Phytochemical and Pharmacological Evaluation of *Trapa natans L.*

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APPROVAL

This Project, **Phytochemical and Pharmacological Evaluation of *Trapa natans L.*** , submitted to the Department of Pharmacy, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Master of Pharmacy and approved as to its style and contents.

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DECLARATION

I hereby declare that, this thesis report is done by me under the supervision of **Mr. Md. Mahmodul Islam**, Lecturer, Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University &**, Dr. Mohammed Shafikur Rahman**, Assistant Professor Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, impartial fulfillment of the requirement for the degree of Master of Pharmacy. I am declaring that this thesis is my original work. I am also declaring that neither this thesis nor any part thereof has been submitted elsewhere for the award of master or any degree.

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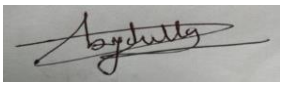

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Author

DEDICATION

I dedicate this work first and foremost to Almighty Allah and secondly to my family especially my parents and to my teachers and my friends.

ABSTRACT

Trapa natans L. which belongs to the family Trapaceae is a small herb well known for its medicinal properties and is widely used worldwide. *Trapa bispinosa or Trapa natans* is an important plant of Indian Ayurvedic system of medicine which is used in the problems of stomach, genitourinary system, liver, kidney, and spleen. The whole plant is used in gonorrhea, menorrhagia, and other genital affections. It is useful in diarrhea, dysentery, ophthalmopathy, ulcers, and wounds. The objective has been to explore and evaluate the Phytochemicals, showed the presence of Reducing sugar, Carbohydrate, alkaloid, Tannin, Steroids, Flavonoid, Saponin, Glycosides, and antioxidant Neuropharmacological , and antidiarrheal activities to provide a suitable lead, which may be utilized in future to peruse a new line of investigation, based on the combined approach of both exploitation and exploration. From the study of antidiarrheal activity it was found that methanolic extract of whole plant of *Trapa natans L.* at a dose 500mg/kg & 1000 mg/kg exhibited significant defecation inhibition of by 66% and 77% while the standard drug lopiramide inhibition was found to be 87% at the dose of 5 mg/kg body weight. The methanolic extract of whole plant of *Trapa natans L.* showed promising DPPH free radical scavenging activity. The positive control ascorbic acid of which IC50 value was 2.59 µg/mL. On the other hand, the Crude methanolic extract of whole plant showed promising DPPH free radical scavenging activity with the IC50 value 14.13 µg/mL. It was found that all the extracts possess central nervous system depressant activity as indicated by decreased exploratory behavior in mice. This study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The obtained results provide a support for the use of this plant in traditional medicine.

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CHAPTER 01

INTRODUCTION

1.1.1 Introduction to Ethnobotany

Ethnobotany is the investigation of how individuals of a specific culture and area make of utilization of indigenous plants. Ethnobotany has its foundations in plant science, the investigation of plants. Natural science, thus, began partially from an enthusiasm for discovering plants to help battle ailment. Truth be told, medicine and plant science have dependably had close ties. A considerable lot of the present medications have been gotten from plant sources. Pharmacognosy is the investigation of restorative and poisonous items from normal plant sources. At one time, pharmacologists looking into drugs were required to comprehend the characteristic plant world, and doctors were educated in plant-determined cures. However, in industrialized countries modern medicine and drug research advanced, chemically-synthesized drugs replaced plants as the source of most medicinal agents. In spite of the fact that examination in plant sources proceeded and plants were as yet utilized as the reason for some medication advancement, the prevailing interest moved to the lab (Veilleux C et al,. 1996)

1.1.2 Phytomedicine in global health care

Plants have been the premise of numerous conventional pharmaceutical frameworks all through the world for thousands of years and keep on providing humankind with new cures. Plant based pharmaceuticals at first administered as unrefined medications, for example, tinctures, teas, poultices, powders, and other natural definitions, now fill in as the premise of novel medication disclosure Phytomedicine, popularly known as herbal medicine, refers to the utilization of plant seeds, berries, roots, leaves, bark, or blooms for therapeutic purposes. It has long reputation as “the people’s medicine” for its accessibility, safety and the ease with which it can be prepared. As indicated by World Health Organization (WHO), from 119 plant-derived medicines, around 74% are utilized as a part of current solution in ways that relate specifically with their conventional employments. WHO also estimates that for primary health care 4 billion people, 80% of the world's population presently uses herbal medicine. Herbal medicine is a typical component in Ayurvedic, Homeopathic, Naturopathic, Traditional oriental, Native American and Indian medicine. Indeed, even among professionally prescribed medications, at least than 25% contain at least than one compound got from higher plants. The rate may be higher on the off chance that we incorporate over-the counter (OTC) drugs (Barrett et al., 1999). In developing nations including Bangladesh, around 75% of the populaces depend on various types of conventional medicine for their essential medicinal services (Matu and Staden, 2003). The high expense of imported

conventional drugs and/ or unavailability to western health care facility, imply that traditional mode of medicinal services is the primary type of health care that is affordable and available to our rural people. On the other hand, even when western health services are accessible, traditional medicine is seen as an effective and an acceptable system from a social point of view. As a result, traditional treatments usually exists side-by-side with western types of medication.

1.1.3 Prospect of natural products and phytomedicine

Various techniques have been used to obtain compounds for drug innovation, including separation from plants and other natural sources, synthetic chemistry, combinatorial science and molecular modeling. Even though the current interest in molecular modeling, combinatorial chemistry and other synthetic chemistry methods by pharmaceutical companies and funding organizations, natural produces and particularly medicinal plants, remain an significant source of new drugs, new drug leads and new chemical entities (NCEs). As indicated by Newman et al. (2003), 61% of the 877 small-molecule NCEs presented as medications worldwide amid 1981– 2002 was encouraged by natural products. These include: natural products (6%), natural products byproducts (27%), synthetic compounds with natural products-derived pharmacophore (5%) and synthetic compounds designed from natural products (natural products mimic, 23%) (Butler, 2004; Geysen *et al*., 2003; Lombardino and Lowe, 2004). New medications got from natural sources have been launched on the market amid the most recent few years.

These new medications have gotten authorization for the treatment of cancer, neurological maladies, infective sicknesses, cardiovascular and metabolic ailments, immunological, inflammatory and related sicknesses, and hereditary disorders, which incorporate a large number of the basic human diseases. Moreover new medications propelled on the market from 2000 to date, there are an assortment of new chemical entities from natural sources experiencing clinical trials, (Newman *et al*., 2003). However, the potential welfares of herbal treatments could lie in their high acceptance by patients, efficacy, comparative safety and low costs (Thomas *et al.*, 2001).

1.1.4 Natural product research and drug discovery

Nature seems to be a healing cornucopia to treat superfluity of illnesses ranging from common cold to different type of illness since the dawn of advancement. Overpowering confirmation has amassed demonstrating that natural items from plants, microorganisms and

marine life forms include significant segment of the aggregate collection of the accessible helpful medications. Results of common inceptions are frequently called natural products. Natural items include: a whole living being (e.g., a plant, a creature, or a microorganism) that has not experienced any sort of preparing or treatment other than a basic procedure of conservation (e.g., drying), some portion of a living being (e.g., leaves or blooms of a plant, a segregated creature organ), a concentrate of a living being or part of a living being, and exudates, and unadulterated mixes (e.g., alkaloids, glycosides, sugars, flavonoids, coumarins, lignans, steroids, terpenoids, and so forth.) separated from plants, creatures, or microorganisms (Samuelsson, 1999). Nevertheless, in most cases the term natural products state to secondary metabolites, small molecules (mol wt <2000 amu) produced by an organism that are not harshly compulsory for the persistence of the organism (Cannell,1998). Natural products have played a vital character in drug finding research, as many medications are either natural products or derivatives thereof. Certainly, it is estimated that about 40% of all medications is either natural products or their semi-synthetic derivatives. This may not be shocking as herbal medicine is a tradition of healthcare since earliest times and natural extracts screening has been one of the origins of drug innovation research, where erythromycin and rifampicin (bacterial infections), statins (hyperlipidemia), quinines and artimesinin (malaria), paclitaxel, vinblastine and vincristine (cancer), are a few renowned natural products-based medications. For microbial infections, over 80% of all medications in scientific use is either natural products or their derivatives, while for anticancer agents over 60% of all drugs is either natural products or derivatives thereof; examples of numerous prospective lead molecules are vincristine, vinblastine, taxol, camptothecin, podophyllotoxin, combretastatins, etc which have been insulated from plants for effective use in cancer treatment (Newman and Cragg, 2007; Butler, 2004; Newman *et al*., 2000).Previously, all drugs and curative agents were derived from natural stuffs, and most of these therapies were acquired from higher plants. Nowadays, numerous new chemotherapeutic agents are synthetically derived, based on "rational" drug design.

1.1.5 Approaches to natural product research and drug discovery

Medicine innovation from plants includes a multidisciplinary tactic linking botanical, ethnobotanical, phytochemical and biological methods. The hunt for bioactive compounds from the natural part of the plant kingdom can be accompanied fundamentally with three methods (Cotton, 1996); the casual method includes the gathering of all plants originate in a particular area of study, phylogenetic targeting means the gathering of all fellows of those

plant families which are recognized to be rich in bioactive compounds, and the ethno botanical methodology is established on the traditional knowledge of medicinal plant use. It has been advised that the ethno-directed specimen is most likely to succeed in recognizing medications for use in the dealing of gastrointestinal, inflammatory and dermatological illnesses. Tactics for investigation in the area of natural products have developed quite meaningfully over the last couple of decades. These can be largely divided into two categories:

1.1.5.1 Older method

- Not on activity but Focused on chemistry of compounds from natural sources.
- Testing of biological activity in animal model after straightforward isolation and identification of compounds from natural sources.
- Chemotaxonomic research.
- Choice of organisms largely based on ethnopharmacological evidence, folkloric characters, or traditional usages.

1.1.5.2 Modern approach

- Bioassay-directed (mainly *in vitro*) isolation and identification of active lead compounds from natural bases.
- Production of natural products archives.
- Creation of active compounds by cell or tissue culture, genetic manipulation, natural combinatorial chemistry and so on.
- Extra concentrated on bioactivity.
- Overview of the conceptions of dereplication, chemical fingerprinting, and metabolomics.
- Collection of organisms based on ethnopharmacological evidence, folkloric reputes, or traditional uses, and also those casually nominated.

1.1.6 Challenges in drug discovery from medicinal plants

Regardless of the accomplishment of medication disclosure programs from plants in the previous 2– 3 decades, future undertakings confront numerous difficulties. The procedure of medication revelation has been assessed to take a normal time of 10 years and cost in excess of 800 million dollars (Dickson and Gagnon, 2004). A lot of this time and cash is spent on the various leads that are disposed of amid the medication disclosure process. It is assessed that just a single in 5000 lead mixes will effectively progress through clinical preliminaries

and be endorsed for utilize. In the medication disclosure process, lead recognizable proof is the initial step.

As medication revelation from plants has generally been tedious, quicker and better techniques for plant gathering, bioassay screening, compound separation and compound improvement must be utilized (Koehn and Carter, 2005). Imaginative systems to enhance the procedure of plant accumulation are required, particularly with the legitimate and political issues encompassing advantage sharing assentions (Rosenthal, 2002). The outline, assurance and usage of fitting, clinically important, high throughput bioassays are troublesome procedures for all medication revelation programs (Knowles and Gromo, 2003; Kramer and Cohen 2004).

The normal issue looked amid screening of extracts is dissolvability and the screening of extracts libraries is ordinarily tricky, however new methods including pre-fractionation of concentrates can mitigate a portion of these issues, (Koehn and Carter, 2005).Thus, there is a need to create joint efforts with manufactured and therapeutic physicists to investigate the conceivable outcomes of its semi-combination or aggregate blend (Ley and Baxendale, 2002). One can likewise enhance the natural products compound advancement by making natural products libraries that consolidate the highlights of normal items with combinatorial science.

1.1.7 Opportunities in drug discovery from medicinal plants

Whenever ethnobotanical or ethnopharmacological approach is used, extra particular necessities that identify with earlier educated assent, acknowledgment of Indigenous Intellectual Property and Indigenous Intellectual Property Rights and also short-and long haul advantage sharing should be considered (Patwardhan, 2005).

So as to screen a great many plant species at one go for as many bioassays could be expected under the circumstances, should have an accumulation of an extensive number of extracts.

