



INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH



ASSESSMENT OF *IN VITRO* ANTIOXIDANT ACTIVITY OF HYDRO ALCOHOLIC EXTRACT OF *SPATHODEA CAMPANULATA* P.BEAUUV

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ARTICLE INFO

Article history

Received 23/02/2024

Available online

08/04/2024

Keywords

Spathodea Campanulata,
Antioxidant,
DPPH,
Ferric Reducing Power,
Phenolic Compounds.

ABSTRACT

Antioxidant nature of *SpathodeaCampanulata* P. Beauv leaves is documented in ancient texts of Ayurveda and is used for symptomatic relief of neurological disorders along with urogenital disorders, analgesic and anti-inflammatory. The diversified activity arises from antioxidant phytochemicals like polyphenols, tannins and flavonoids. The evaluation of antioxidant property was formed *in vitro* by 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) assay and was compared with ascorbic acid as reference standard. Ferric reducing power of the extract was also evaluated by using potassium ferricyanide. Free radical scavenging activity of the extract is in the range of 1.34 & 56.34 percentage inhibition, compared to ascorbic acid (2.32% and 64.00%) in reducing power assay. In DPPH, antioxidant evaluation of extract (23.32 % and 76.00 %) and ascorbic acid (46.21% and 86.45%). In conclusion, the dose dependent property observed may be useful in the formulation of the extract.

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Please cite this article in press as **Mr. Jagadeesha A S et al.** Assessment of in Vitro Antioxidant Activity of Hydro Alcoholic Extract of *Spathodea Campanulata* P. Beauv. *Indo American Journal of Pharmaceutical Research*.2024;14(03).

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INTRODUCTION

Rise in the polluted air, water and food has profound impact on human health leading to a spectrum of morbidity with poor health. The toxicants entering the human system interacts with cell organelles and interferes with ongoing biochemical reactions. Toxicants increase oxidative stress in a major way manifesting as chronic illness and organ failures. It is impractical to prevent exposure of toxicants in air, water and food. However, the living system can be supplemented with antioxidants and nullify the ill effects of toxicants. The supplementation of naturally occurring antioxidants like polyphenols, flavonoids and tannins is a practical approach to overcome the assault by toxicants. Medicinal plants having antioxidant properties are aloe *barbadensis*, *Ginkgo biloba*, *Withania somnifera*, *Piper longum* etc. are established. However, in plant kingdom there are some many plants known to be rich phytochemicals like phenolic compounds, tannins, flavonoids, alkaloids, glycosides. The aim of the research is to find a high penetration through blood brain barrier, better bioavailability and safe use as compared to established antioxidant plants.

METHODOLOGY

Collection and authentication of plant material and preparation of extract

The leaves of the title plant were subjected to continuous hot extraction method using a Soxhlet apparatus. The extracts were concentrated under reduced pressure (temperature at 50°C) and stored in airtight in cool place.[9] The results were summarized in table 1.

Preliminary phytochemical screening

Preliminary phytochemical investigation was carried out on petroleum ether, chloroform, hydro alcoholic and aqueous extract of *Spathodea campanulata* P.Beauv leaves for detection of various phytochemicals.[10] The results are compiled in table 2

I. Quantitative determination of total phenol, flavonoid and tannin content

a) Total Phenolic Content (TPC)

The total phenolic content of the hydro alcoholic extract *Spathodea campanulata* P.Beauv (HAESC) was determined by adopting the method as described in Chikkamath V 2022 et al. [11] Aliquots of the extract were taken in a 10ml glass tube and the volume was made up to 3ml with distilled water. Then 0.5ml of Folin ciocalteu reagent (1:1 with distilled water) and 2 ml sodium carbonate (20%) will be added subsequently in each test tube. A blue color was developed in each test tube and the intensity of the colour is directly proportional to the phenolic content. The blue coloration in the tube is due to the formation of molybdenum blue as a result of complex redox reaction between phenols and phosphomolibdic acid in Folin ciocalteu reagent in alkaline medium. The test solutions were warmed for 1min, cooled and the absorbance was measured at 650nm. The calibration curve was prepared using catechol. The phenolic content of the plant was expressed as mg. equivalent of phenol per gram of extract.

b) Total Tannin content (TC)

Tannin content was estimated using 0.5 ml folin-ciocalteu phenol reagent. 0.1 ml of extract was mixed with 7.5 ml of deionized water in a volumetric flask to make up to volume 10 ml. The admixture was well mixed and kept in dark at room temperature for 30 minutes along with reference standard solutions of tannic acid (10, 20, 30, 40, 50 µg/ml). The absorbance for standard solution was used to make a standard curve at 700 nm. The absorbance of test solution was measured using the standard curve. The tannin content was expressed as mg/g of dried extract. [11]

IV. Antioxidant Activity

A. *In vitro* antioxidant activity:

The following *in-vitro* models were carried out to evaluate antioxidant activity.

