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Research Article

EVALUATION OF THE FOLK CLAIM AND IDENTIFICATION OF THE PHARMACOLOGICAL ACTIVE PRINCIPLES IN *BAUHINIA PHOENICEA* LEAVES

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ABSTRACT

Objective: *Bauhinia phoenicea,* an endemic plant of western Ghats, using in traditional medicine against diabetes, skin allergies, fungal infections, and worm disturbances. To the best of our knowledge, any scientific studies on the medicinal properties of the leaves of this plant are not yet reported. Therefore, as the first step toward unraveling its medicinal property, bioactivity profiling, and gas chromatography-mass spectrometry (GC-MS) analysis were performed.

Methods: The pharmacological activity profiling includes antibacterial, antifungal, anthelmintic, and antioxidant property screening using crude ethanolic extract, which preliminary analyses its folk claim. GC-MS analysis performed to identify the pharmacologically active principles. All analyses were performed according to standard protocols.

Results: Ethanol extract of the leaves of *B. phoenicea* was assayed for the antimicrobial activity against 10 human pathogenic strains using well diffusion assay. The extract showed a significant activity against all pathogens. A maximum zone of inhibition observed in *Salmonella typhi* in their higher concentration (500 µg/ml). The anthelmintic activity of crude drug evaluated on Indian adult earthworms *Pheretima posthuma*, exhibited dose-dependent spontaneous mortality, and evoked responses to pin prick and effects compared with that of albendazole. The drug showed potent antioxidant property in 2,2-diphenyl-1-picrylhydrazyl free radical scavenging and superoxide anion scavenging assays with half maximal inhibitory concentration values 92±0.92 and 62±1.34, respectively. The GC-MS analysis which showed the presence of 19 compounds including hexadecanoic acid and oleic acid.

Conclusion: According to our results, it is concluded that leaf of *B. phoenicea* has significant antimicrobial, anthelmintic, and antioxidant properties supporting the folk medicinal use of this species. The further procedures of isolation and characterization of active principles are in progress.

Keywords: *Bauhinia phoenicea,* Antimicrobial, Anthelmintic, Antioxidant property and gas chromatography-mass spectrometry.

INTRODUCTION

Medicinal plants have an age long remedies to human diseases because they contain components of therapeutic value [1]. The quest for solutions to the global problems of antibiotic resistance in pathogenic bacteria has often focused on the isolation and characterization of new antimicrobial compounds from a variety of sources including the medicinal plant [2]. It may also because that scientists have established that crude extracts of some plants and some pure compounds from such plants can potentiate the activity of antibiotics *in vitro* [3]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multi drug-resistant pathogens [4]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for the potential antimicrobial activity [5].

Bauhinia phoenicea is a liana found in evergreen forests commonly called as "vallimantharam." It is endemic to Western Ghats. Leaves and bark of *B. phoenicea* are used by the traditional practitioners for skin irritations, diabetes, and worm disturbances [6]. In literature, there is no report on the medicinal properties and chemical constituents of the leaves of this species of Bauhinia. Our earlier works describe the pharmacognostic standards of *B. phoenicea* leaves [6] and evaluated the biological activities of the bark of this plant [7]. The pharmacological activity screening of *B. phoenicea* leaf was undertaken as the first step toward unraveling of its medicinal property.

METHODS

Collection and identification of plant material

The fresh leaves of *B. phoenicea* were collected in the months of September 2013 from the botanical garden of St. Mary's College, Thrissur, which is the identified plant given from MS Swaminathan Research Foundation Wayanadu, Kerala, India, and submitted a voucher specimen in our department herbarium. The plant name checked with www.theplantlist.org.

Preparation of extracts

Leaves of the plant were shade dried for several days. The dried plant material was ground to a course powder and 50 g of the powdered plant material was soaked in 95% ethanol (1:5) for 72 hrs. Then, the solvent removed by rotary evaporation. The dried extract was stored in the refrigerator for further studies.

Phytochemical screening

The preliminary phytochemical analysis of the plant extracts was performed using the standard protocol given by Harbone [8].

Antimicrobial assay

Organisms and culture media

The pathogenic strains of bacteria and fungus were obtained from the laboratory, Department of Microbiology, St. Mary's College, Thrissur. Organisms used were *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus,* and *Penicillium notatum.* The bacterial cultures were maintained on nutrient agar (NA), while fungal cultures on Sabouraud dextrose agar (SDA).

