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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTHELMINTIC ACTIVITY OF *BAUHINIA PURPUREA* L

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ABSTRACT

The objective of present work was to evaluate the anthelmintic activity of methanol and aqueous extract of flower of *Bauhinia purpurea L.* using Indian earthworms *Pheretimaposthuma* as test worm. Various concentrations of different extracts were tested in the bioassay, which involved determination of paralysis time and death time of the worm. Piperazine citrate (30mg/ml) was used as a reference standard. The result of present study indicates that the ethyl acetate and methanol extract significantly demonstrated paralysis, and also death of worms. Preliminary phytochemical tests revealed the presence of phytoconstituents such as steroids, tannins, glycosides, alkaloids, carbohydrates, flavonoids, saponins, and phenolic compounds in the aqueous and methanol extract of *B. purpurea*.

KEYWORDS: *Bauhinia purpurea*, phytoconstituents and anthelmintic activity.

INTRODUCTION

Natural products obtained from the plant resources have been the major supplements to combat many serious diseases in the developing countries.[1] With the advancement of modern medicinal systems, herbal medicines have always maintained their popularity in treatment of diseases due to their easy availability and safe effectiveness. The capacity of herbs to produce innumerable bioactive secondary metabolites has increased their usage in healing systems.

Bauhinia genus of family Cesalphiniacea consists of about 15 species that occur in India. Some of them are shrubs or trees, while a few are climbers.[2] India is a developing country which is valued for diversity of species, genetic, habitat. In developing countries like India, flowers are used in all cultural activities at all time.[3] Flowers are broadly known and used for their beauty as well as the color they radiate[4]. Medicinal plants find its application in the treatment of diseases since the dawn of world in the form of traditional medicine.

The different species of *Bauhinia viz., B. reticulata, B. rufescens and B. variegata* have been traditionally used to treat roundworm infections, conjunctivitis, anthrax, ulcerations, dysentery, blood-poisoning, leprosy, lung and skin diseases in Africa; while in India, extracts of the bark of *B. variegata* is used for treatment of cancer. Leaves are used as a plate for food and fodder during lean period[5], bark used as fibre, in dyeing and tannin extraction and its decoction is used as anthelmintic and in diarrhoea. The decoction of root is used for expelling gases, flatulence and gripping pain from the stomach and bowels. The decoction of flower works as a maturant for boils and abscesses. It has been reported that the pharmacological significance was noted due to the presence of various bioactive compounds in the *Bauhinia* species such as flavonoids glycosides, saponnins and tannins, flavonoids and glycosides which are secondary metabolites.[6, 7] Since, the flowers are rich in phytochemicals, it was observed as a principle source in pharmaceutical and nutraceutical industries.

The present study was designed to investigate and evaluate the pharmacological basis for the use of *B.purpurea* in the folk medicine to expel the worms.

MATERIALS AND METHODS

Bauhinia purpurea

Plant collection and authentication: Plant materials are collected from medicinal garden of Venkateshwara Institute of Pharmaceutical sciences, located at charlapally Nalgonda District,

Telangana State, during the month of November-December, 2015. The plant was authenticated by N. Siddulu, Head of the department, Botany, Nagarjuna Government College, Nalgonda, Telangana State, India.

Extraction of plant material: The powdered flower material of *Bauhinia purpurea* L was subjected to successive solvent extraction with Methanol. 10gm of powdered flower material was subjected to Soxhlet extraction for about 10 hours with 250 ml of the Methanol solvent. The extracts obtained were later kept for distillation to remove the excessive solvent. These extracts were mixed and dried. The aqueous extract was fractioned by using different solvents like ethyl acetate and hexane and these extracts were stored in a cool and dry place.[8]

Phytochemical analysis: The extract was tested for the presence of bioactive compounds by adopting standard procedures.[9, 10]

Detection of Alkaloids: Small portions of solvent free chloroform, alcohol & water extracts were stirred separately with a few drops of dilute hydrochloric acids and filtered. The filtrate was tested with various alkaloid reagents.

Wagner's test: The filtrates were treated with potassium iodide (Wagner's reagent) and the formation of reddish brown precipitate indicates the presence of alkaloids.

Mayer's test: The filtrates were treated with Potassium mercuric iodide (Mayer's reagent) and the formation of white colored precipitate indicates the presence of alkaloids.

Dragendorff's test: The filtrates were treated with Potassium bismuth iodide (Dragendorff's reagent) and the formation of orange colored precipitate indicates the presence of alkaloids.

Hager's test: The filtrates were treated with saturated solution of Picric acid (Hager's reagent) and the formation of yellow precipitate indicates the presence of alkaloids.

Detection of Glycosides: Small quantity of alcohol and aqueous extracts were dissolved separately in distilled water and filtered. The filtrate was subjected to various tests to detect the presence of different glycosides.

Brontrager's test: The filtrates were treated with sodium picrate. The formation of yellow or orange colour shows the presence of glycosides.

Legals test: The filtrate was treated with pyridine and sodium nitropruside. The formation of pink to red colour shows the presence of glycosides.

