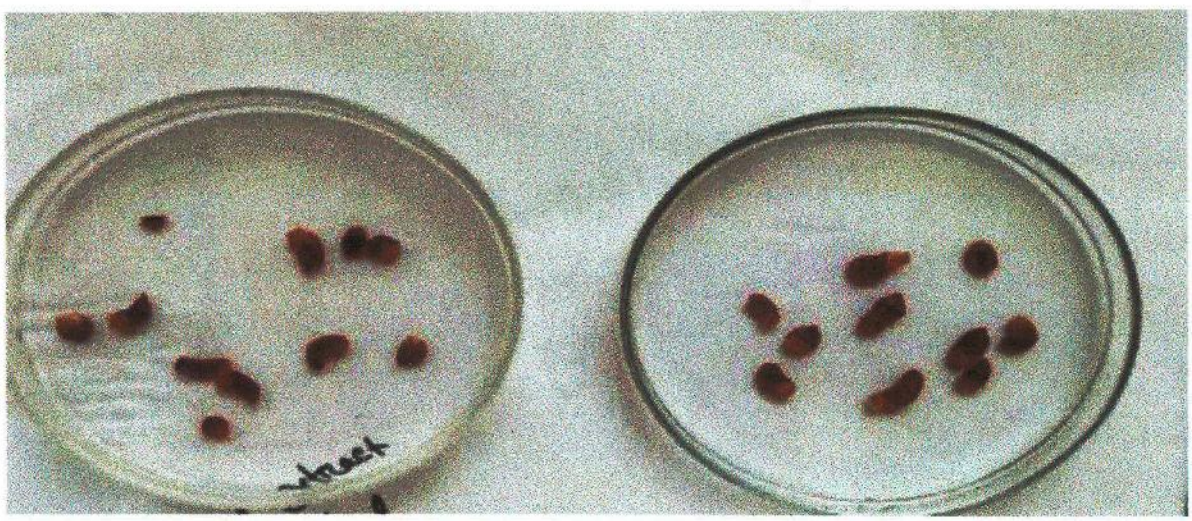


Figure 4.2.3: *Paramphistomum cervi* parasites were collected from Gollamari slaughter house.

@Khulna University

4.2.3.4 Study design

Anthelmintic activity of the raw honey was investigated on live parasite *P. cervi* of cattle. The experimental parasites were randomly selected and divided into three groups denoted as group-I, group-2 and group-III consisting of ten parasites for group-I, ten parasites for group-II and two hundred parasites for group-III. Group I treated with control (0.1% tween-80 in 10 mL PBS). Group II treated with standard drug (Albendazole, 15 mg/mL) and group III treated with test sample (Edible mangrove fruits) at doses of 0.1, 0.2, 0.5, 1, 5 and 10 mg/mL. Ten parasites were placed in each petridish and observed. The time of paralysis was recorded when no movement was observed unless shaken vigorously or dipped in warm water (50 °C) or subjected to external stimuli. The death time was recorded after evaluating that the parasites did not move when shaken vigorously or dipped in warm water (50°C) or subjected to external stimuli. Anthelmentic activity is expressed as the time required for paralysis and death of parasites as compared to control (Sharma et al., 1982).

Table 4.2.3: Experiment profile to assess the effect of the edible mangrove fruits on *Paramphistomum cervi* in anthelmentic test.

Parasite group	Treatment	Dose (mg/mL)	Test performed in
I (Control)	1% tween-80 solution in PBS		Petridish
II (Positive control)	Albebdazole	15 mg/mL	Petridish
III (Test group-I)	*A. corniculatum* Extract	10 mg/mL	Petridish

@Khulna University

III (Test group-II)	*A. corniculatum* Extract	5 mg/mL	Petridish
III (Test group-III)	*A. corniculatum* Extract	1 mg/mL	Petridish
III (Test group-IV)	*A. corniculatum* Extract	0.5 mg/mL	Petridish
III (Test group-V)	*A. corniculatum* Extract	0.2 mg/mL	Petridish
III (Test group-VI)	*A. corniculatum* Extract	0.1 mg/mL	Petridish
III (Test group-VII)	*A. officinalis* Extract	10 mg/mL	Petridish
III (Test group-VIII)	*B. gymnorrhiza* Extract	10 mg/mL	Petridish
III (Test group-IX)	*C. decandra* Extract	10 mg/mL	Petridish
III (Test group-X)	*H. fomes* Extract	10 mg/mL	Petridish
III (Test group-XI)	*N. fruticans* Extract	10 mg/mL	Petridish
III (Test group-XII)	*N. fruticans* Extract	5 mg/mL	Petridish
III (Test group-XIII)	*N. fruticans* Extract	1 mg/mL	Petridish
III (Test group-XIV)	*P .peludosa* Extract	10 mg/mL	Petridish
III (Test group-XV)	*P. peludosa* Extract	5 mg/mL	Petridish
III (Test group-XVI)	*P. peludosa* Extract	1 mg/mL	Petridish
III (Test group-XVII)	*S. apetala* Extract	10 mg/mL	Petridish

@Khulna University

III (Test group-XVIII)	*S. caseolaris* Extract	10 mg/mL	Petridish
III (Test group-XIX)	*S. globosus* Extract	10 mg/mL	Petridish
III (Test group-XX)	*X. mekongenesuis* Extract	10 mg/mL	Petridish

4.2.3.5 Preparation of sample solution:

4.2.3.5.1 Dose of extract -10 mg/mL

To prepare suspension of test extracts at the dose of 10 mg/mL, 200 mg (0.2g) of each extract was measured in a vial. Then 3-4 drops of tween-80 was added and as a suspending agent and final volume was made to 20 ml by adding PBS in the vials.

4.2.3.5.2 Dose of extract -5 mg/mL

To prepare suspension of test extracts at the dose of 5 mg/mL, 100 mg (0.1g) of each extract was measured in a vial. Then 3-4 drops of tween-80 was added and as a suspending agent and final volume was made to 20 ml by adding PBS in the vials.

4.2.3.5.3 Dose of extract -1 mg/mL

To prepare suspension of test extracts at the dose of 1 mg/mL, 20 mg (0.02g) of each extract was measured in a vial. Then 3-4 drops of tween-80 was added and as a suspending agent and final volume was made to 20 ml by adding PBS in the vials.

4.2.3.5.4 Dose of extract -0.5 mg/mL

To prepare suspension of test extracts at the dose of 0.5 mg/mL, 10 mg (0.01g) of each extract was measured in a vial. Then 3-4 drops of tween-80 was added and as a suspending agent and final volume was made to 20 ml by adding PBS in the vials.

@Khulna University

4.2.3.5.5 Dose of extract -0.2 mg/mL

To prepare suspension of test extracts at the dose of 0.2 mg/mL, 4 mg (0.004g) of each extract was measured in a vial. Then 3-4 drops of tween-80 was added and as a suspending agent and final volume was made to 20 ml by adding PBS in the vials.

4.2.3.5.6 Dose of extract -0.1 mg/mL

To prepare suspension of test extracts at the dose of 10 mg/mL, 2 mg (0.002g) of each extract was measured in a vial. Then 3-4 drops of tween-80 was added and as a suspending agent and final volume was made to 20 ml by adding PBS in the vials.

4.2.3.6: Preparation of Standard solution

For the preparation of standard drug solution at concentrations of 15 mg/mL; 300 mg of albendazole powder was taken in vial and triturated with 3-4 drops of tween 80 as a suspending agent and final volume was made to 20 mL by adding PBS in the vial.

4.2.3.7 Methodology

- 20 mL of control, standard and sample were taken in different petridishes;
- 10 parasites were taken in each petridishes;
- Time take for paralysis of each parasite was recorded;
- Time taken for death of each parasite was recorded by stop watch.

4.3: Statistical Analysis: Results obtained in the experiment were expressed as mean ± SD. The data were analyzed statistically using the Statistical Package for Social Sciences (SPSS) database, version 16.0, submitting the data to a simple one factor Analysis of Variance (ANOVA). ANOVA was performed and mean separation was done by SPSS database ($p<0.05$).

@Khulna University

CHAPTER FIVE: RESULTS

5.1: Analgesic activity

5.1.1: Writhing inhibition activity

In acetic acid induced writhing inhibition test, each of the edible mangrove fruits extract [EtOH:MeOH (1:1)] when given orally (250 mg/kg b.w.), significantly ($p < 0.05$) inhibited the frequency of acetic acid induced abdominal constrictions in mice (Fig. 5.1). Among them, *C. decandra* showed the highest activity (44.5%) followed by *H. fomes* (38.1%), *A. corniculatum* (36.4%), *P. paludosa* (34.8%), *X. mekongenesis.*(32.6%) *B. gymnorrhiza* (31.4%), *S. caseolaris* (30.1%), *A. officinalis* (27.5), *S. globosus* (26.3%) and *N. fruticans* (25%), respectively at 250 mg/kg body weight whereas positive control showed 51.3% of inhibition at 25 mg/kg b.w. Dose-dependent inhibitory effect of the topmost two potential fruits (*C. decandra* and *H. fomes)* was conducted for testing dose dependent activity (Figure 5.2), where *C. decandra* extract showed highly potential writhing inhibition activity (63.1%) at 500 mg/kg b.w. in mice. All extracts significantly *($p < 0.05$)* inhibited the writhing of mice.

5.1.2: Response time increasing activity

In hot plate test, the response time (paw licking /jumping response) was highest at 90 min for all treatments. In 0 min all groups showed almost same response time as control group. For all fruit extracts [EtOH:MeOH (1:1)], at 250 mg/kg, *C. decandra* showed the highest response time (17.4 sec.) followed by *H. fomes* (14.2 sec.) *A. corniculatum* (13.6 sec.), *B. gymnorrhiza* (13.6 sec.), *P. paludosa* (12.8 sec.), *A. officinalis* (12.6 sec.), *S. globosus* (12.6 sec.), *X. mekongenesis* (12.2 sec.), *S. caseolaris* (12.0 sec.) and *N. fruticans* (11.6 sec.) respectively at 90 min whereas morphine, a positive control showed 18.2 sec. (Fig. 5.3). The response time for all groups started

@Khulna University

to decline from 90 min. All treated group significantly *($p < 0.05$)* increased the response time compared to control group at 30, 60, 90, 120, 150 and 180 min. Since *C. decandra* showed the highest reaction time followed by *H. fomes*, their dose-dependent effects were shown in Figure 5.4 and 5.5 at 50, 100, 250 and 500mg/kg body weight.

5.2: Athelmentic activity

5.2.1: In vitro anthelmintic activity at 10 mg/ml PBS solution

The anthelmintic activity of edible mangrove fruits extract [EtOH:MeOH (1:1)] was evaluated by observing both the paralysis time and the death time for *Paramphistomum cervi*. At a dose of 10 mg/ml, all fruit extracts significantly showed anthelmintic activity (Figure 5.6). Among them, the *A. corniculatum* extract showed the strongest anthelmintic activity when *B. gymnorrhiza* extract showed the minimum. The paralysis time of the parasite for 10 mg/ml in case of *A. corniculatum*, *A. officinalis*, *B. gymnorrhiza*, *C. decandra*, *H. fomes*, *N. fruticans*, *P. paludosa*, *S. apetala*, *S. caseolaris*, *S. globosus* and *X. mekongenesis* t were 26.2, 194.9, 244.8, 185.1, 246.6, 167, 143.6, 205.9, 210, 163.2, 162.7 minutes, respectively (Figure 5.6). The paralysis time for albendazole (15 mg/mL), a standard drug was 78.4 minutes and the paralysis time for control was 316.8 minutes. The death time of the parasites for 10 mg/ml in case of *A. corniculatum* extract, *A. officinalis*, *B. gymnorrhiza*, *C. decandra*, *H. fomes*, *N. fruticans*, *P. paludosa*, *S. apetala*, *S. caseolaris*, *S. globosus* and *X. mekongenesis* were 46.2, 253.7, 325.9, 257.6, 314.4, 221.3, 200.6, 242.8, 282, 219.6, 224.2 minutes, respectively. The death time for albendazole treated group and control group was 106.3 and 371.29 minute, respectively.

