

**PRELIMINARY PHYTOCHEMICAL
ANALYSIS OF ALTERNANTHERA SESSILIS (L.)**

R. BR. EX DC.

Project submitted

TO

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In partial fulfillment of the requirement in degree of

BACHELOR OF SCIENCE IN BOTANY

Submitted by

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CERTIFICATE

This is to certify that this project work entitled " **Preliminary phytochemical analysis of *Alternanthera sessilis* (L.) R. Br. ex DC.**" is a bonafide piece of project work done by **Fathima Fasarein P. S. (Reg.no:200021023409)** in the Department of Botany, **Bharata Mata College, Thrikkakara** under my guidance and supervision for the award of Degree of Bachelor of Science in Botany during the academic year 2020-2023. This work has not previously formed the basis for the award at any other similar title of any other university or board.

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DECLARATION

I hereby declare that this project entitled " **Preliminary phytochemical analysis of *Alternanthera sessilis* (L.) R. Br. ex DC.**" is the result of work carried out by me under the guidance of **Dr.Surya Sukumaran**, Department of Botany, **Bharata Mata College, Thrikkakara**. This work has not formed on the basis for the award at any other similar title of any other university of board.

FATHIMA FASAREIN P. S.

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ABSTRACT

Alternanthera sessilis are commonly known as sessile joywood and dwarf copper leaf. It is an aquatic plant, widely used as vegetable and also as herbal medicine as it is used in skin diseases, eye diseases, wound healing and as an antidote for snake bite. This is a preliminary phytochemical analysis of *A. sessilis*. Here petroleum ether and water extract used for phytochemical analysis is done by using these extracts. Coumarins, terpenoids and tannins are present in both petroleum ether and water extract. Phytochemicals are chemicals obtained from plants which resist fungi, bacteria etc. Further studies on these compounds may evoke interesting properties of the compounds. Further investigations on the tribal medicine certainly offer contemporary and alternative medicines for the healthcare of the society. Several secondary metabolites are present in *A. sessilis* such as coumarins, terpenoids and tannins compounds which exhibit multiple pharmacological activities.

INTRODUCTION

Traditional medicines and the drugs obtained from natural sources had a greater importance since prehistoric times. Based on the fossil records the usage of natural products as medicines dated back from 60,000 years (Kanagarasu 2017; Umate, 2017). The compounds obtained from natural sources are either from plants, animals, minerals, microorganisms are produced from primary and secondary metabolic pathways (Nayak 2020; Estela Anita in 2016). The basic commodities of human being are food, water, shelter and medicine. Drugs obtained from the nature are inexhaustible and these are used for new drug developments. Since the times the plant-based medicines are obtained from natural sources.

An overview of Alternative medicines in India

India has been practicing various systems of medicine like Ancient Ayurveda, Unani, Siddha, and Homeopathic, Chinese and Tibetan systems of medicine (Rajeev 2022). Other alternative systems of medicine recognized by Indian systems of medicine are acronyms of AYUSH also includes Yoga and Naturopathy not only in India, most of these systems are recognized worldwide for treatment and prevention of disease for Global health maintenance (Lalitha 2016; Vimala 2016). In (ISM) Indian system of Medicine, Ayurveda medicine is one of the oldest medical systems and it has taken part in India's traditional health care systems. According to the basic principles of Ayurveda Human body is composed of five different principles Prithvi (Earth), Tejas (Fire), Vayu (Air), Jal (Water) and Akasha (Sky).

World health organization states that world 70% population depends on unconventional medicines from plant sources in their healthcare systems (Sivakumar

2018). Not only India, Chinese traditional system of medicine had a greater history. Chinese pharmacopeia contains around 5,700 traditional medicines from plant origin. According to WHO around 80% of the people depends on traditional medicine in developing countries and most of the drugs are from plant origin (Nikam and Namdas, 2022). Since from the time dated back Human being had to distinguish the edible and non-edible plants since the times occurring of drugs from natural sources developed gradually (Alok and Abhishek, 2015).

