Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

ISSN 2278-4136
ISSN 2349-8234
JPP 2014; 3 (1): 8-13
Received: 27-03-2014
Accepted: 10-04-2014

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Phytochemical screening, HPTLC studies and screening of antioxidant activity of extracts of leaves of *Spathodea campanulata.*

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ABSTRACT
Objective: Ayurveda is one of the oldest traditional system of medicine practiced widely in India. Toxicity and side effects of allopathic medicines, has led to sudden increase in the number of herbal drug manufactures. The present study aims at phytochemical screening of methanolic and aqueous extracts of leaves of *Spathodea campanulata* Family Bignoniaceae. The powdered dried leaves were initially subjected to determination of physical constants. They were extracted with water and methanol. **Results:** Extracts were subjected to various chemical tests and showed presence of steroids, flavonoids, saponins. Further investigation of the extracts by HPTLC is an attempt to deduce the varied composition of methanolic and aqueous extract of *Spathodea campanulata*. The antioxidant activity was studied by DPPH assay. Both the extracts showed good antioxidant activity. **Conclusion:** The extracts showed presence of important phytoconstituents as revealed by the chemical tests and HPTLC chromatograms. Further investigations can be carried out to make formulations of these extracts to treat various disease conditions.

Keywords: *Spathodea campanulata*, Physical constants, Phytoconstituents, HPTLC, Antioxidant activity.

1. Introduction

During the past decade, the therapeutic use of herbal medicine is gaining considerable momentum in the world. The use of herbal medicine due to toxicity and side effects of allopathic medicines, has led to sudden increase in the number of herbal drug manufacturers. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity [1]. The practice continues today because of its biomedical benefits and has made a great contribution towards maintaining human health. The use of plant derived natural compounds used as alternative sources of medicine continues to play major roles in the general wellness of people all over the world. The curative properties of medicinal plants are due to the presence of various complex chemical substances of different composition which occur as secondary metabolites [2]. Natural products have contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant medicinal activities. The major merits of herbal medicine seem to be their perceived efficacy. Herbal medicines have been widely used and form an integral part of primary health care of many countries and may constitute a reservoir of new antimicrobial substances to be discovered [3]. Similarly many disease conditions are a result of excessive stress and require an individual to be on antioxidant therapy. Antioxidants are abundantly found in a number of trees and in their various plants. In the present study the plant material selected was leaves of *Spathodea campanulata* commonly known as a Rudra palash belonging to family Bignoniaceae. *Spathodea campanulata* L. is native of tropical Africa, with orange scarlet bell shaped flowers, three by two and half inch large, may be seen in full flowering in the month of November. The flower part is most colourful & consists of maximum amount of chromophores responsible for the sunscreen activity. The plant was previously reported to have anti-hyperglycemic, anti-malarial, as well as wound healing activity [4-9]. The present study thus aims to establish a relation between the phytoconstituents present in the leaves and its various pharmacological activities. Techniques like HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time [10].

2. Materials and Methods

2.1 Plant material

The plant specimens for the proposed study were collected from local Spathodea trees growing in Pune and were subjected to authentication to Botanical Survey of India. The specimen was authenticated by Dr. J. Jayanthi.

2.2 Preparation and Extraction of Plant Material

The dried leaves of *Spathodea campanulata* were first powdered coarsely in a grinder and defatted with Petroleum ether (60-80 °C). 100 g of defatted powder was packed in a Soxhlet apparatus and extracted with methanol. Similarly for aqueous extract 100 g of dried powdered drug was defatted using Petroleum ether (60-80 °C) and then extracted with distilled water. The extraction was carried out until the extractive becomes colorless. The methanolic extract was filtered and evaporated under reduced pressure using Rotary vacuum Evaporator. The aqueous extract was filtered, evaporated and dried.

2.3 Phytochemical Screening

The phytochemical investigation of the methanolic and aqueous extracts of leaves of *Spathodea campanulata* was carried out using standard protocol [11]. The extracts were finally weighed. The phytochemical tests were performed on the liquid and dried extracts using standard methods and the physical constants were evaluated. The results are stated in table 1.