@Khulna University

5.2.2: Dose dependent anthelmintic activity

The three mangrove edible fruits are *A. corniculatum*, *N. fruticans* and *P. paludosa* which have the strongest anthelmintic activity. These showed dose dependent anthelmintic activity is shown in Figure 5.7. The paralysis time of the parasite at 0.05, 0.1, 0.2, 0.5, 1, 5, and 10 mg/mL for *A. corniculatum* extract [EtOH:MeOH (1:1)] was 248.1, 173.1, 120.6, 55.5, 38.9, 32.4 and 26.2 minutes, respectively and the death time of the parasites was 297.6, 232.7, 185.9, 86, 57.1, 54.2 and 46.2, respectively. The second potent fruit is *P. paludosa* followed by *N. fruticans* , the paralysis time of the parasites at 1, 5, and 10 mg/mL for *P. paludosa* was, 236.1, 201.5 and 143.6 minutes and for *N. fruticans* was 259.4, 233.8 and 167 respectively. The times until death was 292.6, 257.7 and 200.6 minutes for *P. paludosa* and for *N. fruticans* was 302.1, 272.7 and 221.3 at 1, 5, and 10 mg/mL respectively. The paralysis time for albendazole (15mg/mL), a standard drug was 78.4 minutes and the paralysis time for control was 316.77 minutes. All data were significant when compared to control, and different letters indicate significant differences when compared to positive control at $p < 0.05$.

IC_{50} value of anthelmintic activity for edible mangrove fruits extract was examined (Fig 5.8) and *A. corniculatum* extract showed the strongest action against *Paramphistomum cervi*. All of the fruits extract was significantly ($p < 0.05$) decreased the paralysis time and death time compared to control and different letters indicate significant differences at $p < 0.05$.

5.2.3: Anthelmintic activity of different fractions

In anthelmintic activity test, since *A. corniculatum* showed the most potent effect, its different solvent fractions were undertaken to test action against *Paramphistomum cervi* is shown in Figure 5.9. Among those fractions, methanol fraction of *A. corniculatum* showed the highest

@Khulna University

activity (140.3 min required for paralysis) followed by ethanol fraction (158.8 min), diethyl ether fraction (172.5 min), n-hexane fraction (188.3 min) and chloroform fraction (193.6 min required for paralysis) at 0.1 mg/mL. The death time of the parasite at 0.1 mg/mL for methanol extract, ethanol fraction, diethyl ether fraction, n-hexane fraction, and chloroform fraction of *A. corniculatum* was 194.7, 230.7, 246.9, 259.2 and 254 minutes, respectively. The paralysis time and death time for albendazole (15mg/mL), a standard drug was 78.4 and 106.3 minutes and for control was 316.77 and 371.29 minutes. Furthermore, dose dependent anthelmintic activity was carried out for methanol fraction and ethanol fraction of *A. corniculatum* (Figure 5.10). At 0.05, 0.1, 0.2, 0.4 mg/mL, all data were significant when compared to control, and different letters indicate significant differences at $p < 0.05$.

5.3.1 Correlation between the total amount phenolics and analgesic activity in acetic acid induced writhing test

Fig. 5.11 showed the linear correlation between the total amount phenolics and analgesic activity. The coefficient of determination is such that $0 \leq r^2 \leq 1$, represent the percent of the data is the closest to the line of best fit. **Fig. 5.11** indicated the moderate correlation (r^2=0.761) between the total amount phenolics and analgesic activity.

5.3.2 Correlation between the total amount phenolics and analgesic activity on hot plate method

Linear correlation between the total amount phenolics and analgesic activity of the mangrove fruits sample is shown in **Fig. 5.12.** The figure also indicated moderate correlation (r^2=0.701) between the total amount phenolics and analgesic activity.

@Khulna University

5.3.3 Correlation between the total amount phenolics and anthelmintic activity

Fig. 5.13 showed the linear correlation between the total amount phenolics and anthelmintic activity. The figure showed the coefficient of determination (r^2=0.131), indicated the weak correlation between the total amount phenolics and anthelmintic activity

@Khulna University

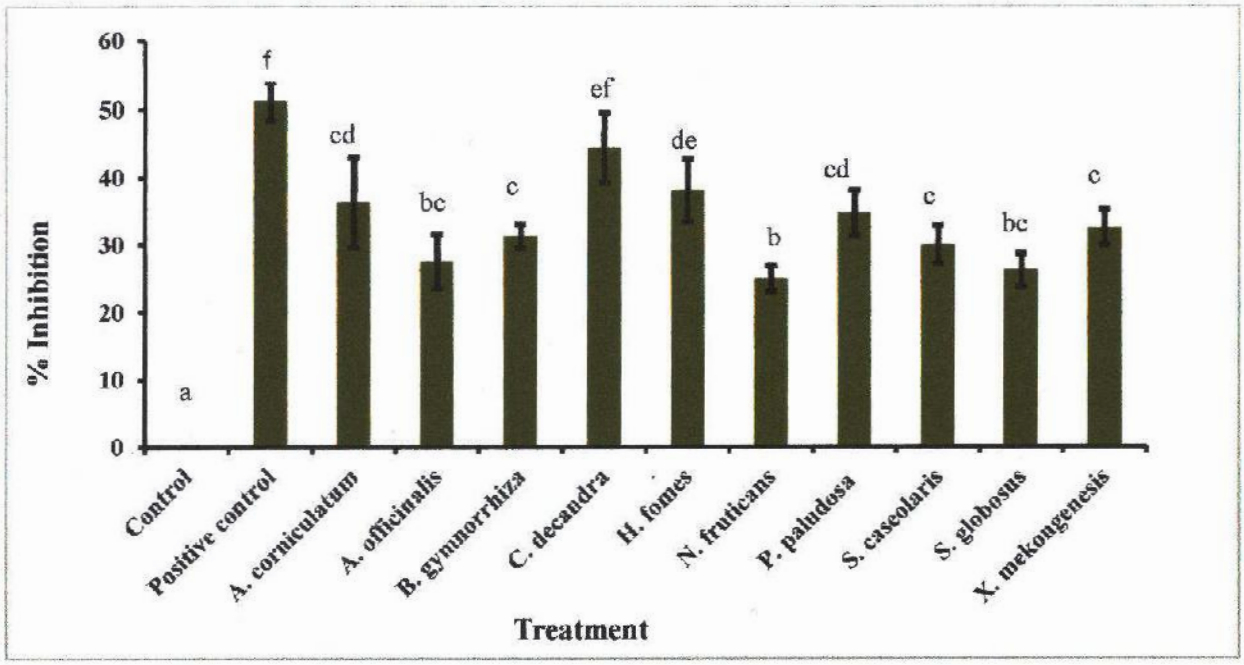


Figure 5.1: Inhibition of writhing (%) in acetic acid induced writhing test of edible mangrove fruits extract [EtOH:MeOH (1:1)] at 250 mg/kg b.w. in mice. All data were significant when compared to control, and different letters indicate significant differences [PC, 25 mg diclofenac sodium/kg body weight] at $p < 0.05$.

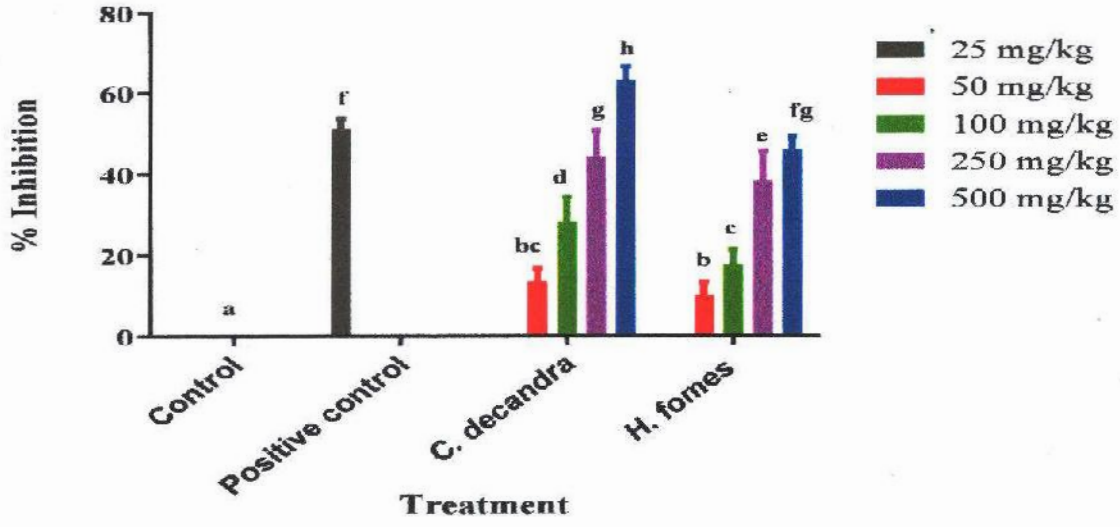


Figure 5.2: Dose-dependent inhibitory effects of *C. decandra* and *H. fomes* at different concentrations on acetic acid induced writhing in mice.

@Khulna University

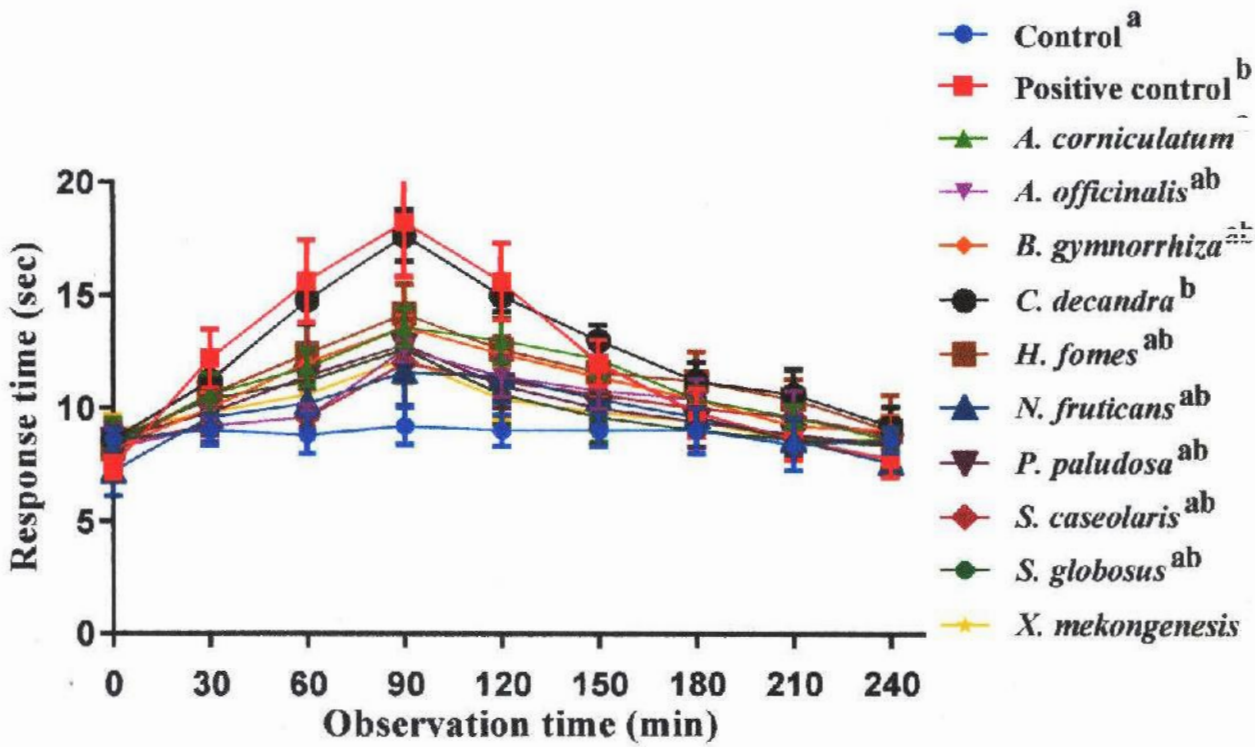


Figure 5.3: Response time increasing of mice with different edible mangrove fruits extract [EtOH:MeOH (1:1)] on hot plate method. Treatment at 250 mg/kg body weight and (positive control, morphine, 10 mg/kg body weight), all data were significant ($p < 0.05$) at 60, 90 and 120 min when compared to control group.