Research on Natural Products

Plants are the important source to develop pharmacologically active compounds. Many successful drugs available in the market are derived from the plant sources. The current research on plants mainly dependent on bioactivity guided fractionation and isolation of many important compounds like anti-diabetic, anticancer etc.. (Chaudhay 1992). The foremost Natural product introduced for the first time in the market is morphine by Merck in 1826, further many compounds were isolated and used as drugs like Cocaine, Codeine, Digitoxin, Quinine and Pilocarpine etc.

Present status of Medicinal Plants in India

Most of the drugs from the herbal source in the market are supplement like dietary products or nutraceuticals (Cardellina, et al., 2002). The secondary metabolite production from plant biogenetic pathways like alkaloids, glycosides etc., leads to new drug discovery from the herbal medicinal plants. E.g., drugs like morphine, codeine, reserpine etc., (Balandin, 1993). The trade of Herbal drugs is about 40 billion in the present market and it may have a drastic raise in percentage in next couple of years (Ephub 2019). Higher growth can be observed in the usage of medicinal plants, isolation and identification of the compounds became easier with advanced versions of NMR, X-Ray Crystallography (Elridge 2002; Pellecchia 2002; Glish 2003 and Blundell 2003).

Herbal medicines are one of the causes for the origin of conventional medicines, these phytopharmaceuticals are herbal preparations which contain a single

active ingredient or a combination of ingredients (Sunil 2006). Herbal Products are free from side effects with higher therapeutic values and minimal drug resistance whereas synthetic formulations develop drug resistance. Eg. *Artemisia annual*, a Chinese medicinal plant used as an alternative source for the control of malarial parasite.

These secondary metabolites usually have pharmacological activities such as anti-inflammatory, antioxidant, anti-cancer, wound healing, antibacterial and liver protection. In the view of varied medicinal properties and chemical diversity of plants, the author selected above four plant species to isolate secondary metabolites for their biological activities.

Weeds are unwanted plants that are found in both Kharif and Rabbi Season. The genus *Alternanthera* is coming under family Amaranthaceae, which consists of 64 genera and 800 species. It has strong taproots. Leaves are simple, opposite, sessile or spatulate. The length of the leaf is 0.6-5cm long and the width is 0.3-1cm wide. Inflorescence seen in the Genera is spikes. Bracts are ovate and persistent . Sepals are white or purplish with long hairs and a strong midrib. Fruits are indehiscent, enclosing the seed. . They are light sensitive. It can be used as a local medicine to treat hepatitis, tight chest, bronchitis, asthma, etc.

The phytochemical study helps to understand the relationships of internal characters and level of structural organization in plants. The World Health Organization (WHO) has emphasized the need to ensure the quality of medicinal plant products by using modern techniques and applying suitable standards (Chaudhay 1992; WHO 1998). So many plants are rich sources of a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids etc.. which are widely used in medical industry and have been found to have several biological properties (Uma *et al.*, 2009).

Phytochemicals are chemical compounds that occur naturally in plants. These plants contain biological significance. These helps to cure diseases such as cancer, stroke and other metabolic syndromes. They are non- nutritive plant chemicals that have protective or disease-preventing properties (Sreedhar and Christy, 2015).

Phytochemical analysis of plants as mentioned in folklore, has yielded a

number of compounds with various pharmacological properties. Plants are rich in a wide variety of secondary metabolites. The modern methods describing the identification and quantification of active constituents in the plant material, may be useful for proper standardization of herbs and its formulations. Chromatographic fingerprinting is a rational option to meet the need for more effective and powerful quality assessment in Indian Traditional Medicine (ITM) and Chinese Traditional Herbal Medicine (TCHM). The HPTLC method can be used for phytochemical profiling and the quantification of compounds present in plants that have an increasing demand in the preparation of herbal products such as medicines and cosmetics. There is an urgent need for standardization of plant products (Pawar et al., 2010).