2.4 HPTLC Profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method of Harborne [12] and Wagner [13] *et al.*

2.4.1 Sample Preparation

Each extract residue was re-dissolved in 1ml of chromatographic grade methanol and water which was used for sample application on pre-coated silica gel 60 F 254 aluminium sheets.

2.5 Developing Solvent System

A number of solvent systems were tried, for extracts, but the satisfactory resolution was obtained in the solvent n Hexane: Ethyl acetate (7:3) for methanolic extract of *Spathodea campanulata*. The solvent system selected for the aqueous extract was Ethyl acetate: N Butanol (6:4).

2.5.1 Sample Application

Application of bands of each extract was carried out (4mm in length and 1 µl in concentration) using spray technique. Samples were applied in duplicate on pre-coated silica gel 60 F254 aluminium sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

2.6 Development of Chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10 x 10 cm saturated with and n Hexane: Ethyl Acetate in the ratio of 7:3 for methanolic extract and Ethyl acetate: n Butanol in the ratio of 6:4 for aqueous extract.

2.6.1 Detection of Spots

The air-dried plates were viewed in ultraviolet radiation to mid-day light. The chromatograms were scanned by densitometer at 450 nm for both the extracts. The R_f values and finger print data were recorded by WIN CATS software.

2.7 Antioxidant activity by DPPH assay [14, 15]

2.7.1 Preparation of DPPH solution

3.3 mg of DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) was dissolved in 100 ml Methanol.

2.7.2 Preparation of standard Ascorbic acid solution

Ascorbic acid stock solution of concentration 100 µg/ml was prepared in distilled water and further dilutions were made to obtain 10, 20, 40, 60, 80 µg/ml of ascorbic acid.

2.7.3 Preparation of extract concentrations.

Extract was weighed and dilutions were made using distilled water for aqueous extract and methanol for methanolic extract to yield concentrations ranging from 10-80 µg/ml.

2.7.4 Preparation for sample

1 ml extract solution was mixed with 1 ml DPPH in Methanolic solution and vortexed thoroughly. The resulting mixture was kept in dark for 30 minutes and absorbance was noted at 517 nm. % inhibition was measured using the formula. Similarly absorbance for standard which was Ascorbic acid was taken and concentration that shows 50% inhibition was noted. The control absorbance was noted using DPPH in methanol as control.

% inhibition = (Absorbance of control – Absorbance of Sample) / Absorbance of Control

3. Results and Discussion

3.1 Physical constants.

The proximate analysis showed satisfactory result with respect to foreign matter, moisture content, Ash value and Extractive values. The water soluble extractive value is 3.73% and methanolic extractive value is 2.09%. The physical constants are given in table 1.

Table 1: Evaluation of Physical constants of powdered leaves of *Spathodea campanulata.*

S.no.	Evaluation Parameter	Value (%)			Mean ± Std Deviation (n=3)
1	Foreign Matter	1	1.05	1.1	1.050±0.005
2	Moisture Content	10.6	10.8	10.3	10.567±0.252
3	Total Ash Value	31	31.2	31.9	31.367±0.473
4	Water Soluble Ash Value	6.45	6.99	6.3	6.580±0.363
5	Acid Insoluble Ash Value	4	4.5	4.9	4.467±0.451
6	Water Soluble Extractive Value	3.4	4.1	3.7	3.73±0.351
7	Chloroform Soluble Extractive Value	0.1	0.15	0.14	0.130±0.026
8	Methanol Soluble Extractive Value	5.9	0.18	0.2	2.093±3.297
9	Ethanol Soluble Extractive Value	2.3	1.3	2.5	2.03±0.6429

3.2 Phytochemical Screening

The methanolic and aqueous extracts of leaves of *Spathodea campanulata* showed presence of carbohydrates, tannins, steroids and flavonoids [16]. The results are reported in Table 2. The presence of steroids and flavonoids may contribute to the antibacterial and wound healing activities.