@Khulna University

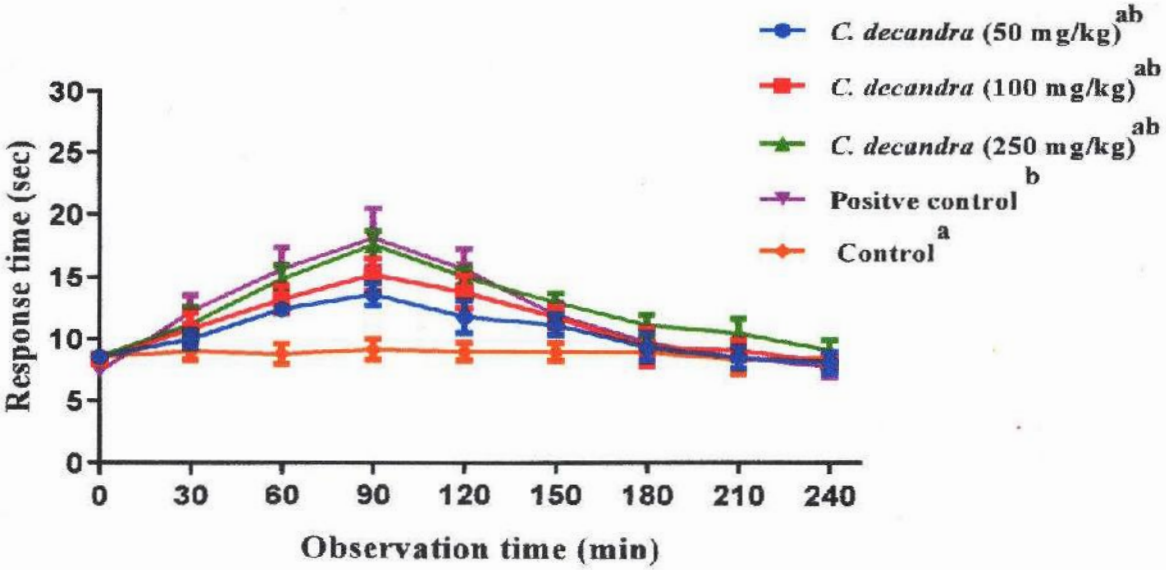


Figure 5.4: Dose-dependent effects of *C. decandra* extract [EtOH:MeOH (1:1)] on response time of mice. All data were significant ($p < 0.05$) at 60, 90,120 and 150 min when compared to control group.

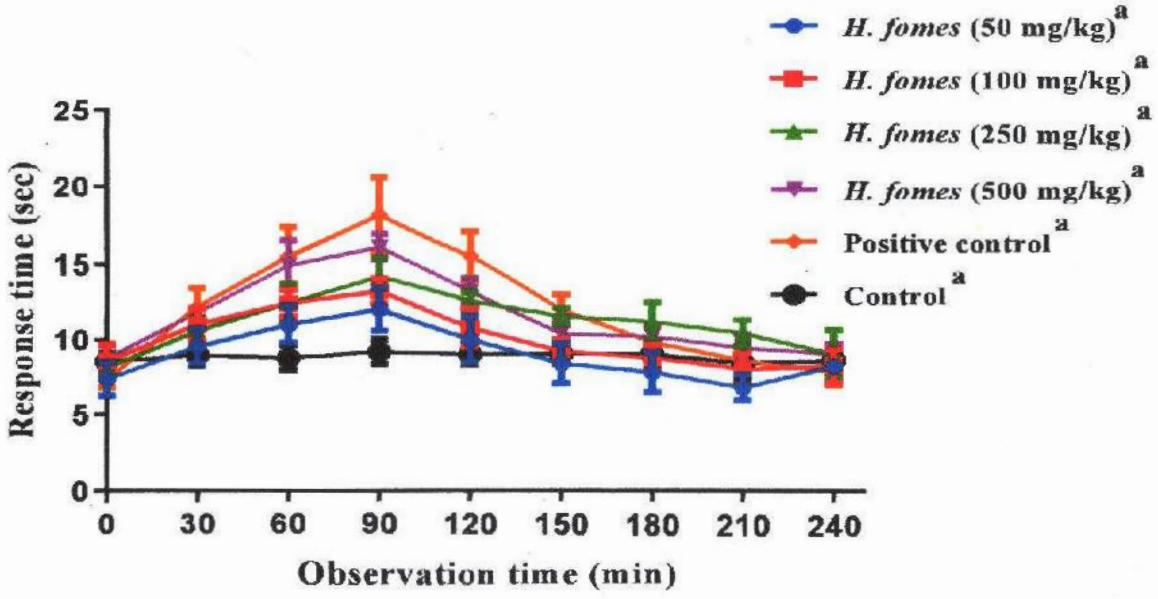


Figure 5.5: Dose-dependent effects of *H. fomes* extract [EtOH:MeOH (1:1)] on response time of mice. All data were significant ($p < 0.05$) at 60, 90 and 120 min when compared to control group.

@Khulna University

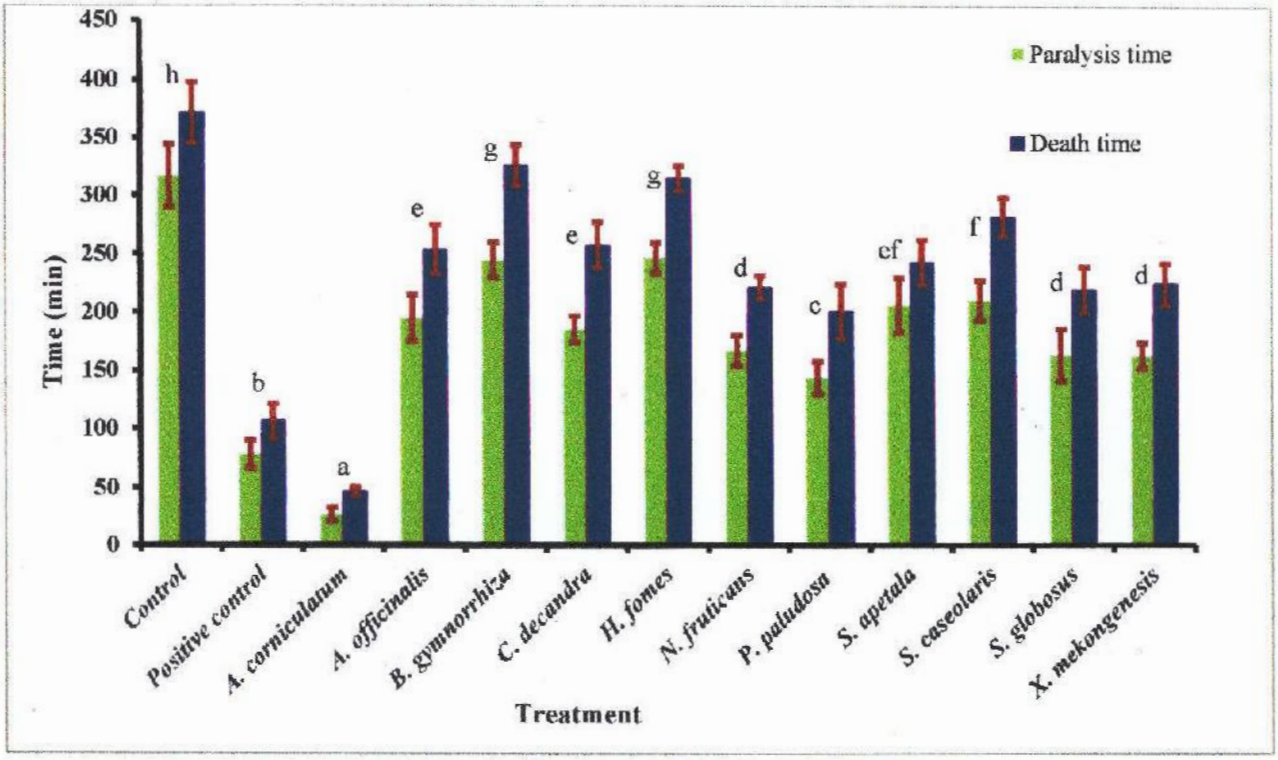


Figure 5.6: Anthelmintic activity of edible mangrove fruits extract [EtOH:MeOH (1:1)] at 10 mg/ml. Different letters indicate significant differences at $p < 0.05$ and albendazole was used as positive control at 15 mg/ml.

KHULNA UNIVERSITY Central Library KHULNA.

@Khulna University

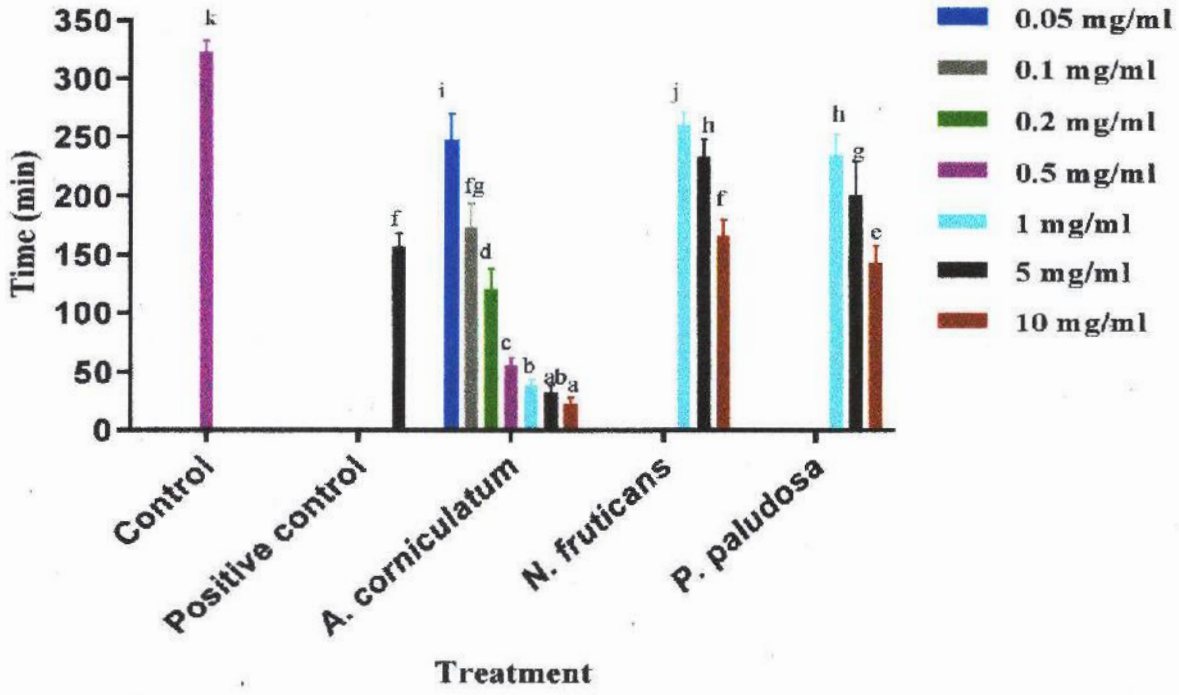


Figure 5.7: Dose-dependent anthelmintic effects of *A. corniculatum, N. fruticans and P. peludosa* at different concentration where positive control [PC, 15 mg albendazole/ml phosphate buffer saline]. All data were significant when compared to control group at $p < 0.05$.

