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Comprehensive Review of Pharmacological Activities of *Trapa bispinosa* Roxb.

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Abstract— *Trapa bispinosa, a perennial aquatic plant commonly known as water chestnut, which belongs to the family Trapaceae. It is a small herb well known for its medicinal properties and is widely used worldwide, also has been extensively studied for its diverse pharmacological properties. This review aims to explore the various bioactive compounds present in T. bispinosa and their potential therapeutic applications. The plant is known to possess antioxidant, anti-inflammatory, antidiabetic, anticancer, antimicrobial, and neuroprotective activities, contributing to its value in traditional medicine. The review systematically discusses the mechanisms underlying these activities, focusing on the bioactive phytochemicals, including flavonoids, alkaloids, and polyphenols, that mediate these effects. Furthermore, the review highlights recent advances in the scientific understanding of T. bispinosa's pharmacological effects, while emphasizing its potential in drug development. The findings provide a comprehensive overview of the promising medicinal properties of T. bispinosa, suggesting that it may serve as a valuable source of natural therapeutics. However, the review also underscores the need for further clinical trials to validate its efficacy and safety for human health applications.*

Keywords— *Herbal drugs; Medicinal plant; Pharmacological activities; Trapa bispinosa.*

I. INTRODUCTION

Plant have been used as a medicines from the ancient times. Throughout the world medicinal plants are widely and successfully used. A plant with active medicinal constituents are used to treat diseases in the traditional systems like Ayurveda, Siddha, Unani. In Asia, the use of medicinal plants are well established and documented. Medicinal plants has got significant role in saving the lives people. In India, a no. of medical plants have been identified and they found to have good medicinal value. In this paper, we selected the plant *Trapa bispinosa* belonging to the family Lyratheace to study it's pharmacological effect [32].

Taxonomic Position

Kingdom: Plantae
Subkingdom: Tracheobointa
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Myrtales
Family: Lythraceae
Genus: Trapa
Species: *Trapa bispinosa*

Plant Description

The small plant *Trapa bispinosa,* a member of the Lyratheace family, is utilized extensively throughout the world for its therapeutic qualities. Folklore medicine has long acknowledged the medicinal benefits of the entire fruit and herb as a means of curing a variety of illnesses. The herbaceous, floating-leaf aquatic plant species *Trapa bispinosa* often grows in water that is about 60 cm deep. With leathery upper leaves up to 5 cm wide and broadly rhomboid, triangular, deltoid, or broadly ovate shapes. The floating leaves are grouped in a rosette and they have short, stiff hairs, noticeable venation, and a sharp serrated edge.

Additionally, the species produces remarkably different-looking submerged leaves. The beautifully divided, alternating submerged leaves can reach a maximum length of 15 cm. Each stem may generate many leaf rosettes, and the floating leaves' petioles include a spongy portion that permits the leaf rosette to float. The plant develops white single flowers at axils with four green sepals and four petals that are 8 mm long. The fruit, which resembles a horned nut and grows underwater, is about 3 cm wide. The plant's flexible stem, which ranges in length from 1 to 5 meters, has thin, linear roots at its nodes, and the lower roots that grew from the propagating seed husk anchor the plant in the sediment [34].

Trapa bispinosa known locally as water chestnut, it is widely planted throughout India. In India, China, and Southeast Asia, *Trapa bispinosa* species are not only vital for aquatic ecosystems but also provide as food for people and animals. It is cultivated in tropical regions in Asia and Africa in ponds and lakes, and it is frequently grown for its edible fruit. Folklore medicine has long acknowledged the medicinal benefits of the entire fruit and herb as a means of curing a number of illnesses. The tropical, subtropical and temperate regions of the world are home to annual aquatic plant *trapa bispinosa*. They naturally grow in Asia, Africa, and Southern Europe. Since the Neolithic era, it has been cultivated in Europe. Ancient Europeans frequently consumed it as food because it was a plant that grew easily; but, since its introduction to North America in 1874, it has become neutralized in some areas of the United States. It is extensively grown throughout Asia and was discovered in moist places, lakes, ponds, and slowly flowing rivers. It prefers nutrient-rich water with an alkalinity of 12–128 mg/L of calcium carbonate and a pH range of 6.7–8.2 [32].

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Figure 1: Whole plant and fruits of *Trapa bispinosa.*

Chemical composition

Trapa bispinosa contain a large no. of non-nutritional antioxidants such as flavoniods, flavone, also total phenolic contents are abundant in plant. Plant tissues, including fruits, vegetables, nuts, seeds, and leaves, have comparatively high levels of flavonoids. Flavonoids are naturally occurring antioxidants. Tannins, flavonids, and glycosides were found in the pericarp extract of T. bispinosa fruits, whereas carbohydrates, saponins, phytosterols, fixed oils, and fat were found in the seed extract of the fruit according to phytochemical screening. Three substances were separated from the hydroalcoholic extract of *Trapa pseudoincisa Nakai*. The substances' chemical structures were identified as 2-β,3α,23-trihydroxyurs- 12-en-28-oic acid, cycloeucalenol, and Ursolic acid. The no. of phytoconstituent are present Riboflavin (vitamin B2), Nicotinic acid/Niacin (vitamin B3), Thiamine (vitamin B1), Pantothenic acid (vitamin B5), Flavonoids, Alkaloids, Ascorbic acid (vitamin C), Retinol (vitamin A), Triterpenoids (Ursolic acid, (cycloeucalenol) [34].

Medicinal uses

Trapa bispinosa have many medicinal uses like it is a nutritive, appetizer, astringent, diuretic, aphrodisiac, tonic, cooling agent, and antidiarrheal ingredient in different types of Ayurvedic medicines. Additionally, it helps with bronchitis, bilious affections, sore throats, lumbago, inflammation, and exhaustion. Folklore medicine has long acknowledged the medicinal benefits of the entire fruit and herb as a means of curing a variety of illnesses and diseases. It has been observed that the entire herb exhibits hepatoprotective, antimicrobial, antibacterial, anticancer, antioxidant, and free radical scavenging properties. The fruits of TB have also been used as an intestinal astringent, aphrodisiac, anti-inflammatory, and anti-leprotic agent, as well as used for urinary discharges, fractures, sore throats, bronchitis, and anemia. Furthermore, the fruit's juice has been utilized to treat dysentery and diarrhea. Additionally, fruits are used to make liniments that are used to treat sunburn, wounds, and rheumatism. It is also thought to have anti-cancer qualities. Stem juice of TB used as the Ophthalmic medicines [36].

II. Pharmacological Activities of *Trapa Bispinosa*

The whole plant of *Trapa bispinosa* have many pharmacological activities like antidiabetic, anti-cancer activity, antimicrobial and cytotoxic activity, antibacterial activity, antiulcer activity, antioxidant activity, anti-inflammatory activity, neuroprotective activity, nootropic activity, antidepressant activity, analgesic activity, neuropharmacological activity, anti-arthritic activity, antiemetic activity, antihelmintic activity, immunomodulator activity and Exerts 5α-Reductase Inhibitory Activity in Castrated Benign Prostatic Hyperplasia etc. pharmacological activites of TB have mentioned in following table [35].

TABLE I. Pharmacological activities of *Trapa bispinosa*

Sr. No.	Plant Part	Pharmacological Activities	Model	Extract Used	Dose	Reference
1.	*Trapa bispinosa* Roxb.	Antimicrobial and Cytotoxic Activity	Antimicrobial activity by disc diffusion method & cytotoxicity Artemia salina (Brine shrimp) lethality	Methanolic, $CHCl_3$, Petroleum ether	200 µg/disc	M. Matiur Rahman et al., 2000
2.	Fruits	Neuroprotective Activity	D-galactose induced ageing	Hydroalcoholic	500mg/kg	Digambar. B. Ambikar et al., 2009
3.	Fruits	Nootropic Activity	Elevated Plus Maze, Passive avoidance response, Object Recognition test	Hydroalcoholic	100, 250 & 500 mg/kg	Ambikar D.B. 2009
4.	Root*	Analgesic Activity	Phenobarbitone induced sleep	Methanolic	200 & 400 mg/kg	A. K. Agrahari et al., 2010
5.	Fruit	Antiulcer Activity	Pyloric ligation and Aspirin plus pyloric ligation	Ethanolic	250 and 500 mg/kg	D. M. Kar et al., 2010
6.	Fruits	Neuropharmacological-al Activity	Effect on motor coordination, Locomotor Activity, Object recognition test, Transfer Latency using EPM, Analgesic activity, Anxiolytic activity using EPM, Sodium nitrite induced respiratory arrest, Hypoxic stress induced neurotoxicity	Hydroalcoholic	100, 250 & 500mg/kg	NS Vyawahare et al., 2010