Universally, there is a need to manufacture natural products extract libraries. The extract libraries offer different points of interest, for example, diminishment in cost and time for rehash accumulation of plants and accessibility of appropriately encoded and protected extract in vast numbers for natural screening as far as high-throughput screenings and acquiring hits inside a brief period. Such libraries could fill in as an intense device and wellspring of extract to be screened for organic exercises utilizing high-throughput examines.

1.1.8 Medicinal plants of Bangladesh

Being normally talented by an appropriate tropical atmosphere and prolific soil, Bangladesh has a rich greenery of tropical plants. Around 5000 types of phanerogams and pteridophytes develop in its timberlands, wildernesses, badlands and roadsides as indigenous, naturalized and developed plants. Out of them, in excess of a thousand have been asserted to have medicinal and/or toxic properties, of which 546 have as of late been counted with their medicinal properties and remedial uses (Ghani, 2003).

Notwithstanding having different other therapeutic properties, 257 of these medicinal plants have been recognized as effectual solutions for diarrhoeal infections and 47 for diabetes. Medicinal plants are an open, moderate and socially suitable wellspring of essential human services framework in Bangladesh. Underestimated, rustic and indigenous individuals, who can't bear the cost of or get to formal human services frameworks, are particularly reliant on these socially recognizable, in fact straightforward, fiscally moderate and by and large compelling customary meds. All things considered, there is across the board enthusiasm for elevating conventional wellbeing frameworks to meet essential social insurance needs. This is particularly valid in this nation, as costs of present day prescriptions winding and governments discover it progressively hard to meet the cost of pharmaceutical-based medicinal services.

In any case, it has been watched that numerous other restorative plants developing in the nation have not been recognized systematically and that there are a significant number of them, which have not been artificially inspected and no consideration has yet been paid to portray them from the pharmacognostic perspective. Along these lines, it is normal that the quantity of therapeutic plants developing or accessible in Bangladesh might be more than what has so far been identified. It has additionally been watched that the endless herbs found in Bangladesh ought to be utilized for advancement of wellbeing and for battling numerous infections. In this way restorative plants of Bangladesh hold great guarantees as potential assets for tranquilize improvement. Be that as it may, keeping in mind the end goal to build up these restorative plants as medications, endeavors ought to be first made to positively recognize them and preclinical investigations on them ought to be completed to set up their asserted remedial properties. These are critical in light of the fact that the organic movement of a plant or its arrangement will help on deciding the restorative focus of its improvement.

1.2 Introduction to *Trapa natans L.*

Trapa natans L., also recognized as a bat nut or devil pod, are the seed pod of an aquatic plant. These dark brown nuts are hard, have smooth downward facing horns, and other ridged areas. The overall shape of the nut is described as a bull's face and horns or the silhouette of a flying bat. Water Caltrops contain a white fibrous nut that has a very mild, starchy flavor, and needs to be cooked before consumption; typically boiled, roasted, or dried and ground into flour (Gupta & Beentje, 2017).

Water chestnut, any of several perennial water plants of the genus Trapa , native to Europe, Asia, and Africa. The name is also applied to their edible, nutlike fruit. The *Trapa natans L.* has submerged leaves that are long, feathery, and root like, and floating leaves, in a loose rosette, that are attached to petioles, or leafstalks, 5 to 10 cm (2 to 4 inches) long. The fruit is 2.5 to 5 cm in diameter and usually has four spiny angles. *Trapa natans L.*, sometimes called Singhara nut, is native to India. The floating leaves, about 5 to 8 cm long, have hairy petioles 10 to 15 cm in length (Britannica, 2018).

It is an annual aquatic plant, growing in slow-current water, depth up to 5 meters, the leaves are float over the water surface, small, arranged in a rosette manner, margin toothed, size 2 to 4 cm, the flowers are small, white flowers consist of four 8 mm long petals and 4 green sepals, and it is located at the center of the plant. The fruits are brownish-black, four-horned, about 3 cm sized nut resemble of the head of buffalo or an ox, or a flying bat, which grows in underwater, contain a single, large, white, starchy seed (Pfingsten,2018).

1.2.1Taxonomy: (USDA, 2018; Mikulyuk & Nault , 2018)

- ***Kingdom:*** *Plantae*
- ***Subkingdom:*** *Tracheobionta*
- ***Superdivision:*** *Spermatophyta*
- ***Division:*** *Magnoliophyta*
- ***Class:*** *Magnoliopsida*
- ***Subclass:*** *Rosidae*
- ***Order:*** *Myrtales*
- ***Family****: Trapaceae*
- ***Genus*** *: Trapa L.*
- ***Species:*** *Trapa natans L.*

1.2.2 Common Names (USDA, 2018; Mikulyuk & Nault , 2018)

- Bengali: Panifol,
- Hindi: Singhada-, Pani-phal,
- English: Water Caltrop, Water Chestnut, Buffalo nut, Singhara, Batnut, Devil pod
- Tamil: Neer kombuchedy-
- Malayalam: Vankottakkaya, Karimbolam-, Kakkamullu
- Japan :Hishi
- China: Lingjiao
- French – Châtaigne d'Eau, Mâcre, Mâcre nageante
- Sanskrit: Kachora

1.2.3 Synonyms (Gupta & Beentje, 2017)

- Trapa bispinosa ROXB.
- Trapa astrachanica (Flerow) N.A.Winter
- Trapa natans L. Var. bispinosa (ROXB.) NAKIN
- Trapa carinthiaca (Beck) V.N.Vassil.
- Trapa colchica Albov
- Trapa conocarpa (F.Aresch.) Flerow
- Trapa maeotica Woronow
- Trapa muzzanensis (Jäggi) Szafer
- Trapa rossica V.N.Vassil.
- Trapa septentrionalis V.N.Vassil.

1.2.4 Geographical distribution (Mikulyuk & Nault , 2018; Diop, 2010).)

- *Trapa natans L*. is widely distributed in Europe, Asia and Africa.

1.2.5 Morphology

Plant: annual aquatic plant with a submerged stem; stems can reach 12-15 ft. in length; very fine roots anchor the plant into the mud.

Leaves: rosette of floating leaves at the water's surface; saw-tooth margins; triangular in shape and connect to an inflated petiole which provides added buoyancy for the leafy portion; additional, feather-like leaves can be found along the submerged stem.

Flowers, fruits and seeds: flowers are four-petaled and white; form in June; are insect-pollinated; fruit is a nut with four ½-inch, barbed spines; seeds can remain viable for up to 12 years, although most will germinate within the first two years.

Spreads: by the rosette and by fruits detaching from the stem and floating to another area on currents or by clinging to birds and other floating objects (Mikulyuk & Nault , 2018).

1.2.6 Habitat and ecology

This plant is an annual (up to 3 m height) floating-leaved plant, growing in stagnant waters, lakes, channels with weak currents, ponds and marshes. It primarily occurs in unpolluted nutrient-rich lowlands, but not in strongly calcareous waters that have a muddy bottom and plenty of light; it is important as food source for birds and provides fish spawning grounds (Mikulyuk & Nault , 2018).

1.2.7. Pharmacognostic Characters

Trapa natans L. contains an extraordinary amount of nonnutritional antioxidants, for example, flavonoids, flavones, and aggregate phenol substance. Flavonoids are available in plant tissues, for example, fruits, vegetables, nuts, seeds, and leaves, in generally high focuses. Flavonoids go about as characteristic antioxidants. Phytochemical screening of seed extract of *Trapa natans L.* fruits reveals the nearness of starches, saponins, phytosterols, settled oils, and fat, while the pericarp extracts of the products uncovered the nearness of tannins, flavonoids and glycosides alkaloids, saponins, steroids, and phenolic compound .The writing uncovers the nearness of saponins, tannins, flavonoids, and glycosides in the pericarp concentrate of natural product.The kernel is tasty and contains starches, proteins, and fundamental minerals. It likewise contains copious B vitamins, E, A, and C vitamins. Seeds also contain thiamine (Adkar, 2014).

1.2.8 Pharmacological properties & Economic Importance

It is utilized in the issues of stomach, genitourinary system, liver, kidney, and spleen. It is unpleasant, astringent, stomachic, diuretic, febrifuge, and clean. The entire plant is utilized in gonorrhea, menorrhagia, and other genital affections. It is valuable in loose bowels, diarrhea, ophthalmopathy, ulcers, and wounds. These are utilized in the approved conditions in pitta, consuming sensation, dipsia, dyspepsia, drain, hemoptysis, loose bowels, diarrhea, oddly, irregular fever, infection, exhaustion, aggravation, urethrorrhea, cracks, erysipelas, lumbago, pharyngitis, bronchitis and general debility, and smothering stomach and heart consuming. (Adkar, 2014).

The economic important benefits described by Gupta & Beentje, 2017 are given below;

1. **Rich in potassium:** It has enough measure of potassium, which counters the impact of sodium and useful for bringing down pulse and additionally for your heart. 5 crude water chestnuts have 5% of day by day prescribed of your potassium admission.
2. **Alleviate sickness:** the drinking of water chestnut juice is useful in backing out of queasiness. Singhara juice is useful for jaundice.
3. **Lowers heart dangers:** It serves to lets down the level of cholesterol and disheartens the ingestion of sugar. 100 grams of it contains 2 g of fiber.
4. **Good for sound rest:** The nearness of Vitamin B-6 is useful for dozing and mitigating your mind-set. It produces neurotransmitters that are viable for your state of mind and rest.
5. **Good for measles:** Boiled water of water chestnuts is useful for measles patients.
6. **Good for thyroid organ:** Due to the nearness of iodine, it is successful in the correct working of thyroid organ.
7. **Hair development:** It has adequate measure of potassium. It is additionally having vitamins B and E. Every one of these supplements are useful for sound hair.
8. **Control free movements:** It has cooling impacts and acts like as coolant.
9. **Anti-viral:** The nearness of enemies of oxidants like poly-phenols and flavonoids , it goes about as hostile to viral, against bacterial, hostile to disease and hostile to parasitic.
10. **Good for fetal development:** During pregnancy, it is useful in the development and improvement of fetal.
11. **Good for skin:** It detoxifies the body and gives generally speaking great appearance. The glue of water chestnuts and lemon juice regards fix skin inflammation.
12. **Regulates water maintenance:** It has the adjusting impacts in the body because of the nearness of enough measure of potassium and lower measure of sodium in this manner helps in direction of water maintenance.
13. **Cure mucus:** Its juice is great in restoring of mucus.
14. **Secretion of mother drain:** It is great in the emission of drain by invigorating the mammary organ.
15. **Sore throat:** Eating the vegetable is useful to fix sore throat.

1.2.9 Photograph of *Trapa natans L.*

Fig-1 whole plant of *Trapa natans L.*

Fig-2 leaves and flowers *of Trapa natans L.*

Fig-3 Fruits of *Trapa natans L.*

CHAPTER 02

PURPOSE OF THE STUDY

Since Bangladesh is a nation of low financial development .So logical investigation and institutionalization of potential unrefined medications is an earnest need to alter our medication area.

Plant secondary metabolites have been utilized for humankind as cures since the start of development. Presently multi day despite everything they assume a vital part in human services for around 80% of the world' populace.

Divers bioactive metabolites like steroids, terpenoids, flavonoids, alkaloids, glycosides, etc.in plants have framed the remedial premise of home grown pharmaceutical. Along these lines accentuation is given on the biological screening of therapeutic plants for facilitate investigation of their active constituents.

In addition, Bangladesh imports a substantial amount of pharmaceutical crude materials including medicinal plants and semi prepared plant items to create drugs and medicines. This enormous foreign exchange can be spared if the indigenous therapeutic plants or their semi-handled items are used by the makers to fulfill their requirements.