Medicinal plants play a major resource base for the traditional medicine and herbal industry but also provide livelihood and health security to a large segment of Indian population. In the past, medicinal plants were the first line of treatment known to man and traditional medicinal practice remain, an important part of the primary healthcare delivery system in most of the developing world (Akerle 1998), Generally, plants constitute a major source of orthodox medicines and the presence of plant secondary metabolites has been attributed for most plants' therapeutic activities (Neumann and Hirsch, 2000). Phytomedicines have shown great promise in the treatment of intractable infectious diseases (Timothy et al., 2008).

Phytoconstituents are the natural bioactive compounds present in plants. These phytoconstituents work with nutrients and fibres to form an integrated part of defence system against various diseases and stress conditions (Koche et al., 2010). These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others (Nonita et al., 2010). Phytoconstituents are naturally occurring in the medicinal plants. leaves, vegetables and roots that have defence mechanism to protect themselves from various diseases. Phytochemicals are primary and secondary phytoconstituents depending on their role in plant metabolism. Chlorophyll, proteins and common sugars are included in primary constituents and secondary constituents have terpenoids, alkaloids and phenolic compounds (Krishnaiah et al., 2007). These secondary

metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycaemic, anti-diabetic, anti-oxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, antimalarial, anticholinergic, antileprosy activities etc. (Negi et al., 2011).

SIGNIFICANCE OF THE STUDY

So many plants are rich sources of a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids etc. Which are widely used in medical industry and have been found to have several biological properties. Thus, this study laid sufficient background

for the further research on extracts from leaves of *A. sessilis* for identification, subsequent

purification and isolation of compounds.

OBJECTIVES

Detection of secondary metabolites from the leaf extracts of *Alternanthera sessilis*.



Picture: 1. Habit of *Alternanthera sessilis* (L.) R. Br. ex DC

REVIEW OF LITERATURE

Rathinam *et al.*, (2017), the study was conducted about the 'Phytochemical characterization of *Alternanthera sessilis* and assessment of its growth promoting potential on the freshwater prawn *Macrobrachium rosenbergii*'. The main purpose of this study is to understand the primary and secondary phytochemical analysis of *A. sessilis* and its influence on growth promotion of *Macrobrachium rosenbergii*. *A. sessilis* using petroleum ether, acetone and ethanol were performed. It contains alkaloids, terpenoids, flavonoids, tannins, cardiac glycosides and quinones.

The study about 'Proximate and phytochemical screening of *A. sessilis* (L.) R. BR. Ex DC. (Amaranthaceae) - An underutilized leafy vegetable' was conducted by Umate and Marathe (2017). This genera belonging to family Amaranthaceae provides nutritive value, which nourishes human population. Its leaves can be eaten as vegetables and this plant can be used as medicines for vitiated blood, skin diseases and ulcers. Phytochemical studies showed positive result for alkaloids, steroids, phenolics, flavonoids, tannins, coumarins, etc. It helps in standardization and detection of adulterants. For finding nutraceutical properties, more investigation and evaluation are needed.

Saswotika and Kalpita (2020), the study was conducted about the 'Preliminary phytochemical analysis of methanol and chloroform leaf extracts of *A. sessilis* (Linn.) R. BR. Ex DC. (Amaranthaceae)'. This study was conducted by the genera *A. sessilis* is rich in nutrition. It is also known as sessile joyweed. This plant is used for treating ulcer, skin diseases, night blindness, etc. The aim of the study is phytochemical analysis of leaf extract of *A. sessilis*. Phytochemicals play vital role in antimicrobial activity. Phytochemical analysis shows the presence of alkaloids, terpenoids, glycosides, carbohydrates, phenolic compounds and saponins in the methanol extract while in chloroform extract shows the presence of alkaloids, terpenoids and phenolic compounds. This helps to plan the strong antimicrobial medications.