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7.	Root*	Psychopharmacological Activity	Phenobarbitone induced sleep	Methanolic	200 & 400 mg/kg	Sanjaya Kumar Panda et al., 2010
8.	Fruit Peel	Antidiabetic Activity	Streptozotocin induced diabetes	Methanolic	100 and 200 mg/kg	Prashanto K. Das et al., 2011
9.	Fruits	Antidiarrheal, Analgesic and Antioxidant Activities	Castor oil induce diarrhea Acetic acid induction for analgesic activity DPPH free radical assay	Ethanolic	500 mg/kg	U.K. Karmakar et al., 2011
10.	Fruits	Antibacterial activity	Disc diffusion method	Methanolic	10 µg/µl, 20 µg/µl and 40 µg/µl	Kamal Krishna Biswas et al., 2012
11.	Kernel of *Trapa bispinosa* Fruit	Antioxidant, Immunoenhancing Studies	DPPH free radical assay & humoral immunity response, cell-mediated delayed type hypersensitivity reaction, and percent change in neutrophil count.	Aqueous Extract	1, 2, 4, 6, and 8 mg/mL.	Ramsankar Sarkar et al., 2012
12.	Fruit Peel and Root	Anthelmintic Activity	*Pheretima posthuman* and *Tubifex tubifex*	Aqueous and Methanolic extract	5, 10 and 20 mg/mL	Vinod k et al., 2013
13.	*Trapa bispinosa* Roxb.	Antioxidant, Laxative effect activity	Total phenolic Content Assay, DPPH assay, Reducing Power Assay, NO Scavenging Activity & Atropine induces decreasing Gastrointestinal time	Methanolic	200mg/kg, 400 mg/kg & 800mg/kg	Mohammad Zia Uddin, 2014
14.	Fruit	Antimycobacterial and Antioxidant Activities	DPPH assay and Total antioxidant activity (FRAP assay). micro-dilution technique & Broth Micro Dilution method.	Ethanolic	1.5 µl & 50 µg/ml	Lipika Aidew et al., 2014
15.	Fruit Peel	Hypoglycemic Activity	Measurement of α-glucosidase and α-amylase inhibition by invitro and Human clinical study	Hot water	125, 250, 500, 1000 µg/mL	Shouko Takeshita et al., 2016
16.	Leaf	Antioxidant and antiproliferative activities	Reducing power, Superoxide anion-scavenging assay, Scavenging assay of hydroxyl radical and DPPH radical, and Determination of total phenolic content (TPC). human cancer cell lines.	95% Ethanol+ Water	10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% methanol, respectively.	J. Xia et al., 2017
17.	Fruits	Anti-inflammatory activity	Formaldehyde induced rat paw edema	Aqueous Ethanolic	100, 200 & 400mg/kg	Aamir Mushtaq et al., 2017
18.	Fruits	Anti-nociceptive activity	Hot plate method	Aqueous Ethanolic	100, 200 & 400mg/kg	Aamir Mushtaq et al., 2017
19.	Dried fruits	Nootropic action against cognitive dysfunction	TL in EPM & Scopolamine Induced amnesia or memory disruption	50% Mixture of hydroalcohol	100,250,500 mg/kg	Ambikar D. B et al., 2017
20.	Fruits	Neurobehavioral deficit Activity	Mercury	Aqueous	150mg/kg & 300mg/kg	Pankaj Phukan et al., 2018
21.	Fruit	Antiemetic Effect	Copper sulfate induced emesis model	Hexane and Ethanolic extracts	150 mg/kg	Syed Waleed Ahmed Bokhari et al., 2020
22.	Pericarp	Improve insulin resistance	STZ-induction	60% Ethanol	100,200,400 mg/kg	Yidi Wang et al.,2021
23.	*Trapa bispinosa* shells	Anti-gastric cancer activity	CCK-8 assay	Ethanol	12.5, 25, 50, 100 &200 µg/mL	Limei Wang et al., 2022
24.	*Trapa Bispinosa*	Anti-arthritic activity	Carrageenan induced arthritis model & Protein denaturation assay	Polyherbal Formulation	100, 200mg/kg & 200- 1000 µg/ml	Amita B Dongare et al., 2023
25.	Pericarp	Exerts 5α-Reductase Inhibitory Activity in Castrated Benign Prostatic Hyperplasia	Invivo induced by Testosterone & Invitro by LNCaP cells	Pericarp extract	0, 50, &100 µg/mL	Takashi Fujita et al., 2023
26.	Fruits	Antidepressant activity	Restraint stress	Ethanolic	200mg/kg & 400mg/kg	Dr. Vanita Kanase et al., 2024

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III. Pharmacological Activity

1. *Antidepressant activity:*

Antidepressant activity of the ethanolic extract of the *Trapa natans* L. fruits was evaluated by Dr. Vanita Kanase et al., at a dose of 200 mg/kg and 400 mg/kg. Standard drug used for this study is Imipramine at a dose 15 mg/kg i.p. daily of 14 consecutive days and subjected to restraint stress on 13th day. In test drug treated group animal was subjected to acute restraint stress on 15th day. Adult male swiss albino mice of 6 number in each group were undertaken for the study and evaluated by tail suspension and forced swim test. Also, different biochemical parameter was studied like Catalase activity, Superoxide Dismutase activity, Malondialdehyde level, Lipid peroxidation assay.

Both doses of ethanolic extract of the *Trapa natans* L. fruits were found to produce a significant ($P < 0.01$) dose-dependant decrease in duration of immobility time in tail suspension and forced swim test. In addition to the behavioral tests, *Trapa natans* L. ethanolic extract also normalized the level of oxidative stress markers such as Catalase, SOD, MDA, and LPO in a dose dependent manner. The result showed significant antidepressant activity at higher dose.

These study suggest that the ethanolic extract of *Trapa natans* L. fruit possess significant antidepressant property, which is mainly due to flavonoids which play an important role in providing Antidepressant-like effect. Along with these, the fruits of Water Chestnut plant contains phenolic compounds such as gallic acid, Caffeic acid, ellagic acid, ferulic acid and flavonoids such as Pinobanksin, quercetin, naringenin, etc all this have antidepressant effect. From these research it can be concluded that the plant *Trapa natans* L. used for the treatment of neurological disorders and may be recommended as a supplement for the antidepressant activity (Dr. Vanita Kanase et al., 2024) [1].

2. *Neuroprotective activity:*

D. B. Ambikar et al., studied the neuroprotective activity of the hydroalcoholic extract of fruits of *Trapa bispinosa* Roxb. on lipofuscinogenesis and fluorenscece product in brain of D-galactose induced ageing accelerated in mice. Ageing is induced by the D- galactose at a dose of 0.5 ml of 5% of D-galactose in 18 female albino mice, it were each group contain 6 animals. Neuroprotective activity was evaluated at a dose of 500mg/kg of TB daily for 15 days. On 15th day after 60 min of administration of TB and D-galactose mice were sacrificed and all subjected to biochemical estimation. Different biochemical parameter are studied like lipofuscien content, lipid peroxidation, glutathione peroxides and catalse activity and fluorenscence.

D-galactose induction resulted in increased fluorescence product, increased lipid peroxidation and decreased antioxidant enzyme like glutathione peroxidase and catalase in cerebral cortex. Later, there was significant ($p<0.001$) reduction in the fluorescence content after co-administration with TB at 500mg/kg. Also, the MDA level decrease and glutathione peroxidase activity was significantly ($p<0.001$) increased as compared to the ageing accelerated mice due to co-treatment of TB. Similar way, CAT activity was significantly ($p<0.001$) increased as compared to D-galactose treated mice. These research, conclude that TB found to be effective antioxidant agent which some extent reverse the D-galactose induced ageing changes resulted due to the oxidative damage.

Oxidative stress and ROX are the major contributor for ageing processes and many age linked neurodegenerative diseases like Alzheimer's and Parkinson's. D-galactose induction increases the excessive formation of ROX and neuronal damage, which result in decline in learning and memory capacity. D-galactose is a reducing sugar that reacts with a free amines group of neuronal proteins and neuropeptides and to form advanced glycation end-products (AGEs). These AGEs undergo chemical oxidation and degradation via AGE-receptor binding and activation of signaling pathways that form free radicals and cause oxidative damage to micro molecules, cell oraganells and mainly mitochondria. Oxidative stress increases the lipid peroxidation it result in formation of the MDA as a by product. It is one of the oxidative biomarkers. The ROX also scavengered by endogenous antioxidant like glutathione peroxidase and catalse. Hence, increased ROX and free radicals decrease the catalse and glutathione peroxidase. Continuous damage of mitochondria due to free radicals causes breakdown of membrane components of lysosomes that result in an indigestible autofluroscent material called lipofuscin granules. D- galactose increases the lipofuscin granules in the cerebral cortex by oxidative damage.