Detection of carbohydrates: Small quantity of alcohol and aqueous extracts were dissolved separately in distilled water and filtered. The filtrate was subjected to various tests to detect the presence of different carbohydrates.

Molisch's test: The filtrates were treated with solution of -Naphthol in alcohol (Molisch reagent) and a few drops of conc. sulphuric acid was added through the sides of the test tube.

The formation of violet ring at the junction of the liquids indicates the presence of carbohydrates.

Fehling's test: The filtrates were treated with a few ml of dilute hydrochloric acid and heated on a water bath for 30 minutes. After hydrolysis the solutions were neutralized with sodium hydroxide solution. To the neutralized solutions, equal quantities of Fehling's A & Fehling's B solutions were added and heated on a water bath for a few minutes. Formation of red-orange precipitate indicates the presence of reducing sugars.

Detection of phenolic compounds and tannins: Small quantities of alcohol and aqueous extracts were diluted separately in water and were tested for the presence of phenolic compounds and tannins.

Ferric chloride test: To the test solutions, a few drops of 5% ferric chloride solution were added. Formation of a blue-black or green-black color indicates the presence of phenolic compounds and tannins.

Potassium dichromate test: To the extract added potassium dichromate solution. Positive result was confirmed by the formation of brown precipitate.

Gelatin test: To the extract added 1% gelatin solution containing 10% sodium chloride. Formation of white precipitate showed positive result.

Detection of Flavonoids

Shinoda test: To the extract added magnesium turnings, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicated positive result.

Zinc hydrochloride test: To the extract added zinc dust, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicated positive result.

Alkaline reagent test: To the test solutions a few drops of sodium hydroxide solution was added. Formation of an intense yellow color that turns less intense on addition of acid indicates the presence of flavonoids.

Test with lead acetate: To the test solution lead acetate solution was added. The formation of yellow precipitate shows the presence of flavonoids.

Detection of triterpenoids: Small quantities of alcohol and aqueous extracts were diluted separately in water and tested for the presence of triterpenoids.

Liebermann-Burchard's test: The ethereal residues were treated with a few drops of acetic anhydride, boiled and cooled. 1 ml of sulphuric acid was added through the sides of the test tube. Formation of a brown ring at the junction of two liquids and green color in the upper layer indicate presence of triterpenoids.

Libermann's test: The filtrate was treated with acetic anhydrine which is heated and then cooled and added hydrogen sulphate to it. The formation of blue colour indicates the presence of triterpinoids.

Detection of proteins: Small quantities of alcohol and aqueous extracts were diluted separately in water and tested for the presence of proteins and free amino acids by subjecting the extracts to various tests.

Ninhydrin test: To the test solutions, a few drops of Ninhydrin solution were added. Formation of a bluish color indicates the presence of amino acids.

Millon's test: To the test solution, few drops of million's reagent was added. The formation of white precipitate which turns to red upon heating indicates the presence of proteins.

Anti-helmintic activity

Worm collection and authentication: The anthelmintic activity was evaluated on adult Indian earthworm Pheretimaposthuma (Annelida). It resembles anatomically and physiologically with the intestinal round worm parasite of human being. Indian earthworms

were obtained from vermiculture area and were identified at NG collge Nalgonda by Ms.J.Neraja, Head of the zoolgy department.

Anti-helmintic Assay: The Anthelmintic activity was carried out as per the method of Mathew *et al.*, and Dash *et al.*, was followed for the screening.[11, 12] Five groups of approximately equal size Indian earthworms consisting of six earthworms in each group were released in 10 ml of desired formulation. Each group was treated with one of the following: Vehicle (1% gum acacia in normal saline). Piperazine citrate (30 mg/ml), methanolic extract (50, 60, 70, 80, 90 & 100 mg/ml) and aqueous extract (10, 30, 50, 70 90 &100 mg/ml) in normal saline containing 1% gum acacia. Observations were made for the paralysis time (PT) and subsequently for death time (DT). The time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50° C).

Statistical Analysis: The data are expressed as Mean ± SEM and analysed by using one way analysis of variance (ANOVA), followed by post hoc Sheffe's test using SPSS computer software version 10. The values were considered significant when $p< 0.05$.[13]

RESULTS AND DISCUSSION

Phytochemical analysis

Preliminary phytochemical analysis of MeOH and aqueous extracts of flower of *Bauhinia purpurea* L. revealed the presence of carbohydrates, glycosides, saponins, phytosterols, triterpenoids, tannin & phenolic compounds, alkaloids, amino acids and fixed oils. The results of phytochemical analysis were shown in Table.No.1.

Anti-helmintic activity

The extracts of flower of *Bauhinia purpurea* L. exhibits moderate to significant anthelmintic activity at the dose of 50-250 µg/ml. The results of phytochemical analysis were shown in Table.No.2. All the extracts were tested for anti-helmintic activity, piperazine citrate was employed as reference standard. It has been observed that all the tested extracts showed mild to moderate anti-helmintic activity. Extracts *EtOAc E* and *MeOH E* extract of flower of *Bauhinia purpurea*L was found to be most active agents among the extracts. Also aqueous extract of flower of *Bauhinia purpurea* L. was showing good antihelmintic activity.

