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Phytochemical and Pharmacological Assessment of the Ethanol Leaves Extract of *Heritiera fomes* Buch. Ham. (Family- Sterculiaceae)

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ABSTRACT

Purpose: The current study was themed to phytochemical and pharmacological investigation to determine anti-nociceptive, antioxidant and analgesic activity to give an appropriate guide for future exploration. **Methods:** Standard test methods were used to explore phytochemical constituents. The acetic acid-induced writhing model was applied to inspect chemical anti-nociceptive effect while thermal nociception was evaluated by hot plate model. DPPH assay was the choice of method for quantitative determination of antioxidant activity. Antimicrobial activity was assessed by disc diffusion assay method. **Results:** Phytochemical exploration of leaves extract demonstrated the presence of Reducing sugars, Saponins, alkaloids, glycosides, tannins, steroids, Flavonoids and Gums. The extract illustrated statistically significant anti-nociceptive activity ($P < 0.01$) in dose dependent manner both chemically and thermally. Significant free radical scavenging activity in 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was illustrated both qualitatively and quantitatively. Quantitatively, it disclosed stable DPPH radical scavenging activity with the IC_{50} value of 26.30 $\mu\text{g/mL}$. The extract also showed significant antimicrobial activity against a number of Gram-positive and Gram-negative bacteria. **Conclusion:** It can be disclosed that the leaves extract of *Heritiera fomes* possess significant anti-nociceptive, antioxidant and antimicrobial activity. The potential of these activities may be due to the presence of phyto-constituents reported in the phytochemical tests and justify its uses as a habitual folk medicine.

Keywords: *Heritiera fomes*, Anti-Nociceptive Activity, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), Antimicrobial Activity.

1. Introduction

H. fomes Buch. Ham. (Family- Sterculiaceae), common name: Sundari (Bangladesh), occurs as a mangrove plant in Sundarban, the world's single largest mangrove forest that extends across Bangladesh and the Indian State of West Bengal. This plant is also found in coastal regions of countries like India, Myanmar, Thailand, and Northern Malaysia. It is an evergreen medium to tall size tree, height ranges from 15 to 25 m. It has a buttressed stem and ancient, longitudinally splintered bark. The dark green leaves are grouped near the ends of the twigs and have short petioles. The species instigates producing pneumatophores at the age of 3 years. Regions with heavy annual rainfall of 1600 mm to 5334 mm and a warm equable climate of 7.22 °C to 37.78 °C are required to grow this species. It flowers in the month of March and April. Unisexual flowers are arranged in panicles. The fruit carpels are extended to 3.81 to 5.08 cm and fall to the land when they ripen in the month of July and August. The seeds ripen in June and July. The plant is used by the rural people as traditional medicine and possesses a variety of pharmacological activities. Leaves, roots, and stem are used for the treatment of gastrointestinal diseases (including diarrhea, dysentery, colic, acidity, indigestion, constipation, stomachache, bloating, and lack of appetite); skin diseases (including eczema, abscess, boils, acne, infections, scabies, itch, dermatitis, rash, sores, scar, warts), hepatic disorders (including jaundice, hepatitis); diabetes and goiter in rural areas ^[1, 2]. Locally, this plant is used to cure fever, and pain as herbal medicine.

In the preceding study, anticancer activity and chromatography characterization of methanol extract of *Heritiera fomes* were accomplished and reported [1]. Methanol extract of *Heritiera fomes* have shown potential antihyperglycemic and antinociceptive effect [2]. Brine Shrimp Toxicity Study of *Heritiera fomes* was also performed [3].

In the present study, an endeavour was made to shield the conventional uses as per technical research. Furthermore, the presences of responsible compounds were distinguished by using a variety of customary chemical tests. Upon adequate literature review, it is found that a slight study has been accomplished to estimate the uses of this plant in traditional medicine. In our study, we therefore tried to assess the Anti-nociceptive, antioxidant and antibacterial activity of the ethanol extract of leaves of *H. fomes*.

2. Materials and Methods

2.1. Sample collection, Identification, and Extraction

Fresh leaves of *H. fomes* were collected from Sundarbans, Bangladesh in January, 2011. After collection it was identified by the professionals at Bangladesh National Herbarium, Mirpur, Dhaka (Accession no.: DACB 35523) and a voucher specimen was also deposited there. The leaves were shade dried for 7 days. After adequate drying, the leaves were cut into small pieces, and then lacerated to crude powder with the help of a suitable mechanical chopper. The powder were stored in an appropriate airtight container and kept in a cool, dry and dark place to shun possible fungal assault.

