Published by SET Publisher

Journal of Pharmacy and Nutrition Sciences

ISSN (online): 1927-5951

Phytochemical and Biological Investigations of Different Partitioned Extracts of *Ficus heteropleura* Blume (Family: Moraceae) Leaves

Partha Protim Nath[1], Md. Shafiul Hossen[2], Sonia Akther Papia[3], Samina Yesmin Trisha[1], Irin Sultana[1] and Mohammed Ibrahim[2,*]

[1]*Department of Pharmacy, Southern University of Bangladesh, Baizid Bostami, Chattogram-4210, Bangladesh*

[2]*Department of Pharmacy, State University of Bangladesh, South Purbachal, Kanchan, Dhaka-1461, Bangladesh*

[3]*Department of Pharmacy, Stamford University of Bangladesh, Siddheswari Road, Ramna, Dhaka-1217, Bangladesh*

Article Info:

Keywords:
Antidiabetic,
Thrombolytic,
Antimicrobial,
Phytoconstituent,
Anti-inflammatory,
Ficus heteropleura,
Membrane stabilizing

Timeline:
Received: September 23, 2024
Accepted: October 22, 2024
Published: November 20, 2024

Citation: Nath PP, Hossen MS, Papia SA, Trisha SY, Sultana I, Ibrahim M. Phytochemical and biological investigations of different partitioned extracts of Ficus heteropleura blume (Family: Moraceae) leaves. J Pharm Nutr Sci 2024; 14: 90-99.

Abstract:

Ficus heteropleura Blume is a plant of the Moraceae family with high medicinal value. Therefore, the plant leaves' fractional extracts have been subjected to preliminary screenings for phytoconstituents and pharmacological studies. The phytochemical analysis was done after fractionating the ethanolic extract with n-hexane, chloroform, and hydro-alcohol. Then, the TLC method, DPPH radical scavenging, disc diffusion, membrane stabilization activity, heat-induced hemolysis, human blood clot lysis, and starch-iodine method were performed. The chemical investigation resulted in the identification of glycosides, amides, reducing sugars, flavonoids, phenols, alkaloids, tannins, saponins, gums, and steroids from the different extracts of the leaves of *Ficus heteropleura* Blume. In the antioxidant assay, hydro-alcoholic, chloroform, and n-hexane extracts at the dose of 100µg/ml produced significant inhibition of 76.11% (IC_{50} = 46.47 µg/ml), 72.83% (IC_{50} = 54.72 µg/ml), and 61.83% (IC_{50} = 77.95 µg/ml), respectively, as compared to the reference standard drug, ascorbic acid, 85.01% (IC_{50} = 32.22 µg/ml). However, only moderate anti-inflammatory activity was found in hydro-alcoholic extract (IC_{50} = 4.24 µg/ml). The extract also showed moderate thrombolytic and membrane-stabilizing effects. Hydro-alcoholic extract had antifungal activity against only Candida albicans (11 mm). However, chloroform plant extract exhibited significant antifungal activity against all included fungi. In the anti-diabetic study, significant inhibition of amylase activity was found by the hydro-alcoholic (73.33%) and chloroform (83.3%) extracts, respectively, whereas the n-hexane was found to have moderate activity (35.44%) compared to acarbose. Therefore, before isolation, characterization, and determination of the mechanism of action of the screened pharmacological activities, further investigation is needed.

DOI: https://doi.org/10.29169/1927-5951.2024.14.11

*Corresponding Author

Tel: +8801816821161
E-mail: mdibrahim_sub@yahoo.com

1. INTRODUCTION

Medicinal plants are crucial in the healthcare system of several developing countries due to the shortage of advanced medical amenities, traditional acknowledgment, and expensive pharmaceutical medicines, as well as the effectiveness of medicinal plants against certain medical conditions that modern therapeutic drugs cannot treat [1]. Also, it has been observed that the adverse effects of synthetic drug use resulted in a spike in health issues [2]. As a result, plant-based medications are gaining appeal globally. *Ficus* is classified twenty-first among angiosperms and is one of the largest genera, with 800 species found worldwide [3, 4]. There are roughly 43 species in Meghalaya alone, out of the 115 species that are spread throughout India [5]. The Indian ficus plants are remarkably antibacterial, anti-arthritic, neuroprotective, analgesic, and antidiabetic [6-10]. Ficus plants are also used to cure nose bleeding, leprosy, coughing, liver illnesses, piles, paralysis, and chest pain using their fruit, bark, roots, leaves, and latex [11, 12].

