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Phytochemical and antibacterial studies on *Leucas vestita* Wall ex Benth.

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ABSTRACT

Objective: In search of alternative herbal medicine for pathogenic microorganism variety of plant species have been identified. However, search of new species are still in progress to reduce the pressure on biological diversity and increase availability of organic compound. In the light of this the present work identified phytochemical property and antibacterial activity of *Leucas vestita*. **Methods:** The ethanol extract of *L. vestita* was used for this study. The phytochemicals present in the extract was identified and the antibacterial activity was tested through disc diffusion method. **Results:** The phytochemical studies revealed the presence of primary and secondary metabolites which ensuring their herbal properties. Antimicrobial activity showed increasing zone of inhibition with increasing concentration of the extract with *Staphylococcus aureus*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Proteus mirabilis* among the other microorganism. Larger zone of inhibition of 14mm was recorded for *K. pneumoniae*. **Conclusions:** The study suggests that this extract can be used as a medicine to control some of these pathogenic bacteria.

1. Introduction

Natural products are believed to be an important source of new chemical substances with potential therapeutic applications. According to World Health Organization 80% of the world population rely mainly on traditional herbal remedies for their medicine. It is a practice as old as mankind and certainly every human culture on every continent of the earth has practiced herbal medicine of one form or another[1].

Medicinal plants are a rich source of antimicrobial agents[2,3]. Antimicrobial activity of plant extracts is due to the essential oil fraction or sulfur containing compounds in the aqueous phase. These compounds are also responsible for the characteristic aroma and flavor of the species. However, a large number of medicinal plants are under threat due to over exploitation since many of the plants are yet to be screened for their medicinal properties.

On the other hand infectious diseases caused by bacteria, viruses, fungi and parasites is still a major threat to public health, despite the tremendous progress in human medicine[4]. The past three decades have seen a dramatic increase in microbial resistance to antimicrobial agents[5]. Such situation stimulates the development of new anti-microbial agents in order to treat the infectious disease in an effective manner.

Although, hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated. Considering the vast potentiality of plants as sources for antimicrobial drugs and antifungal agents, a systematic investigation was undertaken to screen the antimicrobial activity of Leucas vestita along with its preliminary phytochemical testing.

In a continued search for new antimicrobial agents from

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plants for potential use in medicine and in crop protection, the present work provides a report on the phytochemical constituent and antimicrobial efficacy of the Leucas vestita Wall ex. Benth.

2.MATERIALS AND METHODS

2.1.Plant collection and preparation of powder

The plants were collected from the forests of Kodaikkanal Hills, Dindugal district, Tamil Nadu, India. The entire plant was taken for the study. The collected materials were washed with water to remove soil and dust. The plant materials were dried in shade for four to five days and chopped into small pieces. Then the plant materials were pulverized into coarse powder. The powder was used for the extraction.

2.2. Preparation of plant Extract

About 200g of coarse powder was filled in the column of Soxhlet apparatus and extracted with 80% aqueous ethanolic solvent for 16hrs. Then the extract was filtered and evaporated by using a rotary evaporator. The dried extract was stored in a refrigerator.

2.3. Phytochemical studies

The freshly prepared extract was subjected to standard phyto-chemical analysis to test the presence of phyto constituents such as carbohydrates, glycosides, proteins, alkaloids, flavanoids, phenolic compounds, steroids and terpenoids[4,5].

2.4. Antimicrobial activity

2.4.1. Disc Preparation

Sterile discs (Himedia) of 6mm were used to load the crude plant extract. Various concentrations of extract such as 200, 400, 600, 800 and 1000mcg were dissolved in Dimethyl Sulfoxide (DMSO) and loaded in the discs. The standard antibiotic Gentamicin was used as a control due to its broad spectrum of activity against various organisms.

2.4.2. Disc Diffusion method

The Disc Diffusion method[6] is a simple and reliable test to find out the effect of a particular substance on a specific bacterium. The sterile Muller – Hinton agar plates were used for this study. The test micro organisms were seeded into Muller Hinton agar medium by spread plate method. 10 μl (106 cells/ml) of nutrient broth of 24hrs bacterial culture was spread evenly on the solidified medium. After completion of swabbing process, pre – impregnated discs were placed on the surface of the medium with equal distance. Then, it was incubated at 37℃ for 24 hours. After incubation period the zone of inhibition was measured to determine the degree of sensitivity.

2.4.3. Microorganisms tested

In this study strains of common pathogenic microorganisms of both Gram positive and negative were used. The Gram positive strains such as *Staphylococcus aureus* (MTCC 96), *Streptococcus mutans* (Clinical isolate), *Bacillus subtilis* (MTCC 121) and Gram's negative strains such as *Escherichia coli* (MTCC 739), *Enterobacter aerogenes* (MTCC 111), *Klebsiella pneumoniae*. (MTCC432), *Proteus mirabilis* (MTCC 435), *Pseudomonas aeruginosa* (MTCC 424), *Pseudomonas fluorescence* (CIinical Isolate) and *Vibrio cholerae* (CIinical Isolate). Some of the microorganisms were obtained from Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigargh, India and the others were clinical Isolates.

3. Results

The Phyto-chemical studies of the ethanolic extract of *L. vestita* revealed the presence of phyto-chemicals such as proteins, amino acids, carbohydrates, phenolic compounds, alkaloids, tannins, saponins and flavonoids. The results are given in Table -1.

Table – 1

Phytochemical studies of *Leucas vestita*

CONSTITUENTS	TEST	REACTION
ALKALOIDS	Mayer's reagent	–
	Dragondroff reagent	+
	Hager' s reagent	+
	Wagner's reagent	–
CARBOHYDRATES AND GLYCOSIDES	Molisch's test	–
	Fehling's test	–
	Benedict's test	+
	Barford's test	+
	Borntrager's test	–
PHYTOSTEROL	Libermann burchard test	–
	Salkowski test	+
FIXED OILS	Spot test	–
	Saponification test	+
GUMS AND MUCILAGES	Preparation with 90% alcohol	–
FLAVONONE AND FLAVONOIDS	With aqueous NaOH solution	+
	With conc.H2SO4	+
PHENOL	FeCl3 test	–
	Biuret test	+
PROTEINS AND AMINO ACIDS	Ninhydrin test	–
	Xanthoprotein test	+
SAPONINS	Foam test	+
TANNINS	Gelatin test	+

+ : Refers to the presence of a particular constituent

– : Refers to the absence of a particular constituent

The Antibacterial activity of Leucas vestita was analysed against ten bacterial strains (Figure 1). The extract showed visible zone of inhibition against *Staphylococcus*

Table 2
Antibacterial activity of *Leucas vestita*.

Name of the Micro organism	zone of inhibition for Gentamicin (10mcg) (mm.)	Zone of inhibition (mm.)				
		200mcg	400mcg	600mcg	800mcg	1000mcg
Staphylococcus aureus	21	8	8	9	9	11
Strepeptococcus mutans	16	0	0	0	0	0
Bacillus subtilis	23	0	0	0	6	7
Escherichia coli	23	0	0	0	0	0
Enterobacter aerogenes	18	0	7	7	8	8
Klebsiella pneumoniae	23	10	10	11	12	14
Proteus mirabilis	18	8	8	8	9	11
Pseudomonas aeruginosa	23	0	0	0	0	0
Pseudomonas fluorescence	15	0	0	0	0	0
Vibrio cholerae	22	0	0	0	0	0

aureus, *Bacillus subtilis*, *Enterobacter aerogens*, *