International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 27-05-2020; Accepted: 14-06-2020: Published: 28-06-2020
www.botanyjournals.com
Volume 5; Issue 3; 2020; Page No. 618-622

Preliminary phytochemical analysis of methanol and chloroform leaf extracts of *Alternanthera sessilis* (Linn.) R.Br.ex DC. (Amaranthaceae)

Saswotika Nayak[1], Kalpita Bhatta[2*]
[1, 2] Department of Botany, School of Applied Sciences. Centurion University of Technology and Management, Odisha, India

Abstract
Leafy green vegetables take up an important place among the food crops as these provides promising nutrition. *Alternanthera sessilis* having a place with family Amaranthaceae is usually called as sessile joyweed is local to tropical and subtropical regions of the world. It is utilized as a verdant vegetable specially in Sri lanka and some Asian nations. This plant has been used in Indian traditional medicine system to cure skin diseases, night blindness, ulcer etc. Aim of the present study is Phytochemical analysis of leaf extract of *Alternanthera sessilis.* Phytochemicals are the bioactive compound which plays a vital role in antimicrobial activity. Phytochemical screening of methanol extract of *Alternanthera sessilis* shows the presence of alkaloids, terpenoid, glycosides, phenolic compounds, carbohydrates and saponin. But the chloroform extract shows the presence of alkaloids, terpenoid and phenolic compounds. This investigation will assist with planning new and increasingly strong antimicrobial medications of natural origin.

Keywords: *Alternanthera sessilis*, phytochemicals, bioactive compounds, methanol, chloroform

1. Introduction
India isn't just known for its varied culture, customs and traditions yet additionally for its rich biodiversity. The enormous quantities of widely varied vegetation species bear declaration to its rich species wealth. India is known as an emporium of restorative plants in the diverse bioclimatic zones and it is perhaps the most extravagant nation on the planet as respects to hereditary assets of therapeutic plants. Many of the tribal communities of our country like the Irulas, Kanis, Kotas, Kurumbas, Malayaalis, Paliyas, Paniyas, Sholagas, Todas and others rely on the naturally occurring medicines and food.

Nature based medicines (herbal medicine) have numerous advantages over chemical or synthetic medicines. For a long time, humankind has found out about the advantages of plant used to cure maladies. Plant has various bioactive mixes which are utilized as medication or in readiness of new medications, presently a-days 30% of overall medications depend on characteristic items isolated from therapeutic plants. In any case, for certain decades, there has expanding enthusiasm for plant uses and the recognition of their constituents with antimicrobial properties. Numerous endeavors have been made to extricate new antimicrobial bioactive compounds from different sources. It is imperative that some of the most significant medications of recent years, which have altered the advanced clinical practices, have practically all first been isolated from plants. These great medications incorporate penicillin(antibiotics), vincoleucoblastin (anticancer), podophyllotoxin (HPV infections), atropine (anticholinergic), digitoxin (to treat heart failure), stropanthidin (a cardioactive drug), ephedrine (a bronchodilator used to decrease respiratory congestion) and other new therapeutic agents. Natural products can likewise be utilized as building blocks for the synthesis of semi synthetic drugs like plant saponins which can be extricated and effortlessly modified synthetics to deliver sapogenins (aglycones) for the production of steroidal medications. Numerous pharmaceutical organizations show enthusiasm on plant inferred medicines for the most part because of the current far reaching accept that Green medication is protected, safe and more trustworthy than chemical or syntheticmedicine [6].

Phytochemicals are bioactive chemicals present in plants. These are regarded as secondary metabolites which synthesized in all parts of the plant body, bark, leaves, stem, root, flower, fruits, seeds etc. The therapeutic estimation of a plant because of some synthetic substances that produce an unequivocal physiological activity on the human body. The most significant of these bioactive constituents of plants incorporate alkaloids, tannins, starches, terpenoids, steroids and flavanoids. Information on the concoction constituents of plants is alluring, not only for the discovery of therapeutic agents but also because such information may be of value in disclosing new source [7].