1. Reducing power.
2. DPPH radical scavenging activity.

1. Reducing power

Increase in the absorbance indicates increase in the antioxidant activity. In this method antioxidant compound forms a coloured complex with potassium ferricyanide, which is measured at 700 nm. The reducing power of HAESC was determined according to the method of Oyaizu (Oyaizu, 1986).

Procedure:

Different doses of HAESC were mixed in 1 ml of distilled water so as to get 30µg, 60µg, 90µg, 120 µg and 150µg concentration. This is mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ Increased absorbance of the reaction mixture indicates increase in reducing power. The percentage reducing power was calculated by using the following formula: (0.5 ml, 0.1%), and the absorbance (OD) was measured at 700nm. Increased absorbance of the reaction mixture indicates increase in reducing power. The percentage reducing power was calculated by using the following formula:

$$\text{Percentage increase in absorbance} = \frac{\text{Test OD} - \text{Control OD}}{\text{Control OD}} \times 100$$

2. DPPH radical scavenging activity

Free radical scavenging activity of HAESC was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml to the extract in ethanol at a different concentration (100, 200, 300, 400, 500 and 600 µg/ml). The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm by using spectrophotometer (UV-VIS Shimadzu). Reference standard compound being used was ascorbic acid and experiment was done in triplicate. [12]

RESULTS

Preparation of the extract: Percentage yield of crude extract *Spathodea campanulata* P. Beauv was shown in table.1

Table 1. Percentage yield of extract of leaves of *Spathodea campanulata*.

Sl.no.	Solvent	Colour and Consistency	Percentage yield
1	Petroleum Ether	Greenish black and waxy	0.97
2	Chloroform	Greenish black and sticky	1.05
3	Hydro alcoholic	Greenish brown and waxy	18.69
4	Aqueous	Dark green colour	16.00

Preliminary phytochemical Screening:

The preliminary phytochemical study revealed the presence of alkaloids, flavonoids, saponins, tannins, phenolic compounds, steroids and glycosides. It was qualitatively observed that hydro alcoholic extract was rich with polyphenol, flavonoids and tannin. The results were shown in table 2.

Table .2 Preliminary phytochemical constituents of *Spathodea campanulata*.

Phytochemical constituents	Petroleum ether extract	Chloroform extract	Hydro alcoholic extract	Aqueous extract
Carbohydrate	—	—	+++	++
Protein	—	—	+++	+++
Fats and oil	++	+++	—	—
Steroids	+++	+++	—	—
Anthraquinone Glycosides	—	—	+++	+++
Saponins Glycosides	—	—	+	+
Coumarin Glycosides	—	—	—	—
Flavonoids	—	—	+++	+++
Tannin and polyphenolic	—	—	+++	+++
Alkaloids	—	++	+++	—
Vitamins	—	—	—	—

Spectroscopic determination of total polyphenolic and tannin content:

The total Phenolic content of HAESC was 68.65 mg/g whereas, aqueous extract 51.4 mg/g expressed as equivalent to catechol. Tannin content was found to be 2.047 mg/gm and 1.42 mg/gm expressed as equivalent to tannic acid. The results were shown in table 3 and Fig 1 & 2.

Table.3 Quantitative estimation of total phenolic and tannin content of *Spathodea campanulata*.