Table No.1: Phytochemical Screening of extracts of *Bauhinia purpurea. L*

Test	Aqueous extract	Methanolic extract
Test for Alkaloids	++	++
Test for amino acid & proteins	+	+
Test for carbohydrates	+++	++
Test for flavonoid	+++	+++
Test for saponin	++	+
Test for glycoside	+++	++
Test for steroid & sterol	+++	+++
Test for terpenoids	+	++
Test for tannins & phenolic compound	+++	+++
Test for volatile oil	+++	++

"+ Slight changes, ++ moderate, +++ stronger reactions,"

Table No.2: Antihelmintic activity of extracts of *Bauhinia purpurea.L*

S. No	Extract	Time taken for paralysis (min)				Time taken for death (min)			
		Dose (µg/ml)				Dose (µg/ml)			
		50	75	125	250	50	75	125	250
1	**AQE**	4.66± 0.81	3.3±1.03	2.5± 0.54	0.52± 0.34	65±0.89	57.5±1.04	42.5±1.64	14±0.89
2	**MeOH E**	3.5 ±1.04	3.0±0.63	2.33±.516	1.02±1.75	60.83±0.75	52.33±0.816	37±1.41	9.5±0.83
3	**EtOAc E**	4.88±0.75	2.5±0.54	2.6±1.03	0.49±0.27	58.33±0.81	53.55±0.75	28.66±1.03	7.33±1.03
4	**Hex E**	5.8 ± 0.75	2.66±0.516	2.33±0.516	0.82±0.816	62.5±1.04	59.6±1.21	50.66±0.86	47±0.89
5	**Piperazine citrate**	3.55±0.56	2.1±0.59	1.9±0.48	0.32±1.03	48.2±0.59	50.05±1.08	24.55±.52	5.22±1.1

Results expressed as Mean + SEM

CONCLUSION

*Bauhinia purpurea*L is a plant that has shown potential as a source of chemotherapeutic compounds. The present study, therefore investigate the phytochemical constituents of extracts of flower of *Bauhinia purpurea*L by extraction. The results obtained in the present study clearly indicate that the both aqueous and methanolic extract of *Bauhinia purpurea*L are having potent phytochemicals. From the investigational reports indicate that the antihelmintic activity of ethyl acetate and methanolic extracts of *Bauhinia purpurea*L showed significant action towards Indian earth worms. Further research is needed to fractionate the

ethylacetate, methanolic extracts and isolate the molecule(s) responsible for biological activity.

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REFERENCES

1. Shihabudeen MS, Priscilla HHD, Thirumurugan. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. International Journal of Pharma. Sciences and Research, 2010; 1(10): 430-434.
2. Krishnamoorthy A, Manjunath BN, Sastri SB, Deshaprabhu YR. The wealth of India – a Dictionary of Indian Raw materials & Industrial products, first supplement series, Raw materias, New Delhi; NISCAIR, 2004; 1: 119.
3. Jeeva S, Jhonsons M, Aparna JS, Irudayaraj V. Preliminary phytochemical and antibacterial studies on selected medicinal plants, International journal of medicinal and aromatic plants, 2011; 1: 107-114.
4. Joselin J, Thankappan S, Brintha S, Florence AR, Jeeva S. Phytochemical evaluation of Bignoniaceae flowers, Journal of chemical and Pharmaceutical Research, 2013; 5: 106-111.
5. LK Jha. Advances in Agroforestry. APH Publishing Corporations, New Delhi; India, 1995; 669.
6. Kuo YH, Chu PH, et al. Chemical studies of the bark of Bauhinia purpurea. Chemical and Pharmaceutical Bulletin Tokyo; 1998; 46(10): 1630-1631.
7. Kumar A, Datta SK. Anatomy and pharmacognostic evaluation of bark of Bauhinia purpurea. Journal of Plant Anatomy and Morphology; 1985; 2(1): 49-54.
8. Sukdev Swami Handa, Sumanpreet Singh Khanuja, Gennaro Longo, DevDutt Rakesh. Extraction Technologies from Medicinal and aromatic plants, International center for science and high technology; 2008.
9. Harborne JB, Methods of extraction and isolation in Phytochemical Methods. London; Chapman & Hall, 1998; 60-66.
10. Kokate CK, Practical pharmacognosy, 1”ed., New Delhi; VallabhPrakashan: 1986; 111.
11. Dash GK, Mishra B, Panda A, Parto CP, Ganapati S. Indian J. Nat. Prod; 2003; 19(3): 24.

12. Dash GK, Suresh P, Sahu SK, Kar DM, Ganapati S. J.Nat. Remed, 2002; 2(2): 182.
13. RD Vidhyarthi. A textbook of zoology, 14th edition, New Delhi; S.Chand and Co, 1977; 329.