Screening of Anthelmintic Activity of *Heritiera fomes*

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

"Screening of Anthelmintic Activity of *Heritiera fomes*" submitted by Fahmida Haque Riya of spring, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on March, 2022.

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Ethics Statement

This study involves parasite or earthworms' trial.

Abstract

Heritiera fomes is a mangrove medicinal plant which is rich of ethnomedicinal usage against helminths. At present, it is found that *H. fomes* is a good reservoir of compounds such as phenols, tannin, alkaloids which have the possibility to be a better source to treat helminthiasis in living organism which is a great motivation to work on this research. The moto of this finding is to check in vitro anthelmintic activity of different parts (leaf, bark, root) of *H. fomes* on Bangladeshi earthworms *Pheretima posthuma*. Albendazole was used as standard drug to compare the test result. Anthelmintic activity was showed by three concentrations of *H. fomes* (25mg/ml, 50mg/ml and 75mg/ml) of each extract in a dose dependent inhibition. The results have been interpreted as paralysis time and death time of the earth worms. From the obtained result it was found that bark extracts of *H. fomes* exhibited significant anthelmintic activity at the concentration of 75mg/ml where the paralysis time and death time of the worms is 37.67±2.52 min and 47.67±3.51 min respectively, compared to the standard albendazole. This study encapsulates the significance of *H. fomes* in the use for anthelmintic activity and requirement further investigation for the isolation of active constituent which is responsible for the anthelmintic activity.

Keywords: *Heritiera fomes,* anthelmintic activity, Helminths, albendazole, Tween 20, *Pheretima posthuma*.

This work is dedicated to my Supervisor **Dr. Farhana Alam Ripa** and my friend **Nafisa Tabassum Mukta** for their constant support.

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List of Acronyms

THPs	Traditional Heath Practitioners
T and CM	Traditional healing and Complementary medicine
GABA	Gamma-aminobutyric acid
Glucl	Glutamate-gated chloride
DPPH	1,1-diphenyl-2-Picrylhydrazil
FRAP	Ferric Reducing Antioxidant Powder
EHFL	Ethanol extract of *H.fomes* leaves
EHFB	Ethanol extract of *H.fomes* Barks
EHFR	Ethanol extract of *H.fomes* Root
SEM	Standard Error of the Mean
ANOVA	Analysis of Variance
BE	Bark Extract
LE	Leaves Extract
RE	Root Extract
CNS	Central Nervous System
GIT	Gastrointestinal Tract
TAN	Titrate able Acid Number

Chapter- 01
Introduction

1. Introduction

1.1 General Introduction

From the very ancient phase finding healing power in plants and searching for agents to cure various ailments have always played significant part in the journey of discovering new drugs. From antiquity to till date, researchers are always in search of medicinal plants and herbs that can contribute to health care programs and can contribute to heal diseases and alleviate human sufferings. So, in this journey scientists discovered more than 80% medicines that are available as modern medicines and somehow those are directly or indirectly discovered from the medicinal plants. Besides this, according to a source, more than fifty thousand medicinal plants are distributed in 200 families throughout the flora of Bangladesh and up to 33 medicinal plants which are well known for their curative properties are using in the different rural areas as folk medicine nowadays. Apart from that, Sundari plant species are common in up to about 70% of forests. Crude drugs are extracted from many plants which are playing significant activities medicinal industry from the beginning as they have biochemically unique properties and a basis of new natural products because of having significant activities against the living things such as human immunodeficiency virus. However, medicinal plants are called “Herbology or ayurvedic supplements” because the word 'Medicinal Plant' contains a class of herbal plants and is recommended to treat various types of diseases. At present peoples who lives beside the forest areas are totally and completely dependent on plants from mangroves forest for their livelihood as well as their primary healthcare which is known as local traditional health practitioners (THPs). (Mahmud et al., 2014) WHO (World Health Organization) recently published that more than 80% of people around the world directly depend on herbal medicines as their first medicinal uses to treat any diseases. In addition, according to WHO, till now almost about 21000 plant species are introduced and used as medicinal herbs. (Bynum & Porter, 2008). Apart from these numbers and percentages, it was clear in my research that nowadays more

than three-quarters of its global population is directly rely on natural compounds for their health care routines more because those plants have many more medicinal properties such as antioxidant effect, anti-diarrhetic properties, they can also use in skin disease, etc rather than using the synthetic compounds as those synthetic compounds have many side effects.

1.2 Classification of Medicinal Plant:

Medicinal plants have several varieties and based on this aspect medicinal plants can be classified based on their uses, according to their life cycle, according to their active components, botanical classification of Medicinal and Aromatic Plants, and according to their nature of the products. Those are given below-

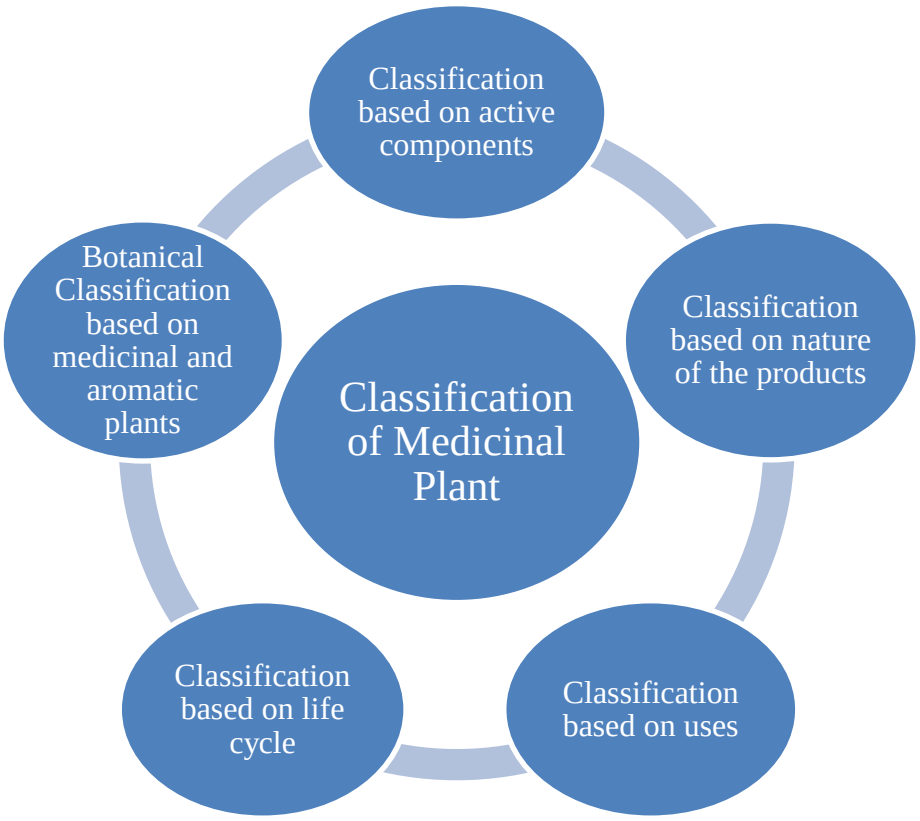

Figure 1: Classification of Medical Plant

1.3 Traditional Medicines of Bangladesh

In Bangladesh, Traditional healing and corresponding medicine is extensively cultured as the primary healthcare in rural areas of Bangladesh among the people with low socioeconomic status due to its quality, safety, and efficacy contributes to reaching the intention of making sure that all of us have to get entry to health care properly. There are two types of traditional healing practices are used in Bangladesh which is folk medicine based on locally-available substances, cultural practices, and religious rites, and the second one is Ayurvedic and Unani systems which are based on the scientific use of pharmaceutical methods and technology. However, according to a study, it was found that only 20–25% of the people have access to modern healthcare facilities, and the remaining 75–80% of the rural population are still dependent on T&CM which is holding a large portion of the healthcare sector in Bangladesh compare to modern healthcare facilities because of its quick effectivity, and their long-held belief on the effectiveness of the traditional medicines. (Haque et al., 2018) Another study found that In Bangladesh, more than 1600 villages and there are greater than 86,000 doctors are present who are called Kaviraj, this means that there are around 2/3 Kavirajes for every 500-800 persons and the most interesting fact is those use different medicinal plants and their parts to treat patients. Those parts of the plants must have gone through the process of maceration or crushing. After that, the juices of the part of plants are collected and processed to assist as the medicine, that will finally be administered orally, topically, apply to the diseased region for treatment (Md. Shahadat Hossan, 2010). Apart from this, one medicinal plant can be used to treat multiple conditions because of its different activities. But those medicines are produced on small scales because the combination of plants or plants parts can give a synergistic effect on the treatment process. (Mohammed Rahmatullah, 2009)

Furthermore, there are huge numbers of plants are present in Bangladesh that used for a different type of diseases either naturally or its synthetic form. Some of them are given below-

