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Preliminary phytochemical screening and antimicrobial potentiality of *Ixora pavetta* leaf extracts

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ABSTRACT
Ixora pavetta (Torchwood tree) commonly found in the Indian subcontinent. The flowers, fruit, root and bark generally used to treat various common ailments. But the medicinal potential of the leaves is less explored. An experiment was conducted to identify the various active compounds present in the leaf extracts and determined their microbial activity. Bioactive compounds from I. pavetta leaves are extracted for about 12-18 h using chloroform, hexane, ethyl acetate, methanol (80%). The solvent extractions were examined for various bioactive compounds. The antimicrobial potentiality of leaf extracts was determined using bacteria Bacillus subtilis (MTCC 441), Escherichia coli (MTCC 439), Proteus vulgaris (MTCC 1688), Salmonella typhi (MTCC 3231), Vibrio cholera (MTCC 3906) and fungi Aspergillus fumigatus, Aspergillus niger, Beauveria bassiana, Candida albicans and Mucor hiemalis. The obtained results revealed the efficiency of methanol and ethyl acetate in extracting bioactive compounds from the leaves of I. pavetta by extracting the leaf methanolic extracts of anrthocyanidines, phenols, quinones, lipids, saponins, tannins, flavanoids, terpenoids, resins and alkaloids. The leaf methanolic extracts of I. pavetta showed potential antibacterial and antifungal activity against P. vulgaris (21 mm) and A. niger (22 mm) respectively. The obtained proved the efficiency of solvent methanol in extracting the various bioactive compounds with potential antimicrobial activity. An increase in water stress. Shoots elongation significantly decreased by concentration of 2-8 MPa whereas no hypocotyl elongation at concentration of 10 and 12 MPa and shoot elongation completely inhibited.
Keywords: *Torchwood, Bioactive compounds, Soxhlet, Solvents, MIC.*

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INTRODUCTION

The role of plants in treating various human diseases is quite unavoidable (1). Use of traditional medicine in healing practices is an age-old practice and still around 3/4th of the global population rely on traditional remedies for healthcare needs (2). In order to synthesize the medicinal drugs about 3, 50, 000 species of the vascular plants are now used (3). Still 85% of the global community uses plant derived drugs for to treat the disease (4). And 80% of synthetic drugs used today are of plant origin (5). The phytochemicals recede in the plant body plays a prominent role in synthesizing the plant-base d drugs (6,7,8). These phytochemicals are the intermediate and end products of the various plant metabolic activities, which generally used in plant defensive mechanism to escape from the external and internal stressors (9,10,11). These phytochemicals further benefitted to the human population in effective eradication of the new world diseases [11]. So far, large population of the world using plant originated drugs to treat various ailments (12,13). Moreover, the side effects due to usage of synthetic drugs tend the scientists to look for remedies of natural origin with safe and effective activity (14). Plants contain enormous number of phytoconstituents with potential biological activity and it is very difficult to use them without knowing their specific activity on specific disease. Screening the active compounds from plant extracts initiates the synthesis of novel drugs of plant origin. Use of various solvents i.e chloroform, ethyl acetate, methanol, n-Hexane etc. in extracting biologically active compounds is a regular practice (15,16). The major part of the plant derived compounds includes phytochemicals, and secondary metabolites, which can be classified in to alkaloids, coumarins, flavanoids, lectins, phenolics, polyphenols, polypeptides, quinones, saponins, tannins and terpenes etc (17,18,19,20).

The *Ixora pavetta* is commonly called the Torchwood tree used to cure cough, anaemia and urinary problems. The medicinal potentiality of the leaf extracts is not much documented and very little information is available. Hence, the present work conducted in order to emphasize the possible secondary metabolites present in the *Ixora pavetta* leaf extracts and to determine their antimicrobial activity.

MATERIAL AND METHODS

Powdered leaf material of the selected plants (150 g) was extracted using chloroform, ethyl acetate, hexane and methanol (80%) for 12-18 h (21) and evaporated using vacuum rotary evaporator (Buchi Labortech Ag, model l, R-215).