Originating from eastern Asia, *Ficus heteropleura* is found in southern China, Myanmar, India, Cambodia, Thailand, Sri Lanka, Laos, Malaysia, Indonesia, and Vietnam. It grows in the country's tropical and subtropical regions and is used for a range of purposes in traditional medicine [13]. It is a member of the Moraceae family and grows up to three meters tall, typically in a shrub setting [14, 15]. According to the previous study, a large number of phytochemicals are found in the leaf of *Ficus heteropleura* [14]. The plant is shielded from biotic and abiotic stress by these compounds. According to the use of plant parts in ancient medical traditions, this protective function of phytochemicals in plants has been utilized recently to treat a variety of disorders [16]. In addition, the plant extract has shown notable analgesic effects [15].

However, comprehensive phytochemical analysis and pharmacological properties of leaves of *Ficus heteropleura* are only sparsely studied. Therefore, in the present study, we endeavored to identify the phytochemicals present in its hydro-alcoholic, chloroform, and n-hexane leaf extract and evaluate the antioxidant, anti-inflammatory, antimicrobial, membrane-stabilizing, thrombolytic, and anti-diabetic effects.

2. MATERIALS AND METHODS

2.1. Soxhlet Extraction of the Plant Powder by Ethanol

The leaves of *Ficus heteropleura* plant were collected from the Chattogram hill tracts (Mithachara, Mirassarai, Chattogram) and identified by the Bangladesh forest research institute. The collected leaves were cleaned properly and shade-dried. The shade-dried plant was subjected to grinding into a fine powder, and then 400 g of leaf powder was kept in the Soxhlet apparatus to be extracted using 1050 mL of ethanol (97%). After extraction, the solvent was removed and concentrated, typically using a rotary evaporator, resulting in 45 g of crude extract. % yield was quantified by applying the following equation:

Yield = (Wt. of specific extract/Total amount of coarse powder) x 100

Yield of leaf extract = (45 ÷ 400) × 100 = 11.25%

2.2. Fractionation of Crude Ethanol Extracts

Fractioning was performed using Kupchan's method with slight modification, resulting in crude ethanol extract dissolved in DDW, fractionated subsequently with n-hexane, and with chloroform. The residual was used as a hydro-alcoholic fraction. All of the fractionated extracts were concentrated and used for further analysis.

Wt. of hydro-alcoholic extract = 11.026 gm.

Wt. of chloroform extract = 13.598 gm.

Wt. of n-hexane extract = 14.376 gm.

2.3. Phytochemical Screening

Hydro-alcoholic crude and its fractions were subjected to preliminary screening of phytochemicals, including flavonoids, alkaloids, reducing sugars, saponins, amides, gum, steroids, tannins, and glycosides. The phytochemical analysis follows Harborne's standard method in “Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis”.

2.4. Antioxidant Study

2.4.1. Qualitative Antioxidant Study using TLC

Methanol solvent was used to dissolve extracts, which were then applied uniformly on TLC plates using an auto-sampler. Sample-loaded plates were developed in a solvent mixture of Chloroform, Methanol, and Water in a ratio of 40:10:1. The plates were then sprayed with 10% H_2SO_4, dried at 110- 120°C, and observed under UV light at wavelengths 254 and 360 nm for detection and marking the presence of fluorescence compound. After that, 0.004 % DPPH solution of methanol was sprayed on the plates and observed under UV light.

2.4.2. Quantitative Assay of Radical Scavenging Activity Using DPPH

With a few modest adjustments, the free radical scavenging activity of the extract against DPPH at different concentrations (20, 40, 60, 80, and 100 µg/ml in ethanol) was measured. In the test tube, equal amounts of plant extract and 0.004% (w/v) DPPH were added. After that, 2 ml of ethanol was added, and the mixture was kept in a dark area for 30 minutes to incubate. A UV-visible spectrometer was used to measure the absorbance of each incubated sample at a wavelength of 517 nm after the reaction was finished. The inhibition % and IC_{50} values of the studied extract samples were used to measure their radical scavenging activity. Every experiment was carried out three times. The formula used to determine inhibition is:

Scavenging activity (%) = (Abs. of control – Abs. of sample)/Abs. of control × 100

2.5. Anti-Bacterial and Anti-Fungal Screening

2.5.1. Disc Diffusion Method

Different solvent fractions of plant *Ficus heteropleura,* viz., hydro-alcoholic, n-hexane, and chloroform extracts, were subjected to antibacterial and antifungal evaluation using the disc diffusion method. Three gram-positive bacteria *B. cereus, B. subtilis, S. aureus,* and three gram-negative bacteria *E. coli, S. dysentariae,* and *S. typhi V. cholera* were used in the antibacterial study. *B. dermatitidis, C. albicans, T. spp.,* and *C. neoformans* were used for antifungal activity**.** The dried extracts of each fungal isolate were dissolved in dimethyl- sulfoxide (DMSO). DMSO was used as a negative control, and Ciprofloxacin and Fluconazole (30 µg/10 µL) were used against bacteria and fungi, respectively, as positive controls.

