



Analysis of phytochemicals and antibacterial activity of Button Weed (*Spermacoce ocymoides burm.F*)

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Abstract

The emergence of bacterial antibiotic resistance is one of the major global issues that affect human health. Consequently, the rise in multidrug-resistant microorganisms has led to an increase in resistant diseases, which is a hazard for public health. The current study aims to examine the chemical makeup and antibacterial properties of the plant extract from the leaves, stem, and root of *Spermacoce ocymoides burm.F*. Gas chromatography in conjunction with mass spectrometry was employed to determine its chemical composition. With the standard strains of *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, and *Salmonella typhi*, the antibacterial activity test was carried out. Serial microdilution was used to determine the minimum inhibitory concentration (MIC) and to confirm how the antibacterial action was modulated. After a 24-hour incubation period at 37°C, the test reading was conducted. The plant extract from *Spermacoce ocymoides burm.F* demonstrated possible synergism against *Salmonella typhi*, *Escherichia coli*, *Micrococcus luteus*, and *Staphylococcus aureus* as well as potentiated the antibacterial effect.

Keywords: *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Salmonella typhi*, inhibitory, antibacterial

Introduction

Since ancient times, the traditional medical system has utilised plants for their therapeutic benefits. Approximately 630 genera and 13,000 species of herbs, shrubs, lianas, small trees, and tall canopy trees make up the biggest family of flowering plants, the Rubiaceae, which is widespread throughout the world. That is common in Africa, Asia, Europe, and tropical and subtropical America. Flowers grow in small, few-flowered clusters at numerous nodes and can reach a diameter of 3-6 mm when they are in the fruiting stage. The bracteoles resemble stipules and have fimbriae that are 1.8–2 mm long. A weakly erect, procumbent, decumbent, or well-branched, 3–40 cm annual herb. Tall with delicate fibrous roots; stems have sparse to moderately dense crisped hairs on the pronounced angles that resemble wings. The petiole is 0-8 mm long and has scattered hairs. The leaf blades are elliptic to elliptic-lanceolate, 0.4-3.6 cm long, 0.25-1.6 cm wide, rounded to acute at the apex, concavely narrowed into the petiole at the base, and glabrous on both surfaces aside from short marginal hairs or pubescent on the main nerve beneath. The traditional medical systems, such as Chinese medicine, Siddha, and Unani, as well as Indian Ayurveda, are still frequently used today. In India, around 60% of the population relies on the conventional medical system, which is the only source of healthcare for the poorest people and about 80% of the world's population, according to estimates from the World Health Organization (WHO). Report on the traditional medicine approach of the WHO Traditional herbal therapy used in the past and remains popular in some parts of the world is based on phytochemicals. Indian traditional medicine uses various plant components to cure conditions like asthma, joint pain, lumber pain, sprains, colds, coughs, eczema, malaria, rheumatism, edoema, and venereal disorders. Plant scientists have been interested in phytochemical research as a result of the advancement of cutting-edge methods. Primary and secondary metabolites

are of synthetic plant origin and contain functional groups. Not only is knowledge of the chemical components of plants desirable for the development of therapeutic treatments, but it is also useful for revealing new sources of these chemicals. According to their roles in plant metabolism, phytochemicals can be broadly categorised into two groups: primary and secondary metabolites. Common sugars, amino acids, proteins, and chlorophyll are examples of primary metabolites, while alkaloids, flavonoids, tannins, and other compounds are examples of secondary metabolites. Phytochemicals are bioactive substances derived from plants. Any portion of the plant body may naturally contain active components, including the bark, leaves, stem, root, flower, fruit, and seeds (Bone, 2014). Phytochemicals, often known as plant chemicals, are what give plants their medical value since they have a specific physiological effect on people. In herbal and homoeopathic treatments, phytochemicals were employed to treat the illness. The phytochemical screening showed that the leaves contain more flavonoids than the stem does. Bacteria are found everywhere, and many of them are harmful. Stress is brought on in host cells as a result of these microbes entering and being recognised by the host defence system. Bacteria have evolved methods over time, some of which include resistance to host defensive mechanisms and the use of antibacterial therapy. The emergence of bacterial antibiotic resistance is one of the major global issues that affects human health. The rise of multidrug-resistant bacteria is a critical issue because it has led to an increase in resistant illnesses. Antibiotic inefficiency and antimicrobial resistance have been major challenges and problems for medicine in this situation. Due to this, numerous studies have been dedicated to finding new antibacterial agents, such as natural compounds derived from medicinal plants or microorganisms. A key source of synthetic and natural pharmaceuticals is medicinal plants. Given the large number of chemical substances it contains, including alkaloids,