Antibacterial and antifungal activity of the plant extract

Well diffusion assay [9] on NA and SDA plates were used to determine the antibacterial and antifungal properties, respectively. Bacteria were inoculated into nutrient broth (NB), while fungus into Sabouraud

dextrose broth (SDB) and incubated at 37°C for 6 hrs. The turbidity of the resulting suspensions was diluted with NB and SDB to obtain a transmittance of 74.3% (absorbance of 0.132) at 600 nm. The percentage is found spectrophotometrically comparable to 0.5 McFarland turbidity standard. This level of turbidity is equivalent to approximately 1.5×10^8 CFU/ml [10]. These bacterial cultures were then inoculated on the surface of NA plates for bacteria and SDA for fungus. Subsequently, wells of 6 mm diameter were prepared on NA and SBD plates using sterile cork borer, and 25 µl sample in different concentrations (100, 250, and 500 µg/ml) were loaded in each well. Antibiotics were used as a positive control (chloramphenicol for bacteria and fluconazole for fungus) [11]. The tests were conducted in triplicates. The plates were incubated at 37°C for 24 hrs. At the end of incubation, zones of inhibition were measured with a transparent ruler. Zones of clearing >6 mm were considered susceptible to the extracts.

Anthelmintic property

The standard albendazole (25 mg/ml) and the test solutions of *B. phoenicea* (25, 50, 100 mg/ml) were evaluated for anthelmintic activity with Indian adult earthworm *Pheretima posthuma,* which is procured from Kerala Agriculture University, Mannuthi, Thrissur. The observations were made for the time taken for paralysis and death of individual worms up to 4 hrs of the test period. Time for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C [12].

Antioxidant property screening

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity of the plant extract was assessed on the basis of the radical scavenging effect of the stable DPPH, by a modified method [13]. The diluted working solutions of the test extracts (10-1000 µg/ml concentration) and 6.34 µM solution of DPPH were prepared in methanol, and 100 µl test, 100 µl DPPH solution, and 800 µl of methanol were taken in a test tube and mixed well. These solution mixtures were kept in dark for 20 minutes and optical density was measured at 517 nm using Cecil-Elect Spectrophotometer. Methanol (900 µl) with DPPH solution (6.34 µM, 100 µl) was taken as control and methanol as blank. The optical density was recorded and % of inhibition was calculated using the formula given below:

Percent (%) inhibition of DPPH activity = A–B/A × 100

Where, A = Optical density of the control and B = Optical density of the sample.

Superoxide radical scavenging assay

In-vitro superoxide radical scavenging activity was measured by nitroblue tetrazolum (NBT) reduction method [14]. This method is based on the generation of superoxide radical by auto-oxidation of riboflavin in the presence of light. The superoxide radical reduces NBT to a blue colored formazan that can be measured at 590 nm.

About 100 µl riboflavin solution, 200 µl ethylenediaminetetraacetic acid, 200 µl ethanol 100 µl NBT solution were mixed in a test tube and diluted up to 3 ml with phosphate buffer. The absorbance of the solution was measured at 590 nm using phosphate buffer as blank after illumination for 15 minutes. This was taken as control reading. For screening of the test sample along with the above solutions added 100 µl sample of varying concentrations (10-1000 µg/ml), and finally, the volume was made up to 3 ml using phosphate buffer and reading taken after 15 minutes of illumination against phosphate buffer as blank. % of inhibition was calculated using the formula given below:

Percent (%) inhibition = A–B/A × 100

Where, A = Optical density of the control and B = Optical density of the sample.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS was carried out on GC Clarus 500 Perkin Elmer. The temperature program was from 200 to 280°C. The chromatographic column was Elite-5MS (5% diphenyl/95% dimethyl polysiloxane), 30 µm × 0.25 µm. 2 µl sample was injected into the non-polar column. Helium was used as the carrier gas at a flow rate of 1 ml/minutes. The run time of sample was 36 minutes.

RESULTS AND DISCUSSION

Phytochemical screening *B. phoenicea* leaf extract

The preliminary phytochemical screening of *B. phoenicea* leaf showed the presence of primary metabolites (such as carbohydrates, starch, sugar, proteins, and amino acids) and secondary metabolites (such as alkaloids, saponins, phenols, steroids, and flavonoids).

Antibacterial and antifungal activity of *B. phoenicea* leaf extract

The antibacterial and antifungal activities of *B. phoenicea* leaf are summarized in Table 1. The leaf extract inhibited almost all bacteria and fungus. It is highly effective against the bacterial species *K. pneumoniae* and *S. typhi* with zone of growth inhibitions 18.3±1.5 mm and 20±2 mm, respectively, at 500 µg/ml concentration. It was least active against *S. pyogenes* with only 9.3±1.2 mm zone of growth inhibition at the same concentration. The inhibition of the positive controls, chloramphenicol, and fluconazole was comparable to that of the plant extract.