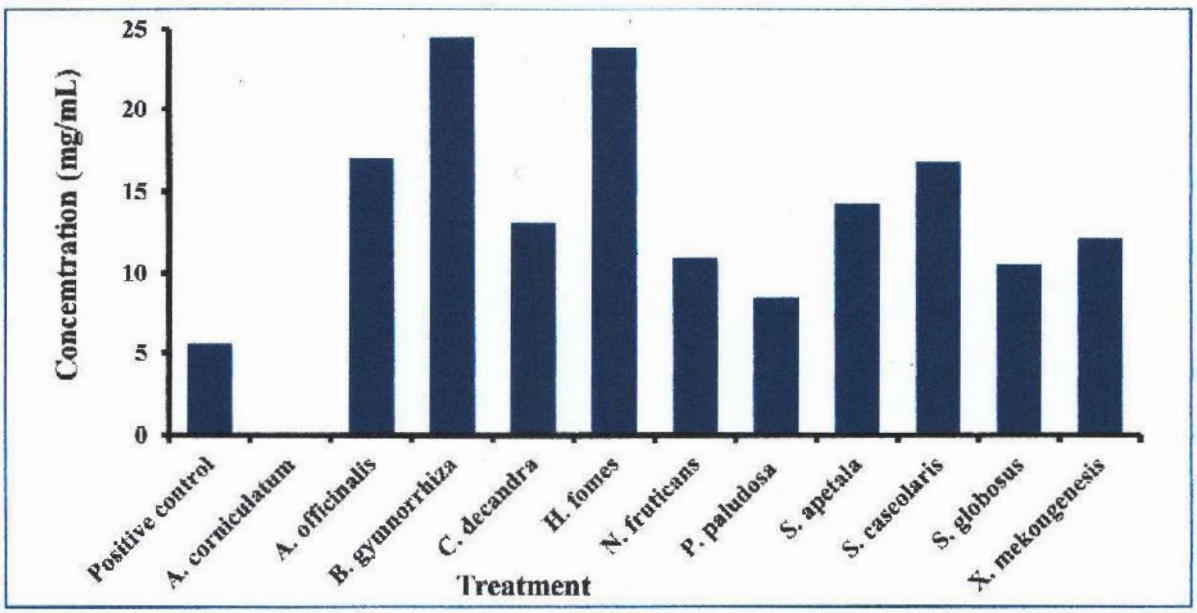


Figure 5.8: IC_{50} value of anthelmintic activity of edible mangrove fruits extract. All data are significantly difference at 5% level when compared to positive control.

@Khulna University

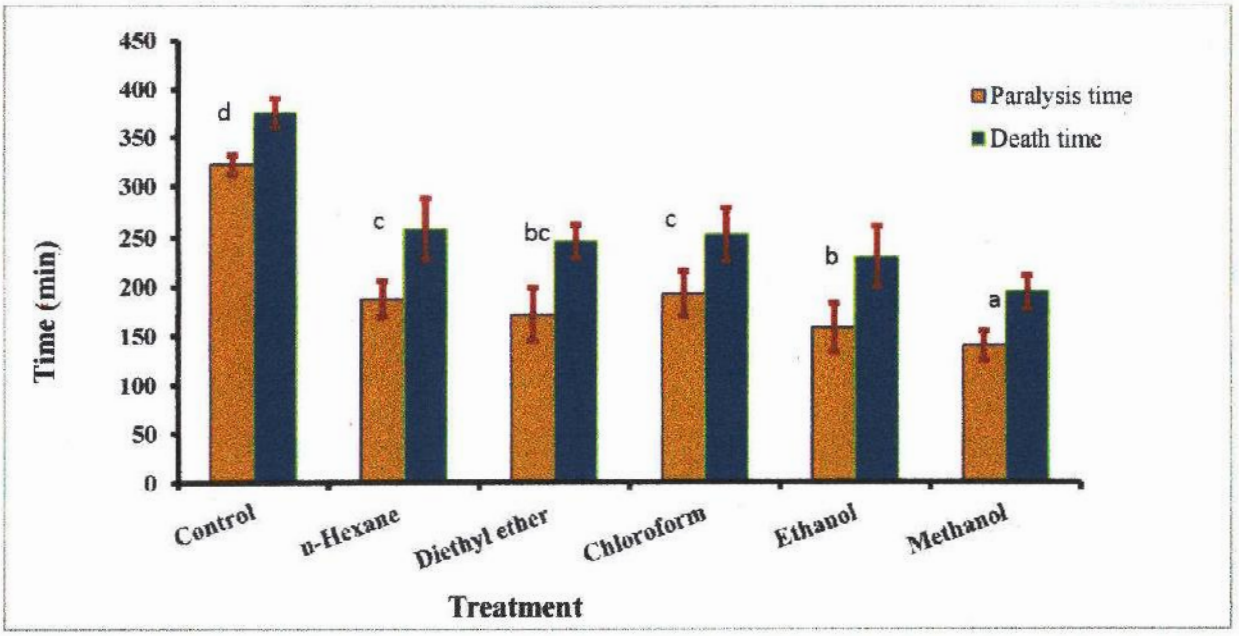


Figure 5.9: Anthelmintic activity of different solvent [n-hexane, diethyl ether, chloroform, ethanol and methanol] fractions of A. corniculatum at 0.1mg/ml. Here different letters indicate the significant difference at $p < 0.05$.

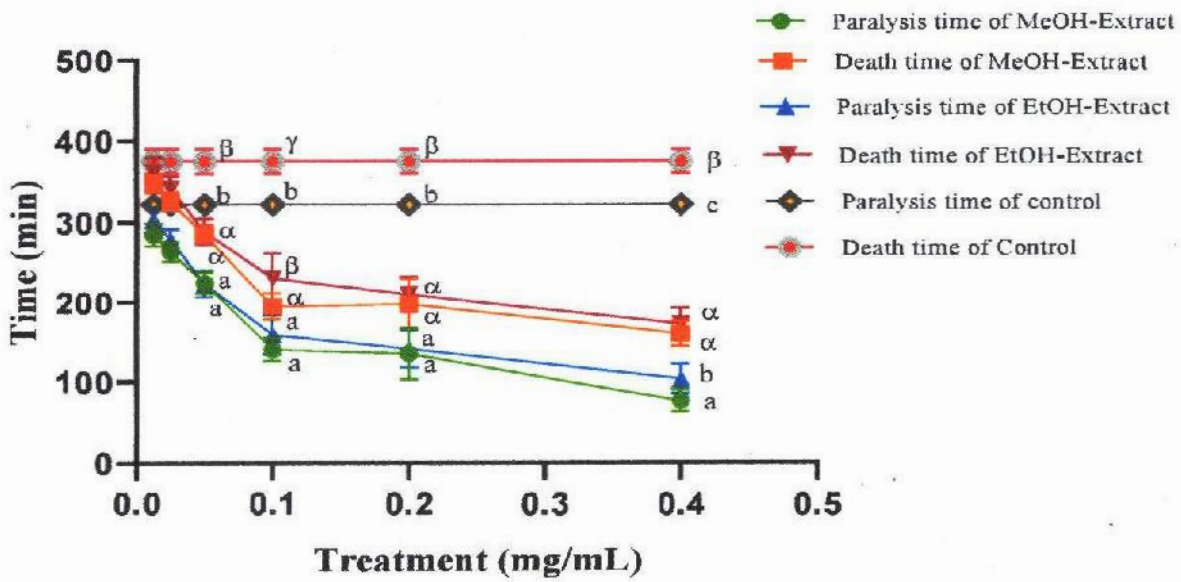


Figure 5.10: Dose-dependent anthelmintic effects of ethanolic and methanolic solvent fractions of *A. corniculatum* at different concentration. At 0.05, 0.1, 0.2, 0.4 mg/ml, all data were significant when compared to control, and different letters indicate significant differences at $p < 0.05$.

@Khulna University

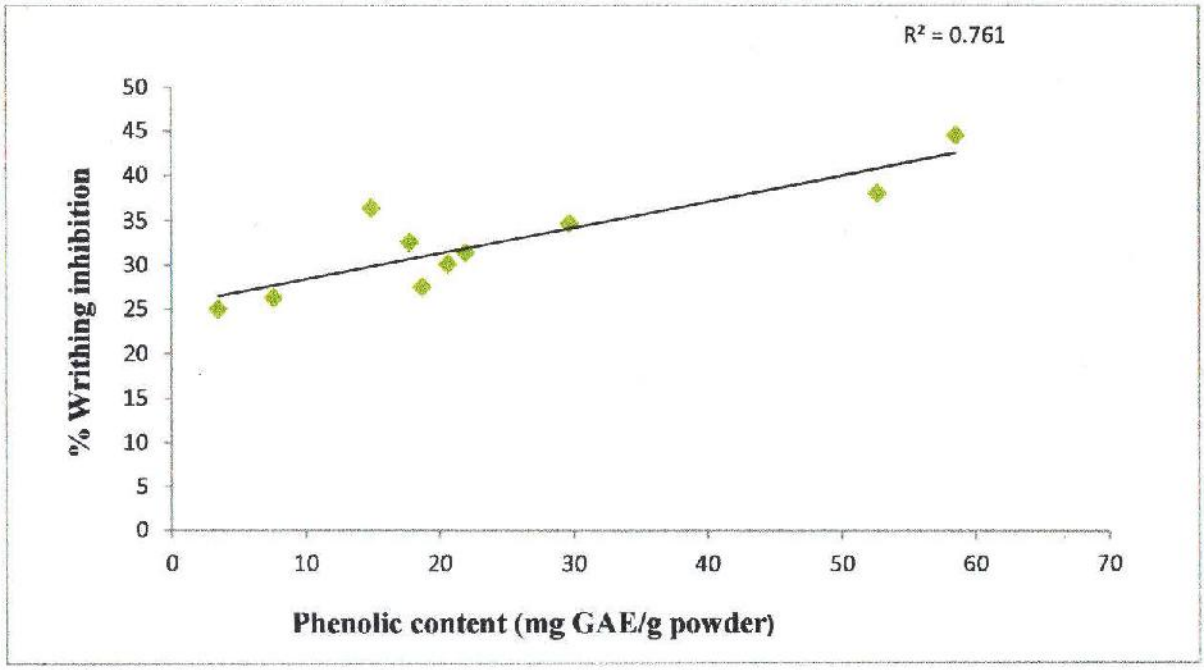


Figure 5.11: Correlation between the amount of total phenolics (GAE/g powder) and analgesic activity by writhing inhibition in acetic acid induced writhing method of the edible mangrove fruits.

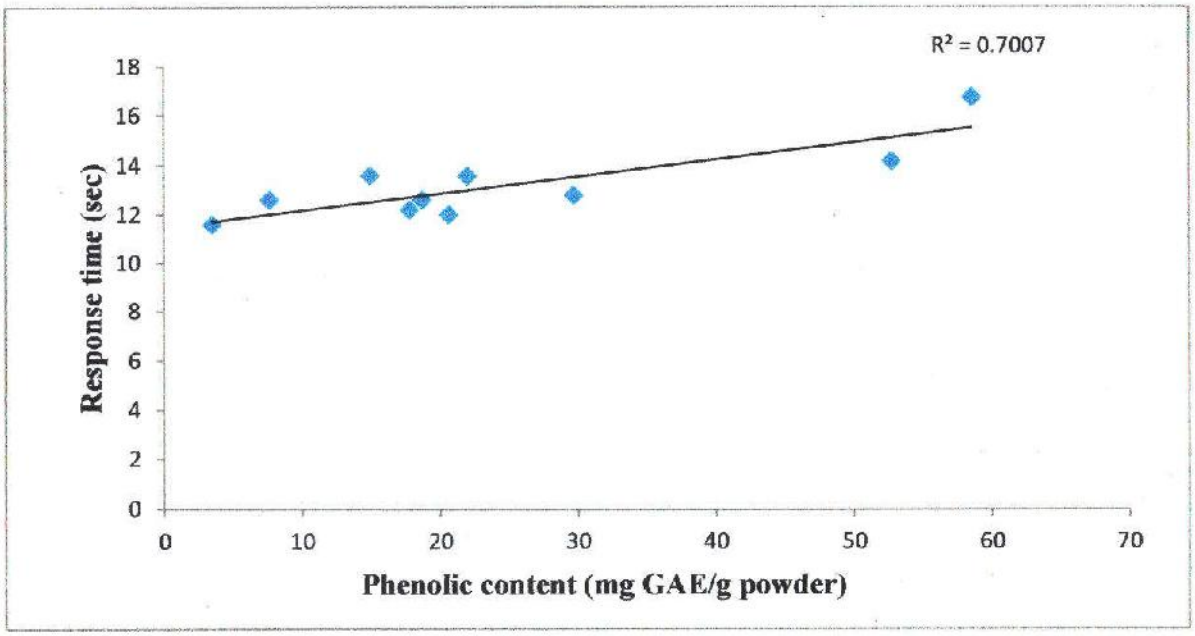


Figure 5.12: Correlation between the amount of total phenolics (GAE/g powder) and analgesic activity by response time (sec) increasing on hot plate method of the edible mangrove fruits.