Cold extraction method was used for extracting. About 250 gm of grinded powder were taken in an appropriate container and saturated in 800 mL of ethanol (99-100%). The container with its contents was kept for a time of 15 days accompanying infrequent stirring and shaking. The whole mixture contents were filtered off with clear cotton plug to eliminate plant garbage. The extract was finally filtered through whatman filter paper. The organic solvent (ethanol) was evaporated with an electric fan at room temperature to get the dried crude extract (yield value 9.03%). Then the dried crude extract was stored in refrigerator at 4 °C.

2.2. Test Animals and Pathogens

For *in-vivo* pharmacological experiment, Swiss-Albino mice of either sex (22 to 27 gm) were used. The mice were collected from animal resources department of ICDDR, B (International Centre for Diarrhoeal Disease Research, Bangladesh). The mice were acclimatized by keeping in the polypropylene cages by providing with right rodent foods under standard laboratory condition (room temperature 25±2 °C, relative humidity 55±5 %, and 12 hours light: dark cycle) in animal house, Pharmacy Discipline, Khulna University for a period of 14 days prior to performing the pharmacological experiment. Animals used in this experiment were treated following the Ethical ideologies and rules for Scientific Experiments on Animals originated by the Swiss Academy of Sciences and the Swiss Academy of Medical Sciences (1995).

Antibacterial activity was executed using both Gram-positive and Gram-negative bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, and *Salmonella paratyphi*. These pathogens were collected from ICDDR, B.

2.3. Chemicals and Reagents

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) was purchased from Sigma Chemical Co. Ltd, (St. Louis, MO, USA). Merck, Germany supplied ascorbic acid and acetic acid. Tween-80 was purchased from Loba Chemie Pvt Ltd, India. Solvents and all other reagents were of analytical grade.

2.4. Standard Drugs

The standard drug diclofenac sodium was collected from Aristopharma Ltd, Bangladesh and used for analgesic activity study. Standard antibiotic discs (Kanamycin 30 µg/disc) were purchased from Oxoid Ltd, UK and sterile blank discs were also purchased from BBL, Cocksville, USA.

2.5. Phytochemical Test

The freshly prepared crude extract was subjected to preliminary phytochemical screening for the identification of foremost functional constituents present in the extract [4-6]. The extract showed the presence of Reducing sugars, Saponins, Alkaloids, Glycosides, Tannins, Steroids, Flavonoids and Gums.

2.6. Anti-nociceptive Activity Test

2.6.1. Acetic Acid Induced Writhing Test

For determining the Anti-nociceptive Activity of the crude sample, acetic acid induced writhing test in mice was used [7, 8]. Experimental animals (mice) were randomly selected, and split into four represented groups as control, positive control, and test groups (I and II) consisting of five mice in each group. Control group was delighted with 1% Tween-80 in distilled water at the dose of 10 mL/kg body weight, and positive control group was treated with diclofenac sodium at the dose of 25 mg/kg body weight. The test sample at the doses of 250 and 500 mg/kg body weight was given to test group I and II respectively. Oral route was the themed to all the treatments. To make proper absorption of the administered treatments, thirty minutes period was given. Then 0.6% acetic acid solution, a writhing inducer, was administered using intra-peritoneal route to each animal. For proper absorption of administered writhing inducer, five minutes interval was given. At that time number of writhing was counted for 10 minutes. Percent inhibition of writhing for both test and control group was calculated and compared.

2.6.2. Hot Plate Method

In the Eddy's hot plate method, test animals (Albino mice, 22 to 25 g) were split into control, positive control and test group I and II. Each group consists of five mice. Control group was treated (orally) with water at the dose of 10 mL/kg body weight. Test groups were administered with test substance at the dose of 250 and 500 mg/kg body weight. Test substance was given orally. The animals of positive control group were served with Morphine (Standard drug) at the dose of 5 mg/kg body weight. Then the mice were placed on aluminium hot plate maintained at a temperature of 55±0.5 °C for a maximum time of 20 second to record reaction time. Reaction time was documented as the amount of time it took to lick its forepaws and jumped from the plate at 0, 30, 60, and 90 minutes after oral administration of crude extract. Standard drug was given intraperitoneally [9, 10].