Alternanthera sessilis is one of the most significant plant utilized for restorative purposes. It belongs to family Amaranthaceae. Alternanthera sessilus is used in Indian traditional medicine for the treatment of eye infections. Various studies have been reported for its anti-microbial, wound healing activities, anti-oxidant activities, antipyretic activity, nootropic activity, hepato-protective activity, hematinic activity, anti-ulcer activity, hypoglycemic activity, anti-diarrhoeal property, and anti-Inflammatory[2]. The enthusiasm for the logical examination of *Alternanthera sessilis* is based on the claims of its effective use for the treatment of many diseases including skin diseases, wound healing, cold, cough, indigestion, diarrhea and boils[1].

Keeping above beneficial properties of *Alternanthera sessilis* the current investigation was planned to evaluate the phytochemical screening of different extracts leaves of Alternanthera sessilis.

2. Plant Profile

Alternanthera sessilis Linn. (Amaranthaceae) is well known as sessile joy weed utilized as verdant vegetable. Equivalent words of *Alternanthera sessilis* incorporate *Alternanthera triandra* Lam., *Alternanthera prostrata* Don., *Achyranthus triandra* Roxb. The genus Alternanthera incorporates around 80 species, local to tropical and sub-tropical areas of Australia, Brazil and South America [9].

Meaning of the Name

Alternanthera- Alterno means alternate
Anthera means flowers
Sessilis- Without a stalk

Taxonomical Classification [8]

Synonym: *Alternanthera glabra, Gomphrena sessilis*
Domain: Eukaryota
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Caryophyllales
Family: Amaranthaceae
Subfamily: Gomphrenoideae
Genus: *Alternanthera*
Species: *sessilis*

Vernacular Names [5]

English: Sessile joyweed, Dwarf copper leaf, joyweed
Sanskrit: Matsyaki, lonica
Hindi: Gudrisag, Garundi
Odia: Madaranga
Tamil: Ponnankanni, Citai, Koduppai
Kannada: Honagone soppu
Bengali: Chanchi, Haicha, Sachishak
Manipuri: Phakchet
Telugu: Ponnagantikura
Marathi: Kanchari
Konkani: Koypa
Malayalam: Meenamgani, Ponnankannikkira

Other Geographical Names [9]

Indonesian: Daun Tolod
Malaysia: Keremak
French: Brede Chevrette, Magloire
Sinhalese: Mukunu-Wenna

Botanical Description [11, 12]

Alternanthera sessilis is a many branched, perennial, sometimes annual herb. It can be erect or prostrate, rooting at the nodes. The stems are greenish, purplish colour, ribbed up to 1m, mostly glabrous. The stems consists of hairs.

Roots: Tap root, white or brown. The plant is prostrate, adventitious roots appear at the nodes creeping on the ground. In aquatic conditions bundles of long white roots are borne from the nodes.

Stems: Stems are cylindrical, many branched, villous in the lines and transversely at the nodes. Solid and thin in dry conditions, the stems are long, hollow and floating in aquatic conditions, reaching 1cm in diameter.

Leaves: Leaves are simple, opposite and decussate. Paired blades are generally the same size. Petiole is indistinct, short or absent. The laminas are narrow and elongated, shape and size varies. Oblanceolate to ellipticlanceolate or linear-lanceolate, apex acute to obtuse, shortly acuminate, base cuneate to attenuate, 1 to 12cm long and 0.25 to 3cm wide

Inflorescence: Inflorescences are sessile spikes, in the axils of leaves, subglobose, to 1cm across with segments to 2.5mm long.

Flowers: Flower are subtended by a white scarious bract, 0.75-1mm long with a pointed end, and 2 bracteoles similar to the bract. The perianth consists of tepals, all equal, ovate-elliptic, pointed at the apex, 1.5 to 2.5mm long, white, glabrous, distinctly mucronate, with a slightly denticulate margin. Five stamens (including 2 without anther) alternate with pseudostaminodes similar to filaments, but usually a bit smaller. At maturity, they equal the ovary and style. The ovary is compressed, sub- orbicular, with a short style.

Fruits: The fruit is a utricle, obcordate to orbicular-obcordate, 2-3mm long, dark brown. It falls with the tepals.

Seeds: Lens-shaped, 0.5-1mm in diameter, testa bright brown, slightly reticulate.

