International Journal of Chemistry and Pharmaceutical Sciences

Journal Home Page: www.pharmaresearchlibrary.com/ijcps

Research Article **Open Access**

Phytochemical screening and quantitative analysis of bioactive components in various extracts of *Alternanthera sessilis linn* Leaves

Lalitha Sree T*[1] and Dr. K. Vijayalakshmi[2]

[1]*Research Scholar, Department of Biochemistry, Bharathi Women's College, Chennai–600108, Tamil Nadu, India.*
[2]*Associate Professor, Department of Biochemistry, Bharathi Women's College, Chennai-600108, Tamil Nadu, India.*

A B S T R A C T

The plant kingdom is a treasure house of structurally diverse phytochemical compounds. The green vegetable *Alternanthera sessilis* is used in medicine for various ailments. The phytochemical analysis of leaf extracts in ethanol, ethyl acetate and aqueous extracts of indigenous medicinally important vegetable *Alternanthera sessilis* was investigated. The phytochemical examination revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenol and carbohydrates. In quantitative analysis, the important bioconstituents such as alkaloids, flavonoids, phenolic compounds, tannins and saponins were tested in all the three extracts. The ethanolic extract showed highest amount of phytochemicals when compared with other extracts. This study provided the basis of the plant usage in traditional medicine.

Keywords: *Alternanthera sessilis*, Phytochemicals, Quantitative analysis.

A R T I C L E I N F O

CONTENTS

Article History: Received 01 March 2016, **Accepted** 19 April 2016, **Available Online** 27 May 2016

***Corresponding Author**
Lalitha Sree T
Research Scholar, Department of Biochemistry, Bharathi Women's College, Chennai–600108, Tamil Nadu, India.
Manuscript ID: IJCPS2940

PAPER-QR CODE

Citation: Lalitha Sree T, *et al.* Phytochemical screening and quantitative analysis of bioactive components in various extracts of *Alternanthera sessilis linn* Leaves. *Int. J. Chem, Pharm, Sci.*, 2016, 4(5): 242-246.

1. Introduction

Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals intermediates bioactive principles and lead compounds in synthetic drugs (1, 2, 3). For the progress of human beings the plant resources play

an important role. They fulfill many needs viz; food, fuel, fiber and medicine. In Indian ancient literature observed that each and every plant on this planet are useful in medicine, industry and allelopathy. The different phytochemicals like Alkaloids, Cellulose, Carbohydrates, Flavonoids, Glycosides, Phenols, Quinones, Saponins, Tannins, Terpenoids, Triterpenoids, Steroids and many others screen out in the plants are key reservoirs of many new essential drugs. Phytochemical analysis is the primary way to the discovery of new useful drugs. Plants are the greatest reservoirs or resources of drugs of traditional systems of medicine, phytochemical intermediates and chemical entities for synthetic drugs. (4, 5, 6).

Alternanthera sessilis (Family: Amaranthaceae) is an aquatic plant. A. sessilis is widely used as vegetable in Asia, and occasionally cultivated for its use in herbal medicine. Traditionally, the leaves of A. sessilis are used in skin diseases, eye diseases, wound healing and as an antidote for snake bite. A decoction of *A. sessilis* is also used to alleviate pain and intestinal inflammation. The plant *A. sessilis* is well known for its stimulant activity and used for removing tiredness, laziness, and sleeps. In some parts of India, including Bihar, poultice of pounded fresh material is used for sprains, burns, and eczema. The plant is also used in the treatment of malaria, diarrhoea, dysentery, postnatal complaints, night blindness, and helminthiasis. Antihelmintic activity is known to be produced when the juice of *A. sessilis* is administered with two spoons of warm water in an empty stomach. (7, 8, 9, 10, 11). The major objective of the work is to analyse the presence or absence of different phytochemicals and also to quantify the bioconstituents in three different extracts of the selected indigenous medicinal plant *Alternanthera sessilis*.

2. Experimental

Collection of Plant Material:

The plant *Alternanthera sessilis* was collected from local market, Chennai. The Plant was authenticated by Dr. P.T. Devaraj, Associate Professor, Department of Plant Biology and Biotechnology, Presidency College, Chennai and the voucher specimen was deposited in the department as herbarium for future reference.