- **Ada (Ginger):** The scientific name of Ada is *Zingiber officinale*. The Rhizome of Ada (Ginger) has many medicinal values and it is used for therapeutic purposes like common cold or cough by cutting into pieces and then those pieces are simmered as tea or drunk with lemon juice and salt. Also, it is used as a treatment for rheumatism, arthritis, ulcer, atherosclerosis, hypertension, etc. It prevents the release of serotonin which assist in halting vomiting. It is the most common in the east region and the most frequently used medicinal plant in Bangladesh.
- **Arjun:** The scientific name of Arjun is *Terminalia arjuna*. The Bark of Arjun is used in medicinal treatment like hypertension and heart disease. Usually, it is soaked in water and drunk regularly.
- **Basak:** The scientific name of basak is *Adhatoda vasica*. The leaf of basak is used to treat cough, phlegm congestion during cold by macerating its leaves.
- **Bel:** The scientific name of bel is *Aegle marmelos*. It is the most commonly used fruit in Bangladesh which is orally taken to treat heatstroke in summer.
- **Chirota:** The scientific name of chirota is *Swertia chirata*. Chirota is a well-known plant to treat diseases like gastric pain, diabetes, liver disease, fever. Here, the whole plant part has medicinal values and therapeutic uses.
- **Roshun (Garlic):** The scientific name of roshum is *Allium sativum*. The Bulb of roshun is used to treat heart disease, flatulence, troubles in urinating because it contains a high amount of sulfur. In addition, it is also known for the help to improve resistance to disease and maintain proper blood pressure. At present, garlic is also used to maintain cholesterol level, acts as an anti-viral, reduce glucose level in the blood, and is also used for fungal infection.

- **Piaj (Onion):** The scientific name of piaj is *Allium ceipa*. The Bulb of piaj has medicinal values such as phlegm congestion in cold and treatment of hair loss. Apart from this, it helps as tissue building agent, menstrual diseases, cardiac tonic as well as antiseptic and aphrodisiac.
- **Holud (Termeric):** The scientific name of holud is *Curcuma longa*. The rhizome part of holud is used to treat skin dullness and wounds in livestock. It contains carotene, calcium, phosphorus, thiamine, iron, and niacin which gives antiseptic activity. Often it is advised as an activating agent to treat gastrointestinal tract diseases.
- **Narkel (Coconut):** The scientific name of narkel is *Cocos nucifera*. The Fresh juice and fruits of narkel have medicinal values and it is used to treat burning in urinating, heatstroke, diarrhea, dysentery.
- **Neem:** The scientific name of neem is *Azadirachta indica*. The Leaf part of the Neem remedies scabies, eczema, skin diseases, and diabetes.

(Chowdhury et al., 2009)

Those are the list of some commonly used plants and plants part in the ear of medicine. In addition to these, there are many more medicinal plants are present in Bangladesh that has been used for ages for the purpose of treatment and human wellbeing.

1.4 Anthelmintic activity of plants

The word “Helminth” is derived from the Greek word “Helminths” which means “worm”. It is a group of eukaryotic organisms which refers to the different types of parasites worm that lives in our body and infects the liver and hepatobiliary system. So, Anthelmintics drugs are used to treat helminths infection. These drugs are the class of anti-parasitic drugs that destroy parasitic worms (helminths) without causing any damage to the host cell. It is also known as vermifuges (those that stun) or vermicides (those that kill) parasites. So, people that are infected with helminths (a condition known as helminthiasis) are treated with anthelmintics drugs. According to WHO, about two billion people are experiencing parasitic worm infections and it is anticipated that by the year 2025, in developing countries about 57% of the population will be infected with helminths. However, people who carry heavy parasite burdens inside their bodies if treatments are not provided timely to them then they can be sick anytime and there is a possibility to immortalize the infection within their community. (Salhan et al., 2002 .) Even after having this much severity, the discovery of anthelmintic drugs in the pharmaceutical industry is very poor because the people and nations who are most likely to suffer from this disease have little money to invest in the drug discovery or therapy of anthelmintic drugs. However, the first discovered anthelmintic drug for human treatment was recognized as veterinary medicines. (Holden-Dye & Walker, 2007) For this reason, plants and extracts from plant parts have significant importance in the discovery of anthelmintic drugs. From 20th century, medicinal plants and researches on the chemistry and pharmacology of these extracts played a major role in the discovery of bioactive constituents such as alkaloids, flavonoids, tannins, and phenolic compounds, etc. (Kundu et al., 2014)

However, Anthelmintics are the drugs that destroy parasitic worms from GIT (Gastrointestinal Tract) and eliminate adult or developing helminths that attack organs and tissue by acting locally. Many plants and their extract play an important role in the treatment of those parasites’ infections which act as natural anthelmintics such as tobacco, walnut, wormwood, clove,

kalonji seeds, garlic, male fern, pineapple, diatomaceous earth, soya, and other legumes and honey, water, vinegar. These mixed together with warm water can act as vermifuges. (Yadav & Singh, 2011)

Apart from this, there is a disadvantage to the anthelmintic drug which is the majority of the drugs that use to treat helminths has limited action, for example- praziquantel is used to treat schistosomiasis which acts by destroying the calcium homeostasis in the human body but it has no activity against nematodes.

Depending on the increasing mobility and mortality helminths can be classified into three categories:

- **Nematodes (roundworms):** These are bisexual and they infect humans by inhabiting intestinal and extraintestinal sites.
- **Trematodes (flukes):** These are leaf-shaped flatworms that grows from immature parasite larvae in human host upon ingestion of raw watercress and water plants. Then these larvae travel through the intestinal wall, abdominal cavity, and liver tissue into the ducts where they lay eggs and cause diseases.
- **Cestodes (tapeworms):** These are elongated, segmented, hermaphroditic flatworms which infect humans by inhabiting the intestinal lumen.

  (Castro, 2022)

1.4.1 List of some plants with Anthelmintic Activity:

Many kinds of research and reviews are conducted and still conducted on plants groups to find out new possible anthelmintic molecule and to determine their possible mechanism of action by applying various screening procedures. From this, a list of plants and their chemical constituents come out which have great anthelmintic activity. From those, some of the plants are listed below-

a) ***Ocimum sanctum Linn.*** **(Family-Lamiaceae):** This is generally known as *Sacred Basil* (Tulsi). The main component of this plant is a volatile oil that has Eugenol (about 51%), β-caryophyllene (37%), and many sesquiterpenes and monoterpenes. Here, these essential oils and Eugenol proved their anthelmintic activity in vitro against *Caenorhabditis elegans* (Nematode). However, the finding research suggested that Eugenol has a putative anthelmintic principle.

Figure 2: Ocimum sanctum Linn (Ravindra et al.,2008)

b) ***Piliostigma thonningii*** **(Schum.) Milne-Redh. (Family-Caesalpiniaceae):** Stem bark of this plant has been proven to treat dysentery, snakebite, toothache, and as an anthelmintic. Ethanol extract of this plant was examined for its anthelmintic activity. It has been observed that a potent dose of plant extract exhibits the activity against *Ascardia galli*, by stimulating the neuromuscular junction principally and the ganglion to a lesser degree. Apart from this, the D-3-O-Methylchiroinositol compound that is singled out from methanolic extract of stem bark proven to have anthelmintic activity.

Figure 3: Piliostigma thonningii (Ranvindra et al., 2008)

c. *Punica granatum Linn*. (Family-Punicaceae): Locally it is known as Anar. Astringent and anthelmintic properties are found in the root and stem bark of this plant. After analyzing the stated anthelmintic activity was found from the alcoholic extract of its stem bark. From the experiment, it was found that the activity of this plant extract is dose-dependent, prevent changes of eggs into filariform larvae of *Haemonchus contortus*. Apart from this, clinically the plant showed significant efficacy against nematodes in calves. However, bioactive compounds such as alkaloids and pellitorine were found in the stem bark of the plant.

Figure 4: Punica granatum Linn. (Ranvindra et al., 2008)

1.4.2 Plant Extracts as a Source of Anthelmintics Compounds:

Natural products are a good source to treat human diseases from the very beginning. The World Health Organization (WHO) estimated that two-thirds of the world's population rely on plants for the treatment of their health issues as primary agents. (Dilrukshi Jayawardene et al., 2021) However, plants produce secondary metabolites that helps with plant immunity like phytochemical terpenes, condensed tannins and flavonoids, and also gives good anthelmintic properties and prominent relationship in control of helminth. (Lanusse et al., 2018) Those natural compounds show promising activity as anthelmintics to treat helminths such as-

- **Terpenes:** Terpenes are the most plentiful group of plant volatiles that inhibit biological targets such as acetylcholinesterase (Ach), GABA (gamma amminobutryic acid), and tyramine receptors. It also interacts with glutamate-gated chloride channels and P-gp.
- **Condensed tannins:** These condensed tannins give anthelmintic effect on nematode by the formation of tannin-protein complexes directly. On the other hand, indirectly it enhances the nutritional status and host's immune response against infection.
- **Flavonoids:** Flavonoids act as p-gp modulators that are the widely distributed phenolic compounds and have high activity against *H. contortus* in vitro. It is found that flavonoids may have great potential in the advancement of new compounds or to detect modulator agents which are capable to extend the duration of existing anthelmintics.

