Flora and Fauna
An International Research Journal of Biological Sciences
Journal recognised by DST New Delhi (NAAS RATING 4.74)
ISSN 2456-9364(Online) 0971-6920(Print)

PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIMICROBIAL EVALUATION OF HYDROALCOHOLIC LEAF EXTRACT OF ICHNOCARPUS FRUTESCENS

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Abstract

Background: Medicinal plants are an important source of bioactive compounds that possess various pharmacological activities. Ichnocarpus frutescens, a plant widely used in traditional medicine, is known for its therapeutic potential due to the presence of several phytochemicals. The present study was undertaken to evaluate the phytochemical constituents, antioxidant activity, and antimicrobial potential of the hydroalcoholic leaf extract of Ichnocarpus frutescens.

Methods: The leaves of Ichnocarpus frutescens were extracted using hexane and hydroalcoholic solvents. Preliminary phytochemical screening was carried out to identify the presence of major secondary metabolites. Quantitative estimation of total phenolic, flavonoid, and alkaloid contents was performed using standard spectrophotometric methods. Antioxidant activity was evaluated by the DPPH free radical scavenging assay, while antimicrobial activity was assessed using the agar disc diffusion method against selected microbial strains including Streptococcus mutans, Klebsiella pneumoniae, and Candida albicans.

Results: The hydroalcoholic extract showed a higher percentage yield (5.38% w/w) compared to the hexane extract (3.36% w/w). Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, and sterols in the hydroalcoholic extract. Quantitative analysis indicated higher phenolic (2.013 mg GAE/100 mg) and flavonoid (2.054 mg QE/100 mg) contents in the hydroalcoholic extract. The extract exhibited significant antioxidant activity with an IC_{50} value of 50.65 µg/ml in the DPPH assay. Furthermore, the hydroalcoholic extract demonstrated moderate antimicrobial activity against the tested microorganisms.

Conclusion: The findings suggest that the hydroalcoholic leaf extract of Ichnocarpus frutescens contains valuable phytoconstituents and exhibits considerable antioxidant and antimicrobial activities. These results support the traditional use of the plant and indicate its potential as a natural source of therapeutic agents...

Keywords: Ichnocarpus frutescens, Phytochemical screening, Hydroalcoholic extract, Antioxidant activity, DPPH assay, Antimicrobial activity.

1. Introduction

Medicinal plants have long been recognized as an important source of therapeutic agents and have been widely used in traditional systems of medicine for the treatment and prevention of various diseases (Sofowora et al., 2013). Natural products derived from plants contain a wide range of bioactive phytochemicals such as alkaloids, flavonoids, phenolics, tannins, saponins, and glycosides, which are known to possess significant pharmacological activities. In recent years, there has been increasing interest in exploring plant-derived compounds due to their antioxidant, antimicrobial, and therapeutic potential, as well as their comparatively lower side effects compared to synthetic drugs (Awuchi; 2020).

Oxidative stress caused by free radicals is considered a major contributing factor in the development of several chronic diseases, including cardiovascular disorders, cancer, diabetes, and neurodegenerative diseases (Jomova et al., 2023). Antioxidants play an essential role in neutralizing free radicals and protecting biological systems from oxidative damage. Plant extracts rich in phenolic and flavonoid compounds have been reported to exhibit strong antioxidant activity.

Therefore, the evaluation of antioxidant potential of medicinal plants has gained considerable attention in pharmaceutical and biomedical research (Jomova et al., 2023).
In addition to antioxidant properties, the emergence of antimicrobial resistance among pathogenic microorganisms has become a major global health concern. The development of resistance against conventional antibiotics has created a need for the discovery of new antimicrobial agents from natural sources. Medicinal plants represent a promising source of novel antimicrobial compounds that may help combat resistant microbial strains (Wali et al., 2019).
Ichnocarpus frutescens (L.) R. Br., belonging to the family Apocynaceae, is a perennial climbing shrub commonly found in tropical and subtropical regions of India. Traditionally, the plant has been used in Ayurvedic and folk medicine for the treatment of various ailments such as diabetes, fever, skin diseases, and inflammatory conditions (Kumarappan et al., 2015).
Previous studies have reported that the plant contains various bioactive constituents including flavonoids, phenolic compounds, alkaloids, and glycosides, which may contribute to its pharmacological activities (Ohiagu et al., 2021).
Considering the medicinal importance of this plant, the present study was undertaken to evaluate the phytochemical constituents, antioxidant activity, and antimicrobial potential of the hydroalcoholic leaf extract of *Ichnocarpus frutescens*. The study aims to provide scientific evidence supporting the traditional use of this plant and to explore its potential as a source of natural antioxidant and antimicrobial agents.