Preparation of the Plant extracts:

Fresh leaves of *A.sessilis* were separated, washed and shade dried for about 10 days. These dried leaves were ground to coarse powder using mechanical grinder. The dried leaves were subjected to sequential extraction using ethanol, ethyl acetate, hexane and water by Soxhlet extraction method using standard procedures. (12, 13, 14). The grounded powder was dissolved using distilled water and filtered and used as an aqueous extract. The extracts obtained using solvents were concentrated using rotary vacuum evaporator and then dried. The collected extracts were stored and then taken up for further investigations. The resulted filtrate was used for both qualitative and quantitative phytochemical analysis.

Qualitative Phytochemical Activity Screening

Test for carbohydrates: The presence of carbohydrates was confirmed when 2ml of plant extract was treated with 1ml of Molisch's reagent and few drops of concentrated sulphuric acid resulted in the formation of purple or reddish color.

Test for tannins: To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins: 2ml of plant extract, 2ml of distilled water were added and shaken in a graduated cylinder for 15minutes lengthwise. It resulted in the formation of 1cm layer of foam indicated the presence of saponins.

Test for flavonoids: To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for alkaloids: To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for quinones: To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

Test for glycosides: To 2ml of plant extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Test for Cardiac Glycosides: To 0.5 ml of the extract, 2ml of glacial acetic acid and few drops of ferric chloride were added. This was under layered with 1ml of Conc. Sulphuric acid. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

Test for terpenoids: 0.5ml of the extract was treated with 2ml of Chloroform and conc. Sulphuric acid. Formation of red brown colour at the interface indicates the presence of terpenoids.

Test for phenols: 2ml of distilled water followed by few drops of 10% ferric chloride was added to 1ml of the extract. Formation of blue or green color indicates presence of phenols.

Steroids and phytosteroids:

To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

Anthraquinones:

To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones.

Test for Coumarins: 1ml of 10% Sodium hydroxide was added to 1ml of the extract. Formation of yellow colour indicates the presence of coumarins.

Ninhydrin Test:

To 2ml of the plant extract few drops of 0.2% Ninhydrin reagent was added & heated for 5 minutes. Formation of blue colour indicates the presence of aminoacids.

Test for Phlobatannins: Few drops of 2% Hydrochloric acid was added to 1ml of the extract. Appearance of red colour precipitate indicates the presence of phlobatannins.

Quantitative Determination of Secondary Metabolites

Estimation of alkaloids:

Alkaloid was determined using Harborne method (15). Five grams of the sample was weighed into a 250 ml beaker and

200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of flavonoids

The total flavonoid content in the sample was estimated by the method of Chang et al. (16). A volume of 0.25 ml of the sample was diluted to 1.25 ml with distilled water. A volume of 75 µl of 5% sodium nitrite was added and after six minutes 0.15 ml of aluminium chloride solution was added. A volume of 0.5 ml of 0.1M NaOH was added after 5 min and made up to 2.5 ml with distilled water. The solution was mixed well and the absorbance was read at 510 nm in comparison with standard quercetin at 5-25 µg concentration. The results are expressed as mg of flavonoids as quercetin equivalent/ gm of dried sample.

Total Phenolic content (TPC)

Total phenolic content of extract was assessed according to the Folin–Ciocalteau method of Slinkard and Singleton (17) with some modifications. Briefly, 0.1 ml of extracts (200, 600 and 1000µg/ml), 1.9 ml distilled water and 1 ml of Folin–Ciocalteau's reagent were seeded in a tube, and then 1 ml of 100 g/l sodium carbonate was added. The reaction mixture was incubated at 25 °C for 2 h and the absorbance of the mixture was read at 765 nm. The sample was tested in triplicate and a calibration curve with six data points for catechol was obtained. The results were compared to a catechol calibration curve and the total phenolic content of sample was expressed as mg of catechol equivalents per gram of extract.

Total tannins content (TTC)

Tannins – phenolics were determined by the method of Peri and Pompi (18). 1ml of the sample extracts of concentration 1mg/ml was taken in a test tube. The volume was made up to 1ml with distilled water and 1ml of water serves as the blank. To this 0.5 ml of Folin's phenol reagent (1: 2) followed by 5ml of 35% sodium carbonate was added and kept at room temperature for 5 minutes. Blue colour was formed and the colour intensity was read at 640nm. A standard graph (gallic acid – 1mg/ml) was plotted, from which the tannin content of the extract was determined. The total tannin content was expressed in mg/g of extract.