The *Trapa natans L.* is one of the important Traditional drugs. *Trapa natans L.* is usually used in Ayurvedic and Unani systems of medication and is used to treat stomach pain, eczema, inflammation, liver and kidney disease, nausea, diarrhea and dysentery and spleen ailments. It is a bitter, astringent, diuretic, and antiseptic plant (Jolly RS, 2017).
The present project work was designed to investigate the scientific basis of the traditional use of this plant for its

- Phyto-chemical screening
- Antioxidant activity
- Neuropharmacological Activities
- Antidiarrheal activity

CHAPTER 03

LITERATURE REVIEW

Antioxidant Activity of Phenolic Compounds Extracted from Fresh and Dried Trapa bispinosa RoxbPulp (Trapa taiwanensis Nakai) by Po-Yuan Chiang*, Jhih-Ying Ciou and Li-Chun Hsieh

Trapa natans L. (Panifol) was utilized as the crude material to explore the impacts of drying medications on the antioxidant activities and phenolic compounds of water caltrop. For methanolic extracts from crisp, solidify dried, and hot air dried tests, at 10 mg/mL, the rummaging capacity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was 87.2%, 61.4% and 52.1%, individually. At 10 mg/mL, the diminishing force was 1.63, 0.81 and 0.52, separately. At 40 mg/mL, the chelating capacity on copper particles was 29.6%, 14.8% and 34.3%, individually. The IC50, half inhibition concentration is the focus, at which the radicals or particles was scavenged or chelated by half. The aggregate phenolics and flavonoids substance were higher in the crisp examples, while the IC50 values were lower. The HPLC investigation demonstrated that four phenolic mixes (gallic acid, quercetin, ferulic acid, and hydroxycinnamic acid were found in water caltrop), and that lone had three phenolic mixes (gallic acid, quercetin, and ferulic acid were found in the wake of drying). While the stop drying treatment could protect more cell reinforcement action of the Trapa bispinosa Roxbpulps than hotair drying treatment, distinctive drying Trapa bispinosa Roxbpulps displayed diverse cell reinforcement systems.

Physico-chemical, Morphological and Pasting Properties of Starches Extracted from Water Chestnuts (*Trapa natans*) from Three Lakes of Kashmir, India by Adil Gani1 , Sham Sul Haq, F.A. Masoodi1, A. A. Broadway and Asir Gani.

Extracts on physicochemical, morphology and gluing properties of starches removed from water chestnuts of three Pools of Kashmir valley (Wular, Anchar and Dal Lakes) were led to decide their application in various nourishment items. The water chestnut starch from Dal Lake had more oval molded granules than water chestnut starches from the Wular and the Anchar Lakes.The one of a kind element of the water chestnut starches were state of starch granules which resembled horn(s) jutting from the surface which did not show up in different starches effectively examined. Proximate investigation of water chestnut starches demonstrated that normal protein content were 0.4%, amylose 29.5 % and fiery remains 0.007 on dry weight premise. Increment in water restricting limit, swelling power and solvency was found over a temperature scope of 50-90oC. Water chestnut starches demonstrated an expansion in syneresis

amid solidify defrost cycles and decrease in glue clearness upon capacity. Starch separated from the water chestnuts of the Dal Lake indicated higher water restricting limit, swelling, solvency, past lucidity, solidify defrost strength, top thickness, last consistency and lower protein content, amylose content, sticking temperature and gel solidness than starches extricated from water chestnuts of the Wular and the Anchar Lakes.

Components of Volatile Oil from Water-caltrop and Their Anti-tumor Effect in vitro by ZHAO Wen-jing, NIU Feng-lan, LI Jing, DONG Qing and HUANG Zhan-you

The volatile oil was separated from *Trapa natans L. (Panifol)*by steam refining; it was then investigated by GC-MS to acquire 16 parts, 8 of which were distinguished. Apocynin was the most bottomless one, representing 81.41% of the total oil. The in vitro inhibitory impacts of the volatile oil on SMMC-7721, MCF-7, Hela, HL-60 cells, and human fringe blood mononuclear cells(PBMC) were researched by means of the MTT technique. The morphological changes of the tumor cells were watched and the apoptosis of HL-60 cells was identified by stream cytometry. The multiplication of the tumor cells could be altogether hindered and the apoptosis of HL-60 cells could be prompted by the volatile oil. The expansion hindrance impact of the volatile oil on HL-60 tumor cells and the enlistment of the apoptosis of HL-60 cells had measurements subordinate element.

Physicochemical, Pasting and Thermal Properties of *Water Chestnut* Flours: A Comparative Analysis of Two Geographic Sources by Nisar Ahmad Mir, Khalid Gul And Charanjit Singh Riar

Relative investigation of physicochemical, sticking and warm properties of water chestnut (*Trapa natans*) flours from Jammu and Kashmir (WCF [K]) and Punjab (WCF [P]) was done. Flour from WCF (P) had higher dampness, fiery debris, mass thickness, genuine thickness and clear thickness while that from Jammu and Kashmir had higher protein, fat, sugar substance and L, a, b esteems. WCF (P) had bigger molecule measure than WCF (K) and a large portion of the sifters held most extreme number of flour particles of WCF (P). Water-restricting limit (90%) and oil binding limit (60%) of WCF (P) were altogether higher than that of WCF (K), 88% and 57%, separately. WCF (P) showed higher swelling power than flour from WCF (K). Warm investigation comes about demonstrated that beginning, pinnacle and end temperature, and

enthalpy of gelatinization were higher in WCF (P). Pinnacle thickness (2,581 cP) and breakdown thickness (870 cP) were observed to be higher if there should arise an occurrence of WCF (K).

Physico-chemical characteristics and sensory quality of Singhara (*Trapa natans L.*): An Indian water chestnut under commercial and industrial storage conditions by Gagan Deep Singh, Sukhcharn Siingh, Navdeep Jindal, Amrinder S. Bawa and Dharmesh C. Saxena

The physicochemical properties of water chestnut (*Trapa natans L. var. bispinosa Roxburgh*.) were researched. Substance of dampness, rough lipid, unrefined fiber, rough fiery remains, and rough protein were 81.12, 0.36, 0.72, 1.33, 1.87%, separately. Add up to solvent solids and titrable acridity decided was 7.2 and 0.142%, separately. The time span of usability of entire water chestnut at surrounding, refrigerated, solidified and fluid conditions was examined. The impact on weight reduction, add up to dissolvable solids, titrable sharpness, add up to sugars, shading what's more, inward visual examination, eating quality and textural properties of white bit were assessed over the capacity day and age. Tests kept at solidified conditions displayed better stockpiling life in contrast with others. The solidified examples represented moderate yet continuous decrease in TSS over the capacity time frame. The aggregate acridity, as well, took after a similar pattern of decrease in an incentive from starting quality 0.144 %. The lessening in acridity was sharp if there should be an occurrence of control, refrigerated and watery examples in contrast with those at solidified conditions.

CHAPTER 04

Mathod and Materials

4.1.1 PHYTOCHEMICAL SCREENING:

Phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds. Although the knowledge of how these substances provide medicinal value to humans reflects a relatively recent scientific understanding, the use of plants and plant extracts to heal, relieve pain and promote good health dates back to before the beginnings of medical science.

The subject of phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plant and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function.

In all these operations, methods are needed for separation, purification and identification of many different constituents present in plants. Thus advances in our understanding of phytochemistry are directly related to the successful exploitation of known techniques, and the continuing development of new techniques to solve outstanding problems as they appear. As a result of modern extraction, and isolation techniques and pharmacological testing procedures, new plant drugs usually find their way into medicine as purified substances rather than in the form of galenical preparations.

The precise mode of extraction naturally depends on the texture and water content of the plant material being extracted and on the type of substance that is being isolated. Generally, two type of procedure are used for obtaining organic constituents-

- Cold extraction and
- Hot extraction

The classical chemical procedure for obtaining organic constituents from dried plant tissue (heartwood, dried seeds, root, leaf) is to continuously extract powdered material in a sox let apparatus with a range of solvents, starting in turn with petroleum ether and chloroform (to separate lipids and terpenoids) and then using alcohol and ethyl acetate .

The extract obtained is then carried out in a rotary evaporator which will concentrate bulky solution down to small volumes. In most cases, mixtures of substances will be present in the extract and these are subjected to chromatographic fraction. If a single substance is present, the crystals can be purified by recrystalization. As standard precaution against loss of material, concentrated extracts should be stored in the refrigerator and a trace of toluene added to prevent fungal growth.

The separation and purification of plant constituents is mainly carried out using Thin Layer Chromatography (TLC) and column chromatography. The choice of techniques depends largely on the solubility properties and volatilities of the compounds to be separated.

4.1.2 Apparatus and Reagents

A. Materials Required

Table-1 Equipment required for extraction

Serial no.	Material	Source
01	Amber Glass Jar (2litres bottle)	Used Reagent Bottle
02	Beakers (0.5 litre)	Borosil, Germany
03	Funnel	Glassco Laboratory Equipments, UK
04	Filter Paper	Whatnam Filter Paper
05	Beaker (100ml)	Borosil, Germany
06	Beaker (50ml)	Glassco Laboratory Equipments, UK
07	Glass rod	-
08	Dropper	-
09	Stand with clamp	-
10	Cotton	
11	Measuring cylinder	Glassco Laboratory Equipments, UK

Apparatus Required

Table-2 Apparatus required for extraction

SL no.	Name of the equipments	Source
01	Rotary evaporator	--
02	Electronic balance	Shimadzu Corporation Limited
03	Oven	MMM, Germany
04	Mortar and pestle	---

C. Solvents Required

Table-3 Solvent used for experiment

SL no.	Name of the solvent	Source
01	Methanol	Merck Germany
02	Ethanol	Merck Germany

D. Chemicals

Table-4 Reagents used for experiment

SL no	Name of the chemicals	Source
01	α-napthol	Merk, Germany
02	Ethanol	Merk, Germany
03	Sulphuric Acid	Active Fine Chemicals
04	Copper sulphate	Merk, Germany
05	Sodium Potassium tartarate	JHD Chemical, China
06	Sodium hydroxide (pellet)	Merck Specialities Private Limited, India
07	Sodium Citrate	Merk, Germany
08	Sodium Carbonate Anhydrous	Active Fine Chemicals

09	Mercuric Iodide	Active Fine Chemicals
10	Potassium iodide	Merck Specialities Private Limited, India
11	Bismuth Nitrate	Merk, Germany
12	Tartaric Acid	JHD Chemical, China
13	Ferric Chloride	Merk, Germany
14	Lead Acetate	JHD Chemical, China
15	Hydrochloric Acid	Merk, Germany
16	Distilled Water	----------

4.1.2.1 Experiment Plant

Trapa natans L. (Panifol) included in Trapaceae was investigated in this study.

Table-5 Experiment Plant

Plant Name	Family	Plant part used
Trapa natans L. (Panifol)	Trapaceae	whole plants

4.1.2.2 Collection

Fresh whole plants of *Trapa natans L.* were collected from the local fields of Nator Zilla, Bangladesh, and the genus as well as family was identified in Bangladesh National Herbarium.

4.1.2.3 Preparation of crude extract

- **Drying and grinding**

  The whole plant material of *Trapa natans L.* bark was subjected to shade drying for about several days. The dried plant material was further crushed to powder and the powder was passed through the mesh and stored in air tight container for further analysis.

- **Extraction (Methanol extraction)**

  The powdered plant materials (Bark) are submerged in methanol solution in an air-tight flat bottomed container for 7 days, with occasional shaking and stirring. The major portion of the extractable compounds of the plant material will be dissolved in the solvent during this time and hence extracted as solution. and then all the extracts were filtered through a cotton plug followed by Whatman filter paper number 1 and then concentrated by using a rotary evaporator at low temperature (40-50)°C and reduced pressure to have greenish extracts.

- **Extraction at a glance:**

Plant collection

↓

Sun Drying

↓

Milling with hand blender

↓

Powder of Coccicinia grandis

↓

Macerated with methanol for 4 days

↓

Heating in reflex condenser for 4 hours at 80^{o}c

↓

Filtration by vacuum

↓

Crude methanol extract

4.1.3 CHEMICAL GROUP TESTS

Testing of different chemical groups present in extract represent the preliminary phytochemical studies. The chemical group test, which are performed as follows (Trease, G.E. and Evans, W.C., 1983). In each test 10% (w/v) solution of extract in methanol was taken unless otherwise mentioned in individual test**.**

4.1.3.1 ***Reagents used for the different chemical group test***

The following reagents were used for the different chemical group test (Trease, G.E. and Evans, W.C., 1983).

- **Mayer's reagent:**
  1.36 gm. mercuric iodide in 60 ml of water was mixed with a solution contains 5 gm. of potassium iodide in 20 ml of water.

- **Dragendroff's Reagent:**
  1.7 gm. basic bismuth nitrate and 20 gm. tartaric acid ware dissolved in 80 ml water. This solution was mixed with a solution contains 16 gm. potassium iodide and 40 ml water.

- **Fehling's solution A:**
  34.64 gm. copper sulphate was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml.

- **Fehling's solution B:**
  176 gm. of sodium potassium tartarate and 77 gm. of sodium hydroxide were dissolved in sufficient water to produce 500 ml. Equal volume of above solution were mixed at the time of use.

- **Benedicts Reagent:**
  1.73 gm. cupric sulphate, 1.73 gm. sodium citrate and 10 gm. anhydrous sodium carbonate were dissolved in water and the volume was made up to 100 ml with water.