Material and Methods

Material

Fresh leaves of *Ichnocarpus frutescens* were collected and authenticated. Analytical grade chemicals and reagents such as hexane, ethanol, methanol, hydrochloric acid, sodium hydroxide, bromocresol green, chloroform, Folin–Ciocalteu reagent, gallic acid, quercetin, atropine, and ascorbic acid were used for phytochemical and antioxidant studies. DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent was used for antioxidant evaluation. Standard antimicrobial drugs including ofloxacin, ciprofloxacin, and fluconazole were used for antimicrobial studies. All chemicals and reagents used in the study were of analytical grade and obtained from standard commercial suppliers. Microbial strains such as *Streptococcus mutans*, *Klebsiella pneumoniae*, and *Candida albicans* were used for antimicrobial activity evaluation.

Methods

Extraction using maceration method

Extraction is an essential step in phytochemical processing for the finding of bioactive secondary metabolite from plant materials.

Defatting of plant materials

50 gram of leaves of *Ichnocarpus frutescens* shade dried plant material were coarsely powdered and subjected to extraction with Hexane in a maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted leaves of *Ichnocarpus frutescens* were extracted with hydroalcoholic solvent (methanol and water; 80:20v/v) solvents by maceration method. Each extraction process was carried out for 24 hours. The filtrate was separated from the residue using Whatmann filter paper. The filtrate from each solvent was collected and evaporated using a water bath at 50°C until a thick extract was obtained. Finally, the percentage yields were calculated of the dried extracts (Mukherjee; 2007, Khandelwal; 2005).

Determination of percentage yield

Percentage yield measures the effectiveness of the entire extraction process. % yield is calculated using the formula below:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Qualitative phytochemical screening

Qualitative phytochemical screening is carried out to investigate the various classes of natural compounds present in the extract. This is accomplished using standard methods (Tiwari et al., 2011). The classes of compounds identified in the extract included phenol, flavonoids, tannins, saponins, alkaloids and protein.

Quantitative studies of phytoconstituents

Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Mishra et al., 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5-25 µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 760 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Parkhe et al., 2019). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25 µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of flavonoids. Two ml of 2% $AlCl_3$ solution was added to 2 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Estimation of total alkaloids content

The total alkaloid content of the extract was estimated using the bromocresol green (BCG) complex formation method. Bromocresol green reagent was prepared by dissolving 69.8 mg of bromocresol green in 3 ml of 2 N NaOH and 5 ml of distilled water with gentle heating until completely dissolved, and the volume was made up to 1000 ml with distilled water. Phosphate buffer (pH 4.7) was prepared by adjusting the pH of 2 M sodium phosphate solution with 0.2 M citric acid. A standard solution of atropine was prepared by dissolving 10 mg of atropine in 10 ml of distilled water. For analysis, 10 mg of plant extract was dissolved in 10 ml of 2 N HCl and filtered. One milliliter of the filtrate was transferred into a separatory funnel and washed with 10 ml chloroform three times to remove non-alkaloidal impurities. The aqueous layer was neutralized with 0.1 N NaOH, followed by the addition of 5 ml bromocresol green solution and 5 ml phosphate buffer (pH 4.7). The resulting mixture was shaken and the formed alkaloid–BCG complex was extracted successively with chloroform (1, 2, 3, and 4 ml). The chloroform extracts were combined in a 10 ml volumetric flask and the volume was adjusted with chloroform. A calibration curve was prepared using atropine standard solutions (40–120 µg/ml) following the same procedure. The absorbance of the chloroform layer was measured at 470 nm using a UV–Visible spectrophotometer against a reagent blank, and the total alkaloid content of the extract was calculated as atropine equivalents (Sharma et al., 2021).