Hence, these studies find that hydralcoholic extract of fruits of TB show significant neuroprotection via reducing the oxidative stress induced by D-galactose in a way of reducing the level of oxidative biomarker and increasing the level of antioxidant in cerebral cortex like Malondialdehyde, fluorescent material Lipofuscin and Glutathione, Catalse respectively (Ambikar DB et al., 2009) [2].

3. *Nootropic activity:*

The effect of hydroalcoholic extract of fruits of *Trapa bispinosa* (TB) was investigated by Ambikar D. B. for its nootropic activity using various experimental models of learning and memory like transfer latency (TL) on elevated plus-maze, passive avoidance response (PAS) and object recognition test. The nootropic activity was evaluated at a dose of 100, 250 and 500mg/kg on the swiss male albino mice. The standard drug used in these study is Piracetam at dose 150mg/kg. The doses were given to the animal on scheduled day and different parameter are also studied.

The result find out that on 2nd day, the administration of TB at all dose and Piracetam at 150mg/kg significantly ($p<0.001$) reduced transfer latency as compared to control group while on 9th day TB 250mg/kg was found effective as compared to 2nd day. TB 250 and 500mg/kg significantly ($p<0.001$) increased the step down latency in the PAS at acquisition and retention test. Also, in the object recognition index the TB 250 & 500mg/kg increased discrimination index, it indicates it's nootropic activity. It find out TB extract showed significant facilitatory effect on learning and memory in mice as well as improvement of memory in cognitive deficit.

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Current behavioral and neurobiological studies have provided evidences for involvement of cholinergic transmission in the learning and memory processes. It is also believed that acetylcholine and acetylcholinesterase activity can affect the learning abilities. Those drugs affecting learning and memory function have modulated Ach content and or AchE activity. In these research, the effectiveness of TB towards the facilitation of learning and retention of learned task suggested the nootropic active by the extract. These study conclude that hydroalcoholic extract of TB possess significant nootropic activity. The present study indicates that chronic pretreatment of TB possesses significant facilitatory effect on aversively motivated learning and memory in mice. It suggest the possible use of TB extract to improve the learning and memory processes (Ambikar DB et al. 2009) [3].

4. Analgesic activity:

A. Anuj k Agrahari et al., studied the analgesic activity of the methanolic extract of the *Trapa bispinosa* root. TB at a dose of 200 mg/kg and 400 mg/kg was evaluated against the standard drug used pentazocine at a dose of 30 mg/kg. Adult Swiss albino mice of either sex are used for the study where each group contain six animals. Analgesic activity was evaluated by tail flick model and tail immersion model.

Both doses of *Trapa bispinosa* roots methanolic extract were found to produce significant ($p<0.001$) analgesic activity in both model. In tail flick method, the extract at 200 mg/kg showed significant ($p<0.001$) activity after 45 minutes, but in tail immersion method, the extract showed significant ($p<0.001$) activity at all tested dose levels after 30-minute interval. The results showed significant ($p<0.001$) analgesic activity against stimuli. Hence, these study suggest the methanolic extract of roots of TB show the analgesic activity (Anuj k Agrahari et al., 2010).

B. U.K. Karmakar et al., studied the analgesic activity of the ethanolic extract of the fruits of *trapa bispinosa* Roxb. The analgesic activity of the TB was studied using acetic acid induced writhing model in mice at a dose of 500 mg/kg. 15 Swiss-Albino mice of either sex were used for the study, where each group contain 5 animals. Diclofenac sodium at the dose of 25 mg/kg was used as reference standard for the activity. After thirty minutes interval was given to ensure proper absorption of the test and standard drug, then the writhing inducing chemical, acetic acid solution (0.7%) was administered intra-peritoneally to each of the animals of a group. After an interval of 5 minutes was given for absorption of acetic acid and number writhing was counted for 15 minutes.

In acetic acid induced writhing test the TB extract, at the dose of 500 mg/kg, exhibited a maximum of 50.17% significant inhibition ($P<0.01$). of writhing reflex compared to the reference drug diclofenac sodium 55.22% ($P<0.01$). Preliminary phytochemical screening showed the presence of flavonoids, alkaloids and gum in the plant extract. So, the observed analgesic activity may be due to these compounds. Hence, the study conclude that ethanolic extract of the fruits of *trapa bispinosa* Roxb. have good analgesic activity due to phytoconstituent present in it (U.K. Karmakar et al., 2011) [4].

5. Neuropharmacological activity:

Neuropharmacological activity was evaluated by Vyawahare N. S by using the hydroalcoholic extract of fruits of *trapa bispinosa* in laboratory animals. *Trapa bispinosa* used at a different dose 100, 250 and 500mg/kg in these study. The standard drug used in these research is Diazepam at a dose 2 mg/kg for reference. The swiss male albino mice are pre-treated with TB for determining the effect of hydroalcoholic extract on various parameter like motor coordination, locomotor activity, Object recognition, transfer latency, anxiolytic activity, analgesic activity, sodium nitrite induced respiratory arrest and hypoxic stress was studied for assessing the neuropharmacological activity.

All doses of TB 100, 250 and 500mg/kg were found to be statistically significant in reducing the fall of time. Diazepam showed highest reduction in fall of time in both the experiments ($p<0.001$). TB at a dose of 100mg/kg did not produce any significant ($p<0.05$) effect on locomotion as compared to control group animal, but at a dose of 250 & 500mg/kg show significant ($p<0.001$). increased effect on the locomotor activity. The TB 250 &500 mg/kg found to decrease time required to occupy the central platform that is decrease the transfer latency in EPM and also increase the discrimination index in the object recognition test, it indicate it have the nootropic activity. TB at 250 & 500 mg/kg significantly ($p<0.001$) increase the reaction time in hot plate, it shows the analgesic activity. Also, the TB show the significant ($p<0.001$) increase in the latency to death in the sodium nitrite induced respiratory arrest. TB at dose 250mg/kg did not show significant ($p<0.05$) prolonged the latency for death but at 500mg/kg show significant ($p<0.01$) prolonged the latencies for death in hypoxic stress.

These antihypoxic effect of TB makes it a best candidate for the treatment of stroke. So, results of these study suggest that the hydroalcoholic extract of *trapa bispinosa* possess significant action on learning and memory, analgesic, antihypoxic activity. It claim TB as nervine tonic (NS Vyawahare et al., 2010) [5].

6. Neurobehavioral Deficit activity:

These study evaluated the effects of aqueous extract of dry fruits of *trapa bispinosa* in alleviating the mercury induced neurobehavioral deficit in animals. A total of 36 adult male Swiss albino mice of weight 25–30 g were equally divided into six groups. Group I received distilled water, Group II received mercuric chloride at 1.5 mg/kg, Group III received TB extract low dose 150 mg/kg, Group IV received TB extract high dose 300 mg/kg, Group V received mercuric chloride plus TB extract low dose, and Group VI received mercuric chloride plus TB extract high dose. All the groups received doses orally through oral gavage tube and the treatment continue for 14 consecutive days. The neurobehavioral effects were evaluated with locomotor activity in the open field test (OFT), spatial learning ability and memory in the Morris water maze test (MWM), immobility in Forced swimming test (FST) and anxiety in Elevated plus maze test (EPM).

In this study, it was observed that Hg-exposed mice significantly decreased the locomotor activity ($p<0.001$), time

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spent in open arms ($p<0.001$), also no. of entries in open arms ($p<0.001$), no. of crossover in OFT ($p<0.001$). Along with these, there will be significant ($p<0.001$) increase in immobility in FST, escape latency ($p<0.001$), and path length ($p<0.001$). The aqueous extract of *trapa bispinosa* significantly ($p<0.001$) reduced the neurotoxic effects of mercury. The aqueous extract of TB showed significant increase the locomotor activity ($p<0.001$), significant increase in no. of open arm entries ($p<0.005$) and significant increase intime spent in the open arm ($p<0.001$). Also, the no. of crossover increase significantly ($p<0.001$) in the OFT these all the parameters are decreased in the mercury induced animals. TB extract show the significant ($p<0.001$) decrease the immobility time, escape latency ($p<0.001$), and path length ($p<0.001$) in Hg exposed mice.

According to the result obtained from the behavioral study of the extract and mercuric chloride, the study indicates that mercuric chloride caused neurobehavioral changes which were significantly reversed by the aqueous extract of *trapa bispinosa* and the TB high dose of the extract was more effective than the TB low dose. The behavioral study also indicated the aqueous extract of *trapa bispinosa* to possess neuroprotective potential with antidepressant and anxiolytic activities and to improve learning and spatial memory in mice. These study conclude that TB be the effective in ameliorating the neurobehavioral deficit induced by Hg exposure (Pankaj Phulan et al., 2018) [6].