Total Saponins

The plant samples were ground and 20 g of each plant sample is put into a conical flask and100 ml of 20% ethanol is added to the plant sample Obdoni et al (19). The sample is heated over a hot water bath for 4 hours with continuous stirring at about 55ºC. The mixture is then filtered and the residue re-extracted with another 200 ml of 20% ethyl alcohol. The combined extracts are reduced to 40 ml over a water bath at about 90ºC. The concentrate is then transferred into a 250 ml separating funnel and 20 ml of diethyl ether is added to the extract and vigorously shaken. The aqueous layer is recovered while the diethyl ether layer is discarded and the purification process is repeated. 60 ml of n-butanol is added and the combined n-butanol extracts is washed twice with 10 ml of 5% sodium chloride. The remaining solution is then heated in a water bath and after evaporation; the samples are dried in the oven to a constant weight.

3. Results and Discussion

Results

Preliminary Phytochemical analysis was performed qualitatively to analyse the presence of various phyto constituents in the different leaf extracts of the powdered plant. Alkaloids, Tannins and Saponins were present in all the four extracts. Terpenoids, Glycosides and Cardiac Glycosides were found to be present in Ethanolic and Aqueous extracts. Flavonoids, Phenols, Coumarins and Carbohydrates were present in Hexane, Ethanol and Aqueous extracts. Anthroquinone, Phlobatannins, Aminoacids and Quinones were found to be absent in all the extracts. From this analysis, it was revealed that the ethanolic extract of the leaves was found to have more constituents compared to other extracts. The results of the preliminary phytochemical analysis were shown in Table 1. In the current study, the plant *Alternanthera sessilis* was analysed for its phytoconstituents quantitatively. Table 2 depicts the quantitative assay of Phytoconstituents. The Phenolic content (501.63 ± 1.41), Alkaloids (32.89 ± 1.33), Flavonoids (41.04 ± 1.07), Tannins (375.08 ± 1.03) and Saponins (79.99 ± 1.14) were found to be maximum in the ethanolic extract of the leaves compared to other extracts.

Discussion

Alternanthera sessilis also known as 'sessile joy weed 'or 'dwarf copperleaf' and has two such popular sayings, one is About the transformation of body into golden luster and the other one is regarding the clarity of the eyes to visualize the stars even in broad day light when this green vegetable is consumed periodically as mentioned in the traditional literatures (20). The previous studies on the phytochemical investigation of the plant *Alternanthera sessilis* documented the presence of phytochemicals like alkaloids, steroids, terpenoids, glycosides, Phenolic compounds, carbohydrates and Saponins in the ethanolic extract which is confirmed in the present study (21). Our recent study also focuses on the presence of the certain phytochemicals and the content of the secondary metabolites in ethanol, hexane, ethyl acetate and aqueous extracts.

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds (22, 23). The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties. Steroids and triterpenoids show the analgesic properties. The steroids

and saponins are responsible for central nervous system activities.(24). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. (25,26). They possess biological properties such as antiapoptosis, antiaging, antioxidant, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities. (27) Flavonoids are large family of polyphenolic components that are found to reduce blood lipid and glucose and to enhance human immunity (28). Tannins are generally defined as naturally occurring polyphenolic compounds of high molecular weight to form complexes with the proteins (29). The high content of bioconstituents reveals the importance of this vegetable for its nutritive and therapeutic value. (30)

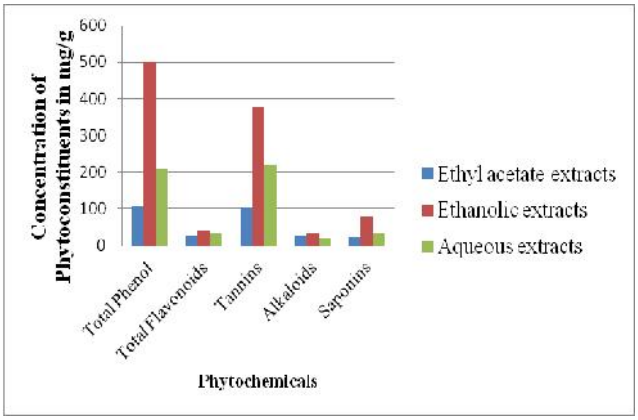

Figure 1: Showing the Quantitative analysis of Phytochemicals in mg/g

Table 1: Result of Preliminary Phytochemical screening for the different extracts of the leaves of *Alternanthera sessilis*