Table 2: Phytochemical screening of Methanolic and Aqueous extracts of *Spathodea campanulata.*

Phytochemical constituents		Methanolic Extract	Aqueous Extract
Alkaloids	Mayer's reagent	-	-
	Dragendorff's reagent	-	-
	Hager's reagent	-	-
	Wagner's reagent	+	-
Phenolic compounds	Ferric chloride test	+	+
	Lead acetate test	+	+
Carbohydrates	Molish's reagent	+	+
	Barfoed's test	+	+
	Fehling's test	+	+
	Benedict's test	+	+
Flavonoids	Lead acetate test	+	+
	Ferric chloride test	+	+
	Sodium Hydroxide test	+	+
	Shinoda test	+	+
Steroids	Liebermann-Burchard test	+	+
	Salkowski reaction	+	+
	Liebermann's test	+	+
Saponins	Foam test	-	-
Tannins	Ferric chloride test	+	+
	Lead acetate test	+	+
	Potassium Dichromate	+	+
	Dilute Potassium Permanganate	+	+
Cardiac Glycosides	Keller- Kiliani test	+	-
	Legal's test	+	-
Anthraquinone Glycosides.	Borntrager's test	-	-
Saponin Glycosides	Foam Test	-	-
	Hemolytic test	-	-
Carbohydrates	Molisch's test	+	+
	Fehlings test	+	+
	Benedict's test	+	+
Proteins	Biuret's test	-	-
	Millon's test	-	+

3.3 HPTLC studies

3.3.1 HPTLC studies of methanolic extract of *Spathodea campanulate*

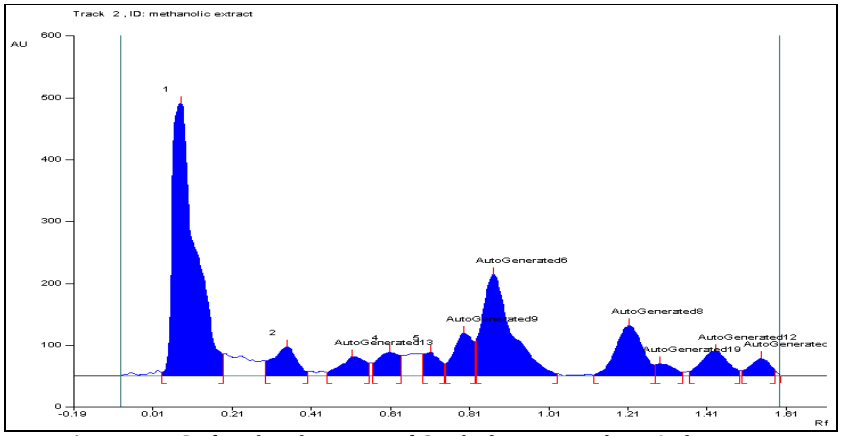


Fig 1: HPTLC of methanolic extract of *Spathodea campanulata.*10 µl injection.

The resolution was obtained at 450 nm. Mobile Phase was n Hexane: Ethyl acetate (7:3) [17-20]. Resolution was obtained into 08 peaks. The peak numbered 6 showed good area under the curve of more than 200 at R_f value 0.91. The volume of injection for the present study was 10 µl.

Same extract was tried with sample volume 20 µl keeping the mobile phase same. Resolution was obtained into 09 peaks with maximum at peak 6 at the R_f value of 0.91 which was very similar to the results obtained when the sample volume was 10µl. Mobile Phase: n Hexane: Ethyl acetate (7:3)