7. Nootropic action against cognitive dysfunction

Nootropic activity against cognitive dysfunction of the ethanolic extract of the *Trapa natans* L. fruits was evaluated by Ambikar D. B. et al., at a dose of 100mg/kg, 250 mg/kg and 500 mg/kg. Standard drug used for this study is Piracetam at a dose 150 mg/kg. Cognitive dysfunction was indued by scopolamine hydrobromide at 1 mg/kg dose. Male swiss albino mice of 5 number in each group were undertaken for the study and nootropic action evaluated by transfer latency (TL) on elevated plus-maze, passive avoidance response (PAS) in scopolamine induced amnesia (SIA) and brain acetylcholinesterase activity in albino mice.

The study finds out that TB 500 mg/kg significantly reduced the TL on 2nd and 9th day while TB 250 mg/kg was found effective on 9th day. TB 250 and 500 mg/kg significantly increased the step down latency in the PAS at acquisition and retention test. The extract also significantly attenuated the amnesic effects of scopolamine on the TL and PAS. The brain acetylcholinesterase (AchE) levels were not altered with the pretreatment TB. Hence, it show Tb does not have the action on cholinergic system. In present investigation TB extract showed significant facilitatory effect on learning and memory in mice. Moreover, it also attenuates the scopolamine induced memory disruption in mice.

These study conclude that hydroalcoholic extract of TB possesses significant nootropic action also study indicates that chronic pretreatment of TB possesses significant facilitatory effect on aversively motivated learning and memory in mice. Moreover, it also attenuates the scopolamine induced memory disruption in mice, to suggest possible use of TB extract to improve the learning and memory processes in poor learners and for improving cognitive deficit observed in various neurological disorders. The nootropic effect may be due to enhanced cholinergic function as TB inhibits scopolamine induced memory interruption (Ambikar D. B et al., 2017) [7].

8. Psychopharmacological activity:

Psychopharmacological activity of the methanolic extract of the *trapa natans* L.var. *bispinosa* Roxb. root was evaluated by Sanjaya Kumar Panda et al., at a dose of 200mg/kg &400mg/kg. Standard drug used for this study is diazepam and Chlorpromazine at a dose of 10mg/kg & 4mg/kg respectively. In these study, rat were used for studying the exploratory behaviour like swimming and pole climbing performance by the phenobarbitone induced sleep.

The methanolic extract of the *trapa natans* L.var. *bispinosa* Roxb. root at a dose of 200mg/kg and 400mg/kg show the significantly minor effect. The plant show the tranquilizing effect due to the presence of various psychoactive substances. Moreover, due to these psychoactive substances in *trapa bispinosa*, it shows the psychopharmacological effect on the different animal model. Hence, the psychoactive substances in the plant *trapa bispinosa* will be used as the psychoactive drug (Sanjaya Kumar Panda et al.,2010) [8].

9. Anti-nociceptive activity:

Aamir Mushtaq et al., studied the anti-nociceptive activity of the aqueous ethanolic extract of the fruits of the *trapa bispinosa*. Total 36 albino mice were used for these study where each group contain 6 animals. The anti-nociceptive activity was evaluated at a three different dose of 100mg/kg, 200mg/kg &400mg/kg by the hotplate method at a controlled temperature 55 to 56°C. Readings were taken at 00minute, 30minutes, 60minutes, 90minutes 120minutes and 180minutes Standard drug used for these study is diclofenac sodium at a dose of 10mg/kg, i.p. Analgesic activity was evaluated by recording the time of licking and jumping of mice by hotplate.

The study investigate the *trapa bispinosa* showed some increase in latency time in certain intervals at the applied dose of 100mg/kg on time scale. At the dose of 200mg/kg, extract showed slightly better increase in latency time between 120 to 180 min. While the dose of 400mg/kg, extract showed significant ($P < 0.001$) increase in latency time between 90min to 180minThe standard drug diclofenac sodium showed significant ($P < 0.001$) latency time. So, it can be conclude that the TB have the significant analgesic activity.

Trapa bispinosa contains alkaloids, anti-oxidants, saponins, tannins, phenols and glycosides. It is also used as a good source of enzymes like amylase, invertase, cellulose, lipase and protease. The fruit of *trapa bispinosa* reduces arachidonic acid formation. As a result formation of prostaglandins gets inhibited and urge of pain is remarkably reduced. This observation was noted at the maximum given dose of TB at 400mg/kg which showed viable results. The antioxidants, enzymes and phytochemical constituents of the plant are responsible for reducing analgesia. Phytochemical studies indicated the presence of phenols, alkaloids, tannins, glycosides, terpenes and terpenoides in plant extract. Thus, alkaloides, flavonoids, terpenes and terpenoids inhibit prostaglandin synthesis and may contribute in analgesic. On the

basis of results study has been concluded that aqueous ethanolic extracts of *Trapa bispinosa* analgesic activity as compared to standard drug diclofenac sodium (Aamir Mushtaq et al., 2017) [9].

10. Antidiabetic activity:

Prashanto K. Das et al., studied the antidiabetic activity by using the peels of the fruits of the *trapa natans* L. at a dose of 100 and 200 mg/kg. the methanolic extract of fruit peels was used for these activity by using the adult male wistar albino rats. The effect of METN on oral glucose tolerance and its effect on normoglycemic rats were also studied. Diabetes mellitus was induced in rats by single intraperitonial injection of streptozotocin at 65 mg/kg body weight. Three days after STZ induction, the hyperglycemic rats were treated with METN orally at the dose of 100 and 200 mg/kg body weight daily for 15 days. s. Glibenclamide at 0.5 mg/kg, was used as reference standard drug for these activity. The activity was evaluated by measuring the fasting blood glucose levels on every 5 day during the 15 days treatment.

METN at the dose of 100 and 200 mg/kg orally significantly ($p < 0.001$) and dose dependently improved oral glucose tolerance, exhibited hypoglycaemic effect in normal rats. Also, METN significantly ($p < 0.001$) show antidiabetic activity in STZ-induced diabetic rats by reducing and normalizing the elevated fasting blood glucose levels as compared to those of STZ control group. The antidiabetic effect of METN at 200 mg/kg dose was found to be comparable to that the effect exerted by the standard drug glibenclamide at the dose of 0.5 mg/kg. The study concluded that *T. natans* fruit peels have promising antidiabetic activity in STZ-induced diabetic Wistar rats.

Phytochemical studies showed the presence of alkaloids, triterpenes, steroids, polyphenol and carbohydrates in METN. Among them polyphenolics are the most reported phytoconstituents showing a wide range of pharmacological effects including antidiabetic activity. The presence of polyphenols or putative constituents may be responsible for promising antidiabetic activity of METN (Prashanto K. Das et al., 2011) [10].

11. Improving insulin resistance activity:

Yidi Wang et al., exploring the ingredients of total dried water caltrop (*Trapa bispinosa* Roxb.) pericarp powder and a dried ethanol extract for their capacity to reduce insulin resistance in the diabetic mice. In these study treatment group received water caltrop (*Trapa bispinosa* Roxb.) pericarp powder at one dose 400 mg/kg or extract at three doses 100, 200 & 400mg/kg for four weeks with inclusion of proper control groups. The type 2 diabetes was induced by the strepatazocine. The standard drug used for these study was glimepiride tablets at 5 mg/kg. The different indexes such as body weight, blood glucose and biochemical parameter were measured for four weeks after administration. Histopathological examination was done on the last of the study.

By the result, treatment of TB could significantly ($p<0.001$) improve the health index of type 2 diabetic mice. The mRNA and protein expression of SIRT1/PGC1α and PI3K/Akt signaling pathways were significantly ($p<0.001$) increased, while the mRNA expression of forkhead transcription factor (FoxO1) was decreased in the treatment group. The body weights of the animals receiving TB pericarp intervention were lower than those of the DC group. The difference in body weight between the WCPP and WCPEL groups compared to the DC group was significant ($P < 0.05$). The WCPEM and WCPEH groups had a strongly significant ($P < 0.01$) lower body weight, which was slightly lower than that of the PC group and close to that of the NC group. As expected, a significant increase in blood glucose levels was observed in the DC control mice, compared to the NC group ($P < 0.01$). After four weeks of treatment of TB, the PC, WCPP, and all three dose groups of WCPE all displayed certain reduction in blood glucose levels compared to DC group ($P< 0.05$). From this it can be concluded that consumption of WCPP has a blood glucose lowering effect in experimental diabetic mice.