S. No.	Tests Parameters	Hexane Extract	Ethyl Acetate Extract	Ethanol Extract	Aqueous Extract
1	Alkaloids	+	+	+	+
2	Terpenoids	-	-	+	+
3	Glycosides	-	-	+	+
4	Flavonoids	-	+	+	+
5	Tannins	+	+	+	+
6	Saponins	+	+	+	+
7	Quinones	-	-	-	-
8	Phenol	-	+	+	+
9	Cardiac Glycosides	-	_	+	-
10	Coumarins	+	-	+	+
11	Steroids	-	-	+	-
12	Anthroquinones	-	-	-	-
13	Aminoacids	-	-	-	-
14	Carbohydrates	+	-	+	+
15	Phlobatannins	-	-	-	-

Table 2: Results of Quantitative assay of Phytoconstituents

S.No	Phytochemical Parameters	Concentration in mg/g of leaf extracts		
		Ethyl acetate extract	Ethanolic extract	Aqueous extract
1	Total Phenols	107.8 ± 1.46	501.63 ± 1.41	208.83 ± 1.34
2	Total Flavonoids	24.65 ± 1.62	41.04 ± 1.07	32.81 ± 1.33
3	Total Tannins	106.06 ± 1.05	375.08 ± 1.03	218.90 ± 1.19
4	Total Alkaloids	28.32 ± 1.22	32.89 ± 1.33	20.56 ± 1.41
5	Total Saponins	22.08 ± 1.64	79.99 ±1.44	35.78 ± 1.65

4. Conclusion

From the results of the study, it could be concluded that the plant contain bioactive components specifically in the ethanolic extract of the leaves. Results of our study further confirms that this green leafy vegetable used in cooking in most parts of Tamilnadu and Southern States of India is a potential source of bioingredients used in traditional medicine for curing various ailments.

5. References

[1] A L Tariq and A L Reyaz. Quantitative phytochemical analysis of traditionally used medicinal plant terminilia chebula. International Research Journal of Biotechnology, 2013, (ISSN: 2141-5153) Vol. 4(5) pp. 101-105.

[2] N S Ncube, A J Afolayan, A I Okah. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afri. J. Biotechnol. ; 2008, 7 (12):1797-1806.

[3] N Praveen, S Nayak, D M Kar, P Das. Pharmacological evaluation of ethanolic extracts of the plant *Alternanthera sessilis* against

temperature regulation. J. Pharm. Res., 2010, 3(6):1381-1383.

[4] Y R Chavan, S V Thite , V T Aparadh, B A Kore. Phytochemical analysis of some weeds. *The Global Journal of Pharmaceutical Research Vol. 2(1), pp. XXXX, 31 Mar 2013.*

[5] C S T Sastry and K Y Kavatheker. Plants for reclaimation of wastelands, 1990. New Delhi, India:

[6] K A Hammer, C F Carson and T V Riley . J Appl Microbiol 1999; 86(6): 985.

[7] Himangsu Mondal, Sanjib Saha, Khalijah Awang, Hemayet Hossain, Abdulwali Ablat, Md Khirul Islam, Ismet Ara Jahan, Samir K Sadhu, Md Golam Hossain, Jamil A Shilpi and Shaikh J Uddin. Central-stimulating and analgesic activity of the ethanolic extract of Alternanthera sessilis in mice Mondal et al, 2014. BMC Complementary and Alternative Medicine 2014, 14:398.

[8] A Ghani. Medicinal plants of Bangladesh: chemical constituents and uses.In Asiatic Society of Bangladesh. 1998:75.

[9] A K Gupta. Reviews on Indian Medicinal Plants, 2004. New Delhi: Indian Council of Medical Research.