Fig 1: *Alternanthera sessilis* plant

Biology and Ecology [10]

Alternanthera sessilis generally grows in constant or periodically high humidity, however it can tolerate extremely dry conditions. This plant grows in mixed association with several other aquatic species. The propagation of the plant is spreads by seeds, which are wind-and water-dispersed, and by rooting at stem nodes. Seedlings appear in April. Fruits appear during August-October.

3. Materials and Methods

3.1 Collection of Plant Materials

Alternanthera sessilis is an annual or perennial herb. Found in the hotter part of India, ascending to an altitude of 1200 m [14].

The fresh and disease-free leaves of *Alternanthera sessilis* were collected from various locations of Jatni block, Odisha in the early morning. The plant is identified by comparing with the Flora of Odisa book (Saxena and Brahman, 1996). The plant materials were collected in a sterile bag, washed thoroughly and transformed into the laboratory. To reduce the chance of contamination, the plant materials were freshly collected and utilized for further studies [4].

3.2. Preparation Of leaves for Extraction

The leaves were washed two to three times thoroughly in double distilled water and shade dried for 2-3 weeks. Periodically the moisture levels of the leaves were observed.

Once it is completely dried, using a mechanical grinder the leaves were powdered. The ground samples were stored in a sterile glass container for further process [15].

3.3. Extraction Procedure

The dried leaves powder of *Alternanthera sessilis* was extracted by using Soxhlet apparatus using methanol anrm. About 30gms of leaf powder was dissolved in 250ml of methanol and chloroform separately using soxhlet apparatus at 30^0C for 48-72 hours. Extracts were concentrated under reduced pressure and then kept at 4^0C in an air tight sample bottle for further study [16].

3.4. Phytochemical screening

Reagent Preparation [17]

- **Molisch's reagent:** 10g of alpha-naphthol + 100ml of 95% ethyl alcohol
- **Hager's Reagent:** Picric acid
- **Wagner's Reagent:** 2.5g of iodine + 12.5g of potassium iodide + 250ml of distilled water
- **Vanilline hydrochloride Reagent:** 1% vanilline solution + 8% HCL solution
- **Anthrone reagent:** 0.2g of anthrone + 100 ml of conc. H2SO4 and shaken well (the solution to be freshly prepared)
- **Biuret reagent:** 1.5g of CuSO4 + 6g of sodium potassium tartrate + 500 ml of water + 300 ml of freshly prepared NaOH solution was added to this solution made up to volume of 1 liter.
- **Ninhydrin reagent:** 0.1% Ninhydrin + 100 ml of acetone.
- **Dragendroff's reagent:** 50 mg of tartaric acid + 200 ml of distilled water + 4.5g of basic bismuth nitrate(shaken for 2 hours) + 100 ml of 40% potassium iodide(solution was shaken vigorously and allowed to stand for 24 hours and filtered).
- **Mayer's reagent:** 1.36g of mercuric chloride + 5g of potassium iodide + 100ml of distilled water.
- **Barfoed's reagent:** 13.3g of copper acetate + 200ml of distilled water + 1.8ml of glacial acetic acid (the solution to be freshly prepared).
- **Seliwanoff's reagent:** 34ml of HCL + 68ml of distilled water + 0.15ml of resorcinol

Phytochemical screening

Preliminary phytochemical screening was performed for various compounds such as carbohydrates, proteins, alkaloids, tannin, saponins, phenolics etc. These compounds are known as secondary metabolites. These are responsible for plants medicinal effect. To check the presence or absence of these secondary metabolites different chemical tests are given below [14].

Test for Alkaloids [17]

Alkaloids are naturally occurring organic compounds. These are mostly containing basic nitrogen atoms. They have heterocyclic structure.

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

- Mayer's Test: Filtrates were treated with mayer's reagent. Formation of a creamy white coloured precipitate indicates the presence of alkaloids.
- Wagner's Test: Filtrates were treated with wagner's reagents. Formation of reddish-brown precipitate indicates the presence of alkaloids.
- Dragendroff's Test: Filtrates were treated with dragendroff's reagent. Formation of red precipitate indicates the presence of alkaloids.
- Hager's Test: filtrates were treated with hager's reagent, presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Test for Phenolic compound [17]

Phenols are aromatic compounds with phenyl group bonded to hydroxyl group. These are crystalline solid that is volatile. These are found in all parts of plant. They improve disease resistance capacity in plant.