In-vitro* antioxidant activity of hydroalcoholic extract of *Ichnocarpus frutescens

DPPH method

Total free radical scavenging capacity of extract was estimated according to the reported method with slight modification (Parkhe et al., 2018). Solution of DPPH (6 mg in 100ml methanol) was prepared and stored in dark place. Different concentration of standard and test (10- 100 µg/ml) was prepared. 1.5 ml of DPPH and 1.5 ml of each standard and test was taken in separate test tube; absorbance of this solution was taken immediately at 517nm. 1.5 ml of DPPH and 1.5 ml of the methanol was taken as control absorbance at 517nm.

The percentage inhibition of free radical DPPH was calculated from the following equation:

$$\% \text{ inhibition} = [(\text{absorbance of control - absorbance of sample})/\text{absorbance of control}] \times 100\%$$

In vitro* antimicrobial activity of hydroalcoholic extract of *Ichnocarpus frutescens

Well diffusion method

Nutrient agar media (NAM) was prepared for growing of bacteria inside the laboratory. The standard size (100mm× 15mm) petri dishes as required for whole experiment. For preparation of NAM, 13 gram powder was mixed with 1000 ml of distilled water and stirred to obtain homogenized mixture. After which, NAM mixture were placed in Autoclave under 15 psi pressure, at 121°C for 25 min for sterilization of media. After that poured the culture media into petri dishes at ratio of 20 ml/dish and was left half covered on the table to let the agar cool down and solidify at room temperature.

Agar well-diffusion method was followed to determine the antimicrobial activity of hydroalcoholic extract of *Ichnocarpus frutescens* (Bauer et al., 1966).

Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with fresh broth culture of bacteria. Wells (6mm diameter) were made in each of these plates using sterile cork borer. 100mg/ml, 50mg/ml and 25mg/ ml solution was prepared in different extracts. About 100 µl of different concentrations of extract were added sterile micropipette into the wells and allowed to diffuse at room temperature for 2hrs. Control experiments comprising inoculums distilled water were set up. The plates were incubated at 37°C for 24 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Results and Discussion

The present study was carried out to evaluate the phytochemical composition, antioxidant activity, and antimicrobial potential of *Ichnocarpus frutescens* leaf extracts.

The percentage yield of extracts indicated that the hydroalcoholic solvent system was more efficient in extracting phytoconstituents from the leaves of *Ichnocarpus frutescens*. As shown in Table 1, the hydroalcoholic extract showed a higher yield (5.38% w/w) compared to the hexane extract (3.36% w/w). This result suggests that polar and semi-polar solvents are more effective in extracting bioactive constituents present in the plant material.

Preliminary phytochemical screening (Table 2) revealed the presence of several important secondary metabolites in the extracts. The hydroalcoholic extract showed the presence of alkaloids, glycosides, flavonoids, diterpenes, phenols, proteins, carbohydrates, saponins, and sterols, whereas the hexane extract showed comparatively fewer phytoconstituents. The presence of these bioactive compounds is significant because many of them are known to possess antioxidant, antimicrobial, and therapeutic properties.

Quantitative estimation of phytoconstituents (Table 3) showed that the hydroalcoholic extract contained higher levels of total phenolic content (2.013 mg GAE/100 mg), total flavonoid content (2.054 mg QE/100 mg), and total alkaloid content (0.560 mg AE/100 mg) compared to the hexane extract. Phenolic and flavonoid compounds are well known for their strong antioxidant activity due to their ability to donate hydrogen atoms and scavenge free radicals.

The antioxidant activity evaluated by the DPPH free radical scavenging assay demonstrated that the hydroalcoholic extract exhibited considerable antioxidant potential (Table 4). The percentage inhibition increased with increasing concentration of the extract, indicating a dose-dependent response. The IC_{50} value of the hydroalcoholic extract was found to be 50.65 µg/ml, whereas the standard ascorbic acid showed a lower IC_{50} value of 20.69 µg/ml, indicating stronger antioxidant activity of the standard. However, the extract still demonstrated significant free radical scavenging activity, which may be attributed to the presence of phenolic and flavonoid compounds.