(Lanusse et al., 2018)

To find out the anthelmintic activity of plants, three different screening techniques are developed now which are: animal-based screening, target-based screening, and nematode phenotypic screening (whole organism-based). Apart from this, two approaches are developed to identify active molecules of plant extract as a major new resource for drug discovery which is forward pharmacological approach and reverse pharmacological approach.

The process of two approaches is-

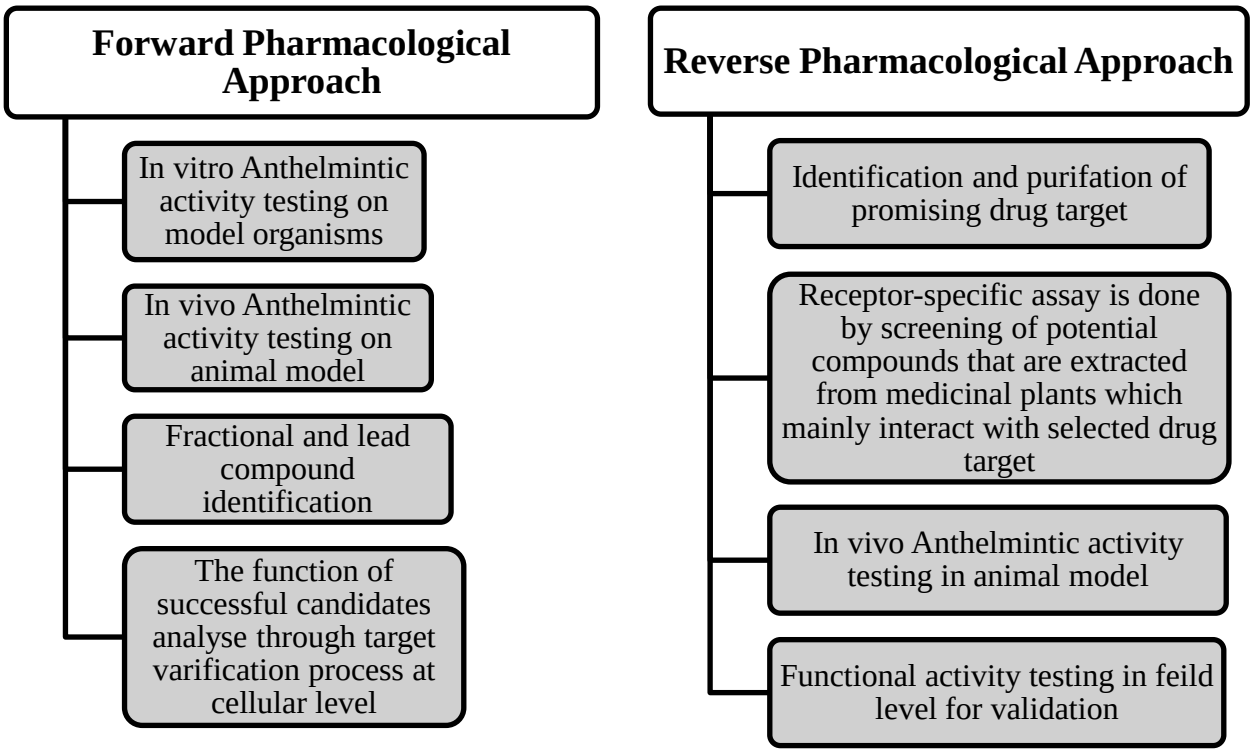

Figure 5: Approaches to identify active molecules of plant extract (Dilrukshi Jayawardene er al., 2021)

From these two approaches, the reverse pharmacological approach has an advantage over the forward pharmacological approach because it requires fewer animal experiments to discover new potential drugs compounds compared to the use of complex drug compounds. It is also saved time and gives good in vivo efficacy. (Dilrukshi Jayawardene et al., 2021)

1.4.3 Mechanism of action of phytoconstituents of medicinal plants

From the abundant phytoconstituents phenols, tannins and alkaloids shows significant anthelmintic activity which's mechanism and action given below-

Phenols: Phenols gives anthelmintic effect by interfering with energy generation in helminth parasites by uncoupling oxidative phosphorylation and may causes death of earth worm.

Tannins: Tannins gives anthelmintic activity by bonding to free proteins in the GIT of host animal or glycoprotein on the cuticle of the parasite and may cause death.

Alkaloids: Alkaloids gives anthelmintic activity by interfering with CNS or interfering with homeostatic and may cause paralysis or death.

(VVM Kumar et al., 2012)

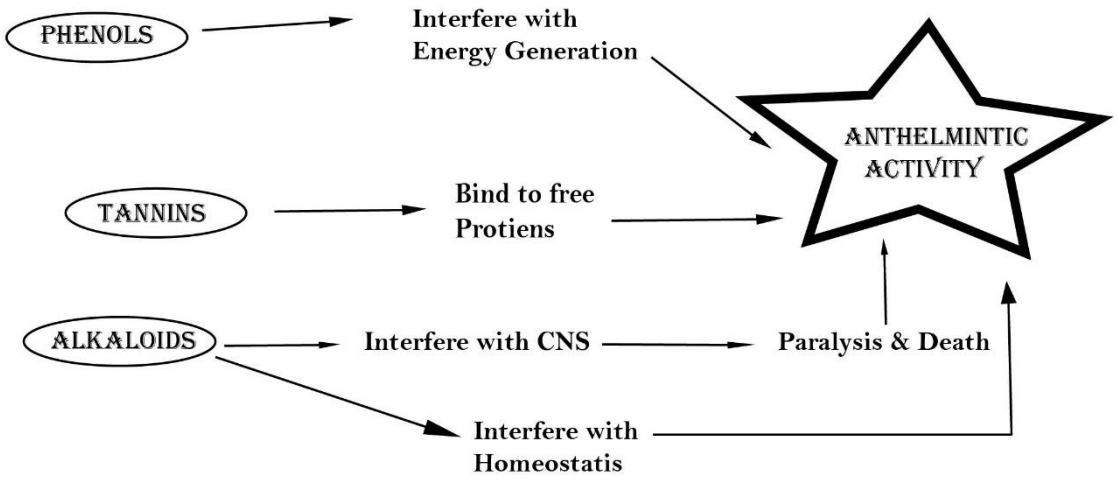

Figure 6: Mechanism of the phytoconstituents of medicinal plants (VVM Kumar et al, 2021)

1.4.4 Modern Anthelmintic compounds

A various number of Anthelmintics are discovered to treat Helminths. Those anthelmintics are characterized by their binding or manipulating the unique target site, especially in the presence of the parasites.

Table 1: Example of Anthelmintic compounds with their mood of action (Martin, 1997)

Examples of Anthelmintic compounds	**Mode of action**
Tetrahydropyrimidines, Imidazothiazoles Levamisole, butamisole, Bephenium, Thenium, Methyridine	Those are nicotinic agonists which act by targeting the nervous system of parasites.
Haloxon dichlorvos	It works as an acetylcholinesterase inhibitor by stopping the breakdown of acetylcholine.
Piperazine (mostly as citrate salt)	It is Gamma-aminobutyric acid (GABA) agonist (T-amino-bu-ric acid) which is found on the receptors of nematode muscles and causes flaccid paralysis. (Martin, 1997)
Diethylcarbamazine citrate	It inhibits arachidonic acid metabolism by stimulating innate immunity.
Praziquantel	It increases the permeability of the cell membrane permeability by blocking the calcium channel which ultimately increases calcium influx and leads to muscular Contracture.
Benzimidazoles	Those drugs act by targeting microtubules of the cytoskeleton. They block the transport of secretory granules and movements of another subcellular organelle.
Diamphenethide	It inhibits malate metabolism by deacetylating the host liver and activating monoamine and diamine. (Martin et al., 1997)
Salicylanilides: closantel, rafoxanide, oxyclozanide, brolianide and substituted phenols: nitroxynil, niclopholan, hexachorophene dibromsalan and niclosamide	Those are bioenergetics and proton ionophores which are act as oxidative phosphorylase uncouplers. It gives a therapeutic effect by binding with high plasma protein. (Martin et al., 1997)
Ivermectin; Abamectin; Doramectin; Moxidectin; Milbemycin D	It acts as Glutamate-gated chloride (GluCl) potentiators and ion channels by increasing muscle CI- permeability. (Martin, 1997)
Clorsulon	It acts by inhibiting phosphoglycerate kinase and mutase and bind with ATP and 3-phosphoglycerate to the phosphoglycerate kinase. (Martin, 1997)