@Khulna University

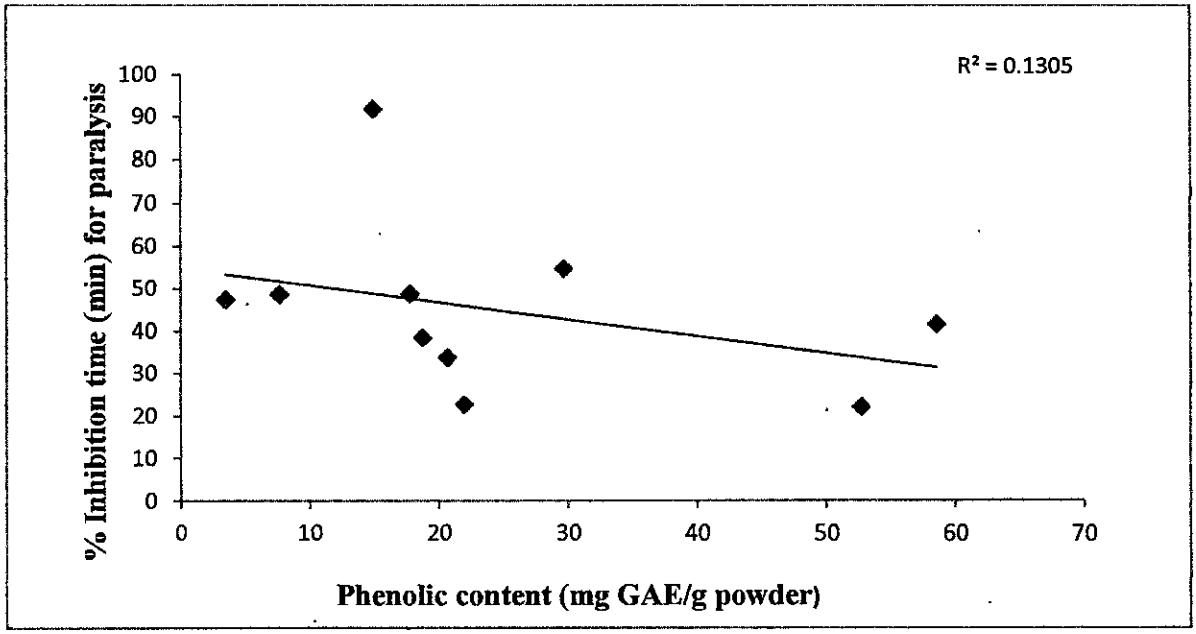


Figure 5.13: Correlation between the amount of total phenolics (mg GAE/g powder) and anthelmintic activity by percentage (%) of inhibition time (min) for paralysis of the edible mangrove fruits (r^2=0.131).

@Khulna University

CHAPTER SIX: DISCUSSION

Mangroves usually grow in estuarine swamps; have unique adaptations to combat environmental stress conditions e.g. high salinity, high temperature, low-nutrient and excessive radiation. Salt stress creates both ionic as well as osmotic stress on plants. Ultimately, plant function is explained by the operation of genes in cells and tissues to regulate plant growth in coordination with environmental constraints (Munns and Tester, 2008). These stresses can be distinguished at several levels (Tester and Davenport, 2003). Therefore, a better understanding of the integration and expression of tolerant mechanisms from the cellular level to the whole-plant level is necessary (Yang et al., 2010) and in vitro culture provides an excellent means to study these physiological genetic processes of plant at cell and tissue levels. Mangroves are thus negatively influenced by siltation and the trees will adapt their anatomical and physiological characteristics (Noor, 2015). As a result, mangroves are enriched with some special secondary metabolites which protect them in adverse conditions. Parida and Das (2002) noticed an increase in polyphenols with the increasing levels of salinity in *Bruguiera parviflora*. In general higher polyphenol accumulation is related to higher antioxidant capacity in most of the halophytes (Ksouri et al., 2007; Alhdad et al., 2013).

Moreover, mangroves are good source of polyphenols like tannins (Naskar and Bakshi, 1995). Phenolics have been considered as classic defense compounds for protecting plants from herbivores, ever since plant secondary metabolites were suggested to have evolved for that reason. In contrast to these concepts, it has been suggested that the main role of many plant phenolics may be to protect leaves from photodamage, not herbivores; they can achieve this by acting as antioxidants; and their levels may vary with environmental conditions in order to

@Khulna University

counteract this potential photodamage (Close and McArthur, 2002). The phenolics especially flavonoids were shown to protect mangroves from UV radiation (Agati et al., 2007).

Many plants have been investigated for the antioxidant activities and the search is gradually increased in recent times since ROS were the salient feature behind many dreadful diseases. The production of ROS and accordingly the antioxidant enzymes were upregulated due to altered expression of these antioxidant genes (Jitesh et al., 2006). Several anti-inflammatory, digestive, antinecrotic, neuroprotective and hepatoprotective (Ropetto and Llesuy, 2002; Perry et al., 1999) drugs have recently been shown to have an antioxidant and/or radical scavenging mechanism as part of their activity. Compounds of phenolic nature have been isolated from several species of mangrove and reported to attribute anti-tumerogenic, cytotoxic, anti-inflammatory, wound healing, anti-ulcer activities. Phenolic compounds like Quinic acid esters were isolated from this plant and have been shown to inhibit collagenase and impart almost no cytotoxicity (Teramachi et al., 2005).

Pain and inflammation are associated with pathology of various clinical conditions like arthritis, cancer, vascular diseases and so on (Weitzmann and Gordan, 1990). In various traditional medicinal systems a number of natural products are used to relieve the symptoms of pain (Dhawan et al., 2007). The present study demonstrates significant central and peripheral analgesic activity of edible mangrove fruits in the Sundarbans. Acetic acid induced abdominal contraction method has been used to evaluate peripherally acting analgesics. In acetic acid induced method, pain is generated indirectly via indigenous mediators like PGE2, PGF2α (Colier et al., 1968), bradykinin, histamine, 5-hydroxy tryptamine (Whittle et al., 1964) in the peritoneal fluid, which stimulates peripheral nociceptive neurons. These nural fibre are sensitive to both nacrotic and non-steroidal anti-inflammatory drugs. The hot plate method has been found to be

@Khulna University

suitable for evaluation of centreally acting analgesis (Woolfe and Mac Donald, 1994). Hot plate test measures nociceptive response latencies of test animals to thermal stimulus, which is sensitive to opioid receptors located in the central nervous system (Yaksh, 1977). The involvement of endogenous substances such as PGs may be minimized by this model. All the fruits (administered at 250 mg/ kg) inhibited acetic acid-induced writhing, and significantly delayed the response time in mice. Among the fruits fraction, *C. decandra* fruit fraction showed the highest analgesic activity through both the peripheral and the central nervous system amongst the ten halophyte species investigated. *C. decandra* is a common habitat in Sundarbans and leaves were known to be antinociceptive (Uddin et al., 2005). Asad et al. reported (2017) that *Sonneratia alba* Leaves are a great source of secondary metabolites responsible for analgesic activity. These observations suggest that edible fruits of the Sundarbans have both peripheral and central analgesic properties. The peripheral analgesic action of these fractions may be mediated via inhibition of cyclooxygenases (COXs), and/or lipoxygenases, whereas the central analgesic action may be mediated through inhibition of central pain receptors.

From our undergraduate thesis work, it was reported that mangrove edible fruits contain different phytochemicals like alkaloid, anthocyanin, flavonoid, polyphenol, reducing sugar, tannin, vitamin C etc. Since, anti-nociceptive activity of many plants has been attributed to their flavonoid (Pathak et al., 1991), tannins (Khaksa et al., 1996), triterpines (Ahmad et al., 1983) and alkaloid (Woolfe et al., 1994). It is, therefore possible that the anti-nociceptive effects observed in the present study may be attributed to the components that are present abundance in the fruits.

Helminthiasis is a disease in which a part of the body is infested with worms such as pinworm, roundworm or tape worm. Typically, the worms reside in the gastrointestinal tract but may also grow into the liver and other organs (Raju and Yesuf, 2010). They produce harmful effects on

@Khulna University

host by depriving him of food, cause blood loss and secrete toxins (Tripathi, 2003; Mahadik et al., 1998). The parasitic worms are categorized into three groups: cestodes, or tapeworms; nematodes or roundworms and trematodes or flukes (Tripathi, 2003; Sharma, 2007). Anthelmintics are drugs that act locally to expel parasitic worm from gastrointestinal tract or systematically remove adult helminthes from invaded organs and tissues. They can either kill (vermin-cides) or expel them (Tripathi, 2003; Kokate et al., 2005). The plants derivatives have the anthelmintic activity mainly due to their phytoconstituents especially due to secondary metabolites. Phytoconstituents, jointly or separately may act by inhibition of tubulin polymerization and blocking glucose uptake (Jain et al., 2011). Any damage to the mucopolysaccharide membrane of worms will expose the outer layer restricting their movement which finally may cause paralysis and ultimately death of parasite (Chandrashekhar et al., 2008). The anthelmintic effects of tannins may be attributed to its capacity to bind free protein available for larval nutrition and thus reducing the nutrient availability resulting in larval starvation or decrease in gastrointestinal metabolism directly through inhibition of oxidative phosphorylation causing larval death (Scalbert, 1991; Athanasiadou et al., 2001). According to Roy et al. (2010), alkaloids may act on central nervous system and caused paralysis of the earthworm. Anthelmintic effects of the honey may be due to presence of the steroidal alkaloids and oligoglycosides which may suppress the transfer of sucrose from the stomach to the small intestine together with their antioxidant effects which is capable of reducing the nitrate generation which can interfere in local homeostasis that is essential for the development of helminths (Borba et al., 2010). Natural compound may also show anthelmintic activities through hyperpolarization of the nerve membrane, and by inhibiting acetylcholinesterase enzymes, damaging neuromuscular coordination in the parasite (Martin, 1997; Guest, 2008). Detection of

@Khulna University

potent nematocidal activity of the mangrove edible fruits in this study is an indication for defense against helminthes parasites. All the fruits significantly showed the anthelmintic activity when compared to control. In this study, *A. corniculatum* fruit has shown to be the most potent as anthelmintics amongst the eleven mangrove species investigated. The sundarbans' edible fruits contain a complex mixture of phytochemical constituents. The constituent(s) of the edible fruits responsible for this anthelmintic activity; however we can speculate metabolites of the fruits as the probable candidate(s) since several antinematodal activities can be seen from natural plant sources have been reported (Park et al., 2004, Jang et al., 2004).

@Khulna University

CHAPTER SEVEN: CONCLUSION

Based on the results found in this study, it can be concluded that the edible mangrove fruits produced in the Sundarbans have analgesic and anthelmintic activity. Possession of various bioactive secondary compounds such as alkaloid, anthocyanin, flavonoid, polyphenol, reducing sugar, saponin, tannin, vitamin C etc. in the fruits might be a reason for the activities. The strongest analgesic activity of the edible mangrove fruits was found in *C. decandra*, whereas *A. corniculatum* fruit showed the strongest anthelmintic activity followed by *P. paludosa*. Therefore, regular consumption of the edible fruits may therefore prevent pain and helminth diseases. Further studies are essential to elucidate the potential bioactive component(s) in the edible fruits from the Sundarbans, and their utilization as dietary supplements and preventive medicine and chemical studies for the purification and identification of responsible bioactive components followed by pharmacological assessment is required to drug development. It is clear that mangroves are potentially of great commercial value. Therefore, mangroves need to be protected from destruction and there is need to recognize mangrove forestation as an acceptable land use system.

@Khulna University

CHAPTER EIGHT: REFERENCES

Aaby K, Hvattum E. 2004. Analysis of flavonoids and other phenolic compounds using high-performance liquid chromatography with colorimetric array detection: relationship to antioxidant activity. *Journal of the Agricultural and Food Chemistry* 52: 4595–4603.

Abeywickrama WSS, Jayasooriya MCN. 2010. Formulation and quality evaluation of cordial based on Kirala (Sonneratia Caseolaris) Fruit. *Tropical Agricultural Research and Extension* 1: 16-18.

Adewunmi CO, Agbedahunsi JM, Adebajo AC, Aladesanmi AJ, Murphy N, Wando J. 2001. Ethno-veterinary medicine: screening of Nigerian medicinal plants for trypanocidal properties. *Journal of Ethnopharmacology* 77: 19-24.