Type 2 DM is accompanied by abnormal blood lipid metabolism, which is typically manifested by increased blood TC, TG, and LDL-C levels, together with decreased HDL-C levels. We tested the lipid parameters of each group of mice after the four weeks of treatment. TC and LDL-C values in the WCPP, WCPE groups were significantly lower than those in the DC group ($P < 0.05$); TG values in the PC and in the WCPEM and WCPEH group, but not in the WCPEL group, were significantly lower than those in the DC group ($P < 0.05$); Lastly, HDL-C values in the PC and in the WCPEH group were significantly higher than those in the DC group ($P < 0.05$). This demonstrates a dose-dependent impact, at least for WCPE, on lipid metabolism. Also, SOD, MDA, and GSH values in the WCPEH group were significantly different from those in the DC group ($P < 0.01$), despite the fact that the notable variations across the WCPP, WCPEL, and WCPEM groups were minimal or nonexistent. In liver function index, there was significant ($P < 0.01$), difference in the extract group relative to the DC group ($P < 0.05$), while the difference in the group WCPP was not significant. It can be seen that the SOD, MDA and GSH values in the WCPEH group were significantly ($P < 0.01$), different from those in the DC group ($P < 0.01$), while the significant differences in the WCPP, WCPEL, and WCPEM groups were low or non-significant. In the liver function index, there was a significant difference in the extract group relative to the DC group ($P < 0.05$), while the difference in the group WCPP was not significant. From the histopathoplogy we conclude that, number of islets and cells in the islets had increased, the degree of islet atrophy was improved, the islet structure was more complete and the cells were more closely arranged.

Crude powder of *Trapa bispinosa* Roxb. pericarp and also in particular dried ethanol extract has been shown to have good therapeutic effects on induced T2 DM mice. The hypoglycemic effect was primarily brought about by reestablishing the pancreas's function, enhancing liver gluconeogenesis, and reducing oxidative stress. These findings provide a basis for the further development and utilization of *Trapa bispinosa* Roxb. pericarp, provide a potential novel alley for treatment of type 2 diabetes (Yidi Wang et al.,2021) [11].

12. Hypoglycemic activity:

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Shouko Takeshita et al., evaluated the effects of the water chestnut peel extract on glycometabolism related enzymes α-amylase and α-glucosidase and the impact of the extract on postprandial blood glucose levels in a human ingestion test. *Trapa bispinosa's* inhibitory effect on glycometabolism-related enzymes was evaluated by invitro test system. In the presence of the test extract they caused a reaction in each of the two pairs – α-amylase and starch, α-glucosidase and disaccharides. After these, then evaluated the measured amount of resulting glucose by invitro test system. Morever, For determining the effect of TB extract on postprandial blood glucose level human clinical study has done. For these study 7 healthy Japanese male and female above age 20 were selected, from these 3 males and 4 females. The subjects ingested bread or rice after TBE intake, and evaluated the subsequent swing in blood glucose levels to assess the impact of TBE on postprandial blood glucose levels on a comparative basis.

The concentration of TBE has an inhibitory effect on α-glucosidase and α-amylase. In humans, if the test extract ingested before meals, the elevation in blood glucose levels 45min, 60min, and 90 minutes after bread intake and 15 minutes after rice intake was significantly lower ($p < 0.05$) than when the test extract was not ingested. In addition, the blood glucose area under the curve, AUC, was significantly lower (bread: $p = 0.012$, rice: $p < 0.01$) in the test extract group for both high-glycemic index foods.

TBE showed an inhibitory effect on α-amylase and α-glucosidase. Further, trials of *trapa bispinosa* extract ingestion in humans suggested its ability to suppress elevation of postprandial blood glucose levels. TBE is expected to become a functional food item that can have composite effects in countering glycation stress by controlling blood glucose levels. This study helped to gain knowledge on the impact of a single ingestion of TBE on the control of postprandial blood glucose levels. Since the long-term ingestion of TBE is believed to lead to suppression of in vivo formation of AGEs (advanced glycation end products).

This study suggests that the ingestion of TBE eases the rise of postprandial blood glucose levels. Its mechanism is thought to be a result of delayed absorption or suppression of glucose in the intestines due to TBE's inhibitory activity on glycometabolism-related enzymes, α-glucosidase and α-amylase. With regards to TBE's ability to reduce glycation stress (Shouko Takeshita et al., 2016) [12].

13. Anti-inflammatory activity:

Aamir Musht AQ et al., studied the anti-inflammatory activity of aqueous ethanolic extract of fruits of Trapa bispinosa. For these study, albino rats were divided into six groups (n=6). Group I & II were only given normal saline 10ml/kg/p.o and formaldehyde 0.1ml/kg s.c. respectively. Group III was given diclofenac sodium 10mg/kg/i.p while groups IV to XII were given plant extracts in different doses 100, 200 and 400mg/kg/p.o, respectively one hour prior to administration of the formaldehyde in sub planter region of left hind paw of rats. Using a vernier caliper, the paw sizes of rats in all groups were measured. The all readings were taken at the intervals of 00hour, 01hour, 02hours, 03hours and 04hours after treatment.

With regard to time scale, the regular control group did not exhibit any variation in paw sizes. The disease control showed visible increase in paw edema while the standard drug controlled the edema at certain level. The readings were taken on time scale in millimeters. When administered at a dosage of 100 mg/kg over time, Trapa bispinosa demonstrated a small reduction in the volume of edema at specific intervals. At the dose of 200mg/kg, extract showed major. decrease in volume of edema on 03hrs and04 hrs of time scale but the dose 400mg/kg showed maximum control and reduction in volume of edema.

Trapa bispinosa contains alkaloids, antioxidants, saponins, tannins, phenols, glycosides. It is also used as a good source of enzymes like amylase, invertase, cellulose, lipase and protease. Damage of tissue in inflammation is due to release of reactive oxygen species from phagocytes which surround the area of inflammation. The fruit of *Trapa bispinosa* reduces arachidonic acid formation. As a result formation of prostaglandins gets inhibited and urge of pain and inflammation is remarkably reduced. This observation was noted at the maximum given dose of *Trapa bispinosa* at 400mg/kg which showed viable results. The antioxidants, enzymes and phytochemical constituents of the plant are responsible for reducing inflammation. From results it clear that plants are enriched with flavonoids. Flavonoids are actually responsible for anti-inflammatory activity (Aamir Musht AQ et al., 2017) [13].

14. Anti-arthritic activity:

Amita B Dongare et al., evaluated the effectiveness of sustained release tablet of herbal plants *Trapa Bispinosa, Cassia Uniflora, Bosevilla Serrata, Cissus Quandragularis* in treating arthritis by using the invivo and invitro models. The 100 µL of 1% freshly prepared solution of carrageenan induced arthritis model was used to evaluate the in vivo anti arthritic activity of sustained release tablet of herbal plants. The in-vitro protein denaturation method was used to examine the anti-arthritic activity. The result was evaluated spectrophotometrically at 660nm against the reference drug diclofenac sodium. The sustained-release tablet of herbal plants was incubated with bovine serum albumin for complete denaturation.

The Sustained released tablet formulation has been shown to be anti-inflammatory activity in this study. A test drug that has significant predictive value for anti-inflammatory agents that act by inhibiting the mediators of acute inflammation reveals that the Sustained released tablet formulation significantly ($p<0.001$) inhibits the rat paw edema that is induced by carrageenan. Inflammation caused by carrageenan can be used to identify oral anti-inflammatory agents. The Sustained released tablet at the dose level of 200 mg/kg decreased the edema significantly ($p<0.001$) at 3rd and 4th hrs after administration of the extract when compared to the control group. The effect was compared to the activity ($p<0.001$) produced by standard drug Diclofenac sodium at 3rd and 4th hr after administration.

By inhibiting protein denaturation, the sustained release tablet displayed significant anti-arthritic activity at 200- 1000

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μg/ml. Comparing the sustained release tablet to the standard diclofenac sodium was used to investigate its effect. A number of studies indicate that protein denaturation is one of the causes of rheumatoid arthritis, which results in the production of autoantigens. At a concentration of 800 μg/ml, the sustained release tablet has the greatest activity. The findings of the study suggest that sustained release tablets can be used to treat arthritis. The study concluded that the sustained released tablet exhibited significant anti- inflammatory activity and hence can be used effectively in the management of arthritis (Amita B Dongare et al., 2023) [14].

15. Antiemetic activity:

The antiemetic effect of hexane and ethanolic extracts of *Trapa bispinosa* Roxb fruit. Was studied by Syed Waleed Ahmed Bokhari et al., at the dose of 150 mg/kg. Metoclopramide and Chlorpromazine (150 mg/kg) were used as standard antiemetic agents. The study was conducted on young and healthy male chicks aged 6-7 days using Copper sulfate (50 mg/kg) for the induction of emesis using oral route. Antiemetic effect was evaluated by observing the reduction in the number of retches in different groups of chicks.

