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Research Article

PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT ACTIVITY AND CYTOTOXIC EFFECT OF DIFFERENT EXTRACTS OF BARK OF BAUHINIA PHOENICEA WIGHT &ARN

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A R T I C L E I N F O

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A B S T R A C T

Aim: To determine phytoconstituents, antioxidant activity and cytotoxic evaluation of the various extracts of bark of *B. phoenicea.*

Background: Though the advances in modern medicines are significant, there remains an ever increasing demand for herbal medicines. Effective and potent herbal medicines require evaluation by standard scientific methods so as to be validated for the treatment of diseases.

Methodology: Qualitative Phytochemical analysis performed to identify various valuable primary and secondary metabolites. The antioxidant potential and cytotoxic efficacy of bark of *B. phoenicea* in various extracts were evaluated. All the analysis were done according to standard protocols

Results: The preliminary qualitative analysis of *B. phoenicea* bark indicated the presence of carbohydrates, starch, sugar, proteins and secondary metabolites like alkaloid, saponin, phenol, steroid and flavonoid. Ethanol and Chloroform bark extract of *B. phoenicea* showed maximum DPPH scavenging with percentage of inhibition of 99.34% and 91.35% at 600µg/ml. Where as in superoxide activity at 200µg/ml ethanol and chloroform extracts exhibited 94.98% and 84.95%. **Coclusion:** The findings of this study indicate that this plant is medicinal with prominent antioxidant and cytotoxic property, which may be due to secondary metabolites.

INTRODUCTION

Medicinal plants have been the subjects of man's curiosity since time immemorial (Constable, 1990). Almost every civilization has a history of medicinal plant use (Ensminger *et al.,* 1983). Medicinal plant is an important element of indigenous medical systems in all over the world. The ethno botany provides a rich resource for natural drug research and development (Farnsworth, 1990). Approximately 80% of the people in the world's developing countries rely on traditional medicine for their primary healthcare, and about 85% of traditional medicine involves the use of plant extracts (Vieira and Skorupa, 1993). Unfortunately, much of the ancient knowledge and many valuable plants are being lost at an alarming rate. Red Data Book of India has 427 entries of endangered species of which 28 are considered extinct, 124 endangered, 81 vulnerable, 100 rare and 34 insufficiently known species (Thomas, 1997).

Bauhinia phoenicea Wight & Arn is a medicinal plant endemic to Western Ghats. It is commonly known as, Vallimantharam. The genus Bauhinia consists of approximately 300 species that are widely distributed in many tropical countries where they are used frequently in traditional medicine (Cechinel, 2009).

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Several species within the genus have been shown to possess anti-diabetic, anti-inflammatory, anti-schistosomal, anti-diarrhoeal, antioxidant and antibacterial activities (Adamu *et al*., 2007; Aliyu *et al*., 2009; Hassan *et al*., 2008). Previous chemical reports on the genus Bauhinia include the isolation of terpenoids, alkaloids, steroids, triterpenes, tannin, quinines, bibenzyls and more frequently flavonoid.

Leaves and bark of *B.phoenicea* is used by the traditional practitioners for skin irritations, diabetes and worm disturbances (Baby and Raphael, 2015). Hence the aim was to investigate the phytoconstituents, the antioxidant activity and cytotoxic efficacy of different extracts of bark of *B. phoenicea.*

MATERIALS AND METHODS

Collection of plant material

The fresh bark of *Bauhinia phoenicia* were collected from the botanical garden of St. Mary's college, Thrissur and identified by the authority of M.S. Swaminathan Research Foundation Wayanadu, Kerala, India. submitted a voucher specimen in our department herbarium. The plant name checked with www.theplantlist.org.

Preparation of extracts

The bark of the plant was shade dried for several days. The dried plant material was ground to a coarse powder and 50gm

of the powdered material was soaked in solvents of increasing polarity starting petroleum ether, chloroform, ethyl acetate, ethanol and distilled water (1:5) for 72hours (Taleb-contini *et al*, 2004). The solvent was then removed by rotary evaporation. Each residue was weighed and the yield percentage was determined. The dried extract was stored in refrigerator for further studies.