2.5.2. Determination of Minimum Inhibitory Concentration (MIC)

The average inhibition value and standard deviation were computed for each experiment, which was carried out in triplicate. The Akinpelu and Kolawole method was used to determine the MIC of the extracts. In the disc diffusion method, to make 500µg/10µl/disc, 10 µl of various extracts were applied to the 5 mm diameter sterilized filter paper discs and allowed for complete evaporation of the solvent. The discs were incubated. Standard and extract-containing discs were separately injected centrally into the agar gel. After that, the plates were inverted and refrigerated at 4°C for roughly 24 hours. The plates were then incubated for eighteen hours at 37°C. Plates were analysed to determine the zone of inhibition (mm) caused by the test groups compared to the standard medications, which are fluconazole (30 µg/10 µl) and ciprofloxacin (30 µg/10 µl), respectively, following the incubation period.

2.7. *In-vitro* Anti-inflammatory Activity

Using iso-saline, a 5% v/v aqueous solution of egg albumin was prepared by reconstitution. To conduct this study, test extracts (2 mL) were mixed with 1 mL of egg albumin (5%) solutions, pH adjusted to 5.6±0.2, heated for 5 minutes at a temperature of 60 °C, and spectrophotometrically measured at 660 nm. Percentage inhibition of protein denaturation was calculated against positive control acetylsalicylic acid using the equation given below:

% Inhibition = (Abs. of control – Abs. of sample)/Abs. of control × 100

2.6. Membrane Stabilization Activity

2.6.1. Preparation of Erythrocyte Suspension

The 10% RBC suspension was prepared by collecting human venous blood and diluting 1 ml of it with 19 mL RBC diluting fluid. A 2 mL diluted solution was combined with 18 ml of isotonic solution to make a 10% RBC suspension**.**

2.6.2. Heat-induced Hemolysis

Various concentrations of different crude fractions were prepared in isotonic solution. 5 mL of acetylsalicylic acid and normal saline were used as positive and negative controls, respectively. One milliliter of a 10% RBC suspension was kept in each treatment tube. After bringing the pH of the reaction mixture (7.4±0.2) under control with phosphate buffer, it was centrifuged for five minutes at 2500 rpm after being incubated for thirty minutes at 56°C in a water bath. At 660 nm, the supernatants' absorbance was measured. There were three attempts at the test. The following equation was used to determine the percentage inhibition or acceleration of haemolysis.

% Inhibition of hemolysis = (Abs control – Abs sample)/Abs control × 100

2.7. Thrombolytic Activity (*In-vitro*)

An *in vitro* thrombolytic model was used to test the thrombolytic activity of *Ficus heteropleura* extracts using 4 mL of venous blood from a healthy human

volunteer who had never taken anticoagulant or contraceptive medication. Five preweighed, sterile microcentrifuge tubes were filled with 0.5 mL of blood after the withdrawal (three for extracts, one for the reference standard, and one for the negative control). After that, the tubes were incubated at 37°C for 45 minutes. The weight of each tube containing a clot was again measured to determine the clot weight (clot weight = weight of tube containing clot minus weight of tube alone) after the serum was completely removed without affecting the clot after it had formed. Streptokinase (10 µl) served as a reference, and corresponding extracts of *Ficus heteropleura* (10 µl) were used to treat the blood clot. The negative control tubes received separate DDW additions. Each tube's clot lysis was observed after 90 minutes of incubation at 37°C. After incubation, the fluid that had been released was removed, and the tubes were weighed again to determine whether the weight had changed as a result of the disruption of the clot. The weight obtained prior to and following clot lysis was compared in order to determine the percentage of clot lysis. Data is expressed using the mean ± standard error of the mean.