Adhikari A Ray M, Sur TK, Kanjilal S, Roy RK, Das AK. 2016. Analgesic actions of Sunderban mangrove, *Rhizophora mucronata L.* leaves. *Journal of Medicinal Plants Studies* 4: 140-143

Agati G, Matteini P, Goti A, Tattini M. 2007. Chloroplast located flavonoids can scavenge singlet oxygen. *New phytologist* 174: 77-89.

Aghaleh M, Niknam V, Ebrahimzadeh H, Razavi K. 2011. Effect of salt stress on physiological and antioxidative responses in two species of Salicornia (*S. persica and S. europaea*) *Acta Physiologiae Plantarum* 33: 1261–1270.

Ahmed F, Selim MS, Das AK, Choudhuri MS. 2004. Anti-inflammatory and anti-nociceptive activities of *Lipia nodiaflora. Die Pharmazie* 59: 329-330.

@Khulna University

Ahmed MM, Qureshi S, Al-Bekairi AM, Shak AH. 1983. Anti-inflammatory activity of *C. tubarculata* alcolic extract. *Fitoterpia- milano,* 46: 357-360.

Ajaiyeoba EO, Onocha PA, Olarenwaju OT. 2001. In vitro Anthelmintic Properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* Extracts. *Pharmaceutical Biology* 39: 217–220.

Akhtar MS, Iqbal Z, Khan MN, Lateef M. 2000. Anthelmintic activity of medicinal plants with particular reference to their use in animals in Indo-Pakistan subcontinent. *Small Ruminant Research* 38: 99 – 107.

Alhdad GM, Seal CE, Al-Azzawi MJ, Flowers TJ. 2013. The effect of combined salinity and water logging on the halophyte *Suaeda maritima* the role of antioxidants. *Environmental and Experimental Botany* 87: 120-125.

Alongi DM. 2011. Carbon payments for mangrove conservation: ecosystem constraints and uncertainties of sequestration potential. *Environmental Science and Policy* 14: 462–470.

Amir F, Golbar G, Payman MK, Hosein F, Amin F. 2009. Antinociceptive effect of promethazine in mice. *Iranian Journal of Basic Medical Science* 12: 140-145.

Arokiasamy M. 1968. Toxicity of *Sarcolobus globosus* as observed in a cat. *Malaysian Veterin Journal* 4: 196–199.

Athanasiadou S Kyriazakis I, Jackson F, Coop RL. 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Veterinary Parasitology* 99: 205-219.

@Khulna University

Atkinson MR Findlay GP, Hope AB, Pitman MG, Saddler HD, West KR. 1967. Salt regulation in the mangroves *Rhizophora mucronata Lam.* and *Aegialitis annulata. Australian Journal of Biological Sciences* 20: 589-99.

Aziz A and Paul AR. 2015. Bangladesh Sundarbans: Present Status of the Environment and Biota. Diversity 7: 242-269.

Bandaranayake WM. 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetlands Ecology and Management* 10: 412–452.

Bandaranayake WM. 1998. Traditional and medicinal uses of mangroves. *Mangroves Salt Marshes* 2: 133-148.

Banerjee D, Chakrabarti S, Hazra AK, Banerjee S, Ray J, Mukherjee B. 2008. Antioxidant activity and total phenolics of some mangroves in Sundarbans. *African Journal of Biotechnology* 7: 805-810.

Baylis GTS. 1940. Leaf anatomy of the New Zealand mangrove. *Transaction of Royal Society of New Zealand* 70: 164-70.

Bently GA, Newton SH and Starr J. 1983. Studies on the anti-nociceptive action of agonist drugs and their interaction with opioid mechanisms. *Britsh Journal of Pharmacology* 79: 125-134.

Biswas M, Biswas K, Karan TK, Bhattacharya S, Ghosh AK, Haldar PK. 2011. 'Evaluation of analgesic and anti-inflammatory activities of *Terminalia arjuna* leaf. *Journal of Phytology* 3: 33-38.

@Khulna University

Boonsong K. and Piyatiratitivorakul S. 2003. Potential use of mangrove plantation as constructed wetland for municipal waste water treatment. *Water Science and Technology* 48: 257–266.

Borba HR, Borba HR, Freire RB, Albuquerque AC, Cardoso ME, Braga IG, Almeida ST, Ferreira MJ, Fernandes GL, Camacho AC, Lima RC, Almeida AC. 2010. Anthelmintic comparative study of *Solanum lycocarpum*. *Nature and Science* 8: 95.

Brown B. 2006. 'Cooking with mangroves 36 Indonesian mangrove recipes' Mangrove Action Project Indonesia, 26-34.

Calixto JB, Scheidt C, Otuki M, Santos AR. 2001. Biological activity of plant extracts: novel analgesic drugs. *Expert Opin Emerg* Drugs 6: 261-79.

Campbel WC. 1993. Ivermectin an anti-parasitic agent. *Medicinal Research Reviews* 13: 61–79.

Cardale S, Field CD. 1971. The structure of the salt gland of *Aegiceras corniculatum*. *Planta* 99: 183-191.

Chaffey DR and Miller FR. 1985. A Forest Inventory of the Sundarbans', Bangladesh. *Land Resources Development Centre: Surrey, UK*, 196

Chandrasekhar CH, Latha KP, Vagdevi HM, Vaidya VP. 2008. Anthelmintic activity of the crude extracts of *Ficus racemosa*. *International Journal of Green Pharmacy* 2: 100-103.

Chen L, Zan Q, Li M, Shen J, Liao W. 2009. Litter dynamics and forest structure of introducted *Sonneratia caseolaris* mangrove forest in Shenzhen, China. Estruarine. *Coastal and Self Science* 85: 18-27.

@Khulna University

Close DC and McArthur C. 2002. Rethinking the role of many plant phenolics – protection from photodamage not herbivores. *Oikos* 99: 166-172.

Coles GC. 1997. Nematode control practices and anthelmintic resistance on British sheep farms. *Veterinary Record* 141(4): 91-93.

Collier HO, Dinneen LC, Johnson CA, Schneider C. 1968. The abdominal constriction.response and its suppression by analgesic drugs in the mouse. *Br J Pharmacol Chemother* 32: 295-310.

Connor DJ. 1969. Growth of grey mangrove (*Avicennia marina*) in nutrient culture. *Biotropica,* 1: 36-40.

Crompton DWT and Nesheim MC. 2002. Nutritional impact of intestinal helminthiasis druging: the humancycle. *Annual Review of Nutrition* 22: 35-59.

Dahibhate NL, Saddhe AA, Kumar K. 2019. Mangrove Plants as a Source of Bioactive Compounds: A Review. *The Natural Products Journal* 9: 86-97.

Dhawan BN et al. 1968. Screening of Indian plants for biogical activity: Part 1. *Indian Journal of Experimental Biology* 6: 232-247.

Das MP, Dhanabalan R, Doss A, Palaniswamy M. 2009. Phytochemical screening and antibacterial activity of aqueous and methanolic leaf extracts of two medicinal plants against bovine mastitis bacterial pathogens. *Ethnobotanical leaflets* 1:15.

Dasgupta N, Nandy P, Sengupta C, Das S. 2015. RAPD and ISSR marker mediated genetic polymorphism of two mangroves *Bruguiera gymnorrhiza* and *Heritiera fomes* from

@Khulna University

Indian Sundarbans in relation to their sustainability. *Physiology and molecular biology of plants* 21:375-84.

Dominguez-Vazquez A and Taylor HR. 1983 Comparison of flubendazole and diethylcarbamazine in treatment of onchocerciasis. *Lancet* 1: 139–143

Downton WJS. 1982. Growth and osmotic relations of the mangrove *Avicennia marina*, as influenced by salinity', *Australian Journal of Plant Physiology* 9: 519-528.

Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, A. Van De Putte LB, Lipsky PE. 1998. Cyclooxygenase in biology and disease. *FASEB Journal* 12: 1063–1073.

Duke N. 2006. Australia's Mangroves, The authoritative guide to Australia's mangrove plants' University of Queensland, Brisbane, Australia.

Duke NC, Benzie JA, Goodall JA, Ballment ER. 1998. 'Geneticstructure and evolution of species in the mangrove genus Avicennia (Avicenniaceae) in the Indo-West Pacific. *Evolution* 52: 1612-1626.

Duman E, Kesim M, Kadioglu M, Ulku C, Kalyoncu NI, Yaris E. 2006. Effect of gender on antinociceptive effect of paraxetine in hot plate test in mice. *Progress in Neuro-Pharmacology and Biological Psychiatry* 30: 292-296.

Eddy NB, Leimbach D. 1953. Synthetic analgesics of dithienylbutenyl and dithienylbutylamines. *Journal of Pharmacology and Experimental Therapeutics* 107: 385-393.

Edeoga HO, Okwu DE. 2005. Phytochemical constituents of some mangrove medicinal plants. *African Journal of Biotechnology* 4: 685-688.

@Khulna University

Ellison J, Nandy P, Sengupta C, Das S. 2010. "*Aegiceras corniculatum*". IUCN Red List of Threatened Species. Version 2013.2. *International Union for Conservation of Nature*.

Emran TB, Rahman MA, Hosen SZ, Rahman MM, Islam AM, Chowdhury MA, Uddin ME. 2012. Analgesic activity of Leea indica (Burm. f.) Merr. *Phytopharmacology* 3:150-7.

Espejo E. 1993. Structure of the rat's behavior in the hot plate test. *Behavioral Brain Research* 56: 171-176.

FAO (Food and Agriculture Organization), 2007, The World's Mangroves 1980-2005, FAO Forestry Paper 153. Forestry Department,

FAO, 1994. Mangrove Forest Management Guidelines. FAO, Rome.

Field CD. 1995. Journey Amongst Mangroves. International Society for Mangrove Ecosystems, Okinawa, Japan.

Flowers TJ and Colmer TD. 2008. Salinity tolerance in halophytes. *New Phytologist* 179: 945–963

Gani MO. 2001. The giant honey bee (Apis dorsata) and honey hunting in Sundarbans reserved forests of Bangladesh. Proceedings of the 37th International Apicultural Congress, Apimondia, Durban.

Ghani A. 2003. Medicinal Plants of Bangladesh, 2nd edition. 1-2, 55-57, 402-500.

Gheldof N, Engeseth N. 2002. Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro

@Khulna University

Hoque AKF, Datta DK. 2005. The Mangroves of Bangladesh. *International Journal of Ecology and Environmental Sciences* 31: 245-253.

Horton J. 2000. Albendazole: a review of anthelmintic efficacy and safety in humans. *Parasitology* 121:113–132.

Hossain M. 2015. Handbook of selected plant species of the Sundarbans and the embankment ecosystem. Sustainable Development and Biodiversity Conservation in Coastal Protection Forests, Bangladesh (SDBC-Sundarbans). Dhaka, Bangladesh 8.

Hossain SJ, Basar MH, Rokeya B, Arif KM, Sultana MS, Rahman MH. 2013. Evaluation of antioxidant, antidiabetic and antibacterial activities of the fruit of Sonneratia apetala (Buch.-Ham.). *Oriental Pharmacy and Experimental Medicine* 13: 95–102.

Hossain SJ, Iftekharuzzaman M, Haque MA, Saha B, Moniruzzaman M, Rahman MM, Hossain H. 2016. Nutrient compositions, antioxidant activity, and common phenolics of Sonneratia apetala (Buch.-Ham.) fruit. *International journal of food properties* 19: 1080-92.

https://dx.doi.org/10.2305/IUCN.UK.2010-2.RLTS.T178796A7608551.en, Downloaded on 10 March 2019.

http://dx.doi.org/10.2305/IUCN.UK.2010-2.RLTS.T178797A7608891.en., Collected on 10 March 2019.

http://dx.doi.org/10.2305/IUCN.UK.2010-2.RLTS.T178803A7610926.en., Downloaded on 10 March 2019.