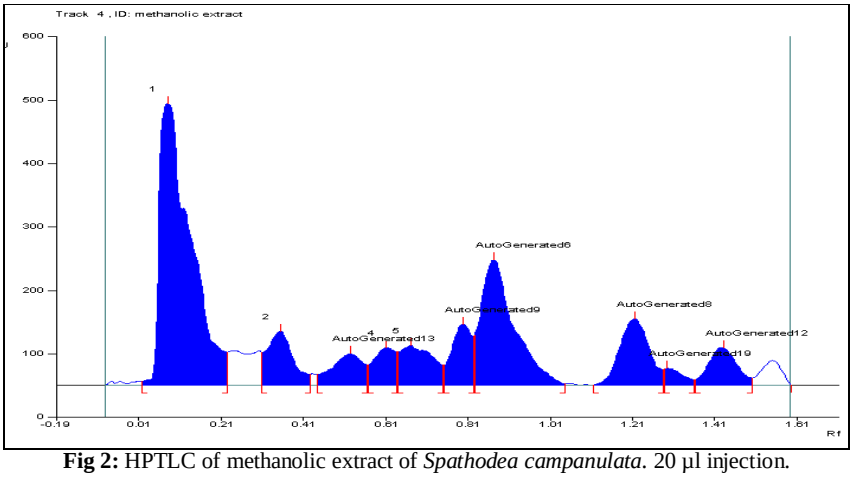


Fig 2: HPTLC of methanolic extract of *Spathodea campanulata*. 20 µl injection.

3.3.2 HPTLC studies of aqueous extract of *Spathodea campanulata*

For the aqueous extracts 10 µl was injected first but there was no elution obtained.

Mobile Phase was Ethyl acetate: N Butanol (6:4) Aqueous extracts of Spathodea showed good resolution at sample volume 20 µl at 450 nm. All four peaks obtained after the injection of 20 µl sample were obtained at 0.81, 1.21, 1.41 and 1.51 had areas of 120 for first two peaks and 100 and 80 for the rest two.

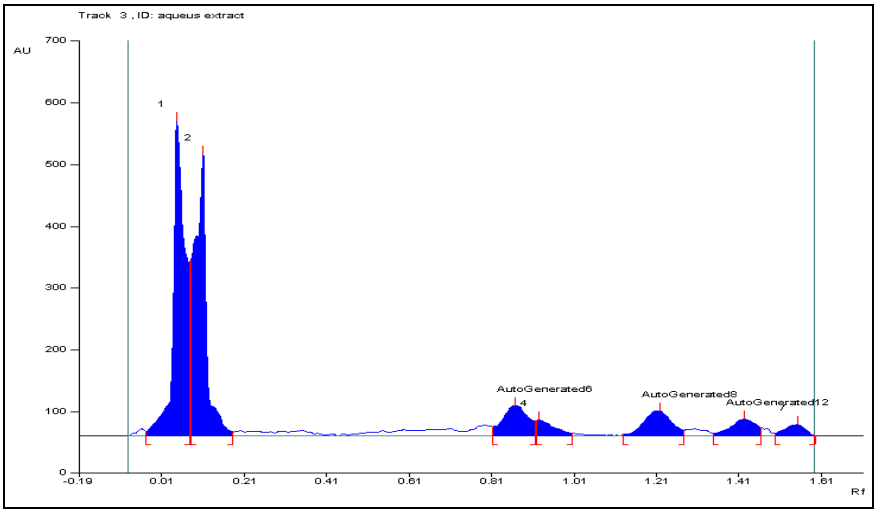


Fig 3: HPTLC of aqueous extract of *Spathodea campanulata*.