2.8. Anti-Diabetic Activity

The α-amylase was prepared by combining 80 µl of 0.050 mg/ml α-amylase solution with 5.2 mL 0.01 M Tris-HCl buffer (pH 6.8) and 320 µl of *Ficus heteropleura* fractional extracts. The activity of α-amylase was measured using the starch-iodine method. After incubating for 20 minutes at 37ºC, a 0.1% starch solution was added, and the mixture was again incubated for 20 minutes at 37ºC. After incubation, 8 millilitres of the 0.01% acidic iodine solution were added, and the absorbance at 578 nm was measured. The inhibition of amylase activity was contrasted with the typical acarbose action. The following formula was used to determine the enzyme's percentage inhibition:

% inhibition of enzyme = (Abs. of sample - Abs. of control)/(Abs. of blank – Abs. of control)×100

3. RESULT

3.1. Phytochemical Screening

The hydro-alcohol, chloroform, and n-hexane leaf extracts revealed the presence of several phyto-compounds such as alkaloids, steroids, flavonoids, saponins, reducing sugars, tannins, phenol, gum, and glycosides (Table **1**).

3.2. Antioxidant Study

In the qualitative test by DPPH-induced TLC plate method, the UV spectrophotometer produced a yellow color band coated by a purple color background both at short (254 nm) and long (360 nm) wavelengths (Figure **1**). Different fractional extracts of leaves of *Ficus heteropleura* showed the presence of anti-oxidant activity measured by DPPH assay test method. At the dose of 100µg/ml, hydro-alcoholic, chloroform, and n-hexane extracts of *F. heteropleura* produced significant inhibition of 76.11% (IC_{50}= 46.47 µg/ml), 72.83% (IC_{50} = 54.72 µg/ml), and 61.83% (IC_{50}= 77.95 µg/ml), respectively, as compared to ascorbic acid 85.01% (IC_{50}= 32.22 µg/ml) (Table **2** and **3**). From the above

Table 1: Chemical Groups for the Different Fractional Extracts of *Ficus heteropleura* Leaves

Chemical Groups	Result		
	Hydro-Alcoholic	n-Hexane	Chloroform
Alkaloids	+	+	+
Steroids	-	-	+
Glycosides	+	-	+
Saponins	-	+	-
Flavonoids	-	+	+
Tannins	-	+	+
Reducing sugars	+	+	+
Gum	-	+	-
Amides	+	+	-
phenol	+	+	+

'+' = presence of respective component. '-' = absence respective component.

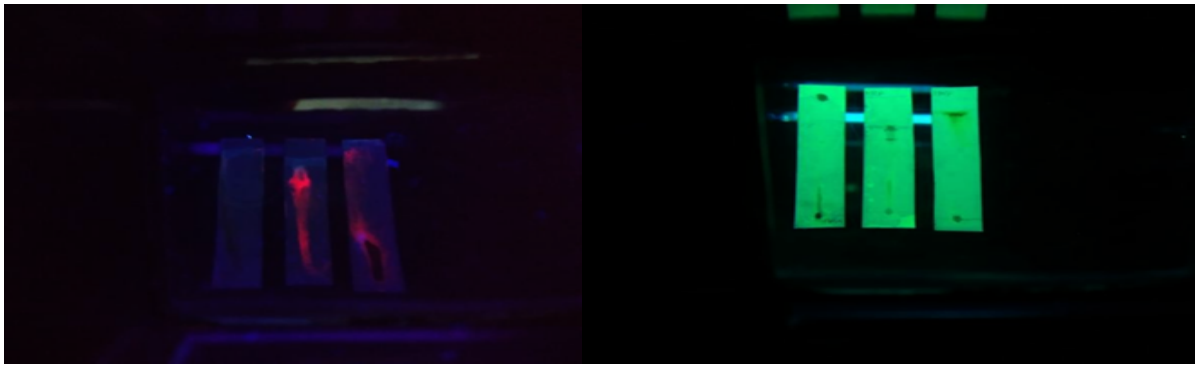

Figure 1: Qualitative screening of antioxidant study.

Table 2: DPPH Inhibitory Action of Standard Drug (Ascorbic Acid) and Hydro-Alcoholic, Chloroform, and n-Hexane Extracts of *Ficus heteropleura*

Concentration (µg/ml)	% Inhibition			
	Ascorbic Acid	Hydro-Alcoholic Extract	n-Hexane Extract	Chloroform Extract
20	43.09	34.89	18.27	30.91
40	55.27	48.24	26.46	43.09
60	63.7	58.55	40.98	54.57
80	74.94	66.28	51.99	62.29
100	85.01	76.11	61.83	72.83

Table 3: IC_{50} for the Ascorbic Acid and Fractioned Extract

Treatment Groups	IC_{50} (µg/ml)
Standard (Ascorbic acid)	32.22
Hydro-alcoholic extract	46.47
Chloroform extract	54.72
n-Hexane extract	77.95

experiment, it is evident that the hydro-alcoholic and chloroform extracts showed potent antioxidant activity in comparison with the standard, whereas the n-hexane extract showed moderate activity.