- **Molish Reagent**: 2.5 gm. of pure α-naphtha was dissolved in 25 ml of ethanol.

4.1.3.2 Tests procedure for identifying different chemical groups

The following **t**ests were performed for identifying different chemical groups (Ghana, 1998).

Test for Carbohydrates

- **Molisch Test**:

  2ml solution of crude extract was taken in a test tube and 2 drops of freshly prepared 10% alcoholic solution of α-napthol was added to it. Then, sulphuric acid was added to the mixture to the flown side of the inclined tube so that the acid forms a layer beneath the aqueous solution. A red or reddish violet ring would be formed at the junction of the two layers confirming the presence of carbohydrate. Upon standing or shaking, a dark purple solution would be formed.

  The test tube was allowed to stand for 2 minutes; dilution of the sample mixture took place with 5ml of distilled water. A dull violet precipitate would be formed immediately confirming the presence of carbohydrate.

- **Fehling's Test (Standard Test for Reducing Sugars)**

  1ml of a mixture of equal volumes of Fehling's solution A and B was added to a 2ml aqueous solution of the crude extract and was boiled for a few minute. Presence of red or brick-red precipitate would be found immediately which would confirm the presence of carbohydrate.

Tests for Tannins

- **Ferric Chloride Test**

  5ml aqueous solution of the crude extract was taken in a test tube and 1ml of 5% Ferric solution was added. Presence of Greenish black precipitation confirmed the presence of tannins.

- **Potassium dichromate Test**

  1ml of 10% Potassium Dichromate solution was added to 5ml aqueous solution of the crude extract in a test tube. Presence of yellow precipitate confirmed the presence of tannins.

- **Lead acetate Test**

  Addition of few drops 1%solution of lead acetate to a 5ml aqueous solution of the crude extract would show yellow or red precipitate confirming the presence of tannins.

Test for Flavonoids

0.5ml of alcoholic solution of the extract of the sample was taken in a test tube and small piece of zinc ribbon or zinc dust with 5-10 drops of concentrated hydrochloric acid was added. The solution was boiled for a few minutes. Development of red to crimson colours would indicate the presence of flavonoids.

Test for Saponins

- .**Frothing Test**

  0.5ml of alcoholic solution of the extract of the sample was diluted to 10ml using distilled water and was shaken in a graduated cylinder for 3-5 minutes. Production of persistence frothing would confirm the presence of frothing.

Test of Steroids

- **Salkowski Test**

  2ml chloroform solution of crude extract and then 1ml of sulphuric acid was added. Emergence of red color would confirm the presence of steroids.

Tests for Alkaloids

- **Mayer's Test**

  0.2ml of concentrated Hydrochloric acid was added to 2ml aqueous solution of the crude extract. Then 1ml of Mayer's reagent was added. Formation of yellow colour precipitate would indicate presence of alkaloids.

- **Dragendroff's Test**

  0.2ml of concentrated hydrochloric acid was added to 2ml aqueous solution of the crude extract. Then 1ml of Dragendroff's reagent was added. Formation of orange brown precipitate would indicate the presence of alkaloids.

- **Hager's Test**

  0.2ml of concentrated hydrochloric acid was added to 2ml aqueous solution of the crude extract. Then 1ml of Hager's reagent was added. Formation of yellow crystalline precipitate would indicate the presence of alkaloids.

Tests for Glycosides

- A small amount of an alcoholic extract of the fresh or dried plant material was taken in 1ml of water. Then, a few drops of aqueous sodium hydroxide were added. A yellow color was considered as an indication for the presence of glycosides.
- A small amount of an alcoholic extract of the plant material was taken in water and alcohol and boiled with Fehling's solution. Brick-red precipitate was considered as an indication for the presence of glycosides.
- Another portion of the extract was dissolved in water and alcohol and boiled with few drops of dilute sulfuric acid, neutralized with sodium hydroxide solution and boiled with Fehling's solution. Brick red precipitate was considered as an indication for the presence of glycosides.

Test for Steroids

- **Sulphuric acid test**

  1 ml solution of chloroform extract was taken and then added1ml Sulphuric acid Red color indicates the presence of steroid.

Test for gums

5 ml solution of the extract was taken and then molish reagent and sulphuric acid were added. Red violet ring produced at the junction of two liquids indicate the presence of gums and carbohydrate.

Table-6 Different chemical group tests performed and the results are mentioned

Sample	Test solution	Observation	Inference
Test for Alkaloids: # 2 ml solution of the extract and 0.2ml of dilute hydrochloric acid	0.1 ml of Mayer's Reagent.	Yellowish buff colored precipitate was obtained.	Presence of alkaloid.
# 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid.	0.1 ml of Dragendroff's reagent.	Orange brown precipitate was observed.	Presence of alkaloid.
Test For Glycosides: #A small amount of an alcoholic extract was taken in 1ml of water.	A few drops of aqueous NaOH was added.	A yellow color was not found.	Presence of glycosides.
# A small amount of an alcoholic extract was taken in water and alcohol.	Boiled with Fehling's solution.	Brick-red precipitate was not found.	Presence of glycosides.
Test for Steroids: # 10 mg extract dissolved in 1 ml chloroform.	1 ml sulfuric acid.	No Chloroform layer acquired reddish brown color .	Presence of steroid.
Tests for Gums : # 5 ml solution of extract.	Molish reagent and sulfuric acid.	Red –violet ring produced at the junction of two liq.	Absence of gums.
Tests for Flavonoids: # 1 ml solution of ethanolic extract.	Few drops of conc. HCl was added to the extract	Immediate red color was not formed.	Presence of Flavonoids.
Tests for Saponins: # 1 ml solution of the Extract was diluted with distilled water to 20 ml.	Shaken in a graduated cylinder for 15 minutes.	No centimeter layer of foam.	Absence of saponins.

Tests for Tannins: # 5 ml solution of extract.	1 ml of 10% Lead acetate solution.	Yellow precipitate was obtained	Absence of tannins.

4.2 ANTIOXIDANT EVALUATION BY DPPH METHOD

4.2.1 *Principle*

The present study was aimed at evaluating the *In vitro* free radical scavenging activity of *Trapa natans L. (Panifol)* using 1,1-diphenyl-2-picrylhydrazyl (DPPH) by the method of Brand-Williams *et al.,* 1995. 2.0 ml of a methanol solution of the extract at different concentration were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of ascorbic acid by UV spectrophotometer.

The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptive of the DPPH radical at 517 nm reduces when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. DPPH radical scavenging activity is described as IC_{50} which is the concentration of samples to produce 50% reduction of the DPPH.

Fig-4 Reactions involved in antioxidant reactions.

4.2.2 Materials and methods

DPPH was used to evaluate the free radical scavenging activity of various compounds and medicinal plants. (Awika et al.2003

4.2.2.1 *Materials*

Table-7 Materials used in antioxidant test

1,1-diphenyl-2-picrylhydrazyl	UV-spectrophotometer
Ascorbic acid	Beaker (100 & 200 ml)
Distilled water	Test tube
Methanol	Light-proof box
Amber reagent bottle	Pipette (5 ml)
Foil paper	Micropipette (10-1000 µl)

4.2.2.2 Methods

- ✓ At first ten test tubes were taken and washed properly.
- ✓ 10mg plant extract was taken in test tube and dissolved with 10ml methanol with continuously stirring. Here the concentration was 1000 µg/ml. This solution was use as sample mother solution.
- ✓ 10mg ascorbic acid was taken in 10ml volumetric flask and dissolved with methanol with continuously stirring and placed in a dark placed. Here the concentration was 1000 µg/ml. This solution was used as mother solution for standard.
- ✓ 2ml methanol was taken in each ten test tube and 2 ml Plant extract was taken from mother solutions in first test tube then serial dilution was done by using following concentration:- 500,250,125,62.5,31.25,15.625,7.813,3.906,1.953a,and 0.9775µg/ml.
- ✓ Same work done for ascorbic acid each ten test tube by using following concentration:500,250,125,62.5,31.25,15.625,7.813,3.906,1.953,and0.9775µg/ml., which was used as a standard.

- ✓ 2mg DPPH was taken in 100ml volumetric flask and dissolved with methanol with continuously stirring, and placed in a dark placed and placed in a dark placed for 15 minutes. Here the concentration was 20 µg/ml.
- ✓ After 15 minutes 2 ml DPPH were added to all 20 test tubes of sample and ascorbic acid and kept in dark place for 30 minutes.
- ✓ A Control solution was Prepared by using 3ml DPPH and 2ml methanol, which is used as a blank.
- ✓ After 30 minutes, absorbance was taken for both plant extract and ascorbic acid at 517nm. Methanol was used to set the spectrophotometer and also to set zero.
- ✓ Al the process was done for triplet time for accouracy.
- ✓ Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula:

$$\%\ of\ Inhibition = \frac{\mathrm{Ab} - \mathrm{Aa}}{Ab} * 100$$

Where, A_b is the absorbance of the control (without test samples) Aa is the absorbance of test samples.

Extract concentration providing 50% inhibition *(IC_{50})* was calculated from the graph plotted inhibition percentage against extract concentration.

Ascorbic acid was used as positive control. Tests carried out in triplicate and average value was taken.

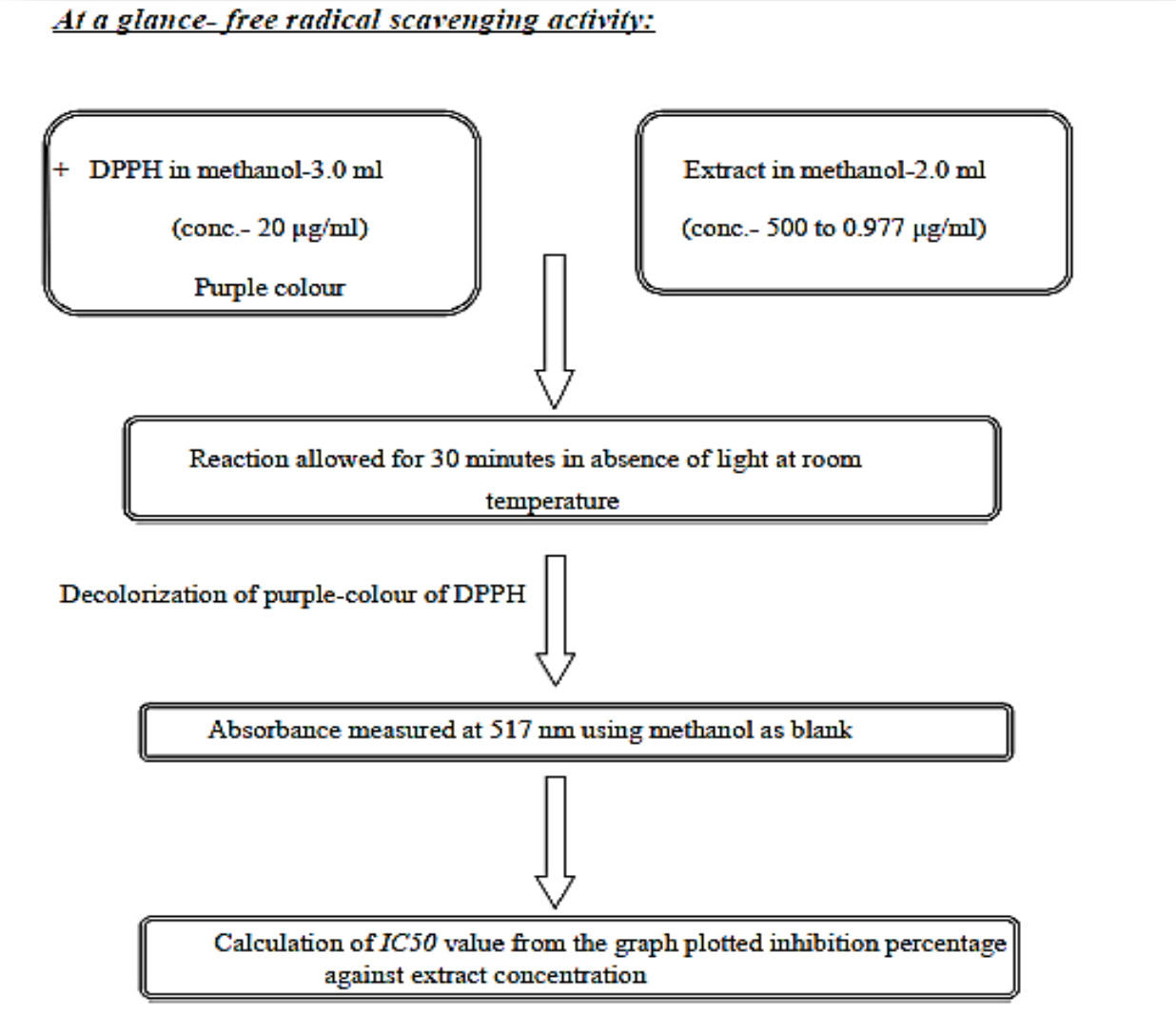

Fig-5 Schematic representation of the method of assaying free radical scavenging activity

4.3 Study of CNS depressant activity of plant extract

4.3.1 Principle:

CNS Depressant drugs are the agents which inhibit the excitement or slow down the activity of brain. For the treatment of anxiety, panic attack, insomnia etc. Mostly CNS Depressant agents activate GABA neurotransmitter. This helps in slowing brain activity.