glycosides, saponins, resins, oleoresins, sesquiterpenes, lactones, and oils, it is regarded as a chemical factory (essential and fixed). There is rising interest in the chemical makeup of plant-based medicines nowadays. Many bioactive components have been isolated, and their pharmacological action has been investigated. The antibacterial substances found in plants might limit bacterial development by methods other than those now in use. *Spermacoce Ocymoides Burm.F.* is also referred to as button weeds that resemble basil and has purple leaves. Due to its products' numerous folkloric and ethnomedical claims, it is intriguing from a scientific and medical perspective. Additionally, the plant has characteristics that are effective against wound healing.

Materials and methods

Collection of the plants

The fresh root, stem and leaf were collected in February 2022 from valathasampatty, Salem District of Tamil Nadu.

Preparation of plant extract

1. The leaves, stem and root of the plant were shade dried and made into powder.
2. 5 g of powder were soaked in 75 ml of (Aqueous, Methanolic) solvents for 72 hours.
3. 20 ml crude extracts taken after distillation. They were used as plant extracts.

Evaluation of phytochemical potentials of *Spermacoce Ocymoides Burm.F.*

Qualitative Phytochemical Screening

Using the techniques outlined by Trease and Evans (2002), Sofowara (2008), and in the Practical Pharmacognosy book (Kokate, 2005).

Detection of alkaloid

A small quantity of the extract was treated with few drops of dilute hydrochloric acid and filtered. The filtrate was tested with alkaloid reagent such as

- Mayer's reagent (Cream precipitate)
- Dragendroff's reagent (Reddish brown precipitate)
- Hager's reagent (Yellow precipitate)
- Wagner's reagent (Reddish brown precipitate)

Detection of flavonoids

The presence of flavonoids was determined by four methods.

1. 5 ml of dilute ammonia solution was added to the aqueous filtrate of the plant extracts followed by the addition of concentrated H₂SO₄. A yellow coloration observed in the extract indicated the presence of flavonoids. The yellow colour disappeared on standing.
2. A small quantity of the extract was treated with sodium hydroxide solution. Formation of yellow colour indicates the presence of flavonoids.
3. A small quantity of the extract was dissolved in alcohol and to that magnesium metal and concentrated hydrochloric acid was added. Magenta colour shows the presence of flavonoids.
4. A small quantity of the extract was heated on a water bath after acidification for 15 minutes and then extracted with chloroform. To the chloroform layer added few pieces of zinc granules followed by a drop of

concentrated HCl. Formation of pink colour indicate the presence of flavonoids.

Detection of sterols

Small quantity of extract was suspended in 5 ml of chloroform separately. The above obtained chloroform solution was subjected to the following tests

LibermannBurchard's test & Salkowski test

Detection of saponins

20 ml of distilled water were used to boil a small amount of plant extract, which was then filtered. To create stable, long-lasting foam, 10 ml of the filtrate was combined with 5 ml of distilled water and forcefully shaken. Three drops of extra virgin olive oil were added to the foam, which was forcefully agitated before an emulsion's creation could be seen.

Detection of tannins and phenolic compound

Small quantity of extract was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration which indicate the presence of tannins. A few drops of alcohol and ferric chloride solution were mixed with the plant extract and formation of blue-green or red colour indicates the presence of phenol.

Detection of glycosides

The freshly made sodium nitroprusside solution was added after dissolving 5 ml of the extract in pyridine. Pink to red colour development suggests the presence of glycosides.

Detection of carbohydrates

A minimum amount of extract was suspended in 5 ml of distilled water. The suspension was subjected to the following chemical tests followed by the Molisch's test, Fehling's test & Benedict's test

Detection of protein and amino acids

To 5 ml of extract, 2 drops of freshly prepared 0.2 % ninhydrin reagent was added and heated. The appearance of blue colour indicates the presence of proteins, peptides or amino acids.

Detection of triterpenoids

A layer was created by carefully adding concentrated H₂SO₄ (3 ml) to 5 ml of leaf extract combined with 2 ml of chloroform. The interface developed a reddish-brown colour to demonstrate successful terpenoid detection.

Detection of oils and gums

Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and gums.