The antimicrobial activity of the hydroalcoholic extract was evaluated against selected bacterial and fungal strains, including *Streptococcus mutans*, *Klebsiella pneumoniae*, and *Candida albicans*. The results (Table 6) showed that the extract exhibited moderate antimicrobial activity, with the zone of inhibition increasing with concentration. At 100 mg/ml, the hydroalcoholic extract produced zones of inhibition of 12 mm against *Streptococcus mutans*, 14 mm against *Klebsiella pneumoniae*, and 14 mm against *Candida albicans*. Although the activity was lower compared to standard drugs such as ofloxacin, ciprofloxacin, and fluconazole (Table 5), the extract still demonstrated promising antimicrobial potential.

The observed antimicrobial and antioxidant activities of *Ichnocarpus frutescens* may be attributed to the presence of various phytoconstituents such as phenols, flavonoids, alkaloids, and saponins, which are known to possess biological activities. These findings support the traditional medicinal use of *Ichnocarpus frutescens* and suggest that the hydroalcoholic extract of its leaves could serve as a potential source of natural antioxidant and antimicrobial agents.

Table 1: % Yield of leaves extract of *Ichnocarpus frutescens*

Sr. No	Extracts	% Yield (W/W)
1.	Hexane	3.36 %
2.	Hydroalcoholic	5.38 %

Table 2: Result of phytochemical screening of extracts of *Ichnocarpus frutescens*

S. No.	Constituents	Hexane extract	Hydroalcoholic extract
1.	**Alkaloids**		
	Wagner's Test:	-ve	+ve
	Hager's Test:	-ve	-ve
2.	**Glycosides**		
	Conc. H_2SO_4 Test:	-ve	+ve
3.	**Flavonoids**		
	Lead acetate Test:	+ve	+ve
	Alkaline reagent test:	+ve	-ve
4.	**Diterpenes**		
	Copper acetate Test:	-ve	+ve
5.	**Phenol**		
	Ferric chloride Test:	-ve	+ve
	Folin ciocalteu Test:	+ve	+ve
6.	**Proteins**		
	Xanthoproteic Test:	-ve	+ve

7.	**Carbohydrate** Fehling's Test: Benedict's Test	 +ve -ve	 +ve +ve
8.	**Saponins** Froth Test:	 -ve	 +ve
9.	**Tannins** Gelatin test:	 -ve	 -ve
10.	**Sterols** Salkowski Test:	 -ve	 +ve

+Ve = Positive, -Ve= Negative

Table 3: Estimation of total phenol and flavonoids content of *Ichnocarpus frutescens*

S. No.	Extracts	Total phenol content	Total flavonoids Content	Total alkaloids Content
		Gallic acid equivalent (GAE)	Quercetin equivalent (QE)	Atropine equivalent (AE)
		mg/ 100 mg		
1.	Hexane	0.808	1.036	-
2.	Hydroalcoholic	2.013	2.054	0.560

Table 4: % Inhibition of ascorbic acid and hydroalcoholic extract of using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	***Ichnocarpus frutescens***
1	10	41.85	19.32
2	20	50.98	25.27
3	40	62.74	49.85
4	60	67.87	58.83
5	80	75.63	70.24
6	100	89.24	80.78
IC 50 value		**20.69**	**50.65**

Table 5: Antimicrobial activity of *standard drug* against selected microbes

S. No.	Name of drug	Microbes	Zone of Inhibition (mm)		
			10 µg/ml	**20 µg/ml**	**30 µg/ml**
1.	Ofloxacin	*Streptococcus mutans*	17±0	20±0	22±0
2.	Ciprofloxacin	*Klebsiella pneumoniae*	30±0.3	35±0	40±0.5
3.	Fluconazole	*Candida albicans*	26±0	30±0	32±0

Table 6: Antimicrobial activity of hydroalcoholic extract of *Ichnocarpus frutescens*

S. No.	Name of microbes	Zone of inhibition (mm)		
		25mg/ml	**50 mg/ml**	**100mg/ml**
1.	*Streptococcus mutans*	8±0.5	11±0.94	12±0.57
2.	*Klebsiella pneumoniae*	9±0.74	13±0.86	14±0.5
3.	*Candida albicans*	9±0.47	10±0.5	14±0.86