Table 1: Antibacterial and antifungal activity of the plant extract

S. No:	Organism	Zone of inhibition				
		Standard		100 µg	250 µg	500 µg
		Chloramphenicol (25 µg)	Fluconazole (15 µg)			
1	*K. pneumoniae*	39±1.2	ND	8.3±0.057	13.3±1.2	18.3±1.5
2	*S. typhi*	30.7±5	ND	8.3±0.57	14.3±2.08	20±2
3	*P. aeruginosa*	9.3±1.2	ND	R	7.3±0.57	10
4	*B. cereus*	32±4	ND	R	10.6±1.2	13.7±1.5
5	*S. pyogenes*	17.6±2.5	ND	8.6±1.2	8.6±1.5	9.3±1.2
6	*S. aureus*	36.6±1.2	ND	9.6±0.57	9.6±0.57	12±2
7	*A. niger*	ND	19±1.6	14±1.9	16±2.3	19±1.8
8	*P. notatum*	ND	16±1.5	15±2.1	17±1.2	19±1.3
9	*C. albicans*	ND	R	12±1.8	14±1.3	15±1.8
10	*A. flavus*	ND	12.3±1.15	14.6±0.57	16.3±1.15	19.3±0.57

ND: Not determined, R: Resistant, *K. pneumoniae*: *Klebsiella pneumoniae, S. typhi*: *Salmonella typhi, P. aeruginosa*: *Pseudomonas aeruginosa, B. cereus*: *Bacillus cereus, S. pyogenes*: *Streptococcus pyogenes, S. aureus*: *Staphylococcus aureus, A. niger*: *Aspergillus niger, P. notatum*: *Penicillium notatum, C. albicans*: *Candida albicans, A.flavus*: *Aspergillus flavus*

Due to the reported development of resistance by bacteria and fungi to various commercially available antimicrobial agents, the leaf extract of plants is potential sources of new compounds which may be developed as effective drugs against micro-organisms. Further, the use of this plant may offer a new source of antifungal agent against the pathogenic fungus such as *C. albicans*, *A. niger*, and *P. notatum* all these fungal species were inhibited by the crude drug in dose-dependent manner. At the concentration 500 µg/ml *C. albicans*, *A. niger* and *P. notatum* showed 19±1.8, 19±1.3 and 15±1.8 mm of growth inhibition, respectively. *C. albicans* is not easily inhibited by other drugs.

Anthelmintic property of *B. phoenicea* Leaf extract

It was seen that the ethanolic extract of *B. phoenicea* leaf possesses dose-dependent anthelmintic activity as compared to a standard drug albendazole. The mean paralyzing time of *P. posthuma* with the dose of 25, 50 and 100 mg/ml was found to be 52.5, 49 and 45.8 minutes, respectively. In the meantime albendazole at a dose of 25 mg/ml cause paralysis in 53.4 minutes only, i.e. the plant drug is the more effective than the commercial drug in the same concentration itself. The mean death time of *P. posthuma* with the dose of 25, 50 and 100 mg/ml was found to be 122.6, 116 and 109.25 minutes, respectively. In the case of albendazole, no death was observed in the above helminth during the 4 hrs of the experiment (Table 2).

Antioxidant property screening of *B. phoenicea* leaf extract

DPPH radical scavenging assay

DPPH is a stable free radical at room temperature often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. The percentage of DPPH radical scavenging activity of ethanolic extract of *B. phoenicea* presented in Table 3. The ethanolic extract of *B. phoenicea* leaf exhibited significant DPPH free radical scavenging activity with half maximal inhibitory concentration (IC_{50}) value 92±0.92.

Superoxide radical scavenging assay

The superoxide radical scavenging assay also shows significant radical scavenging with IC_{50} value 62±1.34. The activity was increasing with the increasing concentrations of test solution (Table 3).

GC-MS analysis of ethanolic extract

Compounds identification: Compounds present in the extract of *B. phoenicea* were identified on the basis of their retention indices. Identification confirmation was by comparison of their mass spectra with NIST Library version year 2005. The presence of the chemical constituents in the crude extract of *B. phoenicea* was shown in Table 4.

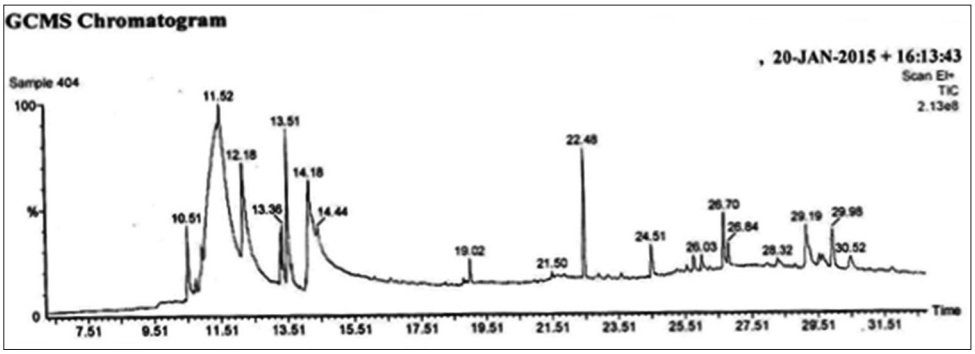

Fig. 1: GCMS Chromatogram of *B.phoenicea* leaf extract.