Preliminary Phytochemical Screening

Qualitative analysis of phytochemicals from the *I. Pavetta* leaf extracts identified using the standard protocols (20,21).

Quantitative phytochemicals estimation

Alkaloids (mg of AE/g)

1 mL of HCl (2N) was added to 1 mg of methanolic leaf extract dispersed in dimethylsulfoxide (DMSO) and filtered. The contents were then transferred to a separatory funnel and bromocresol green solution (5 mL) and phosphate buffer (5 mL) were added. The mixture was stirred with chloroform (1-4 mL) and collected in a 10 mL volumetric flask. Atropine standards ranging from 20 µg/ml to 100 µg/ml at intervals of 20 µg/ml were used as reference controls. The optical density of the solution was measured at 470 nm (22).

Coumarin (mg EC/g)

Coumarin content was determined using standard protocols (23,24). A cocktail was prepared with 500 µl of plant extract, distilled water (2 ml) and 500 µl of lead acetate (5% w/v) and thoroughly mixed. 7 ml of DH_2O was added to this mixture and thoroughly mixed. 8 ml of HCl (0.1M v/v) was added to 2 ml of this solution and maintained at 23°C for 30 minutes. The light absorption of the solution was recorded at 320 nm.

Cardiac glycosides (%)

Cardiac glycosides were determined according to Shams and Abubakar (25). H2O (60ml) and lead acetate (12.5%) 8 ml were added to 8 ml of the plant extract, stirred, and then filtered. 8 ml of Na_2HPO_4 (47%) was added to 50 ml of the filtrate to precipitate excess Pb 2+ ions. 10 ml of the filtrate was treated with Valier's reagent (10 ml), and the OD was measured at 495 nm after 1 hour of incubation.

$$\% \text{ Total glycosides} = \frac{\text{Optical Density} \times 100}{77}$$

Flavonoids (mg QE /g)

A 0.5 ml of leaf extract. 5 ml 2% ethanol $AlCl_3$ were mixed and incubated for 1 hour. Color intensity was later measured at 420 nm. Leaf extracts were measured at a final concentration of 0.1 mg/mL (26). Quercetin used as standard.

Total Phenols (mg GA/g)

The total phenolic content of methanolic leaf extracts was determined (27) using gallic acid as standard. A reaction mixture was prepared from leaf extract (0.5 ml), 10% Folin-Ciocalteu reagent (2.5 ml), 7.5% NaHCO3 (2.5 ml) and distilled water. The reaction cocktail was incubated at 45 °C for 45 minutes. Later colour density was measured at 765 nm.

Crude Saponin (%)

To the 20% aqueous ethanol (100 ml), 20 g of leaf extract was added and heated 55°C for 4 hours with constant stirring. The solution was then filtered and the filtrate was extracted with 20% ethanol (200 ml). Evaporate the extract and adjust the final volume to 40 ml. This concentrate was extracted with diethyl ether (20 ml). The aqueous layer was separated and purified with n-butanol (60 ml). Then, it was washed twice with 5% NaCl aqueous solution (10ml). The solution was dried and the amount of saponin was calculated [28, 29].

Steroids

Steroids present in leaf extracts were measured (30). 2 ml of 4N sulfuric acid was added to 1 ml of the leaf extract followed by adding of 2 ml of 0.5% (w/v) ferric chloride, 0.5ml 0.5% w/v potassium(III) hexacyanoferrate. The cocktail was placed in a hot water bath and heated to 70 ± 20 °C for 30 min with intermittent shaking. The colour intensity was calculated at 780 nm.

Tannins (mg/g)

The amount of tannins present in the methanol extract of leaves was determined (31). To the leaf extract (0.1 ml) distilled water (7.5 ml) and Folin-Ciocalteu phenol reagent (0.5 ml) were added, followed by 1 ml of sodium carbonate solution (35%). The contents were diluted to 10 ml by the addition of DH_2O. Now the

cocktail was gently shaken and incubate at 23 °C for 30 min. The Absorbance was determined at 700 nm. Tannic acid used as the standard.

