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Research Article

ANALYSIS OF THE ANTIOXIDANT PROPERTY, CYTOTOXICITY, AND ANTI-TUMOR EFFICIENCY OF *BAUHINIA PHOENICEA*

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ABSTRACT

Objective: *Bauhinia phoenicea* Wight and Arn. is a medicinal plant endemic to Southern Western Ghats. In the traditional systems of medicine, it is using against various ailments including some oxidative disorders. Detailed studies on the pharmacological activities of this plant are not yet reported. Hence, this paper aimed to prove the efficacy of this plant as a natural antioxidant source.

Methods: The sequential extracts of the dried leaf powder were assayed for an antioxidant property using 2,2 diphenyl 1-picrylhydrazyl free radical scavenging assay, *in vitro* cytotoxicity of the extracts was screened using Trypan blue exclusion method. The antitumor activity of the selected fractions was studied using ascites tumor affected mice and noted the percentage of increase in lifespan.

Results: The free radical scavenging activity of all extracts was increasing with increasing concentration of the drug, the least IC_{50} value was showed by ethanol fraction (41 µg/ml). The plant drug was not toxic to the normal cells and was highly toxic to tumor cell lines. Maximum in vitro cytotoxicity was observed in chloroform fraction (98% cell death at 100 mg/ml) and the least IC_{50} value was exhibited by the aqueous fraction (34 mg/ml). Both the aqueous and chloroform fractions increased the lifespan of ascites tumor bearing mice, aqueous fraction in 100 mg/ml concentration shows 71.9% increase in lifespan which is near to the result showed by the commercial anticancer drug cyclophosphamide (72.5%).

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

Keywords: *Bauhinia phoenicea*, Antioxidant property, Cytotoxicity, Antitumor property.

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INTRODUCTION

Cancer is potentially fatal disease mainly caused by environmental factors which mutate genes coding critical cell regulating proteins. It causes 1 in 8 deaths all over the world and rapidly becoming a global pandemic. The aberrant cell resulted in causes dissemination of the disease and finally leads to patient death by behaving abnormally and destroys surrounding normal tissues [1]. Production of free radicals in the body beyond its antioxidant capacity has been related to a number of oxidative stress diseases including cancer [2]. Diets with plenty of fruits and vegetables are protective against oxidative stress-related diseases. It is linked to the presence of antioxidant principles which are responsible for much of their flavor and color [3].The present-day cancer treatments include chemotherapy and radiation therapy which causes several deleterious side effects. Therefore, natural therapeutics with less or no toxicity is an emerging field of research nowadays.

Bauhinia phoenicea belongs to the family Caesalpiniaceae is a liana found in evergreen forests. It is endemic to the Western Ghats. In the traditional systems of medicine, it is using against various ailments including some oxidative disorders [4]. Many species of *Bauhinia* have demonstrated antidiabetic activity [5-7]. Leaves and Bark of this plant have proved antimicrobial, antioxidant, and anthelmintic properties [8,9].

In our search for new natural sources of antioxidant agents, we have conducted an antioxidant activity screening of some traditionally important medicinal plants [8-11], from which the most active *B. phoenicea* was selected for detailed study. To the best of our knowledge, no detailed study on the pharmacological activity of this plant has been carried out.

METHODS

Collection of plant sample

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

Preparation of plant extract

The leaves were dried at 45–50°C for 2 weeks and powdered using mixer grinder. Dried powder was then extracted sequentially with petroleum ether, benzene, chloroform, acetone, ethanol, and distilled water using column chromatography. The extracts were concentrated to dryness using a rotary evaporator, and the extractive values were determined.

Cell lines

Dalton's lymphoma ascites (DLA) cell lines were procured from Amala Cancer Research Institute, Thrissur, Kerala, India. The mice were injected with a suspension of cells (1×10^6) intraperitonealy, and the cells were aspirated from the peritoneal cavity on the 15th day.

Animals

Swiss Albino mice (non-pregnant females of 6–8 weeks) were purchased from Small Animal Breeding station, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala. They were kept in well-aerated cages with controlled conditions of light and humidity for 14 days for acclimatization. The mice were fed with normal mouse chow (Sai Durga Food and Feeds, Bangalore, India) and water *ad libitum*. All experiments in the study were carried out with the prior approval of Institutional

Animal Ethics Committee and were conducted as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals constituted by the Animal Welfare Division of Government of India.

Antioxidant property screening using 2,2 diphenyl 1-picrylhydrazyl (DPPH) free radical scavenging assay

The potential of the *B. phoenicea* to scavenge the free radicals generated was screened according to the method described by Braca *et al.* [12].DPPH free radicals give a strong absorption maximum at 515 nm and are purple in color. As the odd electrons of DPPH paired with hydrogen from the antioxidant compound to form the reduced compound DPPH-H, the color of the solution turns from purple to yellow.