- Phenol Test: 1 ml of extract was added with Ferric chloride solution, formation of yellow precipitate indicates the presence of phenols.
- Ellagic acid Test: 1 ml extract was added with few drops of 5% (v/v) Glacial acetic acid and 5% (w/v) sodium carbonate solution. The formation of muddy yellow, olive brown, and chocolate colour indicates the presence of phenols.
- Hot water test: Hot water was taken in a test-tube, leaf mixture was dip into it. Warm it for few minutes. The development of black or brown colour ring at the junction of dipping indicates the presence of phenols.

Test for Tannins [19]

Tannins are polyphenolic biomolecules. The astringency from tannin cause dry and pucker feeling in mouth (consumption of wine, raw fruit and tea etc.)

- Ferric Chloride Test: About 0.5gm of dried powder sample were boiled in 20ml of water in a test tube and filtered. Few drops of 0.1% Ferric chloride solution was added to it. Formation of blue black or brownish green colour indicates the presence of Tannins.
- Gelatin test: In a dry test tube 1ml of extract was taken. 1% gelatinsolution was added to it followed by the addition of 10% NaCl. Formation of white precipitation indicates the presence of Tannins.
- Vanillin hydrochloride test: Extract was treated with few drops of vanillin hydrochloride reagent, formation of purplish-red colour indicates the presence of Tannin.

Test for Saponins [15, 17]

Saponins are chemical compounds found in various plant species. Saponin consist of an aglycone link to one or more carbohydrate chain. These are plant steroid compounds or triterpenoids having bitter taste. These are form foam in aqueous solution and lyse erythrocytes.

- Foam Test: 1 ml extract was added to water and shaken well. Formation of honey comb like foam indicates the presence of Saponins.
- Blood Test: Few drops of blood added to 1 ml of extract and observe under microscope. The lyse of blood cells under microscope indicates the presence of Saponins.

Test for Sterols [17. 16]

These are of large class of organic compounds. These occurs widely in plants and animals and are characterized by

the presence of 1,2-cyclopentanophenenthrane ring system. Examples: Steroids

- Salkowski Test: 1 ml extract added to Conc. H2SO4. Formation of wine-red colour indicates the presence of Sterols.
- Liebermann- Burchard's Test: To 1 ml of extract acetic Anhydride and Conc. H2SO4 was added along the sides of test tube. Formation of red ring at the junction of two layers indicates the presence of Sterols.

Test for Glycosides [17, 14, 15]

Glycosides are made up of one or more sugar combined with alcohol, phenol or combined molecules. Those of sugars known as glucosides or fructo-sides.

- Keller- Killiani Test: 1 ml extract was mixed with few drops of glacial acetic acid and boiled for a min and cooled. To this solution 2 drops of ferric chloride solution was added. The contents were transferred to another tube containing Conc. Sulphuric acid. At the junction of 2layers, Formation of reddish-brown ring indicates presence of Glycosides.
- Molisch's Test: 1 ml extract was added with Molisch reagent, 1 ml of Conc. sulphuric acid was added along the sides of the tube. At the junction of 2 layers,Formation of reddish violet ring indicates the presence of Glycosides.
- Baljet's Test: To 5ml of extract 5 drops of sodium picrate were added. Change in yellow to orange colourindicates the presence of glycosides.

Test for Terpenoid [14]

Terpenoids are also called as isoprenoids. Terpenoids add flavour to plants.

- Salkowski test: To 5ml of extract 2ml of chloroform, and 3ml concentrated H2SO4 was added. Formation of reddish-brown colouration of the interface indicates the presence of terpenoids.

Test for Carbohydrate [17]

These are the substances with general formula of (CH2O). These are found in wide variety of natural and processed foods.