Table 2: Anthelmintic property of *B. phoenicea* leaf extract

Observation	Distilled water	Albendazole (25 mg/ml)	Drug (25 mg/ml)	Drug (50 mg/ml)	Drug (100 mg/ml)
Time taken for paralysis (minutes)	-	53.4±4.5	52.5±4.7	49±4.9	45.8±4.7
Time taken for death (minutes)	-	-	122.6±3.6	116±3.6	109.25±3.7

B. phoenicea: *Bauhinia phoenicea*

Table 3: Antioxidant screening of *B. phoenicea* leaf extract

S. No.	Concentration of plant extract (µg/L)	Percentage of inhibition	
		DPPH	NBT
1.	10	6.3±0.43	2.69±0.5
2.	15	13.4±1.21	20.8±1.06
3.	25	21.3±0.92	29.27±2.4
4.	50	34.5±0.58	47.5±1.91
5.	75	42.7±1.36	52±2.1
6.	100	52.9±1.83	58.09±2.4
7.	250	60.7±1.35	66.53±0.81
8.	500	81.6±0.57	75.39±1.24
9.	750	92.3±0.83	88.04±0.38
10.	1000	94.5±1.72	94.86±0.57
IC_{50}		92±0.92	62±1.34

IC_{50}: Half maximal inhibitory concentration, *B. phoenicea*: *Bauhinia phoenicea*, DPPH: 2,2-diphenyl-1-picrylhydrazyl, NBT: Nitroblue tetrazolum

Table 4: Compounds present in the *B. phoenicea* leaf

No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1.	10.51	3,7,11,15-tetramethyl-2-hexadecer-1-ol	$C_{20}H_{40}O$	296	2.36
2.	11.52	à-D-glucopyranose, 4-O-à-D-galactopynnosyl-	$C_{12}H_{22}O_{11}$	342	52.10
3.	12.18	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.52
4.	13.31	9,12-octadecadicnoic acid, methyl ester, (E, E)-	$C_{19}H_{32}O_2$	294	0.86
5.	13.36	Oleic acid	$C_{18}H_{34}O_2$	282	1.03
6.	13.51	Phytol	$C_{20}H_{40}O$	296	5.06
7.	14.18	9,12-octadecadicnoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280	12.41
8.	19.02	1,2-benzenedicarboxyllc acid, mono (2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	0.73
9.	21.50	13,16-octadecadiynotc acid, methyl ester	$C_{19}H_{30}O_2$	290	0.34
10.	22.48	Squalene	$C_{30}H_50$	410	3.32
11.	24.51	2H-1-benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*, 8R*)]], (&-Tocopherol)	$C_{27}H_{46}O_2$	402	1.26
12.	26.03	8,11,14-eicosatricnok acid, (Z, Z, Z)-	$C_{20}H_{34}O_2$	306	0.58
13.	26.70	Vitamin E	$C_{29}H_{50}O_2$	430	1.53
14.	26.84	8-dodecen-l-ol, acetate, (Z)-	$C_{14}H_{26}O_2$	226	0.56
15.	29.19	5,8,11,14-eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	318	2.92
16.	28.32	8,11,14-eicosatricnok acid, (Z, Z, Z)-	$C_{20}H_{34}O_2$	306	0.64
17.	29.19	2H-Pyran, 2-(7-heptadecynyloxy) tetrahydro-	$C_{22}J_{40}O_2$	336	2.92
18.	29.98	Cholestan-3-ol, 2-methylene-, (3à,5à)	$C_{28}H_{48}O$	400	1.81
19.	30.52	Cyclopropaneoctanok acid, 2-([2-[(2- ethylcyclopropyl) methyl] cyclopropyl] methyl]-, methyl ester	$C_{22}H_{38}O_2$	334	1.07

B. phoenicea: *Bauhinia phoenicea*

CONCLUSION

This study reveals that the leaves of *B. phoenicea* possess prominent antimicrobial, anthelmintic, and antioxidant properties. Phytochemical studies portray the presence of several biologically active secondary metabolites and GC-MS profiling of ethanolic extract revealed the presence of possible compounds in the plant with their molecular weights and structural formula, which may be the reason for its biological properties and its traditional applications. This study reports for the first time the pharmacological properties of *B. phoenicea* leaf and the possible chemicals present in it.

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