All the groups were compared with negative control it was seen that there was a significant reduction ($p \leq 0.05$) in number of retches in all the group's i.e both the standard and treated group. All showed significant ($p \leq 0.05$) reduction in retching as compared to negative control. When Standard I (Metoclopramide) was compared with Standard II and both the treated groups it was found that Metoclopramide significantly reduced ($p \leq 0.05$) the number of retching (95.1%) inhibition as compared to Chlorpromazine (79.2%) inhibition and ethanolic extract of trapa fruit (87.8%). However there was insignificant difference between the hexane extract of trapa fruit and metoclopramide (both showed 95.1% inhibition). Similarly when Standard II (Chlorpromazine) was compared with both the treated groups, the number of retching's were significantly increased ($p \leq 0.05$) as compared to ethanolic and hexane extract of trapa fruit. Among the treated groups ethanolic extract of trapa fruit significantly increased ($p \leq 0.05$) the number of retching's as compared to hexane extract. The whole study suggest that the fruit of *Trapa Bispinosa* Roxb. possess anti-emetic activity.

The phytoconstituents responsible for antiemetic effects are alkaloids and triterpenoids. The mechanism of action of TB might be through the inhibition of serotonin and dopamine interacting with 5-HT4 receptor in addition with 5-HT3 and referred to as serotonin antagonist. Ethanolic extract showed sedation with antiemetic effect that might show dopamine antagonist like activity within the CTZ, in this manner restricting emetic input to the medullary spewing center and side-effects of dopamine antagonists such as sedation. The alkaloid present in the fruit possess anti-cholinergic effect which play a role in inhibiting emesis. Alkaloids usually produce this effect by acting at the M3 and M5 receptors and mediating the cholinergic activity within the vestibular input to the vestibular nuclei and probably also within brainstem pathways integrating vomiting such as the nucleus tractus solitarus.

The hexane extract of TB fruit prove to useful against chemotherapy induced vomiting, pregnancy vomiting, gastroenteritis vomiting and anxiety induced vomiting. The effects obtained from this study showed that natural remedy might also be used as an alternate to allopathic medicine for nausea and vomiting (Syed Waleed Ahmed Bokhari et al., 2020) [15].

16. Antiulcer activity:

Anti ulcer activity of 50% ethanolic extract of fruit of *trapa bispinosa* Roxb. in animals was studied by D. M. Kar et al., at a dose of 250 & 500mg/kg of TB twice daily for two days. The wistar rats were divided randomly into four groups of six animals each. Standard drug used for the study was rantidine at a dose of 25mg/kg once daily. Two model were studied for antiulcer activity that are pylorus ligation and aspirin induced ulcer. One hour after the last treatment, pylorus ligation was done under ether anesthesia on animals for ulcer induction. Also, the ulcer was induced by aspirin administration a 200mg/kg orally. The animals were killed after 4 hr, the stomachs were opened along the greater curvature and carefully observed for severity of ulceration. In these study, antiulcer activity was studied by estimation of free acidity, total acidity, total protein, carbohydrates, hexoses and hexosamine. Also, the estimation of Fucose, Pepsin & Sialic acid.

The results of the study suggest that the 50% ethanolic extract of the fruits of *trapa bispinosa* possess significant antiulcer activity. In both pyloric ligated ulceration and aspirin induced ulceration, the test extract of TB in both the dose levels 250mg/kg & 500mg/kg was found to reduce ulcer index to a significant extent in a dose dependent manner. In pylorus-ligated model, free acidity decreases with no significant change in the gastric volume, pH, total acidity and pepsin in both dose levels of the test extract TB. The presence of dissolved mucous substances in the gastric juice seems to be a reliable index of an effective mucosal barrier. The protein content of the gastric juice suggests the mechanism of leakage of plasma protein into the gastric juice responsible for ulcer. The test extract at both the dose levels produced a significant decrease in protein content and an increase in the carbohydrates content in a dose dependent manner The increased carbohydrate content by the test extract may presumed to be responsible for altering mucous secretion which in turn alter status of mucosal barrier. In aspirin plus pylorus ligated model the initial damage by aspirin, aggravates the damage further with increase acid secretion and decreased mucous production with increase pepsin activity. Hence, the pretreatment with 50% ethnolic extract of TB may have role in the suppression of severity of ulcer due to presence of carbohydrates and other components present in the extract (D. M. Kar et al., 2010) [16].

17. Anti-gastric cancer activity:

The molecular mechanism of the anti-gastric cancer activity of 1,2,3,6-tetra-O-galloyl-β-D glucose isolated from the *trapa bispinosa* Roxb. shell in vitro was studied by Limei Wang et al., at increasing concentration 12.5, 25, 50, 100 & 200 μg/mL. One monomer compound 1,2,3,6-tetra-O-galloyl-β-D glucose was separated from the ethanol extract of the trapa bispinosa shell

by fractional extraction, silica gel, Sephadex LH-20 gel column chromatography and liquid phase separation. The human gastric cancer cell line SGC7901 was used for the research. Anti-gastric activity was evaluated by determining the effects of the compounds on short-term proliferation by CCK-8 assays. Anti-gastric activity determined by cell cycle analysis, annexin-V FITC/PI assay, assessment of the mitochondrial membrane potential, intracellular calcium determination, transcriptomic data analysis, RNA extraction and quantitative RT-PCR. The results of the CCK-8 assay showed that 1,2,3,6-tetra-O-galloyl-β-D-glucose could significantly inhibit the proliferation of gastric cancer SGC7901 cells, and the effect was close to that of anti cancer drug 5-fluorouracil. Here, the carbohydrate 1,2,3,6-tetra-O-galloyl-β-D-glucose affect the cell cycle of SGC7901 cells. At the TB dose of 200 μg/mL and an incubation time of 48 hr, SGC7901 cells remained in the G1 phase and apoptosis occurred. Also, the intracellular calcium ion concentration increased and the mitochondrial membrane potential decreased. Moreover, transcriptome sequencing analysis showed that the differentially expressed genes were mainly enriched in the P53 signalling pathway are associated with the apoptosis. The results of quantitative PCR and western blot showed that 1,2,3,6-tetra-O-galloyl-β-D glucose could induce the apoptosis of SGC7901 cells by the up-regulating the expression levels of gene P21, PUMA, PERP and IGF-BP3 genes, down-regulating the CyclinD gene, increasing the expression levels of cytochrome C, caspase-3 and caspase-9 protein and decreasing that of the protein BCL-2. Hence, *trapa bispinosa* shells show the anti-gastric cancer activity due to the 1,2,3,6-tetra-O-galloyl-β-D-glucose and the high conc. of the polyphenol and has an effect against human gastric cancer SGC7901 cells in vitro (Limei Wang et al., 2022) [17].

18. Antidiarrheal activity

U.K. Karmakar et al., studied the antidiarrheal activity of ethanolic extract of *trapa bispinosa* Roxb. fruits at a dose of 500mg/kg. The standard reference drug used for the study is Loperamide at 3mg/kg orally. Total 15 swiss-Albino mice of either sex were used for the study, where each group contain 5 animals, where each group contain the 5 animals. The model of castor oil induced diarrhea was used for screening the antidiarrheal activity of ethanolic extract of fruits of *trapa bispinosa*. 0.3ml of castor oil was administered for diarrhea induction. Later, the animals examined for the presence of diarrhea every hour in five hours study after the castor oil administration. Number of stools on any fluid material that stained the adsorbent paper was counted at each successive hour during the experiment (05 hrs). The latent period of each mouse in every group was also counted.

The ethanolic extract of TB caused an increase in latent period 1.52 hr i.e. delayed the onset of diarrheal episode at the dose of 500 mg/kg body weight. The extract also decreased the frequency of defecation at the same dose where the mean numbers of stool at the 1st, 2nd, 3rd, 4th and 5th hours of study were 1.2, 3.2, 4.4, 2.8 and 2.2 respectively.

Hence, the fruit extract showed a significant ($P<0.001$) antidiarrheal activity against castor oil induce diarrhea in mice in which it decreased the frequency of defecation and increased the mean latent period at the dose of 500 mg/kg body weight, at show the significant antidiarrheal activity, due to the different phytochemical present in the ethanolic extract of TB like reducing sugars, tannins, alkaloids, saponins, gum, flavonoids (U.K. Karmakar et al., 2011) [18].

19. Laxative effect activity:

Mohammad Zia Uddin, studied the laxative effect of methanolic extract of *trapa bispinosa* at a different conc.200mg/kg, 400mg/kg & 800mg/kg. Swiss albino mice of either sex, and weight 20-25gm were used for the study. The animals are separated into 8 different group where each group contain 5 animals. Bisacodyl at 5mg/kg, p.o. used as positive control and the antimotility drug is atropine at 10mg/kg, i.p. were used for the study. After, the animals were given 0.3 mL of charcoal meal of distilled water suspension containing 10% gum acacia, and 20% starch. For the determination of laxative effect, charcoal meal GI transit test was done upon the administration of the crude extract of *Trapa bispinosa*. Lastly, the animals were sacrificed after 30 min and the abdomen was opened to excise the whole small intestine. The length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured to obtain the charcoal transport ratio or percentage.