The CNS depressant effects of *Trapa natans L.* plant extracts were observed by comparing with the standard diazepam in the experimented rodents mice. CNS depressant activity was determined by using three techniques. They are:

- Sleeping time test
- Hole board method
- Open field technique

4.3.2 Reagents, chemical & equipment

Table-8 Reagents, Chemicals and equipments used for CNS depressant test

Reagents Chemicals and Equipment's	**Source**
Thiopental sodium	Square Pharmaceuticals Ltd.
Diazepam	Square Pharmaceuticals Ltd.
Distilled water	BDH Chemicals Ltd.
Sterile disposable syringe (1ml, 100 divisions)	JMI Syringes and Medical devices Ltd., Bangladesh
Tuberculin syringe with ball shaped end	
Electronic and digital balance	Denver Instruments M-220/USA
Magnetic stirrer	

4.3.3 Test methods

4.3.3.1 Sleeping time test

For the sleeping time test hypnotic and sedative effect of different extract of *Trapa natans L. (Panifol)* in combination with thiopental sodium was assessed. The test was done as portrayed by Ali et al. (2015). For this reason, animals were partitioned into 6 groups and marked. After measuring weight dosage were calculated for individual mice. Group-1 was considered as negative control and got distilled water (10 ml/b.w., p.o.). Group-2 got diazepam (1 mg/kg, b.w., p.o.), which was considered as positive control while Group-3 to Group-4 got extract orally (Gradually 100 mg/kg and 200 mg/kg b.w. bark extract). After 30 min of oral administration thiopental sodium (20 mg/kg b.w.) was administered intraperitonealy to all groups to induce sleep. Individual mice was put on a table and recorded for the uncoordinated movements. Then the animals were observed for the time to lose their righting reflex, immediately after thiopental

sodium injection (latent period) and the duration of sleep (time between the loss and recovery of reflex) induced by thiopental sodium. Both period (latent period and sleeping time) ware recorded.

The flow chart of procedure for evaluation of CNS depressant effect of *Trapa natans L.* plant by sleeping time test is shown below:

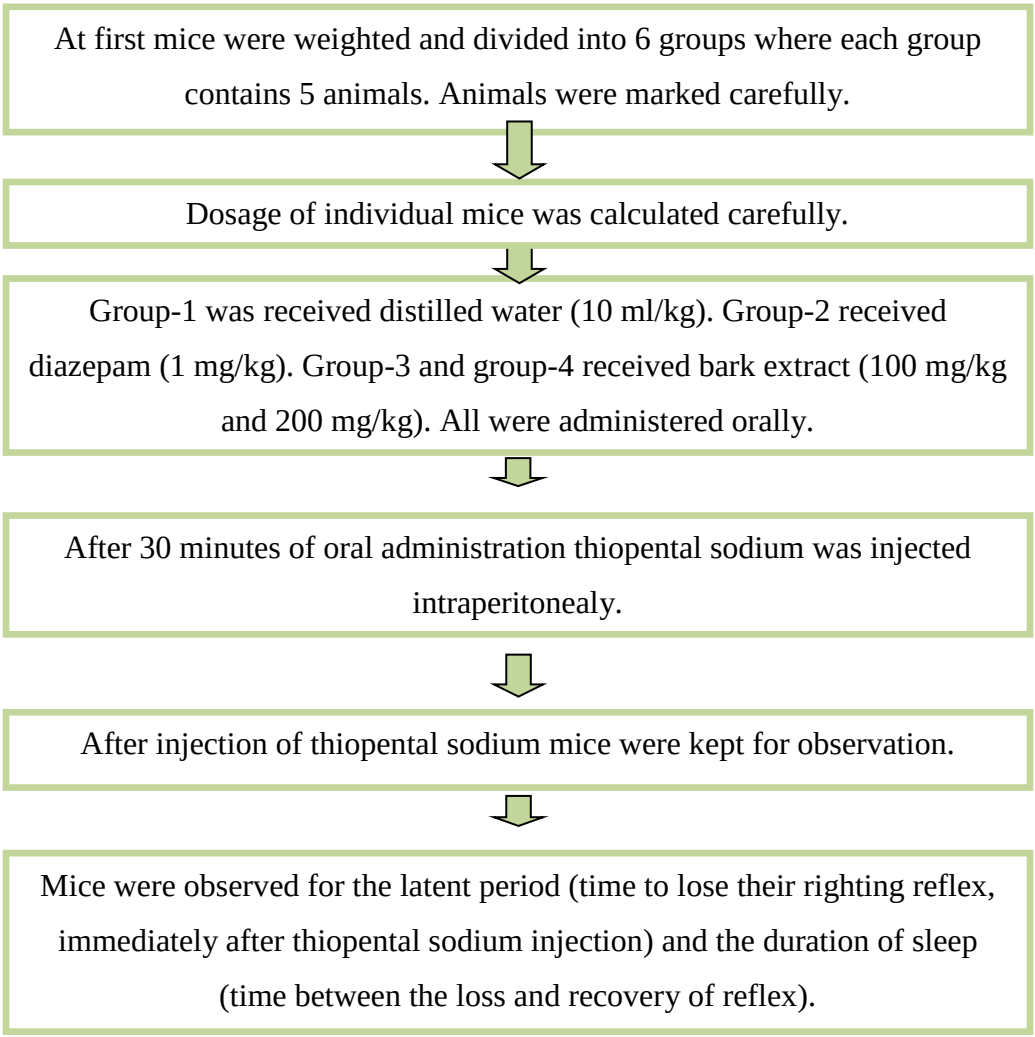

Fig-6 Flow charts of procedure for CNS depressant activity test on mice by sleeping time test.

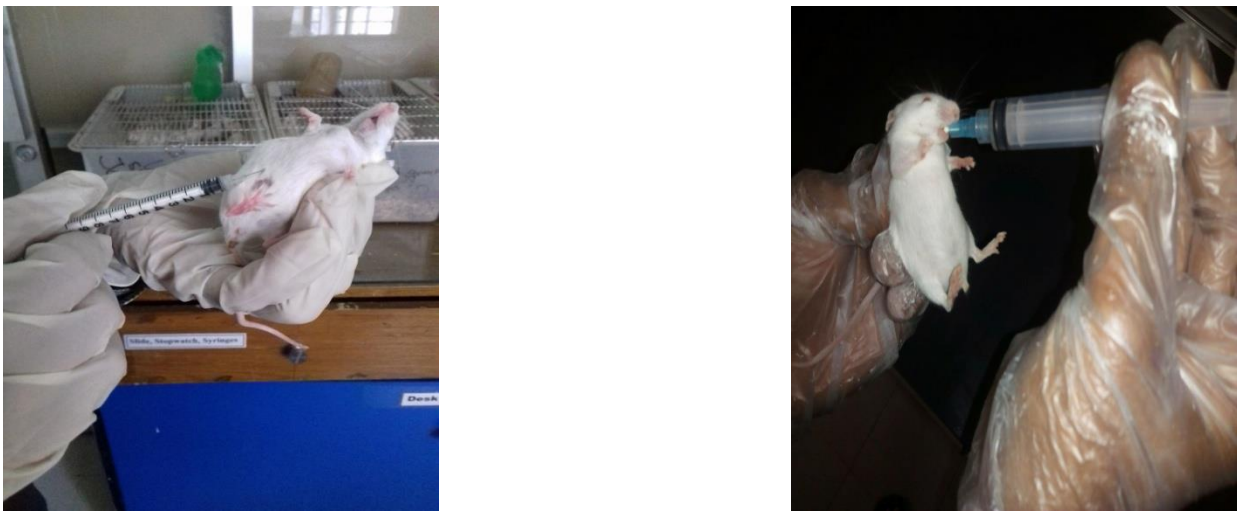

Fig-7 Mice feeding and injection.

Fig-8 CNS depressant activity test on mice by sleeping time test method.

4.3.3.2 Hole board test

The hole-board test was performed according to the previously described method by Sheikh et al (2016). with slight modifications. For this test, we used a flat platform of 45 cm × 45 cm in diameter with 16 evenly spaced holes. This platform also had a wall of 5 cm hight. All animals were divided into 4 groups, control and standard and test groups. Each group was containing 5 mice. Group-1 was considered as negative control and received distilled water (10 ml/b.w., p.o.). Group-2 received diazepam (1 mg/kg, b.w., p.o.), which was considered as positive control while Group-3 & Group-4 received extract orally (Gradually 100 mg/kg and 200 mg/kg b.w. bark extract).

After 30 minutes oral administration of control, standard and extracts each animal was kept on the center of the platform and allowed to move on the platform. The number of head dips into the holes by individual mice was counted for 10 min.

The flow chart of procedure for evaluation of CNS depressant effect of *Trapa natans L.* plant by hole board test method is shown below:

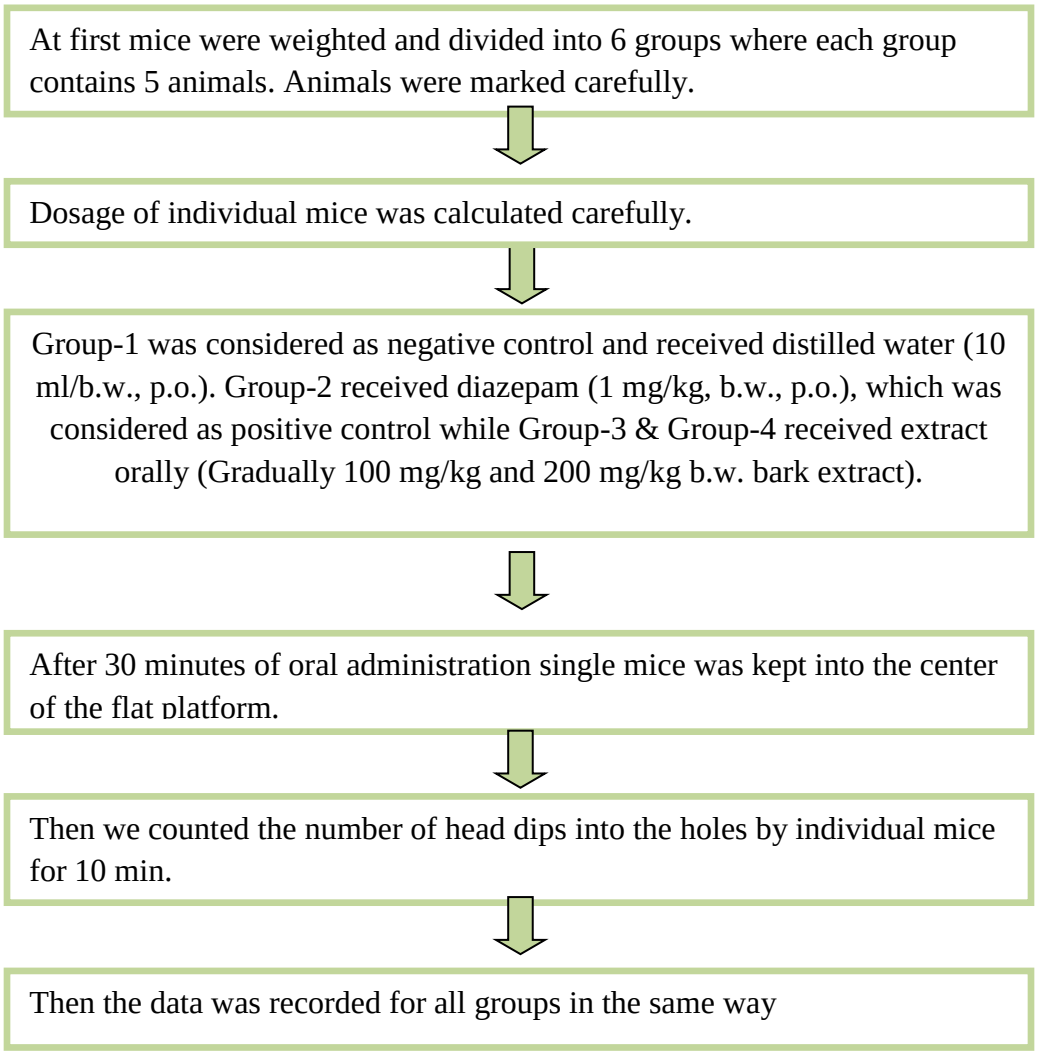

Fig-9 Flow chart of procedure for evaluation of CNS depressant effect by hole board test method