1.5 Classification of Anthelmintic Compounds

Based on the chemical structure Anthelmintics are classified into:

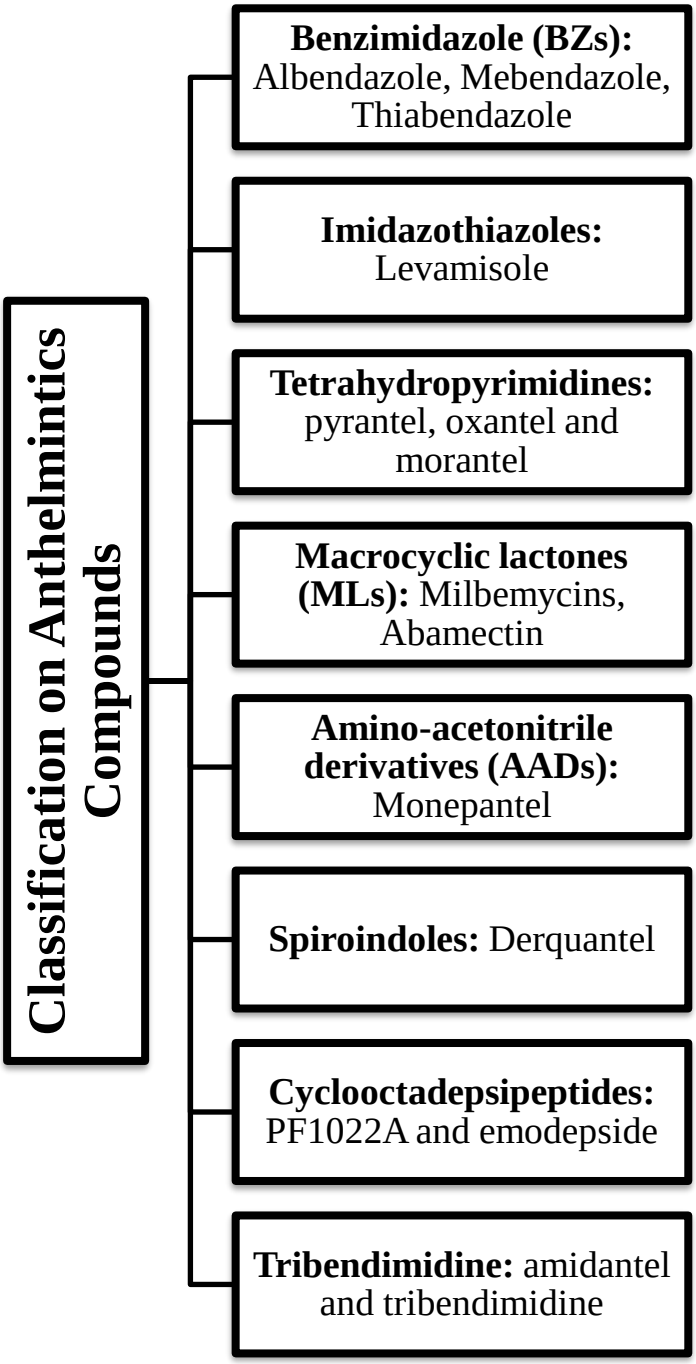

Figure 7: Classification of Anthelmintic compounds (Abongwa et al., 2017)

1.6 Description of *H. fomes*

The *Heritiera fomes* is the most well-known mangrove tree which is found in Sunderbans located at the southern portion of the Gangetic delta bordering the Bay of Benga. *H. fomes* is commonly known as Sundari. (Abdur Rahman & Abdur Rahman, 1996) It is the most dominated species in the Sundarbans which is spread up to about 70 percent of the forest. Different therapeutic activity for diseases like diabetes, skin diseases, cardiovascular diseases, liver diseases is found from different parts of *H. fomes*. Sundarban is the world's largest single mangrove forest which extends throughout Bangladesh and the Indian State of West Bengal. This plant is an evergreen tree found in coastal regions of countries like India, Myanmar, Thailand, and Northern Malaysia. It grows in the regions with heavy annual rainfall of 1600-5334 mm and also it requires a warm unruffled climate with temperatures between 7.22°C to 37.78°C. However, a short description of the various parts of plants are given below (Halder, Ghosh Chatterji, and Sanyal, 2014)-

- **Tree:** The length of the trees is up to 20 m tall, trunk about 50 cm in diameter at the base. The shape of the tree is like buttressed, the color of the trees is like blaze dark-red and the young branches are covered with shining golden-brown scales.
- **Leaves:** The leaves size of the trees are 5-12 x 3-6 cm, shaped like elliptic, the upper surface of leaves is green, the lower surface of the leaves is shining with silvery scales, and tapering at both ends.
- **Root:** The structure of the roots is like pneumatophores which help the plant to grow out from the water surface and facilitate the aeration for root respiration. It also blinded with root sucker.
- **Flower:** The flowers of *H. fomes* are like auxiliary panicles, unisexual, heavily pubescent, and golden-yellow with a reddish tingle inside. Flowers of Sundori tree mainly grow in March and April. (Aslam Hossain et al., 2013)
- **Fruit:** The size of ripe carpels is 3 to 4 cm across, sub-globose, corrugated, woody,

indehiscent, furrowed on the inside, and less prominently winged on the outer side. The fruits of this tree mainly ripen in July and August. (Aslam Hossain et al., 2013)

- **Bark:** The bark of the Sundori tree is splinter and it also has dark green leaves which are grouped near the ends of twigs. (Aslam Hossain et al., 2013)
- **Seeds:** The seeds of the Sundori tree ripen in June and July.

Figure 8: Leaf of H. fomes (Mahmud et al., 2014)

Figure 9: Root of H. fomes (Mahmud et al., 2014)

Figure 10: Bark of H. fomes (Mahmud et al., 2014)

1.6.1 Scientifically classification of *H. fomes* (Vanden Berghe, 2014)

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Malvales

Family: Malvaceae

Genus: Heritiera

Species: *H. fomes*

1.6.2 Synonyms and Different names:

H. fomes is had some different names in different places. They are:

Synonyms: *Heritiera minor Roxb.*

Traditional Name: sunder, sundri, jekanazo, and pinlekanazo.

1.6.3 Geographical Location of *H. fomes*

H. fomes is mainly found in coastal forests. The field ranges are mainly started from the Eastern coast of India, Bangladesh, and Malaysia to Myanmar and Thailand and are native to marshy regions in The Indo-Pacific. In Bangladesh, the tree is found in the Sundarbans and West Bengal, in the Ganges-Brahmaputra Delta, on the coasts of Bangladesh's Chittagong, and in Arakan (an area noticed in Myanmar) (M. N. I. Khan et al., 2020a). In Sundarbans, the plant is mainly found in numerously in the Sundarbans freshwater area and spread throughout the forest mainly in lower or heavy saltwater. The *H. fomes* top grows properly in direct sunlight and even in full shade in the forest. On the other hand, the plasticity of leaf grows indifferent in ambient light at different canopy levels which is a notable property of the tree. However, in saline conditions, it grows in less quantity and blot soil is rarely deluged by the tide compared to the

other plants of mangrove forest. In addition, it is the first species in ecosystems that can also be cultivated on artificial soils and are distributed on the low banks that form around the corners of newly emerged saucer-shaped islands (Khan et al., 2020). Another feature of this plant is it grows best far away from the high strips. This species does not cause flooding (Siddiqi, 2020) and it is restricted as a restrictive moderate to heavy submersion (Roxb, 2009). This plant can't grow in tropical where water hibernates or on high soils that never overflow (Roxb, 2009). The undergrowth is missing from the thick timberlands of *H. fomes*. Under mild cover, normal reclamation appears more effective than when the shade appears to be as well available. (Khan et al., 2020)