The study about Anita and Cynthia, 2016) 'Phytochemical and anti-microbial analysis of "Lupo" *A. sessilis*' was revealed phytochemical from the aqueous, acetone

and ethanolic extracts was screened and the anti-microbial and anti-fungal potentials of the expressed juice of Lupo (*A. sessilis*). The extract was subjected to test for alkaloids, steroids, anthraquinones, flavonoids and tannin. The result showed alkaloids, flavonoids, steroids and anthraquinones were present in all extracts of *Alternanthera sessilis*, while tannins is only present in aqueous extract. *A. sessilis* when extracted using water contains all the five phytochemical tested. And is beneficial when mixed in food products. Because it can supply alkaloids and steroids that exhibit physiological effect on the heart muscles. It can also supply natural purgatives or cathartics due to the presences of anthraquinones. Flavonoids have anti-viral, anti-fungal, anti-inflammatory and cytotoxic activities. Tannins present in aqueous extract have potential value as anti-cancer and as astringents.

According to (Rajeev *et al.*, 2022) conducted study on 'The Genus *Alternanthera* about phytochemical and ethano pharmacological perspectives'. This study was conducted the genus *Alternanthera* was used traditionally for the treatment of hypertension, pain, inflammation, diabetes, cancer, microbial and mental disorders. Phytochemical analysis shows the isolation of alkaloids, saponins, steroids, flavonoids, triterpenoids, glycosides, and phenolic compounds from 9 species. This study was mainly conducted for identifying *Alternanthera* species with therapeutic potential for future investigation.

The study about the 'Phytochemical screening and quantitative analysis of bioactive components in various extracts of *A. sessilis* Linn leaves.' was conducted by Lalitha and Vijayalakshmi (2006). The plant kingdom is a treasure house of structurally diverse phytochemical compounds. *A. sessilis* is an aquatic plant belonging to Amaranthaceae family. *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. Leaf extracts in ethanol, ethyl acetate, aqueous extracts of indigenous medicinally important vegetable *A. 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 three extracts. The ethanolic extract showed highest amount of phytochemicals when compared with other extracts. This study provides the basis of the plant usage in traditional medicine.

The study conducted about the 'Qualitative and quantitative estimation of phytochemicals of *A. sessilis* (L) r. br. ex. dc and *Alternanthera philoxeroides* (Mart.) Griseb' by (Vimala & Krishnan 2016) to evaluate the phytochemicals of *Alternanthera sessilis* and *A. philoxeroides*. This study revealed the presence of alkaloids, flavonoids, tannins, saponins, phenolic compounds, steroids, glycosides, quinone in both the plant extracts. The extracts of both these plants showed phytochemicals which have bioactivity and are the source of herbal drugs.

The study about 'Phytochemical screening and antimicrobial activity of ethanolic leaf extract of *A. sessilis* and *A. philoxeroides* was conducted by (Sivakumar & Sunmathi, 2016). In this study they evaluate the phytochemical screening and microbial activity of ethanolic leaf extract of *A. sessilis* and *A. philoxeroides*. They tested four gram positive bacterial species, four gram negative bacterial species and one fungi with ethanolic extracts of both plants using well diffusion method. In both plant extracts, the studies revealed the presence of alkaloids, flavonoids, aminoacids, carbohydrates, phenols, steroids, terpenoids, saponins and glycosides in both plant extracts and the bacterial strains and fungi were more sensitive to *A. sessilis* than *A. philoxeroides*. The leaf extract of *A. sessilis* and *A. philoxeroides* showed significant antimicrobial activity.

The study about 'Preliminary phytochemical analysis of *A. sessilis* leaves' was conducted by (Nikam & Namdas, 2022). In this study they evaluate the qualitatively primary phytochemical analysis of *A. sessilis*. The leaves of *A. sessilis* were screened to understand the phytochemical potential with aqueous, acetone, methanolic and ethanolic extracts. The study revealed that the plant contains secondary metabolites such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, saponins, tannins, terpenoids, quinones, coumarins etc. The plant contains bioactive components specifically in the ethanolic extract of the leaves. This investigation may focus research

field to develop clinical studies which might be of great scientific contribution for the society.