Qualitative phytochemical Analysis

The preliminary phytochemical analysis of the plant extracts was performed using standard protocol, then the crude extract was fractionated according to their polarity and yield in each fraction were determined(Harborne., 1998).

Antioxidant property screening

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 1, 1-diphenyl2-picrylhydrazyl (DPPH), by a modified method (Braca *et al*., 2002). The diluted working solutions of the test extracts (50 µg/ml -600 µg/ml concentration) and 6.34 µM solution of DPPH were prepared in methanol, and 100µl of drug to be tested, 100µl DPPH solution and 800µl 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 (900µl) with DPPH solution (6.34µM,100µl) 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.
The IC_{50} (the microgram of extract to scavenge 50%of the radicals) value was calculated.

Super oxide radical scavenging assay

In-vitro super oxide radical scavenging activity was measured by NBT reduction method (Lokesh *et al*., 2009). 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 minutes. 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 -200 µg/ml) and finally the volume was made up to 3 ml using phosphate buffer and the reading was taken after 15 minutes of illumination. % 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. The IC_{50} value was also calculated.

In-vitro cytotoxicity study

Any compound, which is cytotoxic to cells, inhibits the cell proliferation and kills the cells. Trypan blue has the ability to penetrate in to the dead cells and give it blue color. This method gives an exact number of dead and viable cells (Kuttan *et al*., 1985).

The chloroform and ethanol extract was studied for short term *in vitro* cyto-toxicity using Dalton's ascites cells (DLA). Cells were aspirated from the peritoneal cavity of tumor bearing mice and it was washed three times using PBS. The viability of cells were checked using trypan blue (cell viability should be above 98%).

The cell suspension ($1x10^6$ cells in 0.1ml) was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using phosphate buffered saline (PBS). Control tubes containing only cell suspension. These assay mixtures was incubated for 3h at 37^0C and then 1ml of trypan blue was added after incubation and the number of dead cell was counted using a haemocytometer (Shrivastava and Ganesh, 2010). Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The numbers of the stained and unstained cells were counted separately.

The percentage cytotoxicity was calculated using the formula given below:

$$\% \text{ cytotoxicity} = \left[\frac{\text{No.of dead cells}}{\text{No.of viable cells+No.of dead cells}}\right] \times 100$$

RESULTS

Yeild of extract

Comparatively, distilled water extract of bark exhibited higher extraction yields (4.16%). The extraction ability of different extractable components from bark followed the order: distilled water (4.16%) > ethanol (3.57%) > chloroform (0.42%)> ethyl acetate (0.36%) > petroleum ether (0.32%). The variation in yield may be due to the polarity of the solvents used in the extraction process.

Phytochemical screening of B.phoenicea bark extract

Phytochemical screening results (Table 1) of the bark extracts in petroleum ether, chloroform, ethyl acetate, ethanol and distilled water showed the presence of phenol, alkaloid and saponin in all solvents. Terpenoid, starch and amino acids were absent in all solvents. Cardiac glycoside, flavanoid, phlobatannin, sugar, protein and ketose were present only in aqueous extract.

Table 1 Phytochemical constituents in different bark extracts of *B. phoenicea*

Primary/ Secondary Metabolites	Petroleum Ether	Chloroform	Ethyl acetate	Ethanol	Distilled water
Quinone	–	–	–	+	+
Cardiac glycoside	–	–	–	–	+
Steroid	–	–	–	+	+
Flavanoid	–	–	–	–	+
Alkaloid	+	+	+	+	+
Phenol	+	+	+	+	+
Saponin	+	+	–	+	+
Tannin	–	–	–	–	+
Coumarin	+	–	–	+	–
Terpenoid	–	–	–	–	–
Phlobatannin	–	–	–	–	–
Sugar	–	–	–	–	+
Protein	–	–	–	–	+
Ketose	–	–	–	–	+
Starch	–	–	–	–	–
Carbohydrate	+	+	–	+	+
Aminoacid	–	–	–	–	–

+ indicates the presence of the metabolite.
- indicates the absence of the metabolite

Carbohydrate present in all extracts except ethyl acetate. Quinone and steroid present in ethanol and distilled water extracts, tannin in chloroform and distilled water extracts and coumarin in petroleum ether and ethanol extracts.