- Fehling's test: 1 ml of Fehling A and 1 mlof Fehling B was mixed in atest tube. 2 ml of extract was addedto the test tube. The formation of red precipitateindicates the presence of carbohydrate (Reducing Sugar).
- Molisch's test: 5 ml of sample was taken in a testtube. Two drops of Molisch reagent was added to it and mixedthoroughly, 3 ml of concentrated sulphuricacid was addedalong the sides with a dropper. Formation of reddishviolet ring at the junction of two liquids indicates the presence of Monosaccharide.
- Barfoed's test: In a test tube5 ml ofBarfoed's reagent was taken. 0.5 ml of extract was added to it, mixedwell and boiled for 2 min. The tube was kept in a testtube rackand examined for precipitate after 10–15 min.Formation of red precipitate clinging to the bottom-most part of thetest tubeindicates the presence of monosaccharide.
- Seliwanoff's test: In a test tube5 ml of Seliwanoff's reagent was taken. 5 drops of the extract were added andheated the contents to just boiling. The formation of red colourindicates the presence of fructose.

Test for Protein [17, 19, 15]

Proteins are complex nitrogenous compounds, occurs in plant and animal cells. Proteins on hydrolysis with strong inorganic acids or by enzymes produce a mixture of amino acids.

- Biuret test: To 3ml of extract few drops of 10% sodium chloride solution and 1% copper sulphate was added. Formation of violet/purple colour indicates the presence of protein. And On adding alkali it becomes dark purple in colour.
- Xanthoprotein test: To 3ml of extract few drops HNO3 was added. Formation of intensely yellow colour indicates the presence of proteins in the sample extract.
- Ninhydrin test: To the 1ml of extract 2% ninhydrin solution was added. Formation of violet colour indicates the presence of protein.

4. Result and Discussion

Plants are the extraordinary significance to the wellbeing of the people and networks from days of yore. Plant kingdom gives a gigantic supply of different phytochemical with likely remedial properties. The information in table – 1 depicted the preliminary phytochemical investigation reports of the various extracts, indicates that the methanolic extract from the leaves of *Alternanthera sessilis* was found to contain alkaloids, terpenoids, steroids, glycosides, phenolic compound, carbohydrate and saponin whereas chloroform extract shown the presence of alkaloids, terpenoids, phenolic compounds as phytoconstituents. The major phytochemical of interest present in the methanolic extract of leaves of plant *Alternanthera sessilis.* The mixes as known to be organically dynamics, inthis mannerhelp the antimicrobial activities through various systems. The P^H of the chloroform and methanol extracts of the leaves was found to be 7 and 6 respectively.

Table 1: Preliminary phytochemical analysis of the leaves of *Alternanthera sessilis.*

		Extracts	
Sl. No.	**Phytochemicals**	**Methanol**	**Chloroform**
1	Alkaloids	+	+
2	Steroids	+	-
3	Terpenoid	+	+
4	Glycosides	+	-
5	Tannins	-	-
6	Proteins	-	-
7	Phenolic compound	+	+
8	Carbohydrate	+	-
9	Saponins	+	-
10	Fixed oil	-	-
11	P^H	6	7

'+' indicates the presence and '-' indicates absence.

Fig 2: Preliminary phytochemical screeening

5. Conclusion

Ethnomedicinal plants are rich wellspring of secondary metabolites for example alkaloids, Flavanoids, terpenoids, steroids, glycosides so forth which assume imperative roles in preventing various diseases. The antidiuretic, anti-inflammatory, anti-analgesic, anticancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the previously mentioned bioactive compounds. Therapeutic plants are utilized for finding and screening of the phytochemical constituents which are useful for the assembling of new medications. Phytochemical screening of methanol extract of *Alternanthera sessilis* shows the presence of alkaloids, terpenoid, glycosides, phenolic compounds, carbohydrates and saponin. Though the chloroform extract shows the presence of alkaloids, terpenoid and phenolic compounds. These phytochemicals exhibit antimicrobial activities due to several reasons like saponin disturb the permeability of the bacterial outer membrane, flavonoid and glycosides harms the cytoplasmic membrane and cause loss of intracellular components etc. This phytochemical screening study established a significant scope to develop a broad spectrum use of *Alternanthera sessilis* in herbal medicine and as a base for the development of novel potent drugs and phytomedicine.