Particulars	Phenolic content		Tannin content	
Extracts	Hydro alcoholic	Aqueous extract	Hydro alcoholic	Aqueous extract
Standard curve	Catechol		Tannic acid	
Absorbance	650 nm		720 nm	
Amount of content in extract per gram	68.65mg/g	2.047mg/g	51.4mg/g	1.42mg/g
R ² value	0.992		0.994	

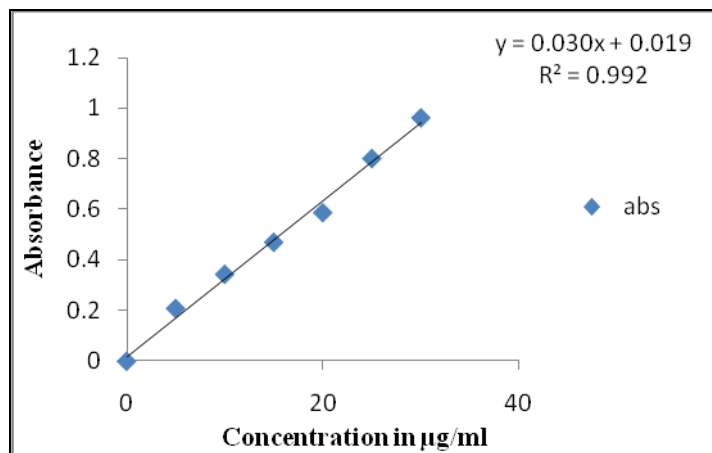


Figure 1. Standard graph of Catechol.

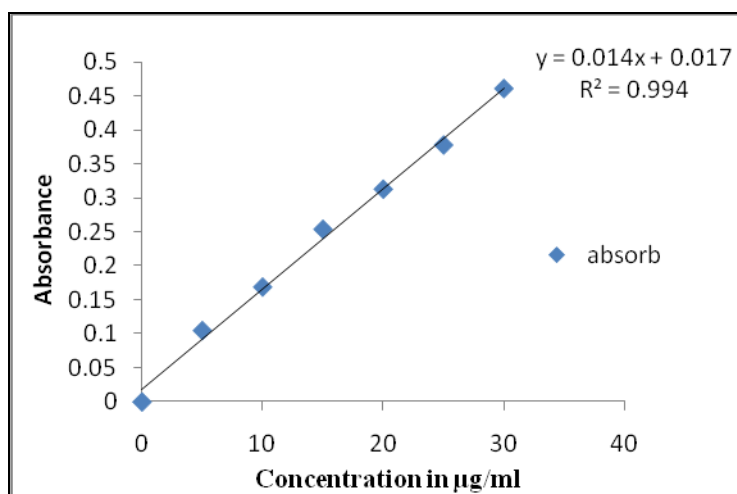


Figure 2. Standard graph of tannic acid.

IN –VITRO ANTIOXIDANT ACTIVITY

1. Reducing power activity of HAESC

It is observed that HAESC demonstrated concentration-dependent increase in the reducing property. Ascorbic acid (100µg/ml) has 2.32% reducing property. The test extract showed a concentration dependent increase in reducing property. However, (600µg/ml) of HAESC showed comparable reducing power i.e. 56.34 %. The results are summarized in table 5 and graphically depicted in figure 3.

2. DPPH (2, 2 –diphenyl-1-picrylhydrazyl) assay of hydro alcoholic HAESC

It is observed that HAESC have demonstrated concentration dependent increase in the DPPH radical scavenging activity. Where, at 600 µg/ml Ascorbic acid 64% DPPH radical scavenging activity. However, HAESC even at 600µg/ml shows lesser inhibition i.e. 56.34% than standard in the antioxidant model. The results are summarized in table 4 and graphically depicted in figure 4.

Table.4 Effect of HAESC on reducing power activity.

Sl.no	Concentration	Standard (Ascorbic acid)		HAESC	
		Mean±SEM	Percentage inhibition	Mean±SEM	Percentage inhibition
1.	Control	0.346±0.0112	--	0.423±0.005	--
2.	100µg/ml	0.342±0.0036	2.32%	0.328±0.0054	1.34%
3.	200µg/ml	0.362±0.0007	4.43%	0.347±0.0021	2.76%
4.	300µg/ml	0.391±0.0021	13.45%	0.352±0.0056	6.54%
5.	400µg/ml	0.448±0.0069	30.67%	0.361±0.0087	24.89%
6.	500µg/ml	0.457±0.0034	42.00%	0.465±0.0032	34.89%
7.	600µg/ml	0.545±0.0043	64.00%	0.480±0.0115	56.34%