DPPH free radical scavenging assay

The result of the free radical scavenging potential of the different extracts tested by DPPH assay is given in the table.2.

Table 2 Percentage Inhibition of DPPH free radical and IC_{50} Values by different bark extracts of *B.phoenicea* at 517nm Values are presented as mean ± standard deviation (n=3)

Sl. No	Concentration (µg/ml)	Percentage of inhibition of DPPH free radical				
		Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Distilled water
1.	50	3.06±0.49	7.83±0.66	6.35±0.25	54.93±0.48	5.08±0.36
2.	100	6.4±0.52	13.16±0.78	8.82±0.33	60.18±0.42	10.30±0.24
3.	150	11.51±0.52	21.35±0.29	22.91±0.26	67.34±0.37	13.8±0.37
4.	200	15.19±0.49	25.97±0.44	32.54±0.43	70.46±0.58	19.25±0.32
5.	250	21.01±0.39	39.67±0.21	34.49±0.41	74.17±0.34	21.08±0.28
6.	300	27.26±0.35	47.27±0.82	38.56±0.27	78.74±0.40	25.93±0.45
7.	350	31.17±0.44	53.42±0.72	42.53±0.38	87.13±0.40	29.27±0.24
8.	400	35.11±0.44	60.07±0.27	45.79±0.39	89.25±0.64	33.25±0.93
9.	450	37.71±0.31	77.14±0.44	50.10±0.30	91.95±0.31	38.81±0.24
10.	500	42.30±0.27	83±0.36	54.04±0.28	94.95±0.24	44.08±0.43
11.	550	45.95±0.47	87.84±0.62	57.87±0.41	97.99±0.18	50.84±0.20
12.	600	50.96±0.35	91.35±0.69	60.16±0.15	99.34±0.03	53.17±0.15
	IC_{50} (µg/ml)	591.24	318.33	449.16	21.82	542.24

Table 3 Percentage Inhibition of superoxide free radical and IC_{50} Values of bark extracts of *B.phoenicea* at 517nm Values are presented as mean ± standard deviation (n=3)

Sl. No.	Concentration(µg/ml)	Percentage of Inhibition (%)				
		Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Distilled water
1.	10	25.44±0.80	47.13±1.25	13.59±1.26	52.10±1.08	33.01±0.87
2.	50	31.55±0.50	55.74±2.65	23.59±1.34	61.02±2.33	41.09±0.88
3.	100	38.05±1.77	69.31±1.34	35.68±1.34	73.01±0.51	49.45±0.51
4.	150	44.83±1.84	76.69±1.02	43.35±2.34	86.08±1.84	59.29±1.77
5.	200	51.61±1.34	84.95±1.52	54.27±1.35	94.98±1.84	64.59±0.88
	IC_{50} (µg/ml)	187.40	22.90	179.30	8.43	119.55

Reduction of the DPPH radicals was observed by a decrease in absorbance where a change in the colour to yellow denotes quenching of the free radicals by the plant extracts. The dosage of extract is expressed in µg of dry weight of the extract per ml of the assay mixture. All the five extracts exhibited considerable DPPH radical scavenging activity. Ethanol and Chloroform bark extract of *B. phoenicea* showed maximum activity with percentage of inhibition of 99.34% and 91.35%.

IC_{50} value represents the concentration of plant extract where the inhibition of test activity reached 50%. Lower IC_{50}value indicates the high antioxidant potential and property. Lower IC_{50} value was observed for ethanol extract (21.8µg/ml).