3.3. Anti-bacterial and Anti-fungal Screening

Using Ciprofloxacin as a reference, the antibacterial properties of the various fractional extracts of *F. heteropleura* leaves were assessed using the disc diffusion method against three Gram-positive and four Gram-negative pathogenic bacteria. In the antibacterial sensitivity test, it was observed that none of the extract prepared in our study exhibited antibacterial activity against the tested bacteria (Table **4**).

The antifungal properties of different solvent extracts were assessed against four fungi using the standard drug Fluconazole. It was found that the hydro-alcoholic extract showed antifungal activity against only *C. albicans (11 mm).* On the other hand, chloroform plant extract exhibited significant antifungal activity against all 4 fungi, in which the zones of inhibition are *B. dermatitidis* (10 mm), *C. albicans* (14 mm), *T. spp.* (10.5 mm), and *C. neoformans* (13 mm), but the n-hexane plant extract showed no antifungal activity. (Table **5**).

3.4. *In-vitro* Anti-inflammatory Activity

In this study, the hydro-alcoholic extract of *F. heteropleura* showed moderate activity (IC_{50} 4.24 µg/ml) in comparison with acetyl salicylic acid (IC_{50} 1.99 µg/ml). Chloroform extract exhibited very poor activity (IC_{50} 24.23µg/ml), and n-hexane extract showed less significant activity (IC_{50} 10.08 µg/ml) (Table **6**).

Table 4: Antibacterial Activity of Different Fractions of *F. heteropleura*

Bacteria	Zone of Inhibition (MZI ± SEM) mm			
	Ciprofloxacin (30µg/10µl)	Hydro-alcoholic Extract (500µg/10µl)	n-Hexane Extract (500µg/10µl)	Chloroform Extract (500µg/10µl)
Gram (+) ve Species				
B. cereus	16.0 ± 1.0	NF	NF	NF
B. subtilis	16.0 ± 1.0	NF	NF	NF
S. aureus	16.7 ± 1.5	NF	NF	NF
Gram (-) ve Species				
E. coli	15.5 ± 0.50	NF	NF	NF
S. dysentariae	14.7 ± 1.0	NF	NF	NF
S. typhi	13.0 ± 0.50	NF	NF	NF
V. cholera	13.8 ± 0.3	NF	NF	NF

Here, MZI= Minimum Zone of Inhibition; SEM= Standard error of mean; NF= Not Found.

Table 5: Antifungal Activity of Different Fractions of *F. heteropleura*

Fungi	Zone of Inhibition (MZI ± SD) mm			
	Fluconazole (30µg/10µl)	Hydro-alcoholic Extract (500µg/10µl)	n-Hexane Extract (500µg/10µl)	Chloroform Extract (500µg/10µl)
B. dermatitidis	12.3 ± 1.00	Ni	Ni	10 ± 0.3
C. albicans	15 ± 1.73	11 ± 0.5	Ni	14 ± 0.2
T. spp.	11 ± 1.73	Ni	Ni	10.5 ± 0.5
C. neoformans	14.3 ± 1.15	Ni	Ni	13 ± 0.2

Here, MZI= Mean zone of inhibition (mm); zone of inhibitions under 8 mm were considered as less active and were discarded. Ni=No inhibition.

Table 6: IC_{50} for the Ascorbic Acid and Fractioned Extract

Treatment Groups	IC_{50} (µg/ml)
Standard, ASA	1.99
Hydro-alcoholic extract	4.24
Chloroform extract of	24.23
n-Hexane extract of	10.08

3.5. Membrane Stabilization Activity

At the maximum dose of 500 µg/mL, the hydro-alcoholic, chloroform, and n-hexane extracts of *F. heteropleura* reduced the heat-induced total hemolysis of RBCs by 45.85%, 11.61%, and 21.14%, respectively. On the other hand, acetyl-salicylic acid reduced hemolysis by 70.23% at the same dosage. From the obtained IC_{50} value, it can be inferred that the plant's hydroalcoholic extract has a moderate ability to stabilize membranes. However, when compared to the reference medicine, ASA, the extracts in chloroform and n-Hexane showed weak and less significant activity, respectively (Table **7**).