Antibacterial Activity

One 100 ml nutrient agar medium was sterilized (at 121 °C and 15 lbs pressure) for 15 minutes. The DN was brought to room temperature and inoculated with the test microorganism suspension (0.1 ml). The contents were thoroughly mixed and poured into petridishes under sterile conditions. Coagulated plant extract inoculum is prepared by making wells with a diameter of 5 mm. The antibacterial activity of *I. pavetta* leaf extract was evaluated against *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 439), *Proteus vulgaris* (MTCC 1688), *Salmonella typhi* (MTCC 3231) and *Vibrio cholerae* (MTCC 3906). Three concentrations (50 µg, 100 µg, and 150 µg) of the leaf extract were spread in agar wells to measure their antibacterial activity. The antibiotics DMSO (30 µl/ml) and chloramphenicol (30 µg/ml) were used as negative and positive controls.

Antifungal Activity

The antifungal activity of *I. paveta* leaf extract was tested against fungi viz. *Aspergillus fumigatus, Aspergillus niger, Beauveria bassiana, Candida albicans* and *Mucor hiemalis*. Fungal cultures grown overnight at 28 °C were spread on potato dextrose agar plates. DMSO (30 µl/ml) and Nystatin (50 µg/ml) and were used as negative and positive controls. Antifungal activity was measured using three different concentrations of leaf extract (50 µg, 100 µg and 150 µg).

Minimum Inhibitory Concentration (MIC)

A 2 fold dilution method was used to determine the MIC values. The plates and tubes were incubated at 37 °C for 1 day. Antibacterial activity was determined by measuring the zone of inhibition by excluding the well diameter. Tubes without turbidity were recorded as MIC values.

RESULT

The present work was aimed to prepare leaf extracts using various organic solvents from *Ixora pavetta* and to evaluate the qualitative and quantitative screening of phytochemicals along with their antimicrobial activity. The results are discussed in detail hereunder.

Preliminary phytochemical screening

The leaf sample of *I. pavetta* was extracted with various organic solvents i.e. chloroform, ethyl acetate, hexane and methanol in order to determine the phytochemicals present in the leaf samples both qualitatively (Table 1) and quantitatively (Table 2).

Phytochemical screening

The preliminary screening of phytochemicals from *I. pavetta* leaf samples extracted with various organic solvents showed the existence of different phytochemicals (Table 1). The chloroform extracted reported the presence of glycosides, tannins, terpenoids and alkaloids. Phytochemicals such as coumarins, tannins, terpenoids, resins and alkaloids are extracted with ethyl acetate from the *I. pavetta* leaves whereas the leaves extracted with hexane showed the presence of glycosides, lipids, tannins, terpenoids, alkaloids; coumarins, quinones, glycosides, lipids, tannins, terpenoids, alkaloids. Leaf methanolic extracts of *I. pavetta* reported the existence of anrthocyanidines, phenols, quinones, lipids, saponins, tannins, flavanoids, terpenoids, resins and alkaloids.

Table 1. Prelimnary phytochemical screening of *Ixora pavetta* leaf extract

Phytochemicals	Chloroform	Ethyl acetate	Hexane	Methanol
Anthocynidines	-	-	-	+
Phenols	-	-	-	+
Resins	-	+	-	+
Coumarins	-	+	-	-
Quinones	-	-	-	+
Flavonoids	-	-	-	+
Glycosides	+	-	+	-
Amino acids	-	-	-	-
Lipids	-	-	+	+
Saponins	-	-	-	+
Terpenoids/Steroids	+	+	+	+
Tannins	+	+	+	+
Alkaloids	+	+	+	+

Quantitative phytochemical screening

The yield of different secondary metabolites presents in *I. pavetta* leaves extracted through different organic solvents was varied significantly from solvent to solvent (Table 2).