[10] K K Tan, K H Kim. Alternanthera sessilis Red ethyl acetate fraction exhibits antidiabetic potential on obese type 2 diabetic rats, 2013. Evid Based Complement Alternat Med:1–8. Art. ID 845172.

[11] B Sahithi, G P Rajani, K Sowjanya, D Gupta.Anti-inflammatory activity of ethanolic and aqueous extracts of Alternanthera sessilis Linn, 2011. Pharmacologyonline 1:109–1043.

[12] A Sofowra. Medicinal Plants And traditional Medicine In Africa, 1993. Spectrum Books Ltd., Ibadan, Nigeria, pp. 191-289.

[13] G E Trease, W C Evans. Pharmacognosy, 1989. 11th edn., Bailliere Tindall, London, pp. 45-50.

[14] J B Harborne. Phytochemicals Methods, 1973. Chapman and Hall Ltd., London, pp. 49-188.

[15] J B Harborne. Introduction to Ecological Bio Chemistry, 1973. Second ED, Academic Press, New York, NY.

[16] C Chang, M Yang, H J Wen. Estimation of total flavonoid content in propolis by two complementary colorimetric methods, 2002. Food Drug Analysis.10:178-182.

[17] K Slinkard and V L Singleton.Total phenol analysis: Automation and comparison with manual methods, 1977.American Journal of Enology and Viticulture, 8, 4955.

[18] C Peri and C J Pompi. Estimation of different phenolic groups in vegetable extracts,1971. Phytochemistry, 19:2187-2189.

[19] B Obdoni and P Ochuko, Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria, 2001. Global J. Pure Appl. Sci, 8: 203 – 208.

[20] M Thomas Walter, S Merish, M Tamizhamuthu. Review of Alternanthera Sessilis with Reference to Traditional Siddha Medicine, 2014. International Journal of Pharmacognosy and Phytochemical Research; 6(2); 249-254.

[21] Monojit Debnath, Monalisha Nandi, Moulisha Biswas. A Critical Pharmacognostic evaluation and preliminary phytochemical investigation of *Alternanthera sessilis* (l.) r. br. Leaves, 2014. Indian journal of pharmaceutical science & researchvol 4 | issue 2 | 71-74.

[22] Satheesh Kumar Bhandary , N Suchetha Kumari Vadisha S. Bhat , K P Sharmila, Mahesh Prasad Bekal. Preliminary phytochemical screening of various extracts of Punica granatum peel, whole fruit and seeds,2012. NUJHS Vol. 2, No.4, December 2012, ISSN 2249-7110.

[23] P Varadarajan, G Rathinaswamy and Asirvatahm D. Antimicrobial properties and phytochemical constituents of Rheo discolour,2008. Ethnobotanical Leaflet; 12: 841–845.

[24] M Amin Mir, S S Sawhney, M M S Jassal. Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*, *2013.*Wudpecker Journal of Pharmacy and Pharmocology Vol. 2(1), pp. 001 - 005, January 2013.

[25] RNS Yadav and Munin Agarwala. Phytochemical analysis of some medicinal plants,2011. Journal of Phytology, 3(12): 10-14

[26] R Singh, S K Singh, S Arora. Evaluation of antioxidant potential of ethyl acetate extract/fractions of Acacia auriculiformis A. Cunn, 2007. Fod Chem. Toxicol., 45: 1216-1223.

[27] X Han, T Shen, H Lou. Dietry polyphenols and their biological significance, 2007. Int. J. Mol. Sci., 950-988.

[28] K Atoui, A Mansouri, G Bosku, P Kefalas.Tea and herbal infusions: their antioxidant activity and phenolic profile,2005. *FoodChemistry* 89, Page No. 27-36.

[29] Mudasir Sultana, Pawan Kumar Verma, Rajinder Raina, Shahid Prawez & Dar M A. Quantitative Analysis of Total Phenolic, Flavonoids and Tannin Contents in Acetone and n-hexane Extracts of Ageratum conyzoides. International Journal of ChemTech Research, Vol.4, No.3, pp 996-999, July-Sept 2012.

[30] A Stephen and R Suresh. Nutritive & Therapeutic Values of Vegetables from the Markets of Chennai, Tamil Nadu, India. Journal of Academia and Industrial Research (JAIR). Volume 3, Issue 11 April 2015.