Antimicrobial activity of methanolic root, stem and leaf extract of *Spermacoce Ocymoides Burm.F.*

Test bacterial and fungal organisms

The test microorganisms used in the study were *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli* and *Salmonella typhi*. The morphologically identified microorganisms were then subjected to a biochemical test for identification up to biochemical level.

Biochemical identification

The biochemical test was based on the test microorganisms' capacity to generate sugar fermentation and oxidation after being isolated and morphologically characterised. The biochemical tests that were performed were those for citrate, urease, nitrate, gelatin, hydrogen sulphide gas, arabinose, fructose, glucose, inositol, lactose, maltose, mannitol, mannose, raffinose, sucrose, and sorbitol. The test materials were produced in Nestler tubes in accordance with the manufacturer's instructions. Durham tubes were placed in media containing broth, and gas generation was afterwards monitored. The outcome was classified as positive if gas was produced, and negatively if no gas was produced. A one millilitre inoculum of the isolated test microorganisms was added to the media and cultured at 37 degrees Celsius for 24 hours before observation. The solid media-filled tubes were checked for colour changes. Using standards, results where colour change happened were classified as positive and results where colour change did not occur as negative.

Antibacterial assay

1. Preparation of inoculums

On nutrient agar slopes, stock cultures were kept at 4°C. A loopful of cells from the stock cultures were transferred to a test tube containing Muller-Hinton broth (MHB) for bacteria, which was kept at 37°C for 24 hours without being stirred. This created the active cultures for the experiment. To reach optical densities comparable to 2.0×10^6 colony forming units (CFU/ml) for bacteria, the cultures were diluted with fresh Muller-Hinton broth.

2. Preparation of sterile swabs

Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or by dry heat (only for the wooden swabs). It was sterilized by packing the swabs in culture tubes, papers, or tins etc.

3. Sterilization of forceps

Forceps can be sterilized by dipping in alcohol and burning off the alcohol.

Preparation of muller-hinton agar

In a conical flask with a flat bottom, one litter of distilled water was mixed with 38 milligrams of Muller-Hinton agar powder. To fully dissolve the media, the liquid was heated while being stirred frequently for one minute. Aluminum foil was then placed on top of the flask after it had been tightly sealed with cotton wool. The mixture was autoclaved at 121 degrees Celsius for 15 minutes before being allowed to cool to room temperature. Laminar flow was used to pour the media into the Petri dishes, resulting in a consistent depth of 3–4 millimeters. The media-filled Petri dishes were then sealed in sterile plastic bags and kept at a temperature between 2 and 8 degrees Celsius until they were needed.

Antibacterial assay using agar well diffusion method

The well diffusion method was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates could solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly, and the inoculums could dry for 5 minutes. Wells were cut and 20 µl of the different concentration of test drug were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Chloramphenicol disc was used as a positive control.

Results and discussion

The preliminary phytochemical screening test 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 qualitative separation of pharmacologically active chemical compounds. (P. varadarajan et al., 2008). The aqueous and organic extracts of root and stem extracts of *spermacoce ocymoides* Burm.F were analysed qualitatively and the result were presented in the Table 1.

Table 1: phytochemical analysis of root and stem extract of (*Spermacoce ocymoides burm.F*)

S. No	Phytochemicals analysed	Aqueous		Methanol		Chloroform	
		ROOT	STEM	ROOT	STEM	ROOT	STEM
1	Alkaloid	++	ND	++	++	ND	ND
2	Flavonoid	++	+	++	++	ND	ND
3	Triterpenoid	++	++	++	++	++	+
4	Carbohydrates	+	ND	++	+	ND	ND
5	Saponin	++	++	++	+	ND	ND
6	Steroids	++	+	++	+++	++	+
7	Amino acid	ND	ND	ND	ND	ND	ND
8	Tannin	++	+	++	+++		++
9	Gums & Mucilage	ND	ND	ND	ND	ND	ND
10	Chlorogenic compound	+	+	++	+	ND	ND

Using the agar well diffusion method, the antibacterial activity of methanolic extracts of the root, stem, and leaf of *Spermacoce ocymoides* Burm. F. was determined by measuring the diameter of the growth inhibition zone. Table

1-3 displays the activation of *spermacoce ocymoides* Burm plant extracts' antibacterial properties. Root, Leaf, and stem extracts in f methanol shown effective antibacterial properties.

