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Original Article

FIRST STEP TOWARDS UNRAVELING THE MEDICINAL PROPERTIES OF AN ENDEMIC TRADITIONAL MEDICINE, *BAUHINIA PHOENICEA* WIGHT AND ARN BARK

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ABSTRACT

Objective: *Bauhinia phoenicea* Wight & Arn is a medicinal plant endemic to Western Ghats. In traditional medicine, it used against diabetes, skin allergies, fungal infections and worm disturbances. To the best of our knowledge, any scientific studies on the medicinal properties of this are not yet reported. Therefore, as a first step towards unraveling its medicinal property, bioactivity profiling was performed.

Methods: Pharmacological activity profiling includes antibacterial, antifungal, anthelmintic and antioxidant property screening using crude ethanolic extract, which preliminary analyses its folk claim. Qualitative Phytochemical analysis performed to identify various valuable secondary metabolites. All the analysis were done according to standard protocols

Results: The present work focused on the evaluation of its folk claim. Ethanolic extract of bark of *B. phoenicia assayed* for antimicrobial activity against 10 human pathogenic strains. The extract showed significant activity against all pathogens. Maximum zone of inhibition observed in *Candida albicans* and *Aspergillus niger* in their higher concentration (500µg/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 ethanolic extract showed potent DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging and super oxide anion scavenging properties with IC 50 values 90±0.92 and 64±0.5 respectively. The preliminary qualitative analysis of *B. phoenicea* bark indicated the presence of alkaloids, saponins, phenols, steroids and flavonoids.

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

Keywords: *Bauhinia phoenicea*, Antimicrobial, Anthelmintic, Antioxidant, Phytochemistry

INTRODUCTION

The genus Bauhinia consists of approximately 300 species that are widely distributed in many tropical countries where they are used frequently in traditional medicine [1]. Several species within the genus have been shown to possess anti-diabetic, anti-inflammatory, anti-schistosomal, anti-diarrhoeal, antioxidant and antibacterial activities [1-4]. Previous chemical reports on the genus Bauhinia include the isolation of terpenoids, alkaloids, steroids, triterpenes, tannin, quinines, bibenzyls and more frequently flavonoids [5]. *B. phoenicea* is a liana found in evergreen forests commonly called as "vallimantharam". It is endemic to Western Ghats. Leaves and bark of *B. phoenicea* is 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 this species of Bauhinia. Pharmacological activity screening of *B. phoenicea* bark was under taken as a first step towards unraveling of its medicinal property.

MATERIALS AND METHODS

Collection and identification of plant material

The fresh bark 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

Bark of the plant was 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 h. Then the solvent removed by rotary evaporation. The dried extract was stored in refrigerator for further studies.

Phytochemical screening

The preliminary phytochemical analysis of the plant extracts performed using standard protocol given by Harborne [7].

Antimicrobial assay

a. 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 (NA) nutrient agar, while fungal cultures on (SDA) Sabouraud dextrose agar.

b. Antibacterial and antifungal activity of the plant extract

Well diffusion assay [8] on nutrient agar and Saboured dextrose agar plates were used to determine the antibacterial and antifungal properties respectively. Bacteria were inoculated into (NB) nutrient broth, while fungus into (SDB) Sabouraud dextrose broth and incubated at 37 °C for 6 h. 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 [9]. These bacterial cultures were then inoculated on the surface of NA plates for bacteria and SDA for fungus. Subsequently, wells of 6 mm diameter was prepared on NA and SBD plates using sterile cork borer and 25 µl sample in different concentrations (100 µg/ml, 250 µg/ml & 500 µg/ml) were loaded in each well. Antibiotics were used as positive control (Chloramphenicol for bacteria and Fluconazole for fungus) [10]. The tests were carried out

in triplicates. The plates were incubated at 37 °C for 24 h. At the end of incubation, zones of inhibition were measured with a transparent ruler. Zones of clearing greater than 6 mm were considered susceptible to the extracts.

Anthelmintic property

The standard Albendazole (25 mg/ml) and the test solutions of *B. phoenicea* bark (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. Observations were made for the time taken for paralysis and death of individual worms up to 4 h of 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 were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50 °C [11].

Antioxidant property screening

a. DPPH Radical scavenging assay

Free radical scavenging activity of the plant extract was assessed on the basis of the radical scavenging effect of the stable DPPH (1, 1-diphenyl-2-picrylhydrazyl), by a modified method [12]. The diluted working solutions of the test extracts (10 µg/ml-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 was taken in a test tube and mixed well. These solution mixtures were kept in dark for 20 min 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.

b. Super oxide radical scavenging assay

In-vitro super oxide radical scavenging activity was measured by NBT (*Nitro blue tetrazolium)* reduction method [13]. This method is based on the generation of super oxide radical by auto oxidation of riboflavin in presence of light. The super oxide radical reduces NBT to a blue colored formazon that can be measured at 590 nm.

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

RESULTS AND DISCUSSION

Phytochemical screening of *B. phoenicea* bark extract

The preliminary Phytochemical screening of *B. phoenicea* bark showed the presence of primary metabolites like carbohydrates, starch, sugar, proteins and secondary metabolites like alkaloids, saponins, phenols, steroids and flavonoids.