3.6. Thrombolytic Activity

After incubating the clot at 37°C for 90 minutes with a 100µl solution containing 30,000 I.U. of Streptokinase (a positive control), 85.42% of the clot was lysed. When the concentration was 500µg/ml, lysis of the clots treated with 100 µl of hydro-alcoholic, chloroform, and n-hexane extracts was 62.47%, 38.09%, and 16.83%, respectively. When the concentration was 250µg/ml,

Table 7: IC_{50} for Membrane Stabilization Activity of Fractional Extracts of *F. hetero pleura*

Test Groups	Dose (µg/ml)	Total Inhibition of Hemolysis (%)	IC50 (µg/ml)
Acetyl salicylic acid	500	70.23±0.008	1.45
	250	58.61±0.022	
	125	43.42±0.006	
Hydro-alcoholic extract	500	45.85±0.006	3.34
	250	23.51±0.004	
	125	11.33±0.008	
n-Hexane extract	500	21.14±0.004	12.56
	250	10.92±0.014	
	125	3.92±0.016	
Chloroform extract	500	11.61±0.008	6.41
	250	7.56±0.004	
	125	3.58±0.012	

the percentage was once more 43.73%, 27.3%, and 8.4%, respectively. Table **8** shows a statistical breakdown of the effective clot lysis percentage by extract, positive thrombolytic control (streptokinase), and negative control (distilled water). Comparatively speaking, the hydro-alcoholic extract exhibited greater thrombolytic activity than the extract and chloroform.

3.7. Anti-Diabetic Activity

Different fractional extracts of *F. heteropleura* leaves at consecutive doses of 25, 50, 100, 200, and 400 µg/mL inhibited amylase activity in a dose gradient manner. Significant inhibition was observed with hydro-alcoholic (73.33%) and chloroform (83.3%) extracts, whereas a moderate activity was seen with the n-hexane extract, which is 35.44%. However, acarbose at 400µg/mL produced the highest inhibition of 95.45% (Table **9**).

4. DISCUSSION

The findings of the qualitative screening of phytochemicals in *Ficus heteropleura* leaf extracts in three different solvents are displayed in Table **1**. All the chemicals except glycosides and steroids were found in the hexane. All three fractions of the extract contain alkaloids, phenols, and carbohydrates, according to the preliminary phytochemical analysis. Whereas the hydro-alcoholic extract of *F. heteropleura* contains glycosides, alkaloids, phenols, and amides, the n-Hexane extract of *F. heteropleura* contains alkaloids, tannins, saponins, reducing sugars, gums, and amides. The screening test findings showed that leaves contained chemicals with medicinal activity. Additional bioactive metabolites may be isolated from the same extract.

Table 8: *In-vitro* Thrombolytic Activity of Fractional Extracts of *F. heteropleura*

Treatment	Dose (µg/100µL)	Clot Lysis (%)
Streptokinase (Positive control)	30000 IU/100µL	85.4±0.0344
Hydro-alcoholic extract	500	43.7±0.0460
	250	62.5±0.0260
Chloroform extract	500	27.3±0.0647
	250	38.0±0.0193
n-Hexane extract	500	8.4±0.0059
	250	16.9±0.0382

Values are expressed as % of lysis ± SEM (Standard error of mean).

Table 9: IC_{50} for Anti-Diabetic Activity by the Standard (Acarcose), and Different Fractional Extracts of *F. heteropleura*

Treatment Groups	Concentrations (µg/mL)	Absorbance (at 578nm)	% Inhibition
Standard (Acarbose)	400	0.098	95.45
	200	0.078	65.57
	100	0.059	50
	50	0.034	25.29
	25	0.022	17.2
Hydro-alcoholic extract	400	0.095	73.33
	200	0.078	58.43
	100	0.063	47.47
	50	0.03	19.04
	25	0.019	13.51
Chloroform extract	400	0.073	35.44
	200	0.045	22.55
	100	0.03	12.15
	50	0.019	7.89
	25	0.009	4.17
n-Hexane extract	400	0.185	83.3
	200	0.166	60.57
	100	0.139	49
	50	0.11	23.8
	25	0.085	15.32

The systemic antioxidant evaluation of plant extracts under study is supported by the significance of oxidative stress in the development of human diseases [17]. *F. heteropleura* leaves are a rich source of naturally occurring antioxidants, of which phenolic compounds and reducing sugars are especially important in avoiding a wide range of oxidative stress-related health issues, such as cancer, neurological diseases, and cardiovascular illnesses [18]. Because of the phenolic components present, all three extracts demonstrated antioxidant activity. The hydro-alcoholic and chloroform extracts gave significantly higher antioxidant potential compared to the moderate effect observed in the hexane extract. Because leaves have the highest concentrations of phenolic chemicals that may be extracted using hydro alcohol and chloroform, these facts may make sense. The antioxidant capacity of phenolic compounds is based on their ability to scavenge free radicals and can be used as a possible food additive or in the nutraceutical and biopharmaceutical industries.