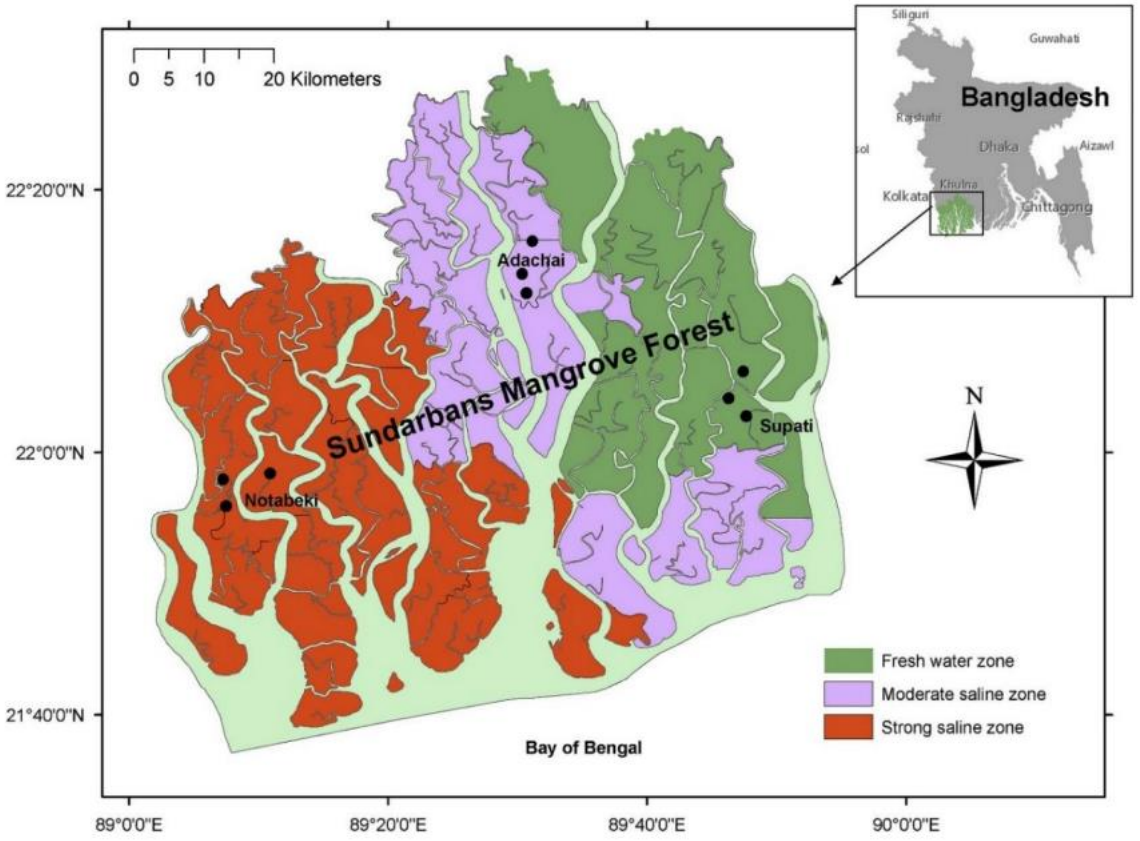

Figure 11: Loaction of H. fomes in bangladesh (Roxb, 2009)

Figure 12: H. fomes in sundarban (Mominul Islam et al., 2018)

1.6.4 Therapeutic uses of the plant:

Therapeutic uses of plants refer to the study of the plant where the study of medicinal plants is used for drug development either pharmacopeial, non- pharmacopeial or synthetic drugs. Different parts of the different plants play a critical role in the development of medicine for the welfare of mankind all over the whole world.

Different parts of *H. fomes* is an ideal plant because of having many pharmacological properties. Barks, leaves, stems of *H. fomes* are commonly used by rural people because of their medicinal property to treat several diseases. However, the therapeutic uses of H. fomes are given below:

- Different parts of *H. fomes* that use in the treatment of different diseases are given below:

Table 2: Medicinal use of different plant parts (Mahmud er al., 2014)

Parts of the plant	**Medicinal uses**	**Mode of preparation**
1. Leafs and Seeds	Use in the treatment of digestive disorders, loss of appetite, bloating	Decoction
2. Timber	Use to treat rectal diseases such as piles	Powder
3. Stem bark	Use to treat skin diseases	Paste
4. Bark	Diabetes and goiter	Hot decoction
5. Twig	Toothache, oral infection	Toothbrush

- *H. fomes* is also well known for its antioxidant, antinociceptive, antihyperglycemic, antimicrobial, and anticancer activities.
- It is also useful to treat cardiac diseases.

(Mahmud et al., 2014)

1.6.5 Phytochemical Constituents and Bioactive reagents of *H. fomes*

H. fomes comprises 0.25% chlorophyll a, 0.09% chlorophyll b, 0.11% carotenoids, 39.45% polyphenols, 21.12% tannins, and titrate able acid number (TAN) which is 34.50. Existing reducing sugars such as saponins, alkaloids, glycosides, tannins, steroids, flavonoids, and gums were determined by phytochemical exploration of leaves extract. Here, the protein content of the leaf is 29.22%. Besides, the bark of *H.fomes* embodies 7-36% tannin and a high amount of proanthocyanins. Following their structures, there are two types of tannins groups are acknowledged which are-

1. water-soluble tannins (hydrolyzable) and

2. proanthocyanidins (condensed tannins).

Procyanidins are present abundantly in stem bark. Trimeric, pen turmeric, and hexametric procyanidins have been derived and determined from the plant. The NMR spectroscopy of $CHCl_3$ extract of the *H. fomes* also revealed β- Sitosterol, stigmasterol, and stigmast-4-en 3-one. (Mahmud et al. 2014)

Table 3: Still now discovered phytochemical constituents of H. fomes (Mahmud et al., 2014)

Plant Parts that have chemical constituents	**Discovered Phytochemical constituents**
1. Leaf of *H. fomes* in percentage (%)	Chlorophyll a- 0.25, chlorophyll b- 0.09, carotenoids- 0.11, polyphenols- 39.45, tannins- 21.12, proteins- 29.22
2. Phytochemical compounds of leaf from the extract	Sugars, alkaloids, glycosides, tannins, steroids, flavonoids
3. Bark	About 7-36% percent of tannin, a good amount of proanthocyanidins
4. The stem bark extract of the sample	Trimeric, pentameric and hexameric procyanidins
5. Obtained NMR spectroscopy of CHCl3 extract	β-Sitosterol, stigmasterol, and stigmast-4-en-3-one

Chemical structures of some phytochemical compounds that have anthelmintic activity are derived by *H. fomes* is demonstrated below:

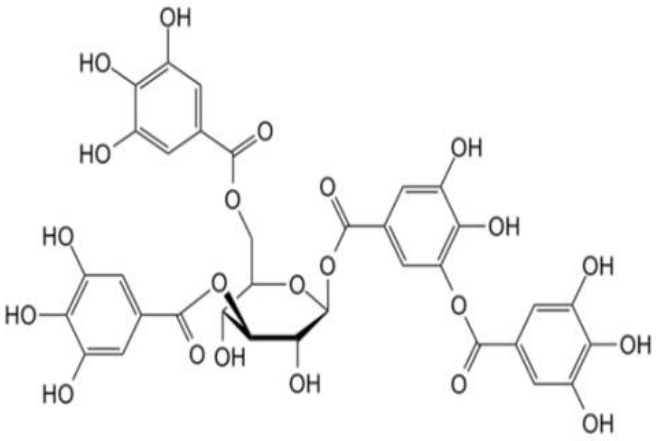

Figure 13: Tannin (Tannic acid) (Drabble & Nierenstein, 1907)

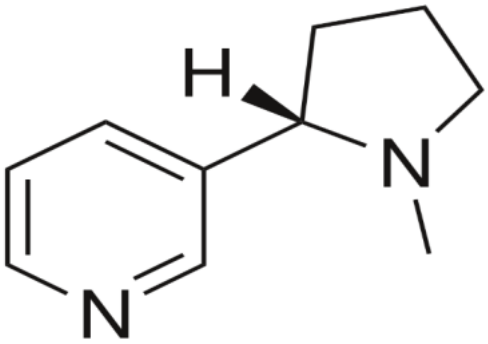

Figure 14: Alkaloid (Peng et al., 2019)

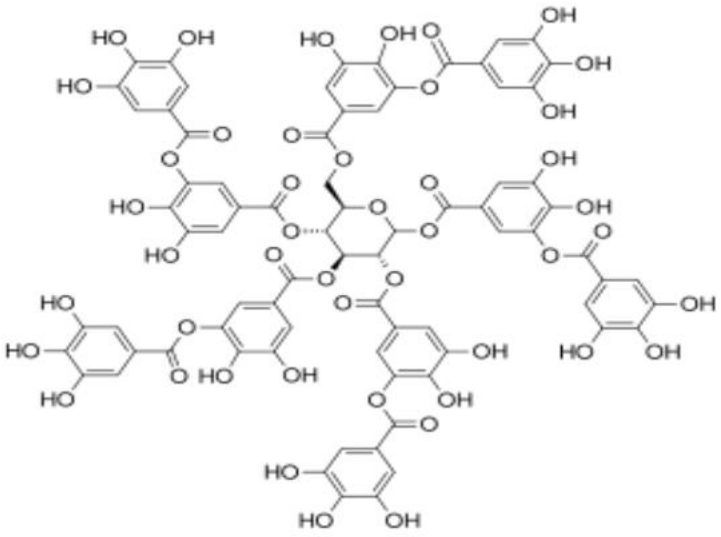

Figure 15: Tannic acid, plant-drived polyphenol (Rasouli, Farzaei & Khodarahmi, 2017)