6. Acknowledgement

The authors thank Centurion University of Technology and Management, Bhubaneswar, Odisha, India for providing laboratory and equipment facilities to carry out the research work.

7. Conflicts of Interest

We declare that there are no conflicts of interest.

8. References

1. Sundar V, Ranjitha D, Ravi L, Mythili S. Discovery of new antifungal phytochemical PDHC (Propane-Diyl-Bis-Hexahydro-Isochromene) Isolated from *Alternanthera sessilis* leaves. International journal of Pharmaceutical Science and Research. 2019; 10(3):1148-1159.
2. Laxmi Sravani V, Abbas Z, Surya P. A review on *Alternanthera sessilis*. Indo American journal of Pharmaceutical science. 2017; 04(09):2845-2852.
3. Sharma V, Agrawal A, Chaudhary U, Singh M. Phytochemical investigation of various extracts of leaves and stems of Achyranthea aspera Linn. International Journal of Pharmacy and Pharmaceutical Sciences. 2013; 5(01):317-320.
4. Abhay kumar Kamble. Phytochemical studies on *Achyranthes aspera*: World Scientific News, 2018; Vol. 04:16-34.
5. Kamble A. Phytochemical studies on *Achyranthes aspera*. World Scientific News, 2018; 04:16-34.
6. http://plants.usda.gov/core/profile?symbol=Alse4.
7. Rao P. Ayurvedic View of Alternanthera Sessilis Linn. with Special Reference to Mathsyakshi. Sci Fed Journal of Herbal Medicine. 2018; 02(01):1-6.
8. Thomas M, Merish S, Tamizhamuthu M. Review of Alternanthera Sessilis with Reference to Traditional Siddha medicine: International Journal of Pharmacognosy and Phytochemical Research. 2014; 6(2):249-254.
9. https://www.cabi.org/isc/datasheet/4404.
10. https://www.eppo.int/ACTIVITIES/plant_quarantine/alert_list_plants/alternanthera_sessilis.
11. Jalalpure S, Agrawal N, Patil MB, Chimkode R, Tripathi A. Antimicrobial and wound healing activities of leaves of Alternanthera sessilis Linn.International journal of green pharmacy, 2008, 02(03).
12. Suganya D, Banupriya R, Umamaheswari A, Elumalai S. Studies on Biological Activity of Aqueous Extract of *Alternanthera sessilis* (Linn) for Developing Potential Herbal Drug Formulation of Ocular Diseases. Med Aromat Plants. 2019; 8(01):178-196.
13. Rajamurugan R, Deepa V, Sivashanmugam M, Raghavan M. Phytochemistry, Antioxidant and antibacterial activities of medicinal plants – A comparative study. International Journal of Current Research and Review, 2013; 05(02).
14. Shukla S, Soni P, Kewat KH. Studies on antibacterial potential and phytochemical screening of different extract of *Achyranthes aspera*. International Journal of Bioassay. 2016; 5(12):5138-5142.
15. Sharma V, Agarwal A, Chaudhary U, Singh M. Phytochemical investigation of various extract of leaves and stems of *Achyranthes aspera* Linn. International Journal of Pharmacy and Pharmaceutical Sciences. 2016; 5(01):317-320.
16. Kushwaha U. Evaluation of qualitative phytochemical analysis of water extract of *Achyranthes aspera* and *Achyranthes aspera 30*. Indian Journal of Research in Homeopathy, 2019; 13:37-40.
17. Parveen S. Phytochemical screening and Antimicrobial Activity of Herbal Plant Extracts- *Achyranthes aspera*. International Journal of Pharmaceutical and Clinical Research. 2018; 10(07)201-209.
18. Tiwari P, Gond P, Khoshale S. Phytochemical analysis of different parts of A*chyranthes aspera*. Journal of Pharmacognosy and Phytochemistry, 2018; 02:60-62.
19. Umate SK, Marathe VR. Proximate and Phytochemical screening of Alternanthera sessilis (L.) R. Br. ex DC. (Amaranthaceae). International Journal of Life Sciences. 2017; 5(4):703-705.