@Khulna University

http://dx.doi.org/10.2305/IUCN.UK.2010-2.RLTS.T178805A7611857.en., Downloaded on 10 March 2019.

http://dx.doi.org/10.2305/IUCN.UK.2010-2.RLTS.T178815A7615342.en., Downloaded on 13 March 2019.

http://dx.doi.org/10.2305/IUCN.UK.2010-2.RLTS.T178816A7615575.en., Downloaded on 13 March 2019.

http://dx.doi.org/10.2305/IUCN.UK.2010-2.RLTS.T178820A7616950.en., Downloaded on 13 March 2019.

http://dx.doi.org/10.2305/IUCN.UK.2010-2.RLTS.T178853A7627935.en., Downloaded on 13 March 2019.

https://en.wikipedia.org/wiki/Aegiceras_corniculatum, Collected on 08 April 2019. https://en.wikipedia.org/wiki/Avicennia_officinalis, Downloaded on 08 April 2019.

https://en.wikipedia.org/wiki/Bruguiera_gymnorrhiza, Downloaded on 08 April 2019.

https://en.wikipedia.org/wiki/Ceriops_decandra, Downloaded on 09 April 2019.

https://en.wikipedia.org/wiki/Heritiera_fomes, Downloaded on 09 April 2019.

https://en.wikipedia.org/wiki/Nypa_fruticans, Downloaded on 09 April 2019.

https://en.wikipedia.org/wiki/Phoenix_paludosa, Downloaded on 09 April 2019.

https://en.wikipedia.org/wiki/Sarcolobus_globosus, Downloaded on 09 April 2019.

@Khulna University

Hussain Z and Acharya G. 1994. 'Mangroves of the Sundarbans', Volume two: Bangladesh. IUCN, Bangkok, Thailand, 256.

Idris AM. 2011. Screening of conditions controlling spectrophotometric sequential injection analysis. *Chemestry Central Journal* 5: 9-10.

Islam MR, Islam MR, Anisuzzaman M, Hossain SJ. 2019. Antidiarrheal, Analgesic, and Anthelmintic Activities of Honeys in the Sundarbans Mangrove Forest, Bangladesh. Preventive Nutrition Food Science 24:. 49-55.

Jain DD. 2015. Maheshwari and R. Somani, 'Screenning the *in-vitro* anthelmentic activity of Alternanthera *sessilis* leaves. *Journal of Advances in Drug Research.* 4: 1402-1415.

Jain P, Singh S, Singh SK, Verma SK, Kharya MD, Solanki S. 2013. Anthelmintic potential of herbal drugs. *International Journal of Research and Development in Pharmacy and Life Sciences* 2: 412-27.

Jang SH, Park Y, Park SC, Kim PI, Lee DG, Hahm KS. 2004. Antinematodal activity and the mechanism of the antimicrobial peptide, HP (2-20), against *Caenorhabditis elegans. Biotechnol Lett* 26: 287-291.

Janmanchi H, Raju A, Degani MS, Ray MK, Rajan MG. 2017. Antituberculosis, antibacterial and antioxidant activities of *Aegiceras corniculatum*, a mangrove plant and effect of various extraction processes on its phytoconstituents and bioactivity. *South African Journal of Botany* 113: 421-427.

@Khulna University

Jayatissa LP, Dahdouh-Guebas F, Koedam N. 2006. A review of the floral composition and distribution of mangroves in Sri Lanka. *Journal of Medicinal Plants Studies* 4: 140-143.

Jitesh M, Prashanth SR, Sivaprakash KR, Parida A. 2006. Monitoring expression profiles of antioxidant genes to salinity, iron, oxidative light and hyperosmotic stresses in the highly salt tolerant gray mangrove, *Avicennia marina* (Frosk.) Vierh by mRNA analysis. *Plant Cell Reproduction* 25: 865-876.

Kassai T. 1999. Veterinary Helminthology. Reed educational and professional publishing Ltd., London 196 – 197.

Kathiresan K, Bingham BL. 2001. Biology of mangroves and mangrove ecosystem. *Advances in Marine Biology* 40: 81-251.

Khafagi I, Gab-Alla A, Salama W, Fouda M. 2003. Biological activities and phytochemical constituents of the gray mangrove Avicennia marina (Forssk.)', Vierh. *Egyptian Journal Botany* 5: 62-69.

Khaksa G, Zolfaghari ME, Dehpour AR, Samadian T. 1996. Anti-inflammatory and anti-nociceptive activity of disodium glycyrrhetinic acid hemiphthalate. *Planta Medica* 62: 326-328.

Khan MA, Qaiser M. 2006. Halophytes of Pakistan: characteristics, distribution and potential economic usages. Sabkha Ecosystems Vol.II: West and Central Asia, pp.129-153.

Kiritikar KR, Basu BD. 1991 Indian medicinal plants. (Second edition) New Delhi, Periodical Experts Book Agency.

KHULNA UNIVERSITY Central Library KHULNA.

@Khulna University

Kirtikar, K. and Basu, B.D. 1935. Indian Medicinal Plants, Vol. II. p. 756.

Kirwan P, Asaolu SO, Molloy SF, Abiona TC, Jackson AL, Holland CV. 2009. Patterns of soil-transmitted helminth infection and impact of four-monthly albendazole treatments in preschool children from semi-urban communities in Nigeria: a double-blind placebo-controlled randomised trial. *Biomedical Central Infectious Diseases* 9: 10-20.

Tsuji K, Ghazalli MN, Ariffin ZU, Nordin MS, Khaidizar MI, Dulloo ME, Sebastian LS. 2011. Biological and Ethnobotanical Characteristics of Nipa Palm (*Nypa fructicans* Wurmb.): A Review. *Sains Malaysiana* 40: 1407–1412.

Kokate CK, Purohit AP, Gohkale SB. 2002. Pharmacognosy. In: Terpenoids, 21st Edition, Nirali Prakashan, Pune, 377-378.

Kotowski W, Maltby E, Szatylowicz J, Miroslaw-Swiatek D, Kotowski W. 2014. Wetlands: Monitoring, Modelling and Management: Proceedings of the International Conference. Washington, DC.

Kris-Etherton PM, Keen CL. 2002. Evidence that the antioxidant flavonoids in tea and cocoa are beneficial for cardiovascular health' *Curr Opin Lipidol* 13: 41–49.

Krogstad DJ, Andengleberg CN. 1998. Introduction To parasitology: Mechanisms of Microbial Disease, Maryland, USA, Williams & Wilkins.

Ksouri R, Falleh H, Megdiche W, Trabelsi N, Mhamdi B, Chaieb K, Bakrouf A, Magné C, Abdelly C. 2009. Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents. *Food and Chemical Toxicology* 47: 2083-2091.

@Khulna University

Ksouri R, Megdiche W, Debez A, Falleh H, Grignon C, Abdelly C. 2007. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritime*. *Plant Physiology and Biochemistry* 45: 244-249.

Kulkarni SK. 1999. Handbook of Experimental Pharmacology, Delhi, *Vallabh Prakashan.*

Kumar KP, Bhowmik SD, Chandra MR. 2010. Medicinal uses and health benefits of honey. *Journal of Chemical and Pharmaceutical Research* 2: 385-395.

Lao RC, Shu YY, Holmes J, Chiu C. 1996. Environmental sample cleaning and extraction procedures by microwave-assisted process (MAP) technol. *Microchemical Journal* 53: 99-108.

Li ZG, Zhao YL, Wu X, Ye HY, Peng A, Cao ZX, Mao YQ, Zheng YZ, Jiang PD, Zhao X, Chen LJ. 2009. Barbigerone, a natural isoflavone, induces apoptosis in murine lung-cancer cells via the mitochondrial apoptotic pathway. *Cell Physiology Biochemistry* 24: 95–104.

Lichtman AH, Cook SA, Martin BA. 1996. Investigation of brain sites mediated cannabinoid induced antinociception in rats: Evidence supporting periaqueductal gray involvement. *Journal of Pharmacology and Experimental Therapeutics* 276: 585-93.

Lima AA, Parial RA, Das MA, Das AK. 2010. Phytochemical and pharmacological studies of ethanolic extract from the leaf of mangrove plant *Phoenix Paludosa* Roxb. *Malaysian Journal of Pharmaceutical Sciences*. 8: 59–69.

@Khulna University

Mahadev ND, Vitthal BP. 2017. An evaluation of anthelmintic activity of *Ricinus communis* Linn. leaves by using different type of solvent. *Journal of Pharmacognosy and Phytochemistry* 6: 1845-1847.

Mahadik KR, Deshmukh KR. 1998. Concise Organic Pharmaceutical chemistry. *Nirali Prakashan*, 19: 81.

Mandal RN, Naskar KR. 2008. Diversity and classification of Indian mangroves. *International Society for Tropical Ecology*, 49: 131-146.

Manilal A. 2015. An in vitro antagonistic efficacy validation of *Rhizophora mucronata*. *Asian Pacific Journal of Tropical Disease*, 5:28-32.

MAP (Mangrove Action Project), Mangrove Ecosystems, 1990.

Martin RJ. 1997. Modes of action of anthelmintic drugs. *Veterinary Journal* 154: 11-34.

Meena MK, Jain AK, Jain CP, Gaur K, Kori ML, Kakde A, Nema RK. 2009. Screening of anti-inflammatory and analgesic activity of *Cassia grandis*. *American Journal of Plant Science* 2: 51-55.

Meot-Duros L, Magne C. 2009. Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. *Plant Physiology and Biochemistry* 47: 37-41.

Mercer DE, Hamulton LS. 1984. Mangrove ecosystems of some economic and natural serebils. *Nature and Resources* 20: 6-13.

Mian RH, Roxrigues KR. 1982. Organic amendment with high tannin and phenolic compound for the control of Meloidogynearenaria in infested soil. *Nematotropica* 12: 71-74.

@Khulna University

Mishra D, Ghosh GO, Kumar PS, Panda PK. 2011. An experimental study of analgesic activity of selective Cox-2 inhibitor with conventional NSAIDs. *Asian Journl of Pharmaceutical and Clenical Research* 4: 78-81.

Moghal MMR, Bhattacharjee A, Seeam SM, Islam ASMM, Bappy MH. 2016. Phytochemical Screening, Cytotoxic and Anthelmintic Activities of Amorphophallus campanulatus (Roxb.), *Avicennia marina* (Forssk.) and *Launaea sarmentosa* (Willd.), *Bangladesh Pharmaceutical Journal* 19: 106-113.

Rahmatullah M, Sadeak SM, Bachar SC, Hossain MT, Abdullah-al-Mamun M, Jahan N, Chowdhury MH, Jahan R, Nasrin D, Rahman M, Rahman S. 2010. Brine shrimp toxicity: Study of different Bangladeshi medicinal plants. *Advance in natural and applied science* 4: 163-173.

Mulla AW. 2010. Evaluation of anti-inflmmatory and analgesic activities of ethanolic extract of roots *Adhatoda vasica* Linn. *International Journal of Pharmacy* 2: 1364-1368.

Mullan DP. 1931. On the occurrence of glandular hairs (salt glands) on the leaves of some Indian halophytes. *Journal of the Indian Botanical Society* 10: 184-189.

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, 59: 651–681.

Mustafa MR, Hadi AHA. 1990. Neuromuscular blocking activity of a glycosidic extract of the plant *Sarcolobus globosus*. *Toxicon* 28: 1237–1239.

Naskar K, Guha BDN. 1995. Vegetation pattern of the Sundarbans, In mangrove swamps of the Sundarbans', An ecological perspective. *Naya Prokash*: Calcutta, India, pp. 27-174.

@Khulna University

Nathan C. 2002. Points of Control in Inflammation. *Journal of Nature* 420: 846-852.

Nikajoo LT. 2009. Analgesic activity of Aqueous and Alcohol root extracts of *Pargularia daemia*. International Journal of Applied Research of Pharmacy and Pharmaceutical Science 1: 33-37.

Noor TA, Batool NA, Mazhar RO, Ilyas NO. 2015. Effects of Siltation, Temperature and Salinity on Mangrove Plants. *European academic research* 2: 14172-14179

Noor YR, Khazali M. 2006. 'Guide of introduction mangroves in Indonesia', PHKA/WI-IP, Bogor, pp. 74-130.

Nypa fruticans. Germplasm Resources Information Network (GRIN). Agricultural Research Service (ARS), United States Department of Agriculture (USDA). Retrieved 9 January 2017.