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

The antibacterial and antifungal activities of *B. phoenicea* bark is summarized in table: 1. the bark extract inhibited almost all bacteria and fungus. It is highly effective against the bacterial species *Candida albicans* and *Aspergillus niger* with zone of growth inhibitions 26.6±0.57 mm and 27.3±0.57 mm respectively at 500µg/ml concentration. It was least active against *Streptococcus pyogenes* with only 12±1.2 mm zone of growth inhibition at the same concentration. Inhibition of the positive controls, Chloramphenicol and Fluconazole were comparable to that of the plant extract.

Due to the reported development of resistance by bacteria and fungi to various commercially available antimicrobial agents, the bark extract of plants are potential sources of new compounds, which may be, developed as effective drugs against microorganisms. From the table given below it is clear that *B. phoenicea* bark is more effective against fungal pathogens than that of bacteria.

The use of this plant may offer a new source of antifungal agent against the pathogenic fungus like *Candida albicans*, *Aspergillus niger* and *Penicillium notatum.* The crude drug inhibited all these fungal species in dose dependent manner. At the concentration 500µg/ml *C. albicans*, *A. niger, A. flavus* and *P. notatum* showed 26.6±0.57, 27.3±0.57, and 19.3±0.57 and 21±1 mm of growth inhibition respectively. Other drugs do not easily inhibit *C. albicans*.

Table 1: Antibacterial and antifungal activity of the plant extract

S. No.	Organism	Zone of inhibition				
		Standard Chloramphenicol (25 µg)	Fluconazole (15 µg)	100 µg	250 µg	500 µg
1	*Klebsiella pneumoniae*	39±1.2	Nd	9±.057	12.3±1.2	14.3±1.5
2	*Salmonella typhi*	30.7±5	Nd	10.3±0.57	13.3±2.08	16±2
3	*Pseudomonas aeruginosa*	9.3±1.2	Nd	8±.057	9.3±0.57	13±.057
4	*Bacillus cereus*	32±4	Nd	12±.057	16.6±1.2	21.7±1.5
5	*Streptococcus pyogenes*	17.6±2.5	Nd	8.6±1.2	10.6±1.5	12.3±1.2
6	*Staphylococcus aureus*	36.6±1.2	Nd	10.6±0.57	15.6±0.57	19±2
7	*Aspergillus niger*	Nd	19±1.6	18.7±1.53	22.6±1.15	27.3±0.57
8	*Penicillium notatum*	Nd	16±1.5	14±1	17.6±0.51	21±1
9	*Candida albicans*	Nd	R	18.3±1.15	22.5±0.87	26.6±0.57
10	*Aspergillus flavus*	Nd	12.3±1.15	14.6±0.57	16.3±1.15	19.3±0.57

Nd-not determined, R-resistant.

Anthelmintic property of *B. phoenicea* bark extract

It was seen that the ethanolic extract of *B. phoenicea* bark possess dose dependent anthelmintic activity as compared to a standard drug Albendazole. The mean paralyzing time of *Pheretima posthuma* with the dose of 25, 50 and 100 mg/ml were found to be 32.5,23 and 10.8 min 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 more effective than the commercial drug in the same concentration itself. The mean death time of *Pheretima posthuma* with the dose of 25, 50 and 100 mg/ml were found to be 72.6,55 and 30 min respectively. In the case of Albendazole, no death was observed in the above helminth (table 2).

Table 2: Anthelmintic property of *Bauhinia phoenicea* bark

	Distilled water	Albendazole(25 mg/ml)	Drug (25 mg/ml)	Drug(50 mg/ml)	Drug(100 mg/ml)
Time taken for paralysis (min)	-	53.4±4.5	32.5±2.7	23±4	10.8±3
Time taken for death (min)	-	-	72.6±2.6	55±3.6	30±3.7

Antioxidant property screening of *B. phoenicea* bark

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 ethanol extract of *B. phoenicea* presented in table 3. The ethanol extract of *B. phoenicea* bark exhibited significant DPPH free radical scavenging activity with IC 50 value 90.±0.92.

Superoxide radical scavenging assay

The super oxide radical scavenging assay also shows significant radical scavenging with IC 50 value 64±0.5. The activity was increasing with the increasing concentrations of test solution (table 3).

Table 3: Antioxidant screening of *Bauhinia phoenicea* bark

S. No.	Concentration of plant extract(µg/l)	Percentage of inhibition	
		DPPH	NBT
1.	10	10.8±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	77.39±1.24
9.	750	92.3±0.83	88.04±0.38
10.	1000	94.5±1.72	94.86±0.57
IC 50		90±0.92	64±0.5

CONCLUSION

The present study reveals that, the bark of *Bauhinia phoenicea* possess prominent anti-microbial, anthelmintic and anti-oxidant properties. Phytochemical studies portray the presence of several biologically active secondary metabolites, which may be the reason for its biological properties. Therefore, there is no doubt that this plant is a reservoir of potentially useful chemical compounds that serve as drugs, provide newer leads and clues for modern drug design. This paper reports for the first time the pharmacological properties of *B. phoenicea* bark and provides scientific evidence for its traditional use.

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CONFLICT OF INTERESTS

Declared None

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