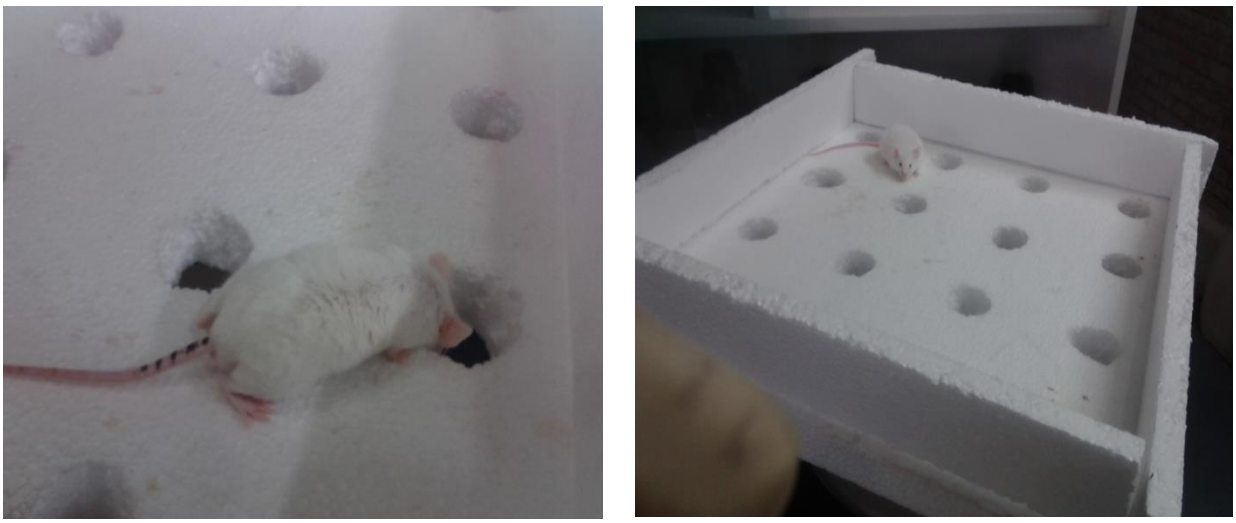

Fig-10 CNS depressant activity test on mice by hole board method.

4.3.3.3 Open field test

Emotion and locomotor activity of mice both can be evaluated by using open field test method. This experiment was carried out as described by Gupta et al. (1971). The open field apparatus consisted of a smooth field of half square meter with a series of squares. All squares alternatively painted in black and white. This test board is looks like a chess board. The apparatus also had a wall of 10 cm height.

The animals were divided into 4 groups, control, standard and test groups. Each group was containing 5 mice. Group-1 was considered as negative control and received distilled water (10 ml/b.w., p.o.). Group-2 received diazepam (1 mg/kg, b.w., p.o.), which was considered as positive control while Group-3 & Group-4 received extract orally (Gradually 100 mg/kg and 200 mg/kg b.w. bark extract). The number of squares passed anyway by the animals was counted for 3min started at 0, 30, 60, 90 and 120 min after oral administration of the test drugs.

The flow chart of procedure for evaluation of CNS depressant effect of *Trapa natans L. (Panifol)* plant by open field method is shown below:

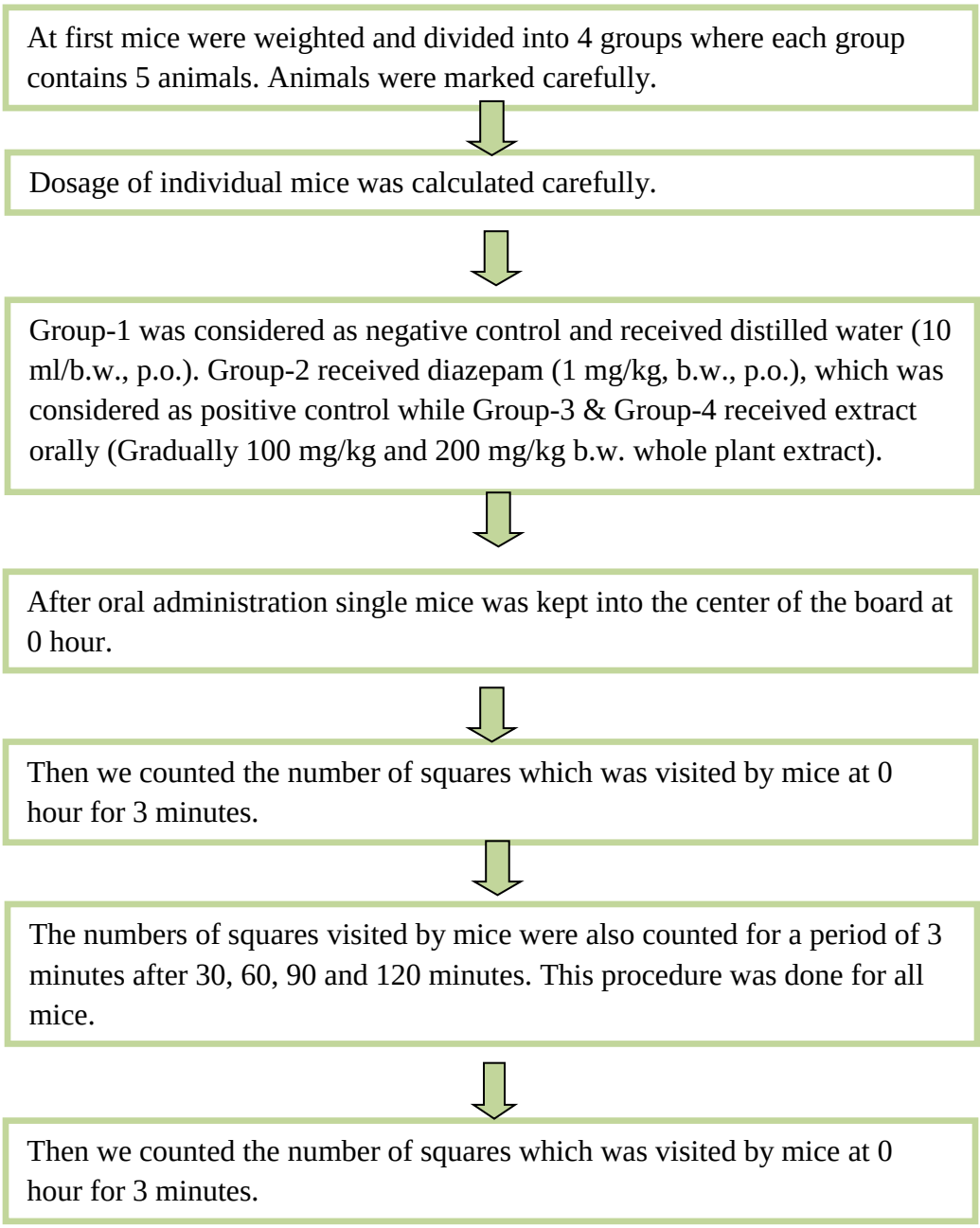

Fig-11 Flow chart of procedure for evaluation of CNS depressant effect by open field method.

Fig-12 CNS depressant activity test on mice by open field method.

4.4 ANTIDIARRHEAL ACTIVITY OF *TRAPA NATANS L. BY.* CASTOR OIL INDUCED DIARRHEA IN MICE:

4..4.1 Principle:

Diarrhea is too frequent, often too passage of poorly formed stools. In pathological term, it happens because of entry of abundance of water in faces. In the ileum and colon dynamic Na+K+ATPase intervened salt retention happen, water takes after isoosmotically. Moreover glucose encouraged Na+ absorption occur in the ileum. This mechanism stays flawless even in extreme bowel issues. Looseness of the bowels related with characinoid (secreting 5-HT) and modularly carcinoma of thyroid (secreting calcitonon). It is mediated by cAMP. Overabundance of bile acids likewise causes looseness of the bowels by actuating adenyl cyclase (Verma , 2001).

Looseness of the bowels was characterized by the nearness of stool or any liquid material that recolored the spongy paper put underneath the pen. Time taken before the first defecation is the ‘Latent period'. The total count stool and latent period time of test group are compared with positive control group. Anti-diarrheal agent increase latent period and decrease total stool count.

4.4.2 Mechanism of diarrheal action of castor oil:

Upon oral administration, castor oil mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglyceride. A small amount of ricinoleic acid is absorbed from the gastrointestinal tract and metabolized like any other fatty acid but most remains in the intestine where it creates its anti-absorptive or secretory effect. The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine. The ricinoleate

salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. The precise with serotonin (5-HT) have been termed as "diarrhoeangenic hormones mechanism "of how ricinoleate salts induce diarrhoea is yet to be unidentified. But most agreed opinion is that it stimulates the intestinal epithelial cell's adenyl cyclase, release prostaglandins and particularly prostaglandins of the E series along".

4.4.3 Materials and Method

Name	Origin
Tween-80	
Luperamide	Square Pharmaceuticals Ltd., Bangladesh
Syringes	
Needle	
0.9% sodium chloride solution	Orion Infusions Ltd., Bangladesh
Castor oil	WELL's Heath Care, Spain
Boxes for mice	
Electronic balance	
Hand gloves	
Castor oils	
Face Musk's	

4.4.3.1 Animal Collection:

Swiss albino mice, which weighed between 16-34g, were used in the present study. The animals were obtained from Pharmacy department, Jahangirnagar University. The animals were acclimatized for Six days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of Daffodil International University, Dhaka, Bangladesh.

4.4.3.2 Environment control:

They are housed in standard polypropylene cages and kept under controlled room temperature (24 ±2°C), relative humidity (RH) 60-70% in a 12 hours light-dark cycle and feed ICDDR'B formulated rodent food and water as these animal are very sensitive to environment changes.

Fig-13 Mice in Cage

4.4.3.3 Experimental design:

- ✓ **Preparation of control:** Normal saline was bought from local market and dissolved in 100ml water. Then 1 ml tween 60 was dissolved in saline solution and the final volume of was made 10 ml.
- ✓ **Preparation of Standard:** To prepare standard at the doses of 5mg/kg per body weight, for 26gm mice 0.65mg loperamide was dissolved in saline solution and the final volume of was made 10 ml.
- ✓ **Preparation of Sample:** To prepare suspension of the test samples at the doses of 400 & 200 mg/kg per body weight, for 26gm mice 52 & 26 mg of samples were measured respectively. The extract was first dissolved in 1 ml methanol then the distilled water was slowly added. The final volume of the suspension was made 10 ml.

4.4.3.4 Methodology:

- ✓ The test animals were randomly chosen and divided into three groups having three mice in each.
- ✓ Of the experimental groups,

  Group-I or the control received only 2ml saline water containing 1% Tween-80.

Group-II or the positive control received 2ml standard antimotility drug, loperamide at a dose of 5mg/kg-body weight as oral suspension.

Group-III, IV or The test groups were treated with 2ml suspension of Methanol extract of *Water Caltrop* whole plant at the oral dose of 500 & 1000mg/kg-body weight.

- ✓ The mice were fed with the samples 1 hour prior to the oral administration of castor oil at a dose of 2ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhea every hour in five hours study after the castor oil administration.
- ✓ Number of stools or any fluid material that stained the adsorbent paper were counted at each successive hour during the 5-hour period and were noted for each mouse.
- ✓ The latent period of each mouse were also counted. At the beginning of each hour new papers were placed for the old ones.

Table-9 Experimental profile to observe the effect of Leaf of *Trapa natans L.* on castor oil induced diarrhea in mice.