2.7. In Vitro Antioxidant Activity Test

2.7.1. Qualitative Test

For qualitative determination of antioxidant activity Thin Layer Chromatographic (TLC) technique was applied [11, 12]. This test was executed by developing TLC plates with polar, medium polar, and non-polar solvent systems. After applying 0.02% ethanolic DPPH on the TLC plate, yellow colour on violet background was viewed, that indicated the attendance of antioxidant components.

2.7.2. Quantitative Assay

DPPH Radical Scavenging Activity

The free radical scavenging activity of the extract was estimated *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH, a stable free radical) assay [11-13]. The DPPH scavenging activity is based on the capability of DPPH to decolorize in the presence of antioxidants. Various concentrations of leave extracts solution (500, 200, 100, 50, 10, 5, and 1 µg/mL) were prepared. 2 mL of each sample solution was taken into seven separate test tubes. Subsequently 6 mL of 0.004% ethanolic DPPH solution was taken separately into these test tubes. For adequate reaction, the sample solutions were kept at dark place for 30 minutes. The lessening of the DPPH radical was concluded by measuring the absorption at 517 nm using UV spectrophotometer against blank preparation. Ascorbic acid was used as standard that was prepared as like as sample preparation. Percent of DPPH radicals scavenging or inhibition was determined using following equation:

$$\text{Percent radicals scavenging or inhibition} = \frac{[(\text{Absorbance of blank} - \text{Absorbance of sample or standard}) / \text{Absorbance of blank}] \times 100}{}$$

The extract concentration providing 50% of radicals scavenging or inhibition (IC₅₀) was calculated from the percent inhibition vs. concentration graph.

2.8. Antimicrobial Activity Test

Disk diffusion Assay

For the assessment of antimicrobial activity of ethanol leaves extract of *H. fomes*, disc diffusion assay method was used against a number of Gram-positive and Gram-negative strains like *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, and *Salmonella paratyphi* [14-16]. Test extract was prepared at the desired concentrations using ethanol. 250 and 500 µg/disc was prepared by saturating sterile blank discs with the test extract using micropipette. Sterile blank discs were also saturated using ethanol only to prepare Control discs (blank). Both sample and control discs (blank) were dried. Standard antibiotic discs (Kanamycin 30 µg/disc), sample containing discs (250 and 500 µg/disc), and control discs (blank) were laid in Petri dishes containing pathogenic nutrient agar medium using sterile forceps. Then Petri dishes were incubated at 37±0.5 °C for 16 h and zone of inhibition was measured by using digital slide callipers.

3. Results

3.1. Phytochemical Test

A number of qualitative phytochemical tests were performed for identification of different biologically active groups in the ethanol extract of *H. fomes*, and finding results are cited in the Table 1.

Table 1: Results of different group tests of ethanol leaves extract of *H. fomes*.

Phytochemical groups	Results
Reducing sugars	+
Combined Reducing sugars	-
Saponins	+
Alkaloids	+
Glycosides	+
Tannins	+
Steroids	+
Flavonoids	+
Gums	+
+ = Presence - = Absence	

3.2. Acetic Acid Induced Writhing Test

The ethanol leaves extract exhibited dose dependent 34.83% and 59.20% writhing inhibition at the doses of 250 and 500 mg/kg body weight respectively. The results were statistically significant (p<0.001) and was extremely comparable with standard diclofenac

sodium, was exhibited 70.65% at the dose of 25 mg/kg body weight.

Table 2: Effects of the ethanol leaves extract of *H. fomes* on acetic acid induced writhing on mice.

Treatment group (n = 5)	Dose (mg/kg, p.o.)	No. of writhing	% Inhibition
Control group	10	40.20 ± 1.16	---
Positive control group	25	11.80 ± 1.02*	70.65
Test group-I	250	26.20 ± 0.86*	34.83
Test group -II	500	16.40 ± 1.44*	59.20

Values are expressed as mean ± SEM, SEM=Standard error of mean, *: $P < 0.001$ vs. control, Student's *t*-test; p o.: per oral.

3.3. Activity in Hot Plate Test

The ethanol leaves extract increased the reaction time in a dose dependent manner to the thermal stimulus. The highest nociceptive inhibition was produced at a dose of 500 mg/kg and has maximum

time needed for the reaction which was extremely comparable to standard morphine sulphate (5 mg/kg). The results were statistically significant ($p < 0.01$) and summarized in Table 3.