Alkaloids

The alkaloid content was ranged from 20.11±0.95 %w/w to 34.15±1.10 %w/w in *Ixora pavetta*. The highest alkaloid content was extracted with methanol both in *I. pavetta* (34.15±1.10 %w/w). After methanol maximum alkaloid concentration was extracted with ethyl acetate (27.10±1.00).

Cardiac glycosides

The cardiac glycoside content of *I. pavetta* varied from 1.10±0.54 g/100 g to 5.68±0.25 g/100 g, and the maximum concentration of cardiac glycosides (5.68±0.25 g/100 g) was observed in methanol extract of *I. pavetta* leaves.

Table 2. Quantitative phytochemical screening of *I. pavetta*

Secondary metabolite	*Ixora pavetta* leaf extract			
	Chloroform	Ethyl acetate	Hexane	Methanol
Alkaloids (% w/w)	20.15±0.95	27.10±1.00	20.11±1.10	34.15±1.10
Cardiac glycosides (g/100g)	3.12±1.40	-	1.10±0.54	5.68±0.25
Coumarins (mg/g)	-	16.21±0.68	10.14±1.58	20.18±0.11
Flavonoids (mg/g)	-	-	1.22±2.12	12.67±0.17
Phenols (mg/g)	-	-	0.81±1.58	2.10±1.10
Saponins (% w/w)	-	-	0.84±1.74	3.19±0.74
Steroids (µg/mg)	30.21±0.25	60.25±0.19	39.27±0.96	69.27±0.96
Tannins (% w/w)	3.21±0.45	5.10±0.22	2.10±1.25	6.10±1.20

Coumarins

The leaf methanolic extracts of *I. pavetta* was ranged from 10.14±1.58 mg/g to 20.18±0.11 mg/g. Among the entire organic solvents' methanol reported to be the best solvent to extract the coumarins (20.18±0.11 mg/g). The *I. pavetta* leaf extracts exhibited more coumarin concentration 20.18±0.11 mg/g with methanol.

Flavonoids

The total flavonoid content in *I. pavetta* was recorded as 1.22±2.12 mg/g and 12.67±0.17 mg/g with hexane and methanol.

Phenols

The essentiality of the phenolic compounds in plant defense mechanism is well documented. The phenolic concentration of *I. pavetta* found to be more when extracted with hexane (0.81±1.58 mg/g) and methanol (2.10±1.10 mg/g).

Saponins

The coumarin concentration of the *I. pavetta* reported to be 0.84±1.74 %w/w and 3.19±0.74 %w/w in hexane and methanol extracts respectively.

Steroids

The steroid content of *I. pavetta* was varied between 30.21±1.40 µg/mg to 69.27±0.96 µg/mg and high steroid content was recorded in methanolic extracts (69.27 µg/mg).

Tannins

The total tannin content was ranged between 2.10±1.25 %w/w to 6.10±1.20 %w/w. In *I. pavetta* maximum tannin concentration was observed with leaf methanolic extracts (6.10±1.20 %w/w).

Antimicrobial activity

A dose dependent antibacterial activity was observed against test organisms. The antibacterial activity of *I. pavetta* leaves extracted with chloroform, ethyl acetate, hexane and methanol were tested against *B. subtilis, E. coli, P. vulgaris*, *S. typhi* and *V. cholerae* and the zone of inhibition was presented in Figure 1. Of all the solvent extracts leaf methanolic extracts of *I. pavetta* recorded relatively more zone of inhibition *P. vulgaris* (Plate 1) followed by leaf ethyl acetate extracts. The positive control nystatin recorded 16 mm (50 µg/ml) and in present study the antifungal activity of plant extracts of *I. pavetta* are on par with the standard drug nystatin and the negative control dimethyl sulfoxide (30 µl/ml) did not show any zone of inhibition.