Comparative evaluation of the extract of TB with the reference motility drug, bisacodyl, and Negative control group showed that the extract significantly ($p<0.001$) increases gastrointestinal motility in mice. A total 8 doses, e.g, 200 mg/kg, 400 mg/kg & 800mg/kg, 400mg/kg + atropine, and 800mg/kg + atropine of crude extract of *Trapa bispinosa* were used for the gastrointestinal transit test and they show significant ($p<0.001$) increase gastrointestinal motility in mice. Atropine along with the test drug and bisacodyl significantly ($p<0.001$) decrease the gastrointestinal transit time. Hence, the study suggest that *trapa bispinosa* is good source for the drugs for the laxative effect (Mohammad Zia Uddin, 2016) [19].

20. Antihelmintic activity:

Vinod K. Verma et al., evaluate the antihelmintic activity of fruit peel and root methanolic and aqueous extract of *trapa natans* L. at three conc. 5, 10 and 20 mg/ml. The effect of this plant was evaluated on adult Indian earthworms *Pheritima posthuma* and aquarium worm *Tubifex tubifex*. Piperazine citrate in 10mg/ml concentration was evaluated as standard reference and distilled water as control. The each extract of different conc. Were studied for the determining the time of paralysis and time of death.

The results of experiment shown that methanolic peel extract showed significant ($p<0.001$) antihelminitc activity out of all tested extracts than the aqueous extract. Result find that time of paralysis as well as death of worms of methanolic peel extract shown as compared to piperazine citrate specially at higher concentration of test drug 20 mg/mL. By employing one-way ANOVA, all data were found to be statistically significant at 5% level of significance ($p<0.005$). The extent of activity shown by the crude peel methanolic extracts was found to be better than methanolic and aqueous root extract and aqueous peel extract of *Trapa natans* plant. However, less than the

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standard drugs Piperazine citrate. It confirmed that the methanolic extract of fruit peels of plant *Trapa natans* is having anthelmintic activity. Hence, fruit peel methanolic extract of *Trapa natans* possess potential antihelmentic activity.

The study have been reported that the presence of flavonoids, tannins and polyphenolic compounds show anthelmintic activity, as they can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and thereby causes death of the worms. Some synthetic phenol anthelmintics e.g. niclosamide, oxyclozanide and bithionolare shown effects to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation and phosphorylation. Tannin content of the peel methanolic extracts of *Trapa natans* produced similar effects therefore it is mainly responsible for antihelmintic activity (Vinod K. Verma et al., 2013) [20].

21. 5α-Reductase Inhibitory Activity in Castrated Benign Prostatic Hyperplasia

The inhibitory effects of *Trapa bispinosa* extract on 5α-reductase in vitro using LNCaP cells and in vivo using a mouse model of castrated benign prostatic hyperplasia was investigated by Takashi Fujita et al., The administration of testosterone implicated in the progression of BPH and prostate cancer. The result of the study show that trapa bispinosa extract showed concentration-dependent inhibitory effects in the 5α-reductase (5αR) activity assay. In a reporter assay using AR-Luc/LNCaP cells, *trapa bispinosa* extract inhibited the activity induced by testosterone. TB pericarp extract also suppressed prostate cell proliferation, prostate-specific antigens, and transmembrane protease serine 2 expression in a castrated benign prostatic hyperplasia mouse model in invivo study. In addition, ellagic acid, but not gallic acid, decreased the 5αR and the AR-Luc activities. Hence, the results of these study suggest a potential role for TB extract in benign prostatic hyperplasia through inhibition of 5αR.

Trapa bispinosa Roxb. pericarp extract (TBE) contains several gallotannins and ellagic acid derivatives and it is great source of antioxidants. Also, it have anti-glycation activities in vitro. TBE is riched with gallotannin derivatives, such as eugeniin; 1,6- or 2,3-Di-O-galloyl-β-D-glucose (DGG); 1,2,3,6- or 1,2,4,6-tetra-O-galloyl-D-glucose (TGG); 1,2,3,4,6-pentagalloyl glucose (PGG); and valoneic acid dilactone. The Gallotannins, including eugeniin, TGG, and PGG, are the phytoconstituent components of *trapa bispinosa* extract, all have been reported to inhibit 5αR enzyme which responsible for testosterone formation. Testosterone have the role in BPH and prostate cancer. Among these all, PGG is the most potent inhibitor of 5αR activity. Hence, the present study investigated the effects of TB pericarp extract on 5αR both in vitro and in vivo (Takashi Fujita et al., 2023) [21].

22. Antibacterial activity:

Antibacterial activities of the fruit extract of two varieties (green and red) of water chestnut by the disc diffusion method from methanol extract were studied by Kamal Krishna Biswas et al., The activities are studied against a number of human pathogenic bacterial species by using the disc diffusion method. The antibiotic Kanamycin (30 µ g) was used as standard.

The result of the study show that, the extract of red variety of water chestnut showed high antibacterial potential (31 mm) against Bacillus subtilis with the concentration of 600 micron. On the other hand, green variety showed highest antibacterial activities (12 mm) against both *Staphylococcus aureus* and *Shigella sonnei* with the concentration of 600 microgram. In the disc diffusion assay, the methanol extract of red variety was found to have a significant ($p<0.001$) antibacterial efficiency compared to the extract of green variety of water chestnut. These findings suggest the efficiency of these extracts to inhibit microbial growth. The methanol extract of red variety of water chestnut fruit was found to be the most potential antibacterial extract that showed inhibitory activity against both gram (+ ve) and gram (- ve) bacteria. This study reveals that water chestnut can be used potentially as a broad-spectrum antibacterial agent. Hence, *T. bispinosa* fruit could be used as a guide in our continuing search for new natural products with potential medicinal properties as it will lead to the development of a phyto-medicine to act against microbes (Kamal Krishna Biswas et al., 2012) [22].

23. Antimicrobial and Cytotoxic Activity

M. Matiur Rahman et al., studied the antimicrobial and cytotoxic activity of *Trapa bispinosa.* The different extract of *trapa bispinosa* whole plant were used for the study like MeOH, EtOAc, $CHCl_3$ and *petroleum ether*. The extracts of Trapa bispinosa demonstrated notable cytotoxic activity and intriguing antimicrobial activity against Gram-positive and Gram-negative test bacteria.

Among the tested extracts of *Trapa bispinosa,* the MeOH extract of this plant at the concentration of 200 µg/disc showed the more potent antimicrobial activity against Gram positive (*Bacillus subtilis*, *B.cereus*, *B.megaterium*, *Staphylococcus aureus* and *Staphylococcus β-haemolyticus*) and Gram negative (*Escherichia coli*, *Klebsiella*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Shigella boydii*, *Salmonella typhi* A and *Salmonella typhi* B-56), while most significant cytotoxic activity was found in $CHCl_3$ extract. Petroleum ether extract showed neither antimicrobial nor cytotoxic activity. These findings may lend support to the traditional use of this plant in diarrhea and dysentery (M. Matiur Rahman et al., 2000) [23].

24. Antioxidant activity:

A. U.K. Karmakar et al., studied the ethanolic extract of fruits of *Trapa bispinosa* Roxb. for antioxidant activity by in-vitro. Antioxidant activity was determined on the basis of their scavenging activity of the stable DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical by qualitative and quantitative assay. In qualitative assay, DPPH (0.02%) is sprayed on the chromatogram of the extract, it forms pale yellow or yellow colour which indicates presence of antioxidant in the sample. And in quantitative assay percentage of inhibition is calculated by comparing with ascorbic acid as standard.

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The extract showed antioxidant activity which was comparable to standard drug ascorbic acid. In this study percentage inhibition of free radical scavenging activity was increased with the increase concentrations of both crude extract and ascorbic acid. The results tend to suggest that the extract might possess some chemical constituents that are responsible for antioxidant activities like reducing sugars, tannins, alkaloids, saponins, gum, flavonoids (U.K. Karmakar et al., 2011) [25].

B. Ramsankar Sarkar et al., evaluated antioxidant activity of the Polysaccharide Isolated from the Kernel of *Trapa bispinosa* Fruit. The antioxidant activity fruit of TB was measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical test. Samples were dissolved in distilled water at 1, 2, 4, 6, and 8 mg/mL. One-milliliter test samples were mixed with 2 mL of freshly prepared DPPH (0.1mM) in 50% ethanol. After shaking, the mixture was incubated at 25 °C for 30 min in the dark, and then the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated, the higher free radical scavenging activity. Ascorbic acid was used as a standard antioxidant material in these study [26].