Animal Group	***Treatment***	***Dose (/kg-body wt.)***	***Route of admin.***
I (Control) n=3	Saline Water containing 1% tween 80	10ml	Oral
II (Positive control) n=3	Loperamide	5 mg	Oral
III & IV *Test sample* n=3	Methanolic Extract of whole plant	500 & 1000mg	Oral
n=no. of mice			

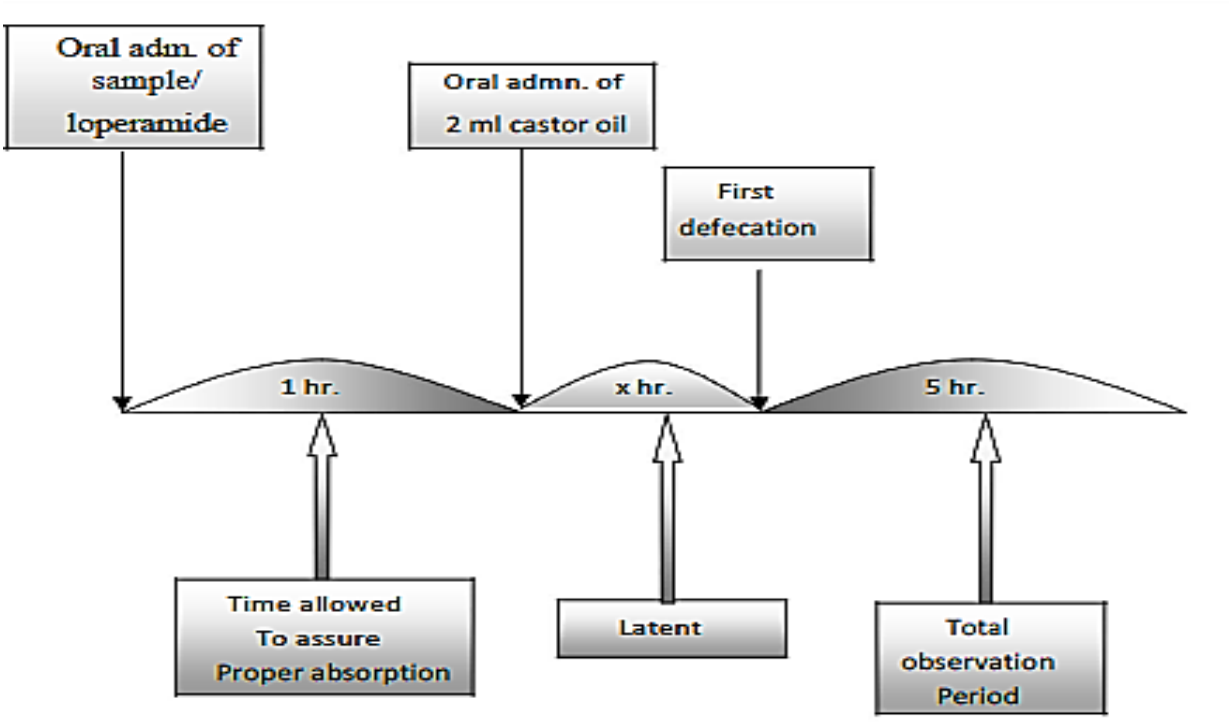

Fig-14 Schematic representation for study design of anti-diarrheal activity

CHAPTER 05

Result & Discussion

5.1 RESULTS & DISCUSSIONS GROUP TESTS

After completing wide range of chemical test for the identification of major classes of therapeutically important compounds like Reducing sugar ,carbohydrates, Tannin, alkaloids, glycosides, flavonoids , Saponin and steroids were found in plant except gum.this study also support the preius reports described by [10] The following table will give us a broad idea about phytochemicals present in these plants (Table-10).

Table-10 Different chemical group tests results

S/N	Test Name	Result
1.	**Test for Reducing sugar**	
	Benedict ‘s test	+
	Fehling’s test	+
2.	**Test for Carbohydrate**	
	Fehling test	+
	Molisch test	+
3.	**Test for Tannin**	
	Ferric chloride test	+
	Llead acetate test	+
4.	**Test for alkaloid**	
	With wagner’s reagent	+
	With mayer reagent	+
	With Hagger reagent	+
5.	**Test for Steroids**	
	Liberman-burchard test	+
	Salkowski test	+
6.	**Test for Flavonoid**	+
7.	**Test for Saponin**	+
8.	**Test for Glycosides**	+
9.	**Test for gum**	-

The experimental findings from the study showed that the methanolic extract has organic compounds which can show extensively pharmacologic activity.

5.2 RESULT AND DISCUSSION OF ANTIOXIDANT ACTIITY

5.2.1 Result

The DPPH test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. Practically, the reaction brings about the reduction of DPPH radicals to the corresponding hydrazine, which is manifested by a color change from violet to yellow, which is monitored spectrophotometrically. It is evident from the tables-11 that the % scavenging of DPPH radical was found to rise with increasing concentration of the samples. The positive control ascorbic acid of which IC50 value was 2.59 µg/mL. On the other hand, the Crude methanolic extract of whole plant showed promising DPPH free radical scavenging activity with IC50 value 14.13 µg/ml (Table-11).

Table-11 Comparison of IC50 Values of standard and Extract

Sample	IC50 (µg/ml)
Crude methanolic extract of whole plant	14.13
Ascorbic acid	2.59

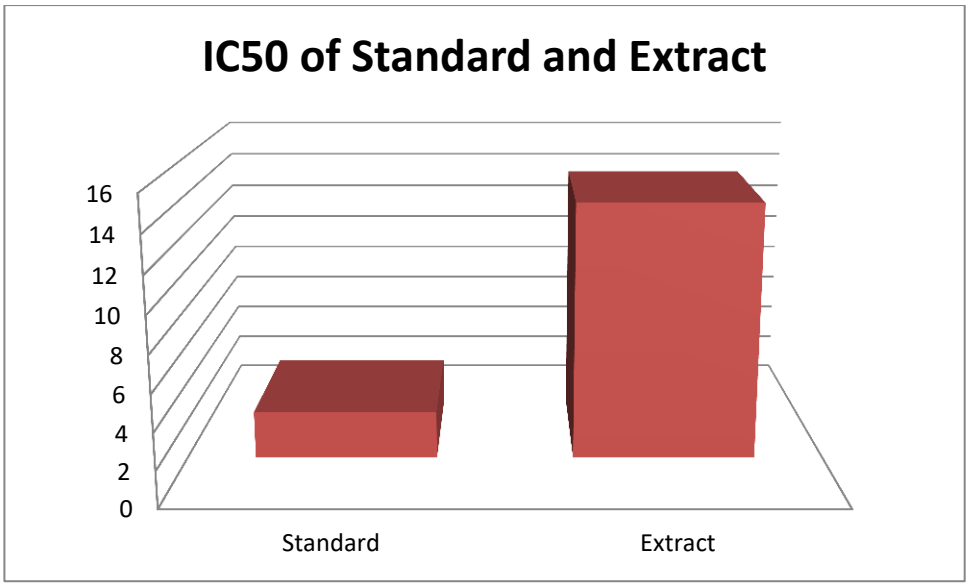

Fig-15 Comparison of IC50 Values of standard and Extracts

Table-12 IC_{50} value of ascorbic acid

S/N	Absorbance of the Control	Concentrations (µg/mL)	Mean Absorbance of Ascorbic acid	% of SCV	IC50 (µg/ml)
1.		500	**0.023**	96.308	2.59
2.		250	**0.041**	93.419	
3.		125	**0.063**	89.888	
4.		62.5	**0.112**	82.023	
5.		31.25	**0.18**	71.108	
6.	0.623	15.625	**0.189**	69.663	
7.		7.813	**0.239**	61.637	
8.		3.906	**0.295**	52.649	
9.		1.953	**0.332**	46.710	
10.		0.977	**0.469**	39.808	

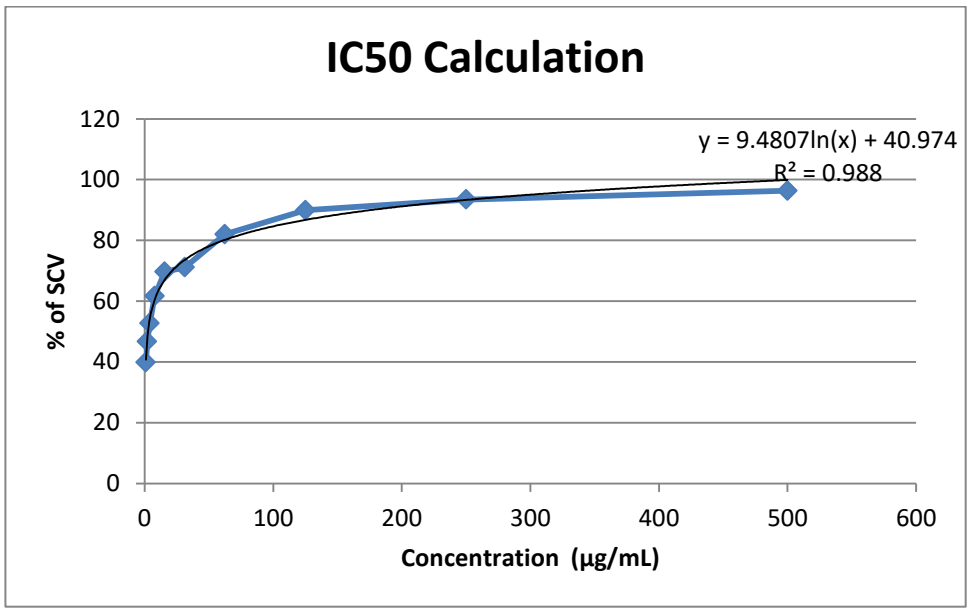

Fig-16 Free radical scavenging activity of ascorbic acid

Table-13 IC_{50} value of *Methanol* extract of whole plant of *Trapa natans L.*

SI No.	Absorbance of the Control	Concentrations (µg/ml)	Mean Absorbance of whole plant	% of SCV	IC50 (µg/ml)
1	0.623	500	0.112	82.02247191	14.13
2		250	0.132	78.81219904	
3		125	0.167	73.19422151	
4		62.5	0.210	66.29213483	
5		31.25	0.236	62.1187801	
6		15.625	0.279	55.21669342	
7		7.813	0.295	52.64847512	
8		3.906	0.312	49.91974318	
9		1.953	0.455	26.96629213	
10		0.977	0.612	1.76565008	

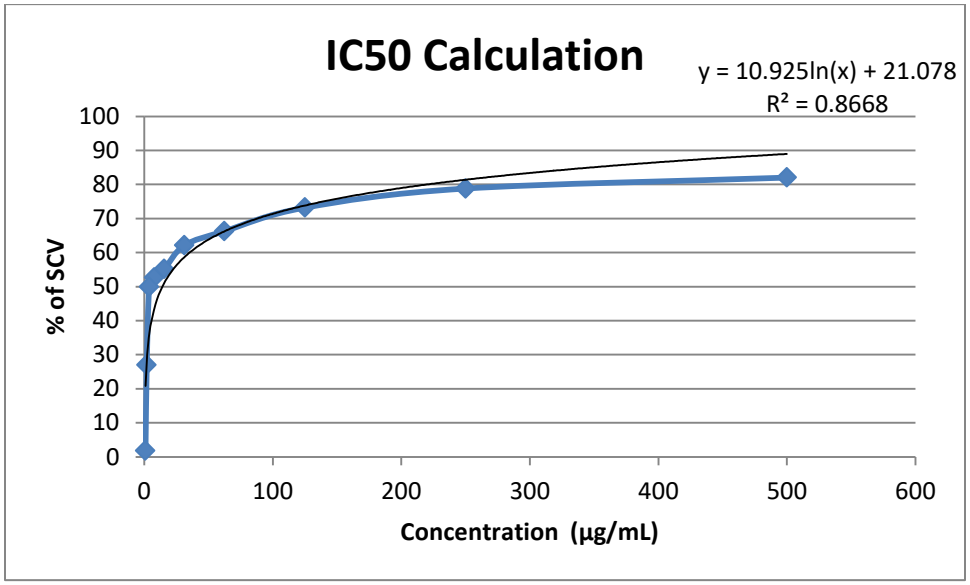

Fig-17: Free radical scavenging activity of methanol extract of whole plant of *Trapa natans L.*

5.2.2 Discussion:

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging strategy is used to imagine the total antioxidant potential of crude plant extract without the utilization of any test. DPPH radical can give hydrogen and electron in order to remove reactive oxygen species and oxidative stress and to inhibit lipid peroxidation of the body. The plants are assessed on the merit and probability of scavenging DPPH free radical and compared in respect to significant natural antioxidant named ascorbic acid to discover the usefulness of the crude plant extract.