The antifungal activity of *I. pavetta* leaves extracted with chloroform, ethyl acetate, hexane and methanol were tested against pathogenic fungi *Aspergillus fumigatus, Aspergillus niger, Beauveria bassiana, Candida albicans, Mucor hiemalis* and the zone of inhibition was presented in Figure 2. Of all the solvent extracts leaf methanolic extracts of *I. pavetta* recorded relatively more zone of inhibition against *A. niger* with 22 mm (Plate 2) at 150 µl of leaf extract. The positive control chloromphenicol recorded 18 mm (30 µg/ml)

and in present study the antibacterial activity of plant extracts of *I. pavetta* are on par with the standard drug chloromphenicol and the negative control dimethyl sulfoxide did not show any zone of inhibition.

Minimum Inhibitory Concentration (MIC)

Both the plant extracts *I. pavetta* reported promising antimicrobial activities against test pathogens compared with reference antibiotics (Table 3). The bacterial pathogens *E. coli* and *P. vulgaris* found to be most sensitive than all other bacteria and they were sensitive at the concentration of 15.12 µg/ml and 16.00 µg/ml respectively. In case of fungi also the methanolic extracts of *I. pavetta* exhibited potent MIC and the minimum inhibitory concentration found to be very low against *C. albicans* and *Aspergillus fumigatus* and this concentration reported to be 18.90 µg/ml and 20.15 µg/ml respectively.