The scavenging activity of the PS increased steadily from 1 to 8 mg/mL, while it reached a maximum plateau from 0.5 to 2 mg/mL for ascorbic acid, which indicates that the scavenging activity of the PS against the DPPH radical was less than that of ascorbic acid. A maximum of 65.5% DPPH radical scavenging activity was observed at 8 mg/mL of the PS. This may be due to the presence of some phenolic or polyphenolic compounds in the crude materials, which are strong scavengers of free radicals. Hence, it conclude that polysaccharide isolated from kernel of fruit of TB have good antioxidant activity (Ramsankar Sarkar et al., 2012).

C. Mohammad Zia Uddin evaluated the antioxidant property of methanolic extract of *trapa bispinosa.* The activity was studied by performing different assay like Total phenolic Content Assay, DPPH Radical-Scavenging Activities, Reducing Power Assay, Nitric Oxide (NO) Scavenging Activity.

The result shows that ethyl acetate fraction of methanolic extract of *Trapa bispinosa* have Highest amount of phenolic content than N-hexane extract of *Trapa bispanosa* have phenolic content, thus it can be a good source of phenol. N-hexane and ethyl acetate extract of *Trapa bispanosa* have good antioxidant activity by DPPH free radical assay. Where, NO scavenging activity of methanolic extract of *Trapa bispanosa* showed least activity. Two different extract showed highest level of reducing power in *Trapa bispinosa (*Mohammad Zia Uddin 2014) [28].

D. Lipika Aidew et al., studied the antioxidant Activities of the fruit of *Trapa natans* L. var. Bispinosa. The antioxidant activity was determined by DPPH radical scavenging assay and Total antioxidant activity (FRAP assay).

The DPPH scavenging values varied from 42.7% to 90.3%, with the highest value found in the ethanol extract of the peel. The value of the FRAP assay of the ethanolic extract of the peel is showing the highest antioxidant activity followed by the methanolic extract of the peel and the ethanol extract of the whole fruit. From the present study it appears that the peel and the fruit of *T. natans* possess potent antioxidant activity. The extracts did not exhibit any cytotoxic activity, even at very high concentrations, making it a promising candidate as a drug component. With the high content of phenolics and flavonoids, the peel could be a potential source of antioxidant compounds (Lipika Aidew et al., 2014) [27].

E. J. Xia et al., investigated the antioxidant activities of the *Trapa bispinosa* leaf by invitro using different assay like Reducing power, Superoxide anion-scavenging assay, Scavenging assay of hydroxyl radical and DPPH radical, and Determination of total phenolic content (TPC). Both ethanol extract and water extract exhibited the antioxidant activity. Ethanol extract exhibited stronger superoxide anion- scavenging capacity than Water extract. Further separation was carried out on ethanol extract by macroporous resin to obtain eleven fractions. Among all fractions, the fraction eluted by 80% methanol had the highest total phenolic content (TPC).

The results demonstrated that both ethanol extract and water extract of the *Trapa bispinosa* leaf have potent antioxidant activity, including the capacity of scavenging intracellular ROS. Different phytoconstituent are responsible for antioxidant activity like Benzoic acid, Pyrogallol, 1,8,10-Trihydroxy-9-anthrone, xanthotoxol, 2-O-p-hydroxybenzoyl-6-O-galloyl glucoside, (2R,3S,10S)-7,8,9,13-tetrahydroxy-2-(3,4-dihydroxyphenyl)-2,3-trans3,4-cis-2,3,10-trihydrobenzopyrano[3,4-c]-2-benzopyran-1-one, Hasubanonine. These results suggest that the *Trapa bispinosa* leaf could also be potential sources for diseases associated with oxidative stress (J. Xia et al., 2017) [24].

25. Immunoenhancing activity:

T. bispinosa fruit aqueous extract (TBAE) shown encouraging immunomodulatory properties. Rats' immunomodulatory response to sheep red blood cells as an antigen was evaluated by examining the humoral immunity response, cell-mediated delayed type hypersensitivity reaction, and percent change in neutrophil count. Immunostimulatory responses were dependently enhanced by oral treatment of *T. bispinosa* (TBAE). The generation of circulatory antibodies (humoral antibody response) was considerably ($p<0.005$) enhanced in response to sheep red blood cells as an antigen, and the mean thickness of the foot pad at 48 hours was observed to significantly ($p<0.005$) increase the delayed type hypersensitivity reaction. Additionally, in reaction to carbon particles, macrophage phagocytosis was upregulated, counteracting immunostimulation. TBAE's immunostimulatory effect was further supported by a significant ($p<0.001$) increase in neutrophil counts when compared to the control. The results of the present study suggested that the aqueous extract of fruits of *T. bispinosa* could stimulate the cellular and humoral response in animals and show the immunoenhacing activity (Ramsankar Sarkar et al.,2012) [29].

26. Antimycobacterial activity:

Lipika Aidew et al., evaluated the antimycobacterial activity of fruits of *trapa natans* L. against two species like

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Mycobacterium tuberculosis and *Mycobacterium smegmatis* a non pathogenic species. The antimycobacterial activity of the extracts of fruits of TN was determined by agar well diffusion method. Rifampicin (8µg/ml) was used as the positive control. The minimum inhibitory concentration was determined using micro-dilution technique & Broth Micro Dilution method.

The ethanolic extract of the peel exhibited largest zone of inhibition against *M. smegmatis* in comparison to the ethanolic extract of the whole fruit and the methanolic extract of the peel. The water extracts of the whole fruit, peel and the methanolic extract of the whole fruit did not show any antimycobacterial activity. The MIC values of the ethanolic and the methanolic extracts of the peel were highest against *M. tuberculosis* and *M. smegmatis* respectively. Hence, The antimycobacterial activity of the ethanolic extract of the peel was much higher than the ethanolic extract of the whole fruit of *T. natans*. The study revealed that the ethanolic extract of the peel was most effective in inhibiting the growth of the bacteria. The scanning electron microscope studies were conducted to find out if there was any plant extract induced morphological change on the bacterial cell wall. Scanning Electron Microscopy of *M. smegmatis* cells treated with the extracts at MIC revealed lysis and shrinkage of the bacterial cell, suggesting that the extracts caused disruption of the mycolic acid rich bacterial cell wall leading to the cell death. From the present study it appears that the peel and the fruit of *T. natans* possess potent antimycobacterial activity. The extracts did not exhibit any cytotoxic activity, even at very high concentrations, making it a promising candidate as a drug component. With the high content of phenolics and flavonoids, the peel could be a potential source of antimycobacterial compounds (Lipika Aidew et al., 2014) [30].

27. Antiproliferative activity:

J. Xia et al., studied the antiproliferative activities of the leaf extracts from *Trapa bispinosa.* y. Anticancer activity was evaluated by testing the antiproliferative effect against four human cancer cell lines. Human lung cancer cell line (A549), human liver cancer celllines (HepG2 and SSMC-7721) and human cervix cancer cell line (HeLa) were used for the antiproliferative study. Ethanolic extract exhibited stronger cytotoxic effect on all cancer cells tested than water extract. Different phytoconstituent are responsible for antiproliferative activity like Benzoic acid, Pyrogallol, 1,8,10-Trihydroxy-9-anthrone, xanthotoxol, 2-O-p-hydroxybenzoyl-6-O-galloyl glucoside, (2R,3S,10S)-7,8,9,13-tetrahydroxy-2-(3,4-dihydroxyphenyl)-2,3-trans3,4-cis-2,3,10-trihydrobenzopyrano[3,4-c]-2-benzopyran-1-one, Hasubanonine. These results suggest that the *Trapa bispinosa* leaf could also be potential sources for diseases associated with cancer having antiproliferative activity (J. Xia et al., 2017) [31].

IV. Conclusion

Medicinal plants play very important role to human beings in preserving our health. The role of medicinal plants in promoting the ability of human health to cope with the unpleasant and difficult situation is well documented from ancient times till date all over the world. There is a growing interest in the pharmacological evaluation of various plants which are used in Indian traditional system of medicine. *Trapa bispinosa* commonly known as water chestnut demonstrates a broad range of medicinal and pharmacological activities that suggest its potential for therapeutic applications. *Trapa bispinosa* can help with wide range of illnesses and ailments. The plant contains enormous different phytochemical constituents like phenols, flavonoids, terpenoids, saponins, tannnis and alkaloids having various medicinal applications. Various bioactive compounds in the plant have been shown to exhibit antioxidant, anti-inflammatory, antimicrobial, antidiabetic, immunomodulatory and anticancer properties. These all diverse pharmacological activities of plant *trapa bispinosa* suggest plant's potential application in the modern medicine for treating the large range of illness as well as it's historical use in many medical systems. Also, plant's anti-inflammatory and neuroprotective properties suggest its promise for treating the inflammatory and neurological disorders. To completely understand the processes underlying this various pharmacological effect and to evaluate the safety and effectiveness of *trapa bispinosa* in therapeutic contexts, more research including clinical trials is required.

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