Alok Kumar *et al.*, 2015, study was conducted about the 'Preliminary phytochemical investigation and pharmacognostic evaluation of *A. sessilis*', *A. sessilis* is widely used to treat the diabetics and skin diseases. They were considered to be used in ethnomedical and therapeutic. Vegetative parts that were collected is used to study macroscopical, anatomical, and preliminary phytochemical studies.

Medicinal plants and plant derived drugs are the alternative medicine and extensively used for centuries to cure various ailments. Around the world about 80,000 plant species have been identified and used as medicinal plant (Joy *et al.*, 1998). Medicinal plants are the first line of treatment and about 75- 80% of people of developing countries and 25% of developed countries depend on plant source for treatment of diseases (Hajiaghaee and Akhondzadeh, 2012). India is the richest, oldest most diverse and versatile in cultural tradition associated with the use of medicinal herbal plants. Herbal plants are the chemical factories as it contain multitude of chemical compounds like alkaloids, flavonoids, glycosides, terpenoids, tannins and saponins etc. Medicinal plants have diverse use in society from medicine to herbal foods, drinks, cosmetics and other daily use articles. In modern times plants play significant role and belief is that they have vast potential for treatment of diseases. Moreover the importance and use of medicinal plants were stated in different religious books. About 176 medicinal plants are mentioned in the Holy Quran (Urbii *et al.*, 2014) and 19 plants in the Holy Bible (Duke, 2007). Among several medicinal plants *A. sessilis* is one of them and widely used since ancient times by traditional healers and also in Homeopathic, Ayurvedic, Chinese and Unani medicinal systems. It is a multipurpose medicinal plant and due to its vast pharmacological property it was used in traditional and modern medicinal treatment so we have selected this plant for study with the aim to understand the pharmacological properties of plant.

MATERIALS AND METHODS

Selection of plants in the present work

The plant species *Alternanthera sessilis* (L.) R. Br. ex DC belongs to Amaranthaceae family, collected from Thrikkakara. The study was undertaken to systematically examine pharmacological and chemical properties. These plants are rich sources of many secondary metabolites like alkaloids, terpenoids, flavonoids, steroids, etc.

Phytochemical analysis (Qualitative)

The plant material was collected from the mother plant (attain the normal growth of flowering and fruiting). The mature leaves were detached and dried in shade at ambient temperature for a period of three weeks. The well dried samples were powdered separately by using an electric blender.

The samples were prepared in 100 ml of 70% methanol separately, in conical flask and kept overnight in an orbital shaker for solvent extraction and the extracts were centrifuged at 10,000 rpm for 10 minutes. The supernatant thus obtained was kept in a water bath to evaporate to dryness for removing the methanol. After evaporation the residue was subjected to phytochemical analysis for detecting the presence of total alkaloids, total quinine, total total tannins and total terpenoids.

1. **Test for Coumarins:** Take 1 ml of the extract in the test tube and add 1.5 ml of 10% NaOH into the extract. Formation of yellow colour were observed which indicates the coumarins.
2. **Test for Quinones:** Take 1 ml of extract in a test tube and add 5 ml of distilled water. Turbidity is observed which indicates the presence of quinones.

3. **Test for Tannins (Braymer's test):** Take 1 ml of extract in a test tube and it was treated with 1 ml of 10% alcoholic ferric chloride solution. Formation of blue or greenish colour was observed which indicates the presence of tannins.
4. **Test for Terpenoids (Salkowski test):** Take 1 ml of extract in a test tube and it is treated with 0.5 ml of conc. HCl. Formation of yellow colour were observed which indicates the presence of terpenoids.

RESULTS AND DISCUSSION

Generally, drugs from natural sources are considered as safe and free from side effects. This statement is emphasized by the aspect considering the unsafe characters and side effects of synthetic drugs on individuals and environment. There are many naturally derived compounds available in the market used for treating many diseases. Herbal medicines used in traditional therapy are safe, so researchers in worldwide had greater attention towards the natural products

In the present study, the phytochemical analysis of *A. sessilis* indicates the presence of coumarins, terpenoids and tannins were present but quinones are absent in petroleum ether and water extract. While quinones are absent in water extract.