Table 3: Effect of ethanol leaves extract of *H. fomes* on hot plate test in mice.

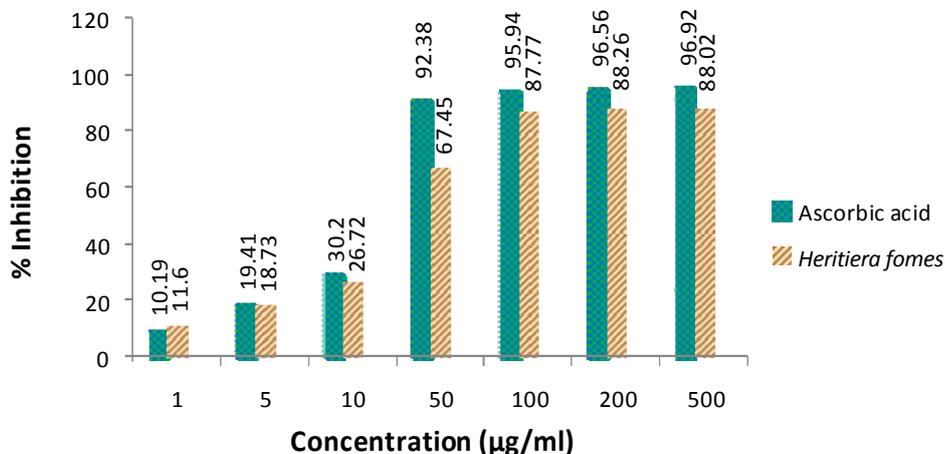
Treatment group (n = 5)	Dose (mg/kg, p.o.)	Response Time (Sec)			
		0 min	30 mins	60 mins	90 mins
Control group	10	3.89 ± 0.12	3.99 ± 0.09	3.93 ± 0.07	3.89 ± 0.11
Positive control group	5	3.97 ± 0.11	8.25 ± 0.08*	9.43 ± 0.30*	7.47 ± 0.14*
Test group-I	250	3.91 ± 0.10	4.56 ± 0.08*	4.99 ± 0.19*	5.68 ± 0.08*
Test group -II	500	4.13 ± 0.13	7.13 ± 0.16*	7.29 ± 0.07*	6.01 ± 0.10*

Values are expressed as mean ± SEM, SEM=Standard error of mean, *: $P < 0.01$ vs. control, Student's *t*-test; p o.: per oral.

3.4. DPPH Radical Scavenging Activity

The sample extract demonstrated DPPH scavenging activity with 26.30 µg/mL of IC₅₀ value which was highly comparable to the

ascorbic acid, a well-known antioxidant showed IC₅₀ value of 14.13 µg/mL.

**Fig. 1:** DPPH radical scavenging activity of ethanol leaves extract of *H. fomes*.

3.4. Antimicrobial Activity

The extract showed significant antimicrobial activity with the zone of inhibition against all the tested pathogens ranging from 3.92 to

7.63 mm and 7.86 to 13.45 mm at the doses of 250 µg/disc and 500 µg/disc, respectively and summarized in Table 4.

Table 4: Antibacterial activity of ethanol leaves extract of *H. fomes* in disk diffusion assay.

Bacterial strains	Type of bacteria	Diameter of zone of inhibition (mm)			
		Blank	Kanamycin (30 µg/disc)	Extract (250 µg/disc)	Extract (500 µg/disc)
<i>Escherichia coli</i>	Gram (-)	-	25.31	3.92	7.86
<i>Salmonella typhi</i>	Gram (-)	-	23.93	7.63	10.43
<i>Salmonella paratyphi</i>	Gram (-)	-	27.39	5.21	9.35
<i>Shigelladysenteriae</i>	Gram (-)	-	23.52	7.54	13.45
<i>Staphylococcus aureus</i>	Gram (+)	-	28.11	6.41	11.16

4. Statistical Analysis

The results were presented as mean ± SEM. Student's *t*-test was used to determine a significant difference between the experimental groups and control group. The test results were statistically significant when $P < 0.01$.