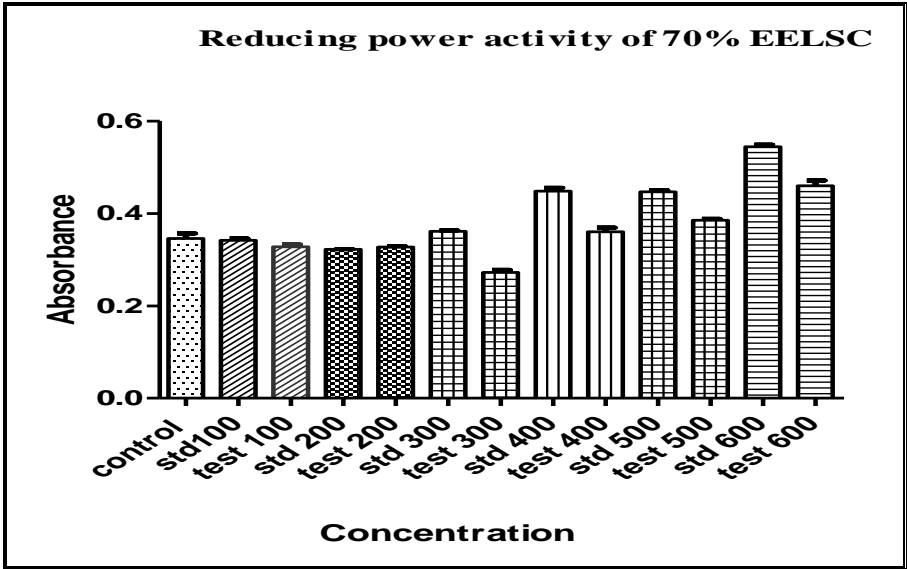


Figure.3 Effect of HAESC on reducing power assay.

Table.5 Effect of HAESC on DPPH scavenging assay.

Sl.no	Concentration	Standard (Ascorbic acid)		HAESC	
		Mean±SEM	Percentage inhibition	Mean±SEM	Percentage inhibition
1.	Control	0.515±0.000	---	0.432±0.0276	---
2.	100µg/ml	0.277±0.0384	46.21%	0.409±0.0279	23.32%
3.	200µg/ml	0.152±0.00440	50.00%	0.346±0.00733	39.90%
4.	300µg/ml	0.161±0.00241	68.45%	0.323±0.0198	45.34%
5.	400µg/ml	0.141±0.00370	77.70%	0.267±0.00487	56.00%
6.	500µg/ml	0.150±0.00187	79.00%	0.224±0.0116	72.00%
7.	600µg/ml	0.131±0.0082	86.45%	0.153±0.00181	76.00%

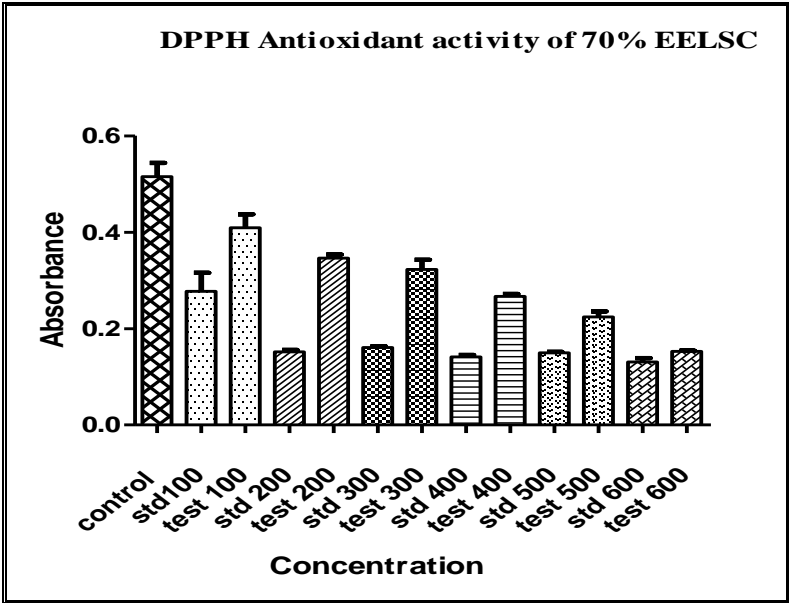


Figure 4. Effect of HAESC on DPPH scavenging activity.