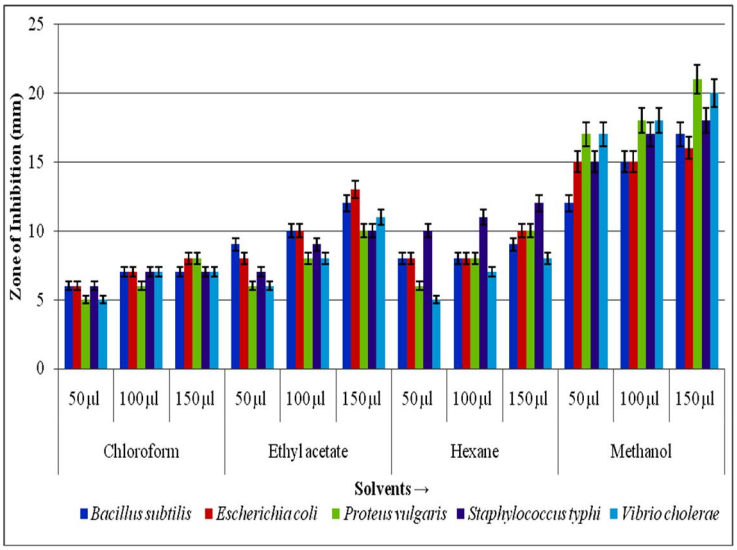

Figure 1. Antibacterial activity of *Ixora pavetta* leaf extract

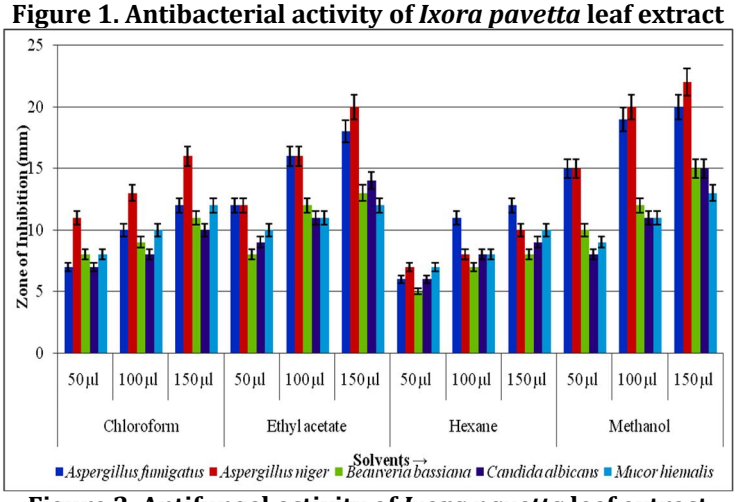

Figure 2. Antifungal activity of *Ixora pavetta* leaf extract

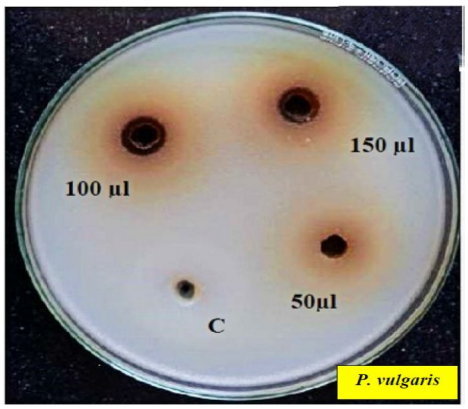

Plate 1. Antibacterial activity *Ixora pavetta* leaf extract against *P. vulgaris*

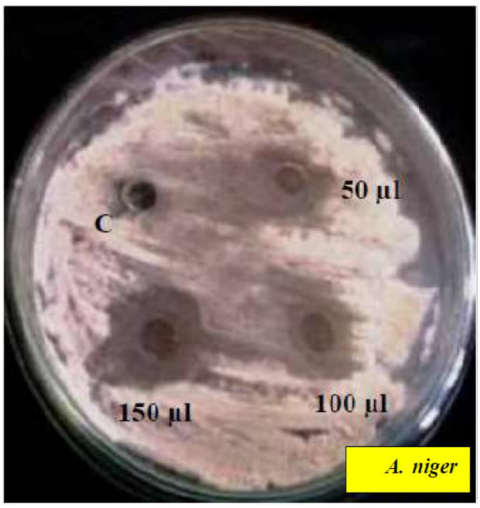

Plate 2. Antifungal activity of *Ixora pavetta* leaf extract against *A. niger*

Table 3. Minimum Inhibitory Concentration of leaf methanol extracts against test bacteria and fungi

Test Organism	Methanol Extracts (µg/ml)		
Bacteria		Fungi	
Bacillus subtilis	16.66	*Aspergillus fumigatus*	20.15
Escheria coli	15.12	*Aspergillus niger*	30.25
Proteus vulgaris	16.00	*Beauveria bassiana*	28.47
Staphylococcus typhi	25.12	*Candida albicans*	18.90
Vibrio cholerae	30.77	*Mucor hiemalis*	30.12

DISCUSSION

Plant generally prepares enormous number of bioactive compounds (32,33,34). The phytochemical studies revealed that leaves extracted with various organic solvents found to be the best extraction practice to extract the secondary metabolites from the plants with ease and speed (Table 1). In case of *I. pavetta* along with methanol, hexane found to be potent in extracting the secondary metabolites but the quantitative extraction is lower than the ethyl acetate and hexane (Table 2). These reports agree with earlier reports (35).

In the present study, promising antagonistic activities against various microorganisms were shown by the chloroform, ethylacetate, hexane and methanol extracts of *I. pavetta*. The methanolic extracts recorded more antibacterial activity, followed by the ethylacetate extracts and hexane extracts whereas the leaf chloroform extracts showed less antibacterial activity (Figure 1). In present study the methanolic extracts of *I. pavetta* (21.00±0.1 mm) showed maximum zone of inhibition against *Proteus vulgaris* (Plate 1). This highest antimicrobial activity is may be due to the existence of good number of secondary metabolites in *I. pavetta* leaves. This study established the antimicrobial efficiency of the leaf methanolic extracts of *I. pavetta*. Similar trend of results was observed in various plants (36).

The solvent extracts of *I. pavetta* also reported potent antifungal activity and the results are on par with the control (Figure 2). In present study methanolic extracts of *I. pavetta* leaves recorded maximum zone of inhibition against *A. niger* (Plate 2) at 150 ml concentration. These results are in accordance with previous reports (37).

CONCLUSION

Of all the studied varieties based on their performance it is concluded that foxtail varieties such as SIA 3156, SIA 3085, TNAU 164 and TNAU 86 found to be potential against drought stress due to PEG treatments.

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CONFLICT OF INTEREST

Authors do not have any conflict of interest.

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