The present study evaluated the crude extract of *F. heteropleura* leaf's minimum inhibitory concentration and antimicrobial activity. The three extracts exhibited no antibacterial action against the study's strains of bacteria. Hexane extract did not affect the test fungus, whereas the hydro-alcoholic extract exhibited a modest level of antifungal activity against candida albicans. Conversely, the chloroform extract had a strong antifungal impact against every type of microbe, which is equivalent to fluconazole, the antifungal drug of choice. The phytochemical examination of the *F. heteropleura* chloroform leaf extract revealed a notable presence of alkaloids, glycosides, tannins, and flavonoids. These substances have an impact on fungi's ability to grow. The phytocompounds found therein may be a valuable source of antifungal medicines, with little to no negative effects on human health.

The hydro-alcoholic extract demonstrated moderate anti-inflammatory benefits, but the other two extracts showed extremely weak or less significant effects

against inflammation, according to an *in vitro* anti-inflammatory test that used egg albumin as a source of protein. Three different extracts of *Ficus heteropleura* were subjected to erythrocyte (RBC) membrane stabilization-induced hemolysis by hypotonic solution. The erythrocyte membrane resembles the lysosomal membrane, and as such, the erythrocyte could be extrapolated to the stabilization of the lysosomal membrane [19]. It is believed that either acute or chronic inflammation is connected to the extracellular activity of lysosomal enzymes. Non-steroidal medications work by either stabilizing the lysosomal membrane or by blocking these lysosomal enzymes [20]. It was observed from Table **6** that the hexane and chloroform extracts showed insignificant anti-inflammatory effects. However, hydroalcoholic extract shows moderate anti-inflammatory activity at a concentration of 500 mg/mL, which is comparatively less than the standard drug acetylsalicylic acid. The anti-inflammatory activity of the extract was concentration-dependent; with increasing concentration, the activity was also increased. The anti-inflammatory effect of the hydro-alcoholic extract may be due to the presence of its phytoconstituent. Therefore, a further study can be executed on hydro-alcoholic extract to confirm its anti-inflammatory effects.

The *in vitro* clot lysis study found hydro-alcoholic leaf extracts to have moderate thrombolytic activity, possibly due to phytochemicals, suggesting potential as natural thrombolytic agents.

As limited research has been found on Ficus heteropleura, we reviewed evidence from other Moraceae species. It is assumed that phytochemical constituents present in the leaf extract either individually or in combination with others may be responsible for the antioxidant, anti-inflammatory, antifungal, thrombolytic, and antidiabetic activity of this species. The study suggests further investigation into the extract's phytochemical and pharmacological properties, including *in vivo* and clinical trials, to fully understand its potential as a therapeutic element.

5. CONCLUSION

The outcomes of this research show that *Ficus heteropleura* leaf extracts have notable differences in the phytochemical and pharmacological activities based on the solvent used for extraction. However, hydro-alcoholic extract among all three possesses the highest pharmacological effect. Therefore, before isolation, characterization, and determination of the mechanism of action of the screened pharmacological activities further investigation is needed.

CONFLICT OF INTEREST

No conflict of interest.

FUNDING

No fund was received to conduct this research.

DATA AVAILABILITY

Data will be available from the corresponding author on request.

REFERENCES

[1] Sharma B, RA. Updated review on Indian Ficus species. Arabian Journal of Chemistry 2023; 16(8): 104976. https://doi.org/10.1016/j.arabjc.2023.104976

[2] Ghule ST, Patil DK. Development of medicinal plants. Kisan World 2001; 28: 33-4.

[3] Frodin DG. History and concepts of big plant genera. Taxon 2004; 53(3): 753-76. https://doi.org/10.2307/4135449

[4] Adebayo EA, Ishola OR, Taiwo OS, Majolagbe ON, Adekeye BT. Evaluations of the methanol extract of Ficus exasperata stem bark, leaf and root for phytochemical analysis and antimicrobial activities. African Journal of Plant Science 2009; 3(12): 283-7.