The diluted working solutions of the test extracts (10–200 µg/ml concentration) with DPPH solution were kept in the dark for 20 min. 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 percentage 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.

Acute toxicity study

Acute toxicity assay was performed in healthy adult non-pregnant female Swiss albino mice (25–28 g b.wt). The mice were divided into two groups of three each and treated with 250 mg/kg drug intraperitoneally. The control group received 2% carboxymethyl cellulose suspension at the same volume.

***In vitro* cytotoxicity screening**

Short-term cytotoxic activity of *B. phoenicea* extract was assayed by determining the percentage of viability of the DLA cells using Trypan blue exclusion methods [13].

The cells were aspirated from the peritoneal cavity of tumor bearing mice. The collected cells were washed using phosphate buffered saline (PBS) and checked for their viability. Different dilutions of the cells were made (10^{-1}, 10^{-2}, and 10^{-3}). The number of cells in the 10^{-3} dilution was counted using hemocytometer, and the cell number was adjusted to 1×10^{7} cells/ml. This cell suspension was added to tubes containing various concentrations of the test in 1 ml PBS and the tubes are incubated at 37°C for 3 h. 100 µl of Trypan blue was added after the incubation period, and the percentage of viability was determined.

Determination of the anticancer effect of *B. phoenicea* on ascites tumor bearing animals

Ascites tumor was induced by injecting DLA cells (1×10^{6} cells/animal) in the peritoneal cavity of Swiss albino mice. 36 animals get divided into six groups; each group consist of 6 animals. Group I was maintained as a negative control (not treated with any drug). Group II–V received 50 and 100 mg/kg b.wt of aqueous and chloroform extracts of *B. phoenicea,* respectively. Animals in the Group VI received cyclophosphamide (10 mg/kg b.wt). The drugs were given intraperitoneally 24 h after the tumor implantation as 5 doses on alternate days. The death of the animals due to tumor burden was noted every day, and the percentage of increase in lifespan (% ILS) was calculated using the formula (T-C/C)×100, where "T" and "C" are the mean survival days of treated and control animals [14].

RESULTS

Extractive values

The yield of the extracts was found to be 3% w/w (petroleum ether), 5.05% w/w (benzene), 4.5% w/w (chloroform), 6% w/w (ethanol), 1.08% w/w (acetone), and 16.06% w/w (aqueous).

Antioxidant property screening using DPPH free radical scavenging assay

The effect of antioxidants present in the plant extract on DPPH free radical scavenging was thought to be due to their hydrogen donating ability. The free radical scavenging effect of *B. phoenicea* increased with an increasing concentration of the extract (Fig. 1). The IC_{50} values of the extracts presented in Table 1.

Acute toxicity study

In the toxicity test dose of 250 mg/kg b.wt, the mice did not cause mortality or any signs of toxicity or change in general behavior during the 14 days of observation.

***In vitro* cytotoxicity analysis**

Both polar and non-polar extracts of *B. phoenicea* found to be cytotoxic toward DLA cells (Fig. 2). Maximum cytotoxicity (98%) was attained at a concentration of 200 µg/ml of chloroform extract. Least IC_{50} value was showed by aqueous extract (Table 1) so chloroform and aqueous extracts were selected for anticancer screening.

Effect of *B. phoenicea* extracts on ascites tumor development

Animals of the ascites tumor control group survived only for a period of 16±2 days. Treatment of *B. phoenicea* chloroform and aqueous extracts at different concentrations increased the survival rate of animals (Table 2).

DISCUSSION

Medicinal plants are nature's gift to human beings to lead a healthful, disease-free life. Most of these plants used today are believed to be much safer and proved an elixir in the treatment of various ailments. Plant-derived compounds have played an important role in the development of several clinical useful anticancer agents [15]. Oxidative stress induced by an imbalance between production of reactive oxygen species and antioxidants has associated with pathogenic disease conditions like carcinogenesis [16].Hence, radical

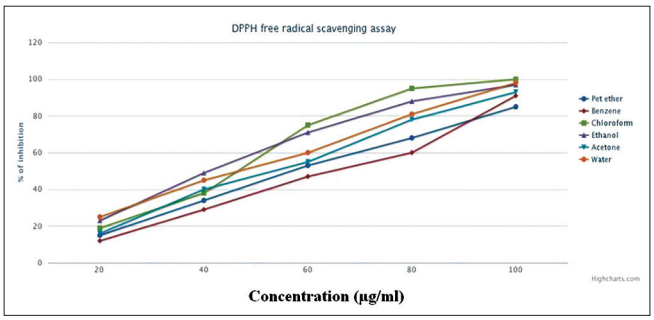

Fig. 1: 2,2 diphenyl 1-picrylhydrazyl free radical scavenging assay of *Bauhinia phoenicea*