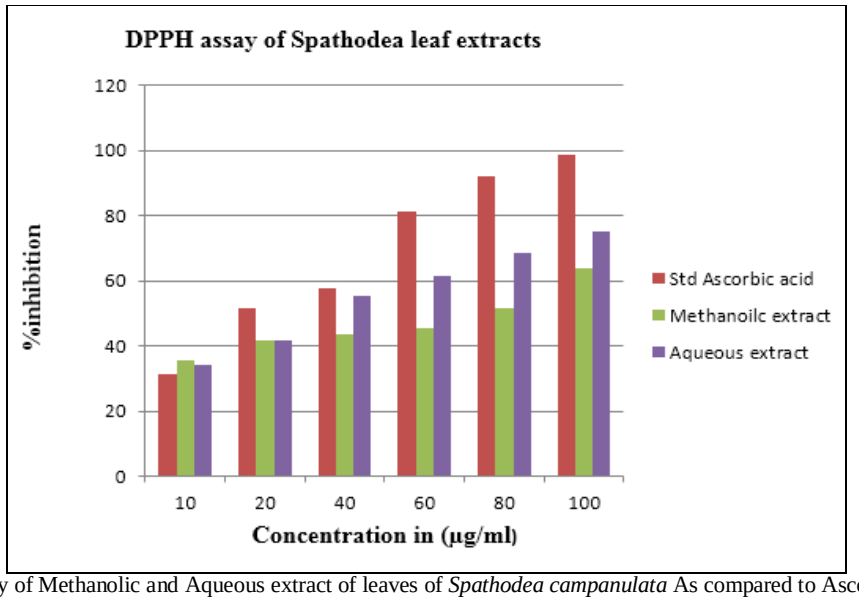
3.4 Antioxidant activity

The activity was found by performing the Free radical scavenging activity of the methanolic and aqueous extracts using DPPH Assay [21] 50% Inhibitory concentration (IC_{50}) for Ascorbic acid was found to be 15 µg/ml. The reading for control was found to be 0.6575. The % inhibitory concentrations are as given in Table no.3.

From the table it is seen that at 40 (µg/ml) concentration the aqueous extract of Spathodea campanulata shows 50% inhibition. While the Methanolic extract shows IC_{50} at 80 (µg/ml). Better antioxidant activity was seen in aqueous extract. This can be attributed to an antioxidant principle soluble in water present in the aqueous extract.

Table 3: DPPH Assay of Methanolic and Aqueous extract of leaves of *Spathodea campanulata*

Concentration in (µg/ml)	% Inhibition of free radical.	
	Methanolic Extract	Aqueous extract
10	35.73±0.214	34.20±0.721
20	41.80±0.267	41.52±0.285
40	43.47±0.155	55.38±0.329
60	45.60±0.359	61.62±0.429
80	51.73±0.666	68.33±0.812
100	63.77±0.252	75.25±0.095



Graph 1: DPPH Assay of Methanolic and Aqueous extract of leaves of *Spathodea campanulata* As compared to Ascorbic acid as standard. Comparative results as seen with Ascorbic acid as standard are given in the graph.

4. Conclusion

Due to the variation in phytoconstituents present in medicinal plants there is possibility of variation in pharmacological activity. Chemical tests carried out to detect the presence of phytoconstituents are therefore required to confirm the presence of important chemical constituents. Screening techniques like HPTLC are also very accurate in helping to determine the presence of chemical constituents. It is an important parameter of herbal drug standardization for the proper identification of medicinal plants. *Spathodea campanulata* has been under pharmacological screening for antibacterial and wound healing and sunscreen effects. Different preparations made from its flowers have been used in different ailments but their exact chemical components were unknown for a long time. To understand the actual chemical constituents responsible for the pharmacological activities of the plant detailed chromatographic studies are needed to be carried. The chemical tests indicate presence of steroids and flavonoids as major constituents in both the aqueous and methanolic extracts. In this study multiple peaks correspond to various pigments and the other compounds like flavonoids, steroids, and anthraquinones. Several peaks observed in this experiment indicate the diverse composition of the extracts. The data and HPTLC fingerprint profile could be used as a valuable analytical tool in the routine quality control and standardization. The antioxidant activity results show that both the methanolic and aqueous extracts have antioxidant activity. When compared with the standard antioxidant Ascorbic acid it was seen that Aqueous extract had IC_{50} nearer to Ascorbic acid. Further characterization of these fractions by applying more sophisticated separation and purification techniques are necessary to find out the exact chemical compounds and their relation to the pharmacological activity. The antioxidant activity makes the extract a useful candidate to be used in anti-ageing and wound healing preparations.

5. Acknowledgement

I wish to express my sincere gratitude to Principal Dr. (Mrs) Kiran Bhise and the Management, of M.C.E. Society's Allana College of Pharmacy for giving me an opportunity to carry out the research work and for their support throughout the work. I would like to express my gratitude towards my internal supervisor Dr R G Singhal, and management of Shobhit University, Meerut for constant guidance and support

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