Nypa fruticans. World Checklist of Selected Plant Families (WCSP). Royal Botanic Gardens, Kew. Retrieved 9 January 2017.

Pardutz A, Schoenen J. 2010. NSAIDs in the Acute Treatment of Migraine: A Review of Clinical and Experimental Data. *Pharmaceuticals* 3: 1966-1987.

Parida A, Das AB. 2002. NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. *Journal of Plant Biology* 45: 28–36.

Park Y, Jang SH, Lee DG, Hahm KS. 2004. Antinematodal effect of antimicrobial peptide, PMAP-23, isolated from porcine myeloid against *Caenorhabditis elegans*. *Journal of Peptide Science* 10: 304-311.

@Khulna University

Pathak D, Pathak K. and Singla AK. 1991. Flavanoids as medicinal agents-recent advances. *Fitoterapia* 62: 371-389.

Patra JK, Das SK, Thatoi H. 2014. Phytochemical profiling and bioactivity of a mangrove plant, *Sonneratia apetala*, from Odisha Coast of India. *Chinese Journal of Integrative Medicine* 21: 367-383.

Perry EK, Pickering AT, Wang WW, Houghton PJ, Perry NS. 1999. Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy. *Journal of Pharmacy and Pharmacology* 51: 527-534.

Perry LM. 1980. Medicinal plants of East and Southeast Asia. MIT Press. Cambridge.

Ponnapalli MG, Annam SC, Ravirala S, Sukki S, Ankireddy M, Tuniki VR. 2012. Unusual isomeric corniculatolides from mangrove, *Aegiceras corniculatum*. *Joural of Natural Product* 75: 275-279.

Prasad N, Yang B, Kong KW, Khoo HE, Sun J, Azlan A, Ismail A, Romli ZB. 2013. Phytochemicals and Antioxidant Capacity from *Nypa fruticans* Wurmb. Evidence-Based Complementary and Alternative Medicine 10: 9.

Provu VV, Nalini G. 2011. Evaluation of anti-inflammatory and analgesic activity of *Tridax procumbens* Linn. Acetic acid and CFA induced pain model. *International Journal of Pharmacy and Pharmaceutical Sciences* 3: 126-130.

Pyrzynska K, Biesaga M. 2009. Analysis of phenolic acids and flavonoids in honey. *Trends in Analytical Chemistry* 28: 893-902

@Khulna University

Rahman MA, Haque E, Hasanuzzaman M, Shahid IZ. 2011. Antinociceptive, antiinflammatory and antibacterial properties of *Tamarix indica* roots. *International Journal of Pharmacology*, 7: 527–531.

Qijie Z, Bosun W, Yongjun W, Ming-Guang L. 2003. Ecological assessment on the introduced Sonneratia caseolaris and S. apetala at the mangrove forest of Shenzhen Bay, China. *Acta Botanica Sinica* 45: 544-51.

Raju JN, Yesuf AE. 2010. Evaluation of anthelmintic activity of rumex abyssinicus jacq and rumex nervosus vahl. *International Journal of Pharmaceutical Sciences* 5: 55-57.

Raju JSSN. 2003. Xylocarpus (Meliaceae): A less-known mangrove taxon of the Godavari estuary, India. *Indian Academy of Sciences* 84: 879–881.

Raju NJ, Patro LR. 2017. Anthelmintic activity of black mangrove of *Aegiceras corniculatum*. *World Journal of Pharmaceutical Research* 6: 1173-1180.

Rang HP, Dale MM. 1993. Pharmacology, 2nd ed., UK, Churchill Livingstone Publisher.

Reddy ARK, Grace JR. 2016. In Vitro Evaluation of Antioxidant Activity of Methanolic Extracts of Selected Mangrove Plants. *Medicinal and Aromatic Plants Journal* 5: 3-7.

Revathi P, Thirumalaikolundusubramanian P, Prabhu N. 2013. Medicinal properties of mangrove plants – an overview. *International Journal of Bioassays* 2: 1597-1600.

Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. 1995. The relative antioxidant activities of plant derived polyphenolic flavonoids. *Free Radical Research* 22: 375-383.

@Khulna University

Rich JR, Rali GS, Oppenmann CH. 1989. Influence of castor bean (Ricinuscommunis) lectin (Ricinin) on mortality of *Meloidogyne incognita*. *Nematotropica* 19: 99-103.

Rivas-Alcala AR, Greene BM, Taylor HR. 1981. Chemotherapy of onchocerciasis: a controlled comparison of mebendazole, levamisole, and diethylcarbamazine. *Lancet* 2: 485–490.

Roome T, Dar A. 2011. Evaluation of antinociceptive effect of Aegiceras corniculatum stems extracts and its possible mechanism of action in rodents. *Journal of Ethnopharmacology* 135: 351-358.

Ropetto MG, Llesuy SF. 2002. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Brazilian journal of medical and biological research* 35: 523-534.

Roxburgh W. 1832 'Flora indica; or descriptions of Indian Plants "*Phoenix paludosa*". 3: 789.

Roy H, Chakraborty A, Bhanja S, Nayak BS, Mishra SR, Ellaiah P. 2010 'Preliminary phytochemical investigation and anthelmintic activity of '*Acanthospermum hispidum*'. *Journal of Pharmaceutical Science and Technology* 2: 217-221.

Salini G. 2015. Pharmacological Profile of Mangrove Endophytes - A Review. *International Journal of Pharmcological Science* 2015; 7:6-15.

Sardar PK, Dev S, Bari MAA, Paul S, Yeasmin MS, Das AK, Biswas NN. 2018. Antiallergic, anthelmintic and cytotoxic potentials of dried aerial parts of *Acanthus ilicifolius* L. *Clinical Phytoscience* 4:34.

Scalbert A. 1991. Antimicrobial properties of tannin. *Phytochemistry* 30: 3875–3883.

@Khulna University

Seepana R, Perumal K, Kada NM, Chatragadda R, Raju M, Annamalai V. 2016. Evaluation of antimicrobial properties from the mangrove *Rhizophora apiculata* and *Bruguiera gymnorrhiza* of Burmanallah coast, South Andaman, India. *Journal of Coastal Life Medicine* 4: 475-478.

Sharma VN. 2007. *Essentials of pharmacology*, New Delhi, CBS Publishers Distributors.

Shettar AK, Vedamurthy AB. 2017. An in-vitro approach for evaluating anthelmintic activity of *Kandelia candel* and *Rhizophora apiculata*. *Journal of Pharmacognosy and Phytochemistry* 6: 05-09.

Siddiqui ASMH. 2016. Flora and Faunal Resources and Ecosystem Conservation in the Sundarbans. *International Journal of Agriculture Innovations and Research* 5: 2319-1473.

Silvana E, María AM. 2015. Development and validation of a dissolution test method for albendazole and praziquantel in their combined dosage form. *Journal of the Brazilian Chemical Society* 26: 729-735.

Simlai A, Roy A. 2013 Biological activities and chemical constituents of some mangrove species from Sundarban estuary: An overview. *Pharmacognosy* 7(14): 170-178.

Singh RP, Banerjee S, Rao AR. 2000, Effect of Aegle marmelos on biotransformation enzyme systems and protection against free-radical-mediated damage in mice. *Journal of Pharmacy and Pharmacology* 11: 52-59.

Subudhi PK, Baisakh N. 2011. *'Spartina alterniflora'*, a halophytes grass model to dissect salt stress tolerance. *In Vitro Cellular and Developmental Biology – Plant* 47: 441–457.

@Khulna University

Sudarwo SA. 2005. Anti-inflammatory and analgesic effect of bromelain in mice and rats. *Universa Medicina* 24: 4-16.

Tagbota S, Townson S. 2001. Antiparasitic properties of medicinaland other naturally occurring products. *The Journal of Advances in Parasitology* 50: 199-295.

Tammu J, Ramana KV, Yuvaraj G, Ramanathan L, Nema RK. 2011. Biological evaluation of anti-inflammatory effects of physalisminima. *Asian Journal of Biochemical and pharmaceutical Reasearch* 3: 1.

Teramachi F, Koyano T, Kowithayakorn T, Hayashi M, Komiyama K, Ishibashi M. 2005. Collagenase inhibitory quinic acid esters from *Ipomoea pescaprae*. *Journal of Natural Production* 68: 794-796.

Tester M, Davenport R. 2003. Na^+ tolerance and Na^+ transport in higher plants. *Annals of Botany* 91: 503–5027.

Tripathi KD. 2001. Essentials Medical Pharmacology, 4th edition, Jaypee Brothers Medical Publishers (P) Ltd.

Tuse TA, Bidkar AA, Bhale SA, Patanka RD. 2011. In-vitro anthelmintic activity of areal roots of *Ficus* benghalensis. *International Journal of Pharmacological Research* 1: 10-13.

Uddin SJ, Shilpi JA, Barua J, Rouf R. 2005. Antinociceptive activity of *Ceriops decandra* leaf and pneumatophore. *Fitoterapia* 76: 261-263

Umamaheswara RV, Nagababu P. 2015. Pharmacological evaluation of *Ceriops decandra* (GRIFF.) ding hou stem extract. *International Journal of Recent Scientific Research* 6: 2783-2789.

@Khulna University

Vadlapudi V, Naidu KC. 2009. Bioefficiency on Mangrove plants *Lumintzera racemosa* and *B. gymnorhiza*. *Journal of Pharmacy Research* 2: 1591-1592.

Venkataraman S, Shanmugasundaram P. 2005. Anti-nociceptive activity of Hygrophila auriculata Heine. *African Journal of Traditional, Complementary and Alternative Medicines* 2: 62-69.

Wahidulla S, Bhattacherjee JJ. 2001. Fatty acids, sterols and hydrocarbons in the leaves from eleven species of mangrove. *Journal of Materials Science* 81: 485.

Walker CLF, Friberg IK, Binkin N, Young M, Walker N, Fontaine O, Weissman E, Gupta A, Black RE. 2011. Scaling up diarrhea prevention and treatment interventions: A lives saved tool analysis. *PLOS Medicine* 8: 33-39.

Walter H, Steiner M. 1936. Die Okologie der Ost-Afrikanischen Mangroven. *Zeitschrift für Botanik* 30: 65-193.

Wangensteen H, Alamgir M, Rajia S, Samuelsen AB, Malterud KE. 2005. Rotenoids and isoflavones from Sarcolobus globosus. *Planta Med.* 71(8): 754–758.

Wangensteen H, Alamgir M, Duong GM, Gronhaug TE, Samuelsen AB, Malterud KE. 2009. Chemical and biological studies of medicinal plants from the Sundarbans mangrove forest. *Advances in phytotherapy research* 1: 59-78.

Wangensteen H, Dang HC, Uddin SJ, Alamgir M, Malterud KE. 2009. Antioxidant and antimicrobial effects of the mangrove tree *Heritiera fomes*. *Natural Product Communications* 4: 371–376.

@Khulna University

Watt JM, Breyer-Brandwijk MG. 1962. The medicinal and poisonous plants of southern and eastern Africa. 2nd edition E. & S. Livingstone, Ltd., Edinburgh and London.

Weitzman SA, Gordon LI. 1990. Inflammation and Cancer, role of phagocyte generated oxidants in carcinogenesis. *Journal of Blood Medicine* 76(4): 655-663.

Wen LY, Yan XL, Sun FH, Fang YY, Yang MJ, Lou LJ. 2008. A randomized, double-blind, multicenter clinical trial on the efficacy of ivermectin against intestinal nematode infections in China. *Acta Tropica* 106: 190-194.

Whittle BA. 1964. The use of changes in capillary permeability in mice to distinguish between narcotic and nonnarcotic alalgesics. *British Journal of Pharmacology and Chemotherapy* 22: 246-253.

WHO Expert Committee (1987) Public Health Significance of Intestinal Parasitic Infections', Bulletin of the World Health Organization 65: 575-588.

WHO (1993) Research guidelines for evaluating the safety and efficacy of herbal medicines (Manila: Regional Office for Western Pacific).

Wong CC, Li HB, Cheng KW, Chen F. 2006. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chemistry* 97: 705-711.

Woolfe G, MacDonald AD. 1994. The evaluation of analgesic action of pethidine hydrochloride. *Journal of Pharmacology and Experimental Therapeutics* 80: 300-307.

@Khulna University