[5] Chaudhary LB, Sudhakar JV, Kumar A, Bajpai O, Tiwari R, Murthy GV. Synopsis of the genus Ficus L.(Moraceae) in India. Taiwania 2012; 57(2): 193-216.

[6] Mahajan MS, Gulecha VS, Khandare RA, Upaganlawar AB, Gangurde HH, Upasani CD. Anti-edematogenic and analgesic activities of Ficus benghalensis. International Journal of Nutrition, Pharmacology, Neurological Diseases 2012; 2(2): 100-4. https://doi.org/10.4103/2231-0738.95936

[7] Patil VV, Patil VR. Evaluation of anti-inflammatory activity of Ficus carica Linn. leaves.

[8] Thite AT, Patil RR, Naik SR. Anti-arthritic activity profile of methanolic extract of Ficus bengalensis: Comparison with some clinically effective drugs. Biomedicine & Aging Pathology 2014; 4(3): 207-17. https://doi.org/10.1016/j.biomag.2014.03.005

[9] Ramakrishna V, Gupt KP, Setty OH, Kondapia AK. Protective effect of Ficus bengalensis L. extract against H2O2 induced DNA damage and repair in neuroblastoma cells. Free Radicals and Antioxidants 2014; 4(1): 3-7. https://doi.org/10.5530/fra.2014.1.2

[10] Anjum NI, Tripathi YC. *In vitro* alpha-amylase and alpha-glucosidase inhibitory activities of fruits of Ficus auriculata Lour. Int J Pharma Bio Sci 2019; 10(4): 134-41. https://doi.org/10.22376/ijpbs.2019.10.4.p134-141

[11] Kirtikar KR, Basu BD. International Book Distributors, Dehradun, India. Indian Medicinal Plant 1999; 3: 2262-3.

[12] Khanom F, Kayahara H, Tadasa K. Superoxide-scavenging and prolyl endopeptidase inhibitory activities of Bangladeshi indigenous medicinal plants. Bioscience, Biotechnology, and Biochemistry 2000; 64(4): 837-40. https://doi.org/10.1271/bbb.64.837

[13] Rastogi RM. Compendium of Indian medicinal plants. Central Drug Research Institute, Lucknow, India 1990; 1: 388-9.

[14] Dhakite P, Arts SNM, Gadpayale J. Phytochemical Screening of Ficus heterophylla L.f., Leaf of Moraceae Family Research Journal of Pharmaceutical, Biological and Chemical Sciences Phytochemical Screening of Ficus heterophylla L.f., Leaf of Moraceae Family 2020.

[15] Hoque M, Rahman MM, Uddin MN. A study of analgesic effect of medicinal plant Ficus heterophylla in Swiss albino mice. World Journal of Advanced Research and Reviews 2023; 19(3): 516-23. https://doi.org/10.30574/wjarr.2023.19.3.1804

[16] Tabassum F, Chadni SH, Mou KN, Hasif KI, Ahmed T, Akter M. *In-vitro* thrombolytic activity and phytochemical evaluation of leaf extracts of four medicinal plants of Asteraceae family. Journal of Pharmacognosy and Phytochemistry 2017; 6(4): 1166-9.

[17] Sieniawska E, Świątek Ł, Sinan KI, Zengin G, Boguszewska A, Polz-Dacewicz M, Bibi Sadeer N, Etienne OK, Mahomoodally MF. Phytochemical insights into Ficus sur extracts and their biological activity. Molecules 2022; 27(6): 1863. https://doi.org/10.3390/molecules27061863

[18] Sirisha N, Sreenivasulu M, Sangeeta K, Chetty CM. Antioxidant properties of Ficus species-a review.

[19] Omale J, Okafor PN. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of Cissus multistriata. African Journal of Biotechnology 2008; 7(17).

[20] Vadivu R, Lakshmi KS. *In vitro* and *in vivo* anti-inflammatory activity of leaves of Symplocos cochinchinensis (Lour) Moore ssp Laurina. Bangladesh Journal of Pharmacology 2008; 3(2): 121-4. https://doi.org/10.3329/bjp.v3i2.956

[21] Shinde UA, Phadke AS, Nari AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Chemical and Biological Investigations of Different Partitionated Extracts of F.heteropleura Blume (Family: Moraceae) Leaves. Page 10 Membrane stabilization activity-a possible mechanism of action for the anti-inflammatory activity of Cedrus deodara wood oil 1999; 70: 251-257. https://doi.org/10.1016/S0367-326X(99)00030-1