5. Discussion

The current study was executed to assess the anti-nociceptive outcome of *H. fomes*, a medicinal substance claimed in traditional medicine to have analgesic and anti-inflammatory activities. The ethanol leaves extract of *H. fomes* established momentous anti-nociceptive activity at two different dose levels in various animal models of pain. Chemical anti-nociceptive effect of this extract was inspected in the acetic acid-induced writhing model while thermal nociception was evaluated by Eddy's hot plate model. These schemes were used so that both the peripherally and centrally mediated anti-nociceptive effects could be explored. Acetic acid-induced writhing test is considered to demonstrate the association of peripheral mechanisms, whereas the hot plate test is considered to demonstrate that of central mechanisms^[17-19].

Peripherally acting anti-nociceptive activity of the leave extract is demonstrated through the activation of locally sensitize peritoneal receptors by acetic acid-induced writhing test, a model of chemonociceptive induced pain, which increases PGE₂ and PGE_{2α} peripherally^[20]. In general, acetic acid causes pain by releasing several endogenous substances like serotonin, bradykinins, histamine, prostaglandins (PGs), and substance P that are liable to produce pain by accelerating nerve endings^[21]. Locally sensitize peritoneal receptors are responsible for the activation of abdominal constrictions response^[22]. This model has also been connected with prostanoids that increase the levels of PGE₂ and PGE_{2α} in peritoneal fluids, as well as lipoxigenase products^[23]. The inhibition of synthesis and release of PGs and other endogenous substances by *H. fomes* may be the most probable pathway of peripherally acting analgesics^b.

The hot-plate test is used to demonstrate centrally acting anti-nociceptive effect that focuses on changes above the spinal cord level. The significant raise in pain threshold produced by leave extract in this test suggests the participation of central pain pathways. A number of composite processes like dopaminergic, opiate, serotonergic and descending noradrenergic systems are

liable for central pain^[25, 26].

The presence of phyto-constituents like steroid, alkaloid and tannins might be responsible for its anti-nociceptive activity. Pain inhibition is associated with the occurrence of alkaloids and steroidal constituents. Tannins cooperates an important role in anti-nociceptive and anti-inflammatory activities by altering the production of cyclooxygenase (COX-1 and COX-2) and lipoxigenase^[20, 27, 28].

The ethanol leaves extract of *H. fomes* was also subjected to determine for its possible radical scavenging activity, a known mechanism by which antioxidants inhibit lipid oxidation. Being a stable free radical, DPPH is regularly used to demonstrate this radical scavenging activity^[29-31]. A freshly prepared DPPH solution reveals a deep purple colour and shows absorption maxima at 517 nm. This purple colour generally renovates to light yellow colour when an antioxidant molecule is present. Thus antioxidant molecules can quench DPPH free radicals by supplying hydrogen atoms or donating electron and convert to 2,2-diphenyl-1-hydrazine, or a substituted analogous. UV spectrophotometer distinguishes an odd electron of DPPH radical and the absorption decreases upon diminution with an antioxidant due to development of DPPH-H. It was a concentration dependent manner and greatly comparable to well establish antioxidant ascorbic acid. Flavonoids defined by the phytochemical exploration may be considerable for this antioxidant activity^[20, 32, 33].

Antimicrobial activity of the ethanol leaves extract of *H. fomes* was explored using disk diffusion assay method against all the tested Gram-positive and Gram-negative bacterial strains based on its folkloric uses in the skin diseases^[34]. Phytochemical constituents such as flavonoids, tannins, alkaloids, terpenoids, saponins and several other aromatic compounds investigated by phytochemical screening provide protection mechanisms against many microorganisms, herbivores and insects. The possible mechanism of antibacterial activity of flavonoids is owed to their capability to composite with soluble and extracellular proteins with bacterial cell walls.^[35-41]

6. Conclusion

Finally, it can be disclosed that the crude leaves extract of *H. fomes* possess significant antioxidant, anti-nociceptive and antimicrobial

activity. The potential of these activities may be due to the presence of phyto-constituents reported in the phytochemical tests and justify its uses as a habitual folk medicine. A comparison among the results guides us to the finale that, constituents of this extract may provide as a source of drugs useful in the chemotherapy of some inflammatory and infectious diseases and also as an antioxidant agent. However, a more extensive study should necessary to establish the exact mechanism(s) of action and its active compound(s) of this extract.

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8. Conflict of Interest

There is no conflict of interest in this study.

9. References

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