After UV-Spectrophotometer investigation, it was discovered that Crude methanolic extract of whole plant have a high level of Free Radical Scavenging activity which show the presence chemical/s having antioxidant properties. The present investigation has been intended to examine the antioxidant activity of the methanol extract of the whole plant of *Trapa natans L*. Antioxidant activity was determined by utilizing 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical assays. The Crude methanolic extract of entire plant indicated strong DPPH free radical scavenging action with an IC50 estimation of 14.13 µg/mL (Table-13) compared with the positive control ascorbic corrosive with an IC50 estimation of 2.59 µg/mL (Table-12).

The great outcome with the past consequence of the presence of flavonoids demonstrates the high presence of antioxidant compound of the plant extract. These antioxidant play not only important role in the body by anti-inflammatory, anti-microbial, free radical scavenging potentiality, these plants upon exposure to environment have immense part in evacuating volatile organic compounds which are possibly destructive as they can be breathed in through the human body unconsciously and their conceivably to induce oxidative stress by producing reactive oxygen and nitrogen species bringing about lipid peroxidation and expelling these compound can help in minimization of the adverse health impact over the human at an extensive scale.

5.3 Result of CNS depressant activity test on mice

5.3.1 Sleeping time test

Thiopental sodium induced sleeping time was affected when *Trapa natans L.*bark was administered at 100 mg/kg and 200 mg/kg dose. The action of extracts show that it reduces latent period and increase or prolong the sleeping time respectively compared with control group.

Table-14 Data of CNS depressant activity test of *Trapa natans L.* plant extracts sleeping time test method:

Group	Dose	Latent period	Sleeping time
Control	10 ml/kg	22.4 ± 2.48	35.4 ± 4.50
Standard	1 mg/kg	3.6 ± 0.60	56.6 ± 8.41
Whole plant extract	100 mg/kg	4.4 ± 0.51	52.0 ± 4.73
Whole plant extract	200 mg/kg	4.0 ± 0.45	56.2 ± 3.26

Values are presented as Mean ± SEM (n=5)

5.3.2 Hole board method

In this technique leave and root extracts were administered to mice at 100 mg/kg and 200 mg/kg dose. As a result, the frequency of head dipping was significantly decreased compared to the control group. The result showed in Table-15 that, the effects of extracts were decreasing of frequency of head dipping in dose depending manner.

Table-15: Data of CNS depressant activity test of *Trapa natans L.* plant extracts by hole board method:

Group	Dose	Number of head dips	% of inhibition
Control	10 ml/kg	49.2±5.90	0
Standard	1 mg/kg	10.2±1.43	79.27
Whole plant extract	100 mg/kg	24.2±5.34	50.81
Whole plant extract	200 mg/kg	18.2±5.60	63.01

Values are presented as Mean ± SEM (n=5)

5.3.3 Open field method

Experimental bark extracts were administered to mice at 100 mg/kg and 200 mg/kg dose. As a result, the movements of mice were reduced in a dose depending manner. Also, it was comparable with diazepam (standard). This movement lowering effect of extract on mice was observed at 30 min interval from zero minute up to 120 minutes. The extracts caused reduction

in movement and this may be connected to CNS depression, as this is common to most antipsychotics that they reduce or depress the movement.

Table-16 Data of CNS depressant activity test of *Trapa natans L.* plant extracts by open field method:

Group	Dose	Number of movement				
		0 min	30 min	60 min	90 min	120 min
Control	10 ml/kg	74.4±6.98	42.6±1.50	31.8±2.40	29.6±3.96	28±3.15
Standard	1 mg/kg	111.8±1.59	78.2±1.16	38.6±1.75	22±2.74	15.2±1.88
Plant extract	100 mg/kg	130.6±6.02	80.8±4.18	61.8±1.73	48±3.49	43.8±2.85
Plant extract	200 mg/kg	77.2±3.79	62.4±3.89	53±5.79	44.2±3.57	37.8±3.77

Values are presented as Mean ± SEM (n=5)

5.3.4 Discussion

Medicinal plants are great asset of commercial drugs for the creation or in the production of lead compounds, it has been chided. The vast majority of the medications which are utilized for depression affect the quality life of sick people. Oppositely, herbal medicines have less less toxicity, good absorption and have a lower side effect profile. That is the reason; this has been utilized since extremely old circumstances (Li et al., 2003). So it is needed to create efforts to represent the new medicinal plants for production of cheaper and less toxic drugs.

In a past report, the presence of alkaloid, tannin, flavonoid and saponins in extracts of *Trapa natans L.* was uncovered by phytochemical tests. These phytochemical compounds presence can be related to the biological activities of *Trapa natans L.*For natural exercises, phenolic and flavonoid mixes are considered as essential secondary metabolites (Dixit et al., 2015). The exploratory extracts have great measure of phenol, flavonoids and tannin substance. These secondary metabolites might be in charge of bioactivities of the extracts.

The locomotor activity is a test to appraise the level of excitability of the CNS (Mansur et al., 1988) and any reduction of this action may be narrowly connected to sedation resulting from depression of the central nervous system (Ozturk et al., 1996). Gamma-amino-butyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs are elucidation their action through

GABAA, therefore it is possible that extracts of *Trapa natans L. (Panifol)* may acts by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts (Kolawole et al., 2007). Many research revealed that plant containing flavonoids, saponins and tannins are useful in numerous CNS disorders (Bhattacharjee et al., 1997). Earlier investigation on phytoconstituents and plants suggests that to be ligands for the GABAA receptors in the central nervous system, many flavonoids and neuroactive steroids were found which led to the assume that they can act as benzodiazepine like molecules (Verma et al., 2010).

In case of the thiopental sodium induced sedative test both the doses of the extracts produced a significant increase in the hypnotic effect, thus suggesting a profound sedative activity. In our study, the acute oral treatment with 100, and 200 mg/kg of methanolic extract of *Trapa natans L.* 30 min before the thiopental sodium injection significantly modified the latency to induce sleep as well as increasing duration of hypnosis induced by thiopental sodium, as depicted in Table-14. As expected, similar types of effects were observed by the administration with diazepam at 1 mg/kg. The results from the CNS depressant tests indicated that it significantly decreased the locomotor activity as shown by the results of the hole board (Table-15) and open field tests (Table-16). While evaluating neuropharmacological activities of *Trapa natans L.*, it was found that all the extracts possesses central nervous system depressant activity as indicated by decreased exploratory behavior in mice.

From our best knowledge, this is the first report of CNS depressant and analgesic activity of *Trapa natans L.*. Now it can be concluded on the basis of results obtained from investigation that the plant may be useful as CNS depressant and analgesic agent. But our work was only preliminary effort. It will require additional detailed advanced investigation.

5.5 RESULT AND DISCUSSION OF ANTIDIARRHEAL ACTIVITY:

In case of castor oil-induced diarrheal test, the MEWP showed a potent antidiarrheal effect in the mice (Table-17).The MEWP extracts in both doses, 500 mg/kg and 1000 mg/kg showed significant defecation inhibition, 66% and 77% where as 87% inhibition for standard lopiramide at the dose of 5 mg/kg. The above discussion indicates that *Trapa natans L.* have potent Anti-diarrheal activities which prove the traditional use of this plant.

Table-17: Analytical evaluation of the data obtained after time (hour) against control group

Code No.	Mice no	1st hour	2nd hour	3rd hour	4th hour	Average	Total Average With SEM	Std. deviation	% Reduction diarrhea
CTL	1	2	5	7	2	4	11.75±.0.08	0.14	0
	2	2	6	7	1	4			
	3	1	4	8	2	3.75			
STD	1	1	0	0	0	0.25	1.50±0.14	0.25	87
	2	1	1	0	0	0.5			
	3	1	1	1	0	0.75			
MEWP-500	1	3	1	0	2	1.5	4.00±.0.08	0.14	66
	2	2	1	1	1	1.25			
	3	3	0	1	1	1.25			
MEWP-1000	1	1	1	1	0	0.75	2.75±.0.08	0.14	77
	2	0	2	2	0	1			
	3	2	2	0	0	1			

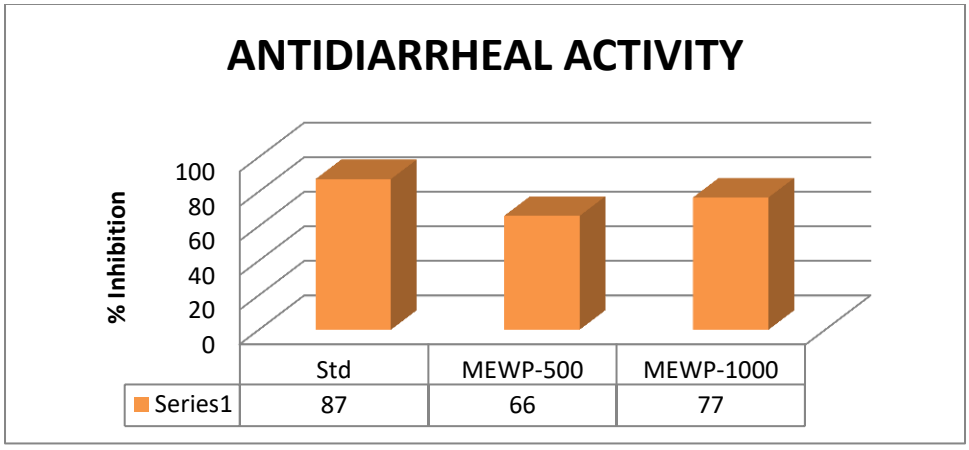

Fig-18: Anti-diarrheal activities of *Trapa natans L.*

CHAPTER 06

Summery of finidings

The project work introduced here manages diverse investigations *Trapa natans L.*(Family-Trapaceae). The examinations were carried on Phyto-science and pharmacology.

The target has been to investigate and assess the Phytochemicals, antioxidant, neuropharmacological and antidiarrheal activities to give a reasonable lead, which might be used in future to scrutinize another line of examination, in light of the consolidated approach of both exploitation and investigation.

The investigational work performed and represented in this project has been separated into two noteworthy parts.

- Phyto-chemical studies
- Pharmacological studies

The authenticity of the plant specimen has been confirmed by Bangladesh National Herbarium, Mirpur, and Dhaka, Bangladesh.

The dried leaves of *Trapa natans L.) w*ere powdered and extracted exhaustively by using continuous extraction process with organic solvent e.g. methanol. The solvent was distilled off and the extract was concentrated.

To get preliminary idea about the active constituents present in extracts different chemical tests were performed and showed the presence of Reducing sugar, Carbohydrate, alkaloid, Tannin, Steroids, Flavonoid, Saponin, Glycosides.

Based on ancient practices, folklore use and pharmacological interests of *Trapa natans L.* the present study was done to evaluate the antidiarrheal activities in the experimental mice (average body weights 16-34 gm.) and thereby to substantiate the claim about the plant to be used in folklore medicine.

From the study of antidiarrheal activity it was found that methanolic extract of whole plant of *Trapa natans L.*at a dose 1000 mg/kg exhibited highly significant defecation inhibition of by 77% and 66% for 500 mg/kg while the standard drug lopiramide inhibition was found to be 87% at the dose of 5 mg/kg body weight.

The methanolic extract of whole plant of *Trapa natans L.* showed promising DPPH free radical scavenging activity. The positive control ascorbic acid of which IC50 value was 2.59 µg/ml. On

the other hand, the Crude methanolic extract of bark showed promising DPPH free radical scavenging activity with IC50 value 14.13 μg/ml

The CNS depressant activity was examined by observing the reduction of locomotors and exploratory activities in the thiopental sodium induced sleeping time, hole cross, hole board and open field tests in mice at the doses of 100 mg/kg and 200 mg/kg b.w. In the present experiment diazepam at 1 mg/kg, bw was used as reference. All results were found statistically significant. We found that, methanol extract of *Trapa natans L.* significantly decreased the induction time to sleep and prolonged the duration of sleeping, induced by thiopental sodium. Besides, the spontaneous motor activity was decreased by all natural products in both hole cross and open field test. Furthermore, it also decreased the number of head dips in hole-board test by mice. Altogether, these results suggest that experimental extracts of *Trapa natans L.* possesses potent CNS depressant and hypnotic properties, which support its use in traditional medicine and suggesting that the plant should be further investigated for its pharmacologically active compound.

Thus a thorough study on both aspects (phyto-chemical and pharmacological) has been made and represented in this project based on the combined approach of both exploitation and exploration which may lead to a new line of treatment by folklore herbal remedies.

CHAPTER 07

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