Table 2: Antimicrobial activity of root extract of (*Spermacoce ocymoides burm.F*)

S. No	Microorganisms	Zone of Inhibition (mm)				
		100µl	75 µl	50 µl	25 µl	+ VE Control
Bacteria – Gram Positive						
1	Staphylococcus aureus	21	18	15	12	25

2	Micrococcus luteus	19	16	13	10	23
Bacteria – Gram Negative						
3	Escherichia coli	20	16	14	12	24
4	Salmonella typhi	17	14	12	10	23

Table 3: Antimicrobial activity of stem extract of (*Spermacoce ocymoides burm.F*)

S. No	Microorganisms	Zone of inhibition (mm)				
		100µl	75 µl	50 µl	25 µl	+ Ve control
Bacteria – Gram Positive						
1	Staphylococcus aureus	20	17	14	12	24
2	Micrococcus luteus	18	15	12	10	23
Bacteria – Gram Negative						
3	Escherichia coli	21	18	16	13	24
4	Salmonella typhi	17	16	13	11	23

Table 4: Antimicrobial activity of leaf extract of (*Spermacoce ocymoides burm.F*)

S. No	Microorganisms	Zone of inhibition (mm)				
		100µl	75 µl	50 µl	25 µl	+ ve Control
Bacteria – Gram Positive						
1	Staphylococcus aureus	22	20	18	16	25
2	Micrococcus luteus	20	17	15	14	24
Bacteria – Gram Negative						
3	Escherichia coli	23	21	18	15	26
4	Salmonella typhi	19	16	13	12	24

The methanolic extracts of the root showed a good significant antimicrobial activity against staphylococcus aureus (21mm), Micrococcus luteus (19mm), Escherichia

coli (20mm), Salmonella typhi (17mm), and Aspergillus niger (13mm) seen in Table-1, graph-1 and plate 1.

Plate- 1&2 Morphological view of leaf, stem and flower of (*Spermacoce ocymoides burm.F*)



Leaf
(*Spermacoce ocymoides burm.F*)



Stem & Flower
(*Spermacoce ocymoides burm.F*)

Plate 3
Antimicrobial activity of leaf, root, stem extract of (*Spermacoce ocymoides burm.F*)



Staphylococcus aureus



Micrococcus luteus

*Escherichia coli**Salmonella typhi*

According to Table 1, Plate 1, and Plate 2, the methanolic extracts of the stem shown strong antibacterial action against *Escherichia coli* (21mm), *Staphylococcus aureus* (20mm), *Micrococcus luteus* (18mm), and *Salmonella typhi* (17mm). As evidenced in Table 3, Graph 3, and Plate 3, the leaf's methanolic extracts demonstrated good, considerable antibacterial action against *Salmonella typhi* (19mm), *Staphylococcus aureus* (22mm), *Micrococcus luteus* (20mm), and *Escherichia coli* (23mm). Studies done in the past on the oil from *Spermacoce ocymoides* Burm's leaves, stems, roots, and seeds. F. Several family members showed possible antibacterial activity. Vaishali, S. (2018). et al. Raphael Conti et al. (2012), T. Rajkumar (2013), Augustinintemafack et al. (2015), and R.A.M. Jainafnachiya et al. (2021).

Summary

This preliminary phytochemical analysis accomplished that specific medicinal plants, including *Spermacoce ocymoides* Burm.F, clearly screened the phytochemical components from various extracts, including the root and stem extract. Exhibited potency and a wealth of bioactive components, particularly alkaloids, flavonoids, triterpenes, carbohydrates, steroids, amino acids, tannins, gums, and mucilage, as well as chlorogenic compounds. Particularly when it comes to *Spermacoce ocymoides* Burm, medicinal plants are essential in the prevention of many ailments is a plant that has historically been used to treat the entire portion, including gonorrhoea, eczema skin disorders, diarrhoea and dysentery, phenolics and tannin steroids, and reducing sugars of *spermacoce ocymoides* Berm, are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs for treatment of bacteria diseases.

Conclusion

In this study, human pathogenic bacterial strains such *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, and *Salmonella typhi* were used to test the antibacterial activity of a methanolic extract of the root, stem, and leaf of *Spermacoce ocymoides* Burm.F. The current study discovered that the plant extract was efficient against the harmful microbes for humans. When compared to the root and stem of the plants used in the study, the leaf demonstrates the highest level of antibacterial activity.

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