Table 4 *In-vitro* cytotoxicity study of chloroform and ethanol bark extracts of *B.phoenicea*

Sl. No.	Drug concentration (µg/ml)	Percent cell death(DLA)	
		Chloroform	Ethanol
1	10	28	12
2	20	32	20
3	50	40	28
4	100	70	52
5	200	100	86

Superoxide radical scavenging activity

The superoxide radical scavenging activity of *B.phoeicea* extracts assayed by NBT reduction method is shown in Table 7. Five extracts of bark were found to be an efficient scavenger of superoxide radical generation. Ethanol and chloroform bark extract of *B. phoenicea* showed maximum scavenging activity with percentage of inhibition 94.98% and 84.95% at 200µg/ml. Lower IC_{50} value was observed for ethanol extract (8.4µg/ml) and highest IC_{50} for petroleum ether (187.40µg/ml).

In a short term cyto-toxicity study using Dalton's ascities (DLA) cells, chloroform and ethanol bark extracts of *B.phoenicea* displayed cyto-toxicity. The percentage of cell death at five different concentrations (µg/ml) of chloroform and ethanol bark extracts of *B.phoenicea* were given in the Table.4. Results showed that both chloroform and ethanol bark extracts of *B.phoenicea* had excellent cytotoxic activity. In 200µg of the extract, 100% cell death was observed for chloroform and 86% cell death for ethanol extract.

DISCUSSION

Medicinal plants are considered as an important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the biological and phytochemical screening of plant extracts and/or extracts from traditional preparations used in popular medicine (Alonso Paz *et al*., 1995; Sohni *et al*., 1995). Qualitative preliminary phytochemical analysis was performed initially with different chemical reagents to detect the nature of phytoconstituents and their presence in different extracts of *B.phoenicea* bark. Primary metabolites carbohydrates, starch, sugar, proteins and secondary metabolites like alkaloid, saponin, phenol, steroid and flavonoid were found to be present in *B.phoenicea* bark according to the previous investigations (Baby and Rapheal, 2014). While in present investigation, phenol, alkaloid, saponin, cardiac glycoside, flavanoid, phlobatannin, quinone, steroid, tannin, coumarin, sugar, protein, ketose and carbohydrate were present. The

phytochemical analysis has established that all the extracts contain active chemicals capable of curing ailments.

The bark of *Bauhinia phoenicea* possesses prominent anti-microbial, anthelmintic and anti-oxidant properties (Baby and Rapheal, 2014). 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.

Five different extracts of *B.phoenicea* showed antioxidant activity in both DPPH and superoxide scavenging assays. Dose dependent antioxidant activity pattern was observed in DPPH and superoxide radical scavenging assays. Ethanol and chloroform extracts showed highest percentage of inhibition in both assays. Further studies may reveal the exact mechanisms of action responsible for the antioxidant activities. This study has highlighted the ethanol extract of bark of *B.phoenicea* could be a potential new natural source for antioxidant activity. Among the different extracts studied, ethanol and chloroform extracts were found to be the best extracts, which were used for DLA cell line study. The cytotoxic effect of chloroform and ethanol extract of bark of *B. phoenicea* was determined against Dalton's ascities (DLA) cell line. Chloroform extract showed an excellent result with 100% cell death at 200µl drug concentration and a very good result also obtained for ethanol extract with 86% cell death in the same concentration.

CONCLUSION

This study has explored the various phytochemicals, including phenols and alkaloids present in the bark of *B. phoenicea*. The antioxidant efficacy indicates that this plant has great scope for isolation and identification of important antioxidant molecules. Ethanol and chloroform extract of bark of *B. phoenicea* has immense value in the prevention and treatment of deadly diseases using good cytotoxic activity. These *in vitro* results should be confirmed *in vivo*.

The species conservation status of *B. phoenicea* has now been identified as 'Vulnerable' based on IUCN 2012. This work revealed some important pharmacological properties of bark of *B. phoenicea*, which raise the importance for the conservation of *B. phoenicea*, Wight. and Arn.

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