The observation made during investigation are in close agreement with the earlier works carried out by (Umate and Marathe, 2017) studied the presence of tannins and coumarins in 'Proximate and phytochemical screening of *Alternanthera sessilis*'. In the below table '+' indicates the presence while '-' indicates the absence.

For curing many diseases, medicinal plants and plant derived drugs were used extensively. Around the world about 80,000 plant species have been identified and used as medicinal plant (Joy et al., 1998). Medicinal plants are the first line of treatment and about 75- 80% of people of developing countries and 25% of developed countries depend on plant source for treatment of diseases (Hajiaghaee and Akhondzadeh, 2012). India is the richest, oldest most diverse and versatile in cultural tradition associated with the use of medicinal herbal plants. Herbal plants contain chemical compounds like alkaloids, flavonoids, glycosides, terpenoids, tannins and saponins etc. Medicinal plants have diverse use in society from medicine to herbal foods, drinks, cosmetics and other daily use articles. In modern times plants play significant role and belief is that they have vast potential for treatment of diseases. The uses of medicinal plants were found in various religious books. About 176 medicinal plants are mentioned in the Holy Quran (Urbii et al., 2014) and 19 plants in the Holy Bible (Duke 2007). Among several medicinal plants *A. sessilis* is one of them and widely used since ancient times by traditional

healers and also in Homeopathic, Ayurvedic, Chinese and Unani medicinal systems. It is a multipurpose medicinal plant and due to its vast pharmacological property it was used in traditional and modern medicinal treatment so we have selected this plant for study with the aim to understand the pharmacological properties of plant.

TEXT	PETROLEUM EXTRACT	ETHER	WATER EXTRACT
COUMARINS	++		++
QUINONES	-		-
TANNINS	++		++
TERPENOIDS	++		++

Table:-1. Phytochemical screening of Petroleum ether and water extract of *Alternanthera sessilis* indicate strong presence of ++ve and weak presence -ve.

TEST FOR COUMARIN



Figure 1: Test tubes with petroleum ether and water respectively.

TEST FOR QUINONE



Figure 2: Test tubes with petroleum ether and water respectively.

TEST FOR TANNIN

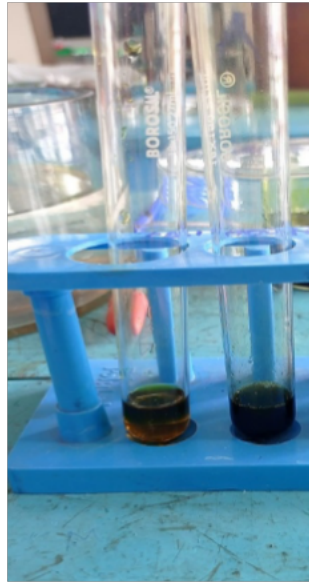


Figure 3: Test tubes with petroleum ether and water respectively.

TEST FOR TERPENOIDS



Figure 4: Test tubes with petroleum ether and water respectively.

CONCLUSION

The phytochemical analysis of *A. sessilis* indicates the presence of coumarins, terpenoids and tannins were present but quinones are absent in petroleum ether and water extract. *A. sessilis* plant is highly nutritive and contain medicinal properties, it can be used for future investigation in pharmaceutical application. Generally, drugs from natural sources are considered as safe and free from side effects. This statement is emphasized by the aspect considering the unsafe characters and side effects of synthetic drugs on individuals and environment. There are many naturally derived compounds available in the market used for treating many diseases. Eg Quinine from *Cinchona* used as antimalarial, Diosgenin from *Dioscorea* as natural alternative to estrogen therapy, Vincristine & vinblastine used as anticancer from *Catharanthus roseus*. Herbal medicines used in traditional therapy are safe, so researchers in worldwide had greater attention towards the natural products. Hence author has selected and worked on four medicinal plants which are being used as traditional medicines in Kerala.

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