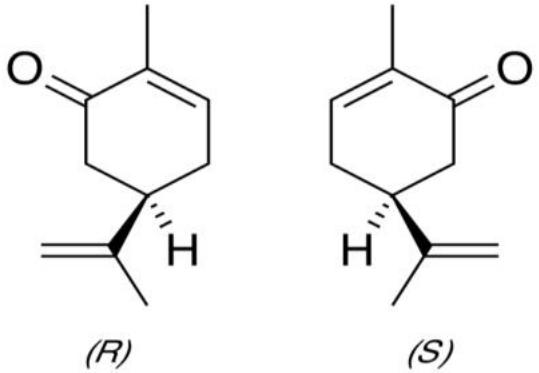

Figure 16: Monoterpenoid (Booth & Bohlmann, 2019)

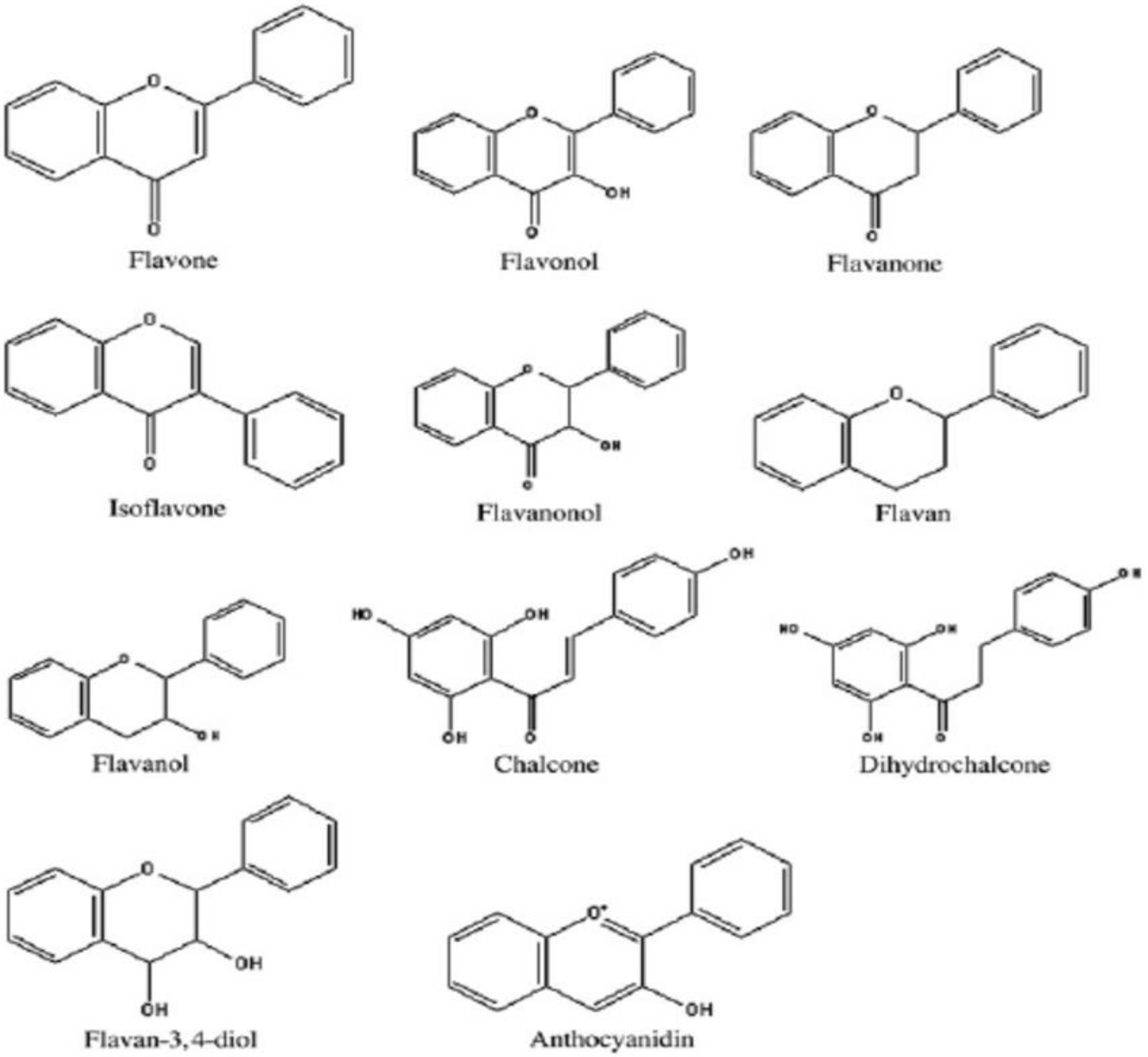

Figure 17: Flavonoids (Mulherjee et al.,2015)

1.6.6 Plant part utilized in the study

For this study, extract of leaves, root, and stem bark of *H. fomes* was used for pharmacological investigation.

1.7 Literature Review

Studies on the plant root, stem bark and leaves were performed before and the reports are given below:

- **Aslam Hossain et al., 2013:** *H. fomes* leaves ethanol extract has been reported to contain anti-nociceptive, antioxidant and analgesic activities where thermal nociception was evaluated by hot plate model, antioxidant activity was evaluated by DPPH assay, and antimicrobial activity was assessed by disc diffusion assay method.
- **Islam et al., 2019:** Ethanolic extract of dried and grinded plant leaves and bark was performed and it shows the antioxidant and anti-inflammatory activity of *H. fomes*. 1, 1-diphenyl-2-picrylhydrazil (DPPH) free radical scavenging assay, ferric reducing antioxidant power (FRAP), and β-carotene bleaching inhibition assay was carried out to confirm the antioxidant activity of the plant. On the other hand, in vitro human RBC membrane-stabilizing assay, and in vivo mice paw edema test was done to observe the anti-inflammatory activity of *H. fomes*. However, the outcomes of all the assays and tests proved that the bark extract of *H. fomes* has significant antioxidant and anti-inflammatory activity.
- **Ali et al., 2011:** Methanol extract of barks of the plant has been reported to have antihyperglycemic activity in oral glucose tolerance tests in glucose-loaded Swiss albino mice, and also reported to contain antinociceptive activity in acetic acid-induced gastric pain writhing in the same mouse model. However, the result of the experiment showed good antihyperglycemic (more potent than glibenclamide to treat diabetes when it was administered at the fixed doses that are mentioned in the article) and

antinociceptive activities which validated the plant part in the treatment of diabetes and pain.

- **Nurunnabi et al., 2020:** This study was conducted to determine the antimicrobial activity of *H. fomes* by isolating and identifying endophytic fungi from the methanol extract leaves, roots, and bark of the plant. Here, in the study, it was stated that among fifty-five strains of endophytic fungi *Pseudopestalotiopsis camelliae-sinensis*, *Pestalotiopsis microspora,* and *Penicillium copticola* were the most active endophytic fungal strains that showed antimicrobial activity against the microorganisms under investigation.
- **Salekeen et al., 2020:** Biochemical analyses of *H. fomes* has been reported a large number of bioactive phytochemicals which act as multi-enzyme inhibition in the arachidonic acid metabolic network by distracting enzymes of a complex inflammatory network. This study opened the door for the future laboratory and clinical testing of anti-inflammatory potentials of *H. fomes* as an exploitable source of safe and potent drug-like molecules.
- **Sarker et al., 2018:** The investigation of the study has established that methanol extract of the leaves (LE) and aerial root (AR) extracts of the plant has antihyperlipidemic, antiatherosclerosis, and cardio-protective activities. This experiment was conducted on alloxan-induced diabetic Sprague Dawley Rats.
- **Patra et al., 2015:** All four solvent (acetone, ethanol, methanol, and aqueous) extracts of the plant parts (leaves and stem powder) indicated antibacterial activity and antioxidant properties of *H. fomes* in the study. However, the phytochemical screening of the extracts has also demonstrated the presence of bioactive compounds which are phenols, cardiac glycosides, terpenoids, tannin, steroids, flavonoids, protein and amino acids, etc.

- **Mahmud et al., 2014:** reported that the extraction of various parts of a plant has several chemicals such as alkaloids, tannins, glycosides, protein, flavonoids, etc which has various pharmacological properties such as anticancer, antidiabetic, anti-nociceptive, antioxidant, anti-obesity, antimicrobial, anti-diarrhea, and anti-MRSA.

1.8 Rationale of the Project:

In Bangladesh, *H. fomes* is the most extensively occurring plant that has been used for several years for its medicinal properties to treat various kinds of diseases such as diabetes, skin disorder and digestive disorder. Till now a lot of chemical constituents were discovered by the qualitative phytochemical test which is glycosides, alkaloid, steroid, tannin, saponin, resin, terpenoids, and carbohydrates. All constituents are important as they play a key role in drug manufacturing and drug discovery.

1.9 Aim of the Project:

This study was performed to investigate in vitro anthelmintic property of *H. fomes*.