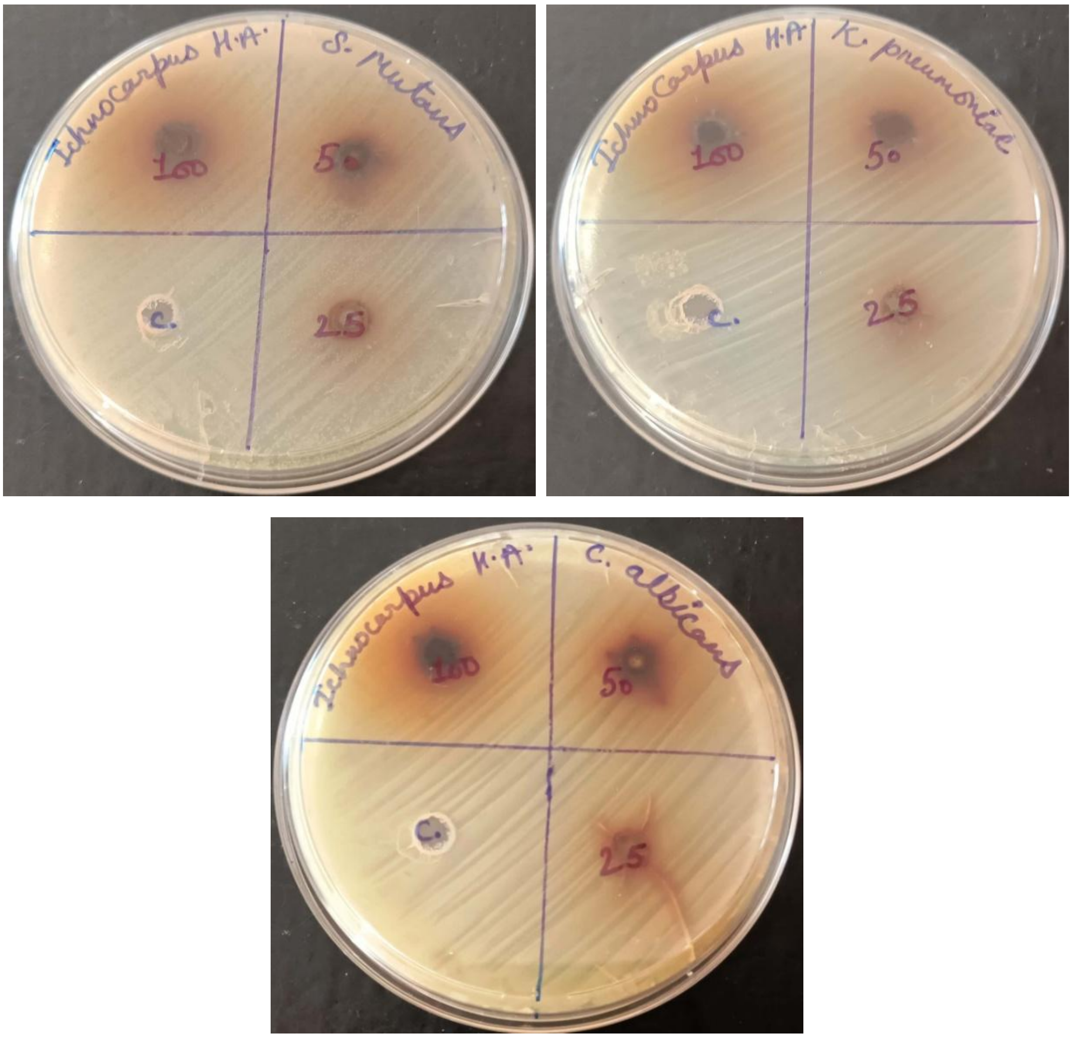

Figure 1: Photoplate of Antimicrobial activity of hydroalcoholic extract of *Ichnocarpus frutescens*

Conclusion

The present study demonstrates that the hydroalcoholic leaf extract of Ichnocarpus frutescens is rich in important phytoconstituents such as alkaloids, flavonoids, phenolics, glycosides, saponins, and sterols. The extract showed higher yield and significant levels of phenolic and flavonoid contents, which contribute to its strong antioxidant potential as evidenced by the DPPH assay. Additionally, the extract exhibited moderate antimicrobial activity against selected bacterial and fungal strains. The findings validate the traditional use of *Ichnocarpus frutescens* and highlight its potential as a natural source of antioxidant and antimicrobial agents. Further studies are required to isolate active compounds and explore their therapeutic applications..

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