DISCUSSION

Human beings constantly struggle against the changing environment condition to maintain optimum health and vigor throughout their life, during all the seasons. The human body depends on the continuous hormonal interaction between internal and external factors. When this interaction has failed, either due to internal deficiency or hostile environmental factors, the balance is disturbed and leads to disharmony and disease. It is increasing being realized now that a majority of the disease/disorders are mainly due to imbalance between pro-oxidant (free radicals) and anti-oxidant homeostatic phenomenon. [13] An antioxidant is a molecule stable enough to donate an electron to a free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property. These low molecular weight antioxidants can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. [14]

Naturally, there is a dynamic balance between the amount of the free radicals generated in the body and antioxidants to quench and/or scavenging them and protect the body against deleterious effects. When the normal level of antioxidant defense mechanism is not sufficient for the eradication of free radicals induced injury. Most of the antioxidants available in the markets are from natural origin for example vitamin-E & C, tocopherols, quercetine, β -carotene etc. Several antioxidants of the plant origin are experimentally proved and used as effective protective agents against oxidative stress.[15] In addition, there are reports that polyphenolic compounds like flavonoids and tannins are useful as antioxidants and organ protectants. Hence, in this study a widely grown plant *Spathodea campanulata* P.beauv reported to possess antioxidant activity.

CONCLUSION

The Preliminary screening of *Spathodea campanulata* P. beauv leaves has shown a significant amount of phenolic compounds, flavonoids and tannins. Hydro alcoholic extract has quantified high amount of total phenolic and tannin content and demonstrated a dose dependant reducing power and DPPH activities in scavenging of free radical. Further studies are needed to be conducted to know the specific phytoconstituents by isolation of the active constituents which are attributed to show antioxidant activity.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

FUNDING

Self funding.

ACKNOWLEDGEMENT

We thank management of TMAE society and the principal of SCS college of Pharmacy,Harapanahalli for providing the facilities to carry out this research work.

REFERENCE

1. Phaniendra A, Jestadi D, Periyasamy L. Free radicals properties sources targets and their implication in various diseases. Indian J Clin Biochem. 2015;30(1):11-26.
2. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods impact on human health. Pharmacogn Rev. 2010;4(8):118-126.
3. Lu JM, Lin PH, Yao Q, Chen C. Chemical and molecular mechanisms of antioxidants experimental approaches and model systems. J Cell Mol Med. 2010;14(4):840-860.
4. Ngouela S, Samo ET, Sondengam BL, Connolly JD, Spathodol. A new polyhydroxysterol from the leaves of *Spathodea campanulata*. J Nat Prod. 1991;54(3):873-876.
5. El-Hela AA. A phenolics from *Spathodea campanulata* Beauv. Leaves. AJPS. 2001;27:152-162.
6. El-Hela AA. A new iridoid glucoside from *Spathodea campanulata* Beauv leaves. AJPS. 2001; 27:115-120.
7. Pianaro A, Jurandir P, Dalva T, Noemia Kazue I, Braz Filho R. Iridoid glucoside and antifungal phenolic compounds from *Spathodea campanulata* root: Semina Cienc Agrar. 2007;28(2):251-255.
8. Markinde JM, Adesogen EK, Amusan O O G. The schizontocidal activity of *Spathodea campanulata* leaf extract on Plasmodium berghei berghei in mice. Phytother Res. 1987;1(2):65-68.
9. James R, Malcolm K, Dariel B, Joanna, Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties. J Microbiol Biol Educ. 2014;15(1):45-46.
10. Wagh AS, Butle SR, plant Profile, phytochemistry and pharmacology of *spathodea campanulata p. beauvais* (african tulip tree) a review. Int j pharm pharm sci. 2018;10(5)1-6.
11. Chikkamath V, Kulkarni VH, Habbu PV, Nagappa AN. *Grewia hirsuta* Vahl hydroethanolic leaf extract phytochemical and acute toxicological Studies. RJPS. 2022;12(1):23-30
12. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey R et al. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules. 2022;27(4):13-26.
13. Sharifi-Rad M, Nanjangud V, Kumar A, Zucca P, Maria E, Varoni, Dini L et al. Lifestyle oxidative stress and antioxidants back and forth in the pathophysiology of chronic diseases. Front Physiol. 2020;11:694.
14. Lien Ai PH, Hua H, Chuong PH. Free radicals antioxidants in disease and health. Int J Biomed Sci. 2008;4(2):89-96.
15. Zafar F, Asif HM, Shaheen G, Ghauri AO, Rajpoot SR, Tasleem MW. A comprehensive review on medicinal plants possessing antioxidant potential. Clin Exp Pharmacol Physiol. 2022;1-13.



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