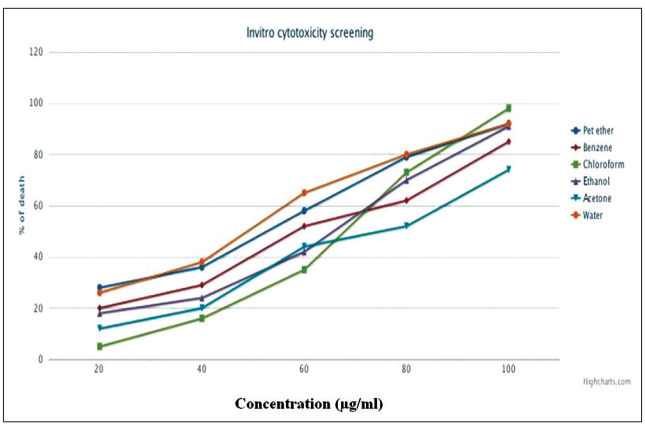

Fig. 2: *In vitro* cytotoxicity screening of *Bauhinia phoenicea*

Table 1: DPPH free radical scavenging assay and *in vitro* cytotoxicity screening using Trypan blue exclusion method

Sl No.	*B. phoenicea* extract	IC_{50} value (µg/ml)	
		DPPH radical scavenging assay	*In vitro* cytotoxicity screening
1.	Petroleum ether	57	40
2.	Benzene	65	48
3.	Chloroform	46	70
4.	Ethanol	41	64
5.	Acetone	53	85
6.	Aqueous extract	46	34

DPPH: 2,2 diphenyl 1-picrylhydrazyl, *B. phoenicea*: *Bauhinia phoenicea*

Table 2: Screening of the anticancer efficiency of *B. phoenicea* using ascites tumor affected mice models

Sl No.	Treatment	Number of days survived	% increase in lifespan
1.	DLA cells alone	16±2	-
2.	DLA+*B. phoenicea* chloroform extract (50 mg/kg b.wt)	20.6±1.5	28.75
3.	DLA+*B. phoenicea* chloroform extract (100 mg/kg b.wt)	26.6±2.80	66.25
4.	DLA+*B. phoenicea* aqueous extract (50 mg/kg b.wt)	22.8±2.13	42.5
5.	DLA+*B. phoenicea* aqueous extract (100 mg/kg b.wt)	27.5±2	71.9
6.	DLA+Cyclophosphamide (10 mg/kg)	27.6±2	72.5

B. phoenicea: Bauhinia phoenicea, DLA: Dalton's lymphoma ascites

scavenging activity is very important, in the searching of natural sources of cancer drugs.

Cytotoxicity is one of the chemotherapeutic targets of antitumor drugs. Most of the clinically proved antitumor agents possess significant cytotoxic activity in cell culture systems. The cytotoxic activity of *B. phoenicea* leaf extracts against DLA cell lines partially explains its significant antitumor activity. The drug shows toxicity toward the tumor cell line and not toxic to normal cells.

The anticancer activity was evaluated using the ascites tumor model. Both chloroform and aqueous extracts of *B. phoenicea* increased the lifespan of affected mice effectively. The highest activity was observed in aqueous extract.

The result of the DPPH free radical scavenging assay shows the potential of *B. phoenicea* as an antioxidant agent, efficiency in the *in vitro* cytotoxicity screening, and as an antitumor agent proves that it can act as a source in the preparation of anticancer drugs. The presence of various secondary metabolites [17] provides some scientific evidence for the biological activities and also account for the pharmacological uses. The present data would certainly help to understand the potency of the plant for medicinal use.

CONCLUSION

The results of the present study reveal that such as vinca alkaloids, podophyllotoxins, and camptothecins the proved natural compounds for cancer treatment *B. phoenicea* will also contribute to cancer treatments in future. Till now, this plant is considering as a disturbance for forest plants, our paper first reporting the anti-cancerous property of this plant. Further investigations are in progress to identify and isolate the active components of this plant.

AUTHORS' CONTRIBUTIONS

Alby Alphons Baby has performed all the experiments in the laboratory. Regi Rahael K has provided the design, intellectual content to choose the plant and acts as a mentor for the works.

CONFLICTS OF INTEREST

The authors are declaring that there are no conflicts of interest regarding the publication of this article.

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