1.10 Objective of the project:

In recent decades, natural products including medicinal plants have obtained major attention in the pharmaceutical research field because of their availability for drug discovery. Nowadays, several pharmaceutical lead molecules are derived from plants because they are cheap in cost of treatment, less toxic, and have fewer side effects compared to synthetic drugs. Apart from this herb medicines are affordable, safe, and culturally acceptable. And for this reason, herbal medicines took all the attention worldwide to the development of the herbal drugs industry.

After performing this experiment, it was confirmed that our experimental plant contains bioactive compounds. We investigate the root, leaves, and stem bark of the plant parts and it already has a lot of medical values in the field of herbal treatment.

However, the main objective of this project is to determine the anthelmintic property of *H. fomes*. And the current investigation was done to isolate anthelmintic activity.

1.11 Present Study Protocol:

This study was conducted to examine the medicinal effect of different extracts of *H. fomes*. The study protocol is needed to describe how to execute the trial. The study protocols are given below:

i. Preparation of *H. fomes* leaves, root, and stem bark extract.

ii. Screening of Anthelmintic activity of crude extracts on Bangladeshi Earthworms (*Pheretima posthuman*).

Chapter- 02
Method & Materials

2. Method & Materials

2.1 Preparation of *H. fomes* Extracts

2.1.1 Collection and identification:

Fresh leaves, bark, and root of *H. fomes* were chosen for the pharmacological investigation. Those were collected from Sundarban, Bagerhat district Bangladesh in December 2019. Identification and authentication of *H. fomes* was done by a taxonomist of Bangladesh Herbarium, Mirpur, Dhaka (DACB Accession No: 50664) and conserved in their laboratory for future investigation.

2.1.2 Preparation of Plant samples:

The experimental plant parts- leaf, root and bark were cleaned with tap water simultaneously to remove all the dirt and then it was again washed with distilled water for sterilizing. Then the leaves, roots, and stems were separated carefully and shade dried for two weeks. After two weeks when the plant parts were dried properly, dried leaves, roots, and stems were pulverized into coarse powder by a laboratory electric blender. After that, the grinder of plant parts was separated and cleaned properly to avoid contamination with previous ground material. Finally, the powder of plant parts was stored separately in airtight containers where the place should have less light, cool, and dry for further analysis.

2.1.3 Extraction of powdered samples:

Three glass jars were taken and washed properly. It was washed with ethanol again and dried. Then 250gm of powder of each sample (leaves, roots, and barks) was weighed and put into the jar. Then 1L ethanol was poured into each of the jars and sealed with its content properly so that air can’t pass through the container. Those three jars were kept for 7 days (a week) at 22-25 ^{o}c which is normal room temperature. Occasional it was shaking and stirring to get better extraction. However, after some days two layers were formed where the upper layer contained ethanol solution, and lower layer contained sedimentation of the plant extract which is the

ethanol-soaked double layer sample solution.

Figure 18: Mixture of three plant extract and ethanol in three different containers

2.1.4 Fractionation of the Extracts:

For fractionation extraction, by using clean cotton *H. fames* extracts were filtered coarsely and the sediments were removed carefully. After that, attained sample solutions were filtered again by using filter paper. Then the filtrated parts were going through fractional separation and three different types of extract which were an extract of leaves, root, and bark were produced. Here the solvent is ethanol. After completing the filtration, the filtrates were collected and split into two portions. This was then concentrated using a rotary evaporator (Heidolph) at 100 rpm. Again, at room temperature, the filtrate was evaporated and placed separately.

After fractional extraction was completed, solvents were evaporated to collect the experimental plant parts into three separate Petri-dishes. Lastly, the extracts were taken into different vials and marked as ethanolic crude extract of *H. fomes* leaves, barks, roots which were written as EHFR, EHFB, and EHFL respectively.

2.1.5 Methodology diagram:

Here, the total extraction process of *H. fomes* was mainly done in three steps which were-

- Collection of plant materials
- Drying
- Extraction

The whole extraction process is given below in a short form-

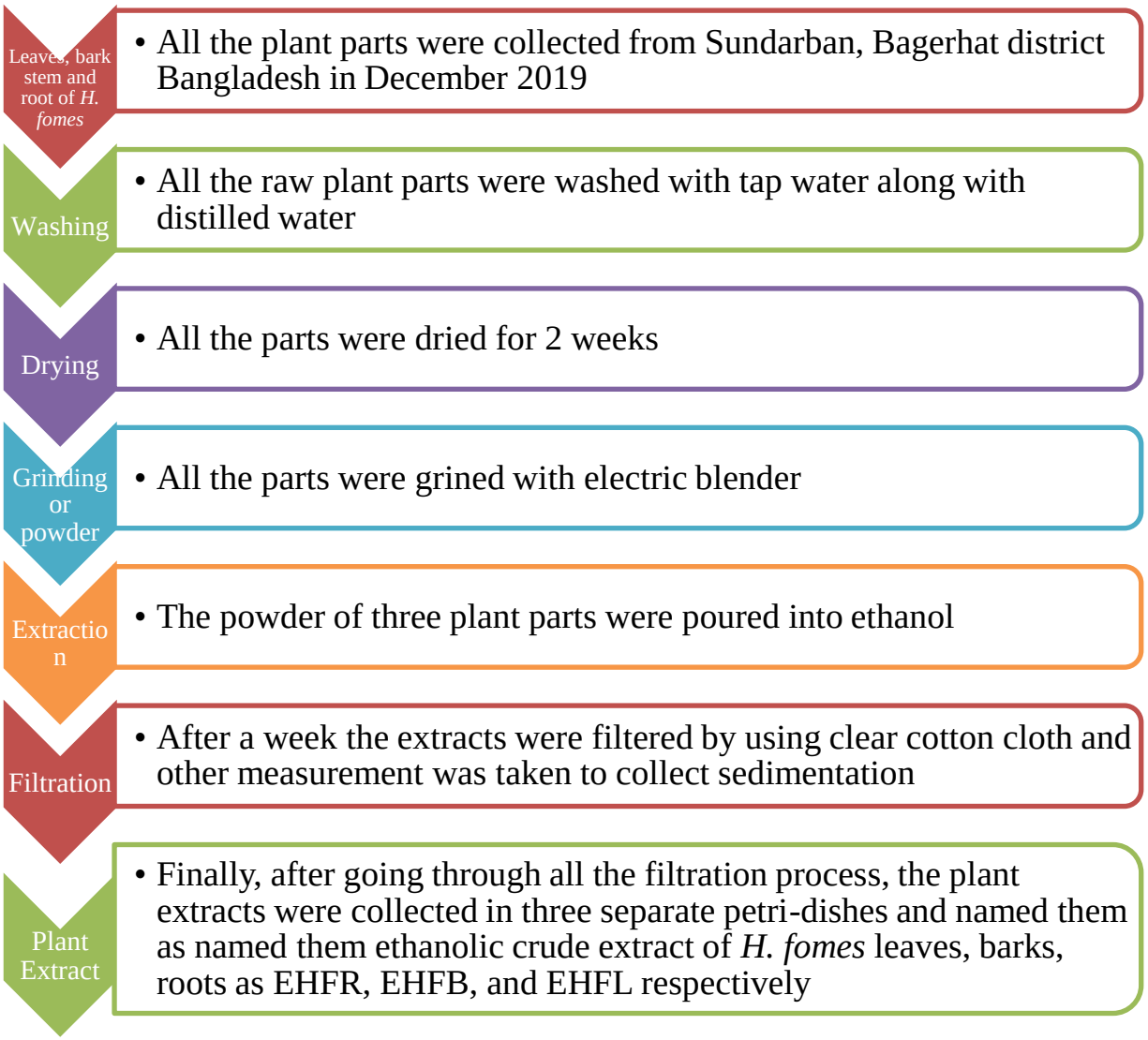

Figure 19: Steps of extraction procedure of H. fomes

2.2 Experimental Procedure

2.2.1 Drug:

Albendazole (a medicine that was used to treat neurocysticercosis) was used for this study.

2.2.2 Chemicals:

- Ethanol
- Saline
- Tween 20

2.2.3 Earthworms collection:

The adult earthworm *Pheretima posthuma (P. posthuma*) was decided to use for the anthelmintic study. Earthworms were collected from the moist soil on the campus of Jahangirnagar University.

The collected worms were rinsed with normal saline to wash out all the debris and unwanted materials for the study of anthelmintic activity. The characteristics of the worms that were used in the experiment are given below-

Length: maximum 3-5 cm

Width: maximum 0.1-0.2 cm

Weight: 0.8-3.04 g

(Hosain et al., 2021)

2.2.4 Preparation of experimental drug and standard drug

For in vitro study, for three different extracts of *H. fomes*, nine ethanolic extracts were prepared at the concentrations of 25mg/mL, 50mg/mL, and 75 mg/mL. After that, Tween 20 (1%) solutions were added to those nine ethanolic extract samples and finally diluted with normal saline, respectively. In our study, the control group was tween-20 along with normal saline, and albendazole was the standard drug.

2.2.5 Anthelmintic Activity

The anthelmintic activity of three different extracts of *H. fomes* was performed by Ghosh et al. 2005 method with slight modifications and Bangladeshi earthworms *P. posthuma* were used for this study because it is anatomically and physiologically similar to the human intestinal roundworm parasite (Vidyarthi, 1967; Lakshmi et al., 2012).

Collected all *P. posthuma* were washed with normal saline. This was done to make them accustomed to the laboratory environment before performing any test and also to remove all their fecal matter.

However, then the standard and test solutions were prepared by following the proper steps. In this experiment, we have used freshly prepared standard and test solutions. Test samples were prepared at the concentrations of (25-75 mg/ml) in Tween 20 (1%) solution and diluted with normal saline.

Then nearly equal-sized earthworms were taken and divided into eleven groups. All those eleven groups were released into 30 ml of the experimental formulation. Here, each group consisted of six adult *P. posthuma*:

1st group - Control (Tween-20 and normal saline)

2nd group - Standard drug (Albendazole)

3rd to 11th group - different solvent extracts of *H. fomes* in three different concentrations (25 mg/ml, 50 mg/ml, and 75mg/ml)

So, from this, it was clear that the first group has tween-20 along with normal saline which was named as the control in the experiment. Group two was treated with 20mg/ml concentrated reference drug Albendazole which was considered standard. And the rest of the groups 3rd-11th were treated with different solvent extracts of *H. fomes* in different concentrations (25 mg/ml, 50 mg/ml, and 75mg/ml). Anthelmintic activity on *P. Posthuma* was observed and time taken for paralysis and time taken for death of the experimented worms were noted. Here, time taken for paralysis was considered when worms could not perceive any kind of movement excluding

when worms were shaken robustly. Time taken for death was taken and recorded when the worms did not show any movement even after shaking the petri dish vigorously and also dipping them into hot water at the temperature of 50ºC where the body color of the worms faded away.

2.2.6 Statistical Analysis

All the values (experimental result) from the experiment are expressed as mean ± standard error of the mean (SEM). Here, all the attained data were compared by using ANOVA (Analysis of variance) which was followed by Student t-test. However, the values could be considered statistically significant, when $P<0.01$.

Chapter- 03
Result

3. Result:

3.1 Results of Anthelmintic Activity of *H. fomes* extracts on adult *P. pasthuma*:

In our trail, the parasites showed loss of motility, response and paralysis and even death corresponding to the concentration of the therapeutic products *H. fomes*. Anthelmintic activity in several concentrations of ethanolic extracts of *H. fomes* and albendazole is considered as reference standard:

Table 4: Evaluation of Anthelmintic activity of H. fomes in different concentrations

Treatment	Conc. Used (mg/ml)	Time taken for paralysis (min) X±SD	Time taken for death (min) X±SD
Control			
Standard	20	35.67±1.53	43.33±2.52
BE	25	48.67±5.13*	67.00±7.55*
	50	42.00±2.00*	52.33±2.52*
	75	37.67±2.52	47.67±3.51
LE	25	57.67±8.08*	65.00±7.09*
	50	46.33±6.66*	59.33±3.05*
	75	41.00±5.29	50.67±4.61
RE	25	51.33±9.45	67.67±8.02*
	50	42.00±7.81	51.00±7.81
	75	40.00±2.64	49.33±4.16

Each value is expressed as Mean±SEM, n=3. All the data were analyzed by ANOVA followed by student t-test. Statistically significant at **P<0.01.

From the table we found the anthelmintic activity of ethanolic bark extract (BE) at 25, 50, 75 mg/mL concentrations showed times taken for paralysis are 48.67±5.13, 42.00±2.00, and 37.67±2.52 min, and time taken for death are 67.00±7.55, 52.33±2.52, and 47.67±3.51 min, respectively.

Anthelmintic activity of ethanolic leaves extract (LE) at 25, 50, 75 mg/mL concentrations showed time taken for paralysis are 57.67±8.08, 46.33±6.66, and 41.00±5.29 min, and the time taken for death are 65.00±7.09, 59.33±3.05, and 50.67±4.61 min, respectively.

Anthelmintic activity of ethanolic root extract (RE) at 25, 50, 75 mg/mL concentrations showed time taken for paralysis are 51.33±9.45, 42.00±7.81, and 40.00±2.64 min, and time taken for death are 67.67±8.02, 51.00±7.81, and 49.33±4.16 min, respectively.

The standard group at the concentration of 20 mg/mL showed a paralysis time of 35.67±1.53 min and a death time of 43.33±2.52 min. Control group (trated with normal saline) worms were observed for 24 h and no paralysis or death was found.

The ethanolic bark extract of *H. fomes* demonstrated paralysis time 37.67±2.52 min and death time of worms 47.67±3.51 at the concentration of 75 mg/ml which is very less among others extracts anthelmintic activity and very close to standard one.

Chapter- 04
Discussion

4. Discussion:

Anthelmintic activity of *H. fomes* was interrupted in contrast to Bangladeshi adult worms *P. pasthuma* that is dependent on dose and has crucial ($P<0.01$) in vitro activity. Nevertheless, from the experiment, it was found that albendazole influenced worms very fast and caused flaccid paralysis which ultimately leads the worms to death by peristalsis. Albendazole mainly binds with free β-tubulin and gives selective prohibition of the polymerization that hinders glucose uptake of microtubule by the parasites. (Rang et al., 2007).

On the other hand, the plant *H. fomes* extracts consist of several secondary metabolites such as phenols, alkaloids, tannins, flavonoids, saponins, steroids, terpenes, etc which are responsible for anthelmintic activity. In our research, we observed that the ethanolic bark extract (EHFB) at the concentration of 75 mg/ml is more potent than others extract of EHFR, EHFL, and EHFB which is very close to the reference standard albendazole. As per findings, it caused quick paralysis and death time of the worms at the concentration of 75 mg/ml which confirms the dose-dependency nature of the extract. Beside this, previous literature showed that tannins, alkaloids and saponins possess anthelmintic properties. Tannins can bind to free proteins in the gastrointestinal tract of host animal or glycoproteins on the cuticle of the parasite and may cause death. Saponins mainly act by parallel irritation of mucus membranes that leads to parasite death. Furthermore, tannins and alkaloids have a direct impact on the viability of the pre parasitic phases and nervous system of helminths. (Vidyarthi, 1967; Lakshmi et al., 2012)

From the above results, it can be said that EHFB extract of *H. fomes* can be used to treat intestinal worm infections because it showed highly therapeutic efficacy. To conclude, our experiment provides evidence of having anthelmintic activity in *H. fomes* that is obtained from the laboratory model. The phytochemical profile of *H. fomes* can be explore in future to identify active constituent responsible for anthelmintic activity.

Chapter- 05
Conclusion

5. Conclusion:

From the very early stage of human society, people are very much dependent on plants for the treatment of diseases but with time modern cultures were introduced and scientists started to invest in many synthetic drugs along with herb drugs to treat many deadly diseases. On this journey, we choose different parts such as the leaf, stem bark, and root of *H. fomes* to investigate its anthelmintic activity.

It consists of many natural compounds such as proteins, polyphenols, tannins, flavonoids, alkaloids, saponins, steroids, and terpenes which show anthelmintic activity in earthworms. However, the different extracts of the experiment plants show significant anthelmintic activity against worms due to having those significant secondary metabolites. We got promising results from our research against *P. posthuma* which has created a way to form new anthelmintic drugs from natural sources. To conclude, it can say that from the investigation it is very much clear that our experimental plant *H. fomes* is a really helpful plant in the discovery of a new anthelmintic drug and our work was just only preliminary effort that will require more involvement, comprehensive exploration, and human-based model to establish our finding for potential therapeutic effects. Apart from this, depiction of active compounds and pre-formulation studies are needed for the expansion of a potential dosage form.

Future work:

During our study we focused on only three extracts of *H. fomes*. In future with other extraction processes and advance screening can reveal more bioactive compounds and specific target components and more potent drug options in regards to parasitic infections. Those steps will help to find out the different structural bioactive compounds and their efficacy. Up until now, there is two types of general mechanisms which are accountable for the anthelmintic effects of herbal medicines. One mechanism directly interacts with bioactive compounds of plants with the parasite and the other has happened through interaction with the host immune system.

However, our study is only focusing on the first mechanism of action which is interaction with the parasite via bioactive compounds of plants. So, the proper screening of the different parts of *H. fomes* can help to find out more different and specific bioactive compounds which may have anthelmintic properties at different concentration. Moreover, the anthelmintic activity against different microorganisms also can be investigated and can be compared with each other. This will help to understand the anthelmintic activity of the extracts on various microorganisms and their therapeutic efficacy.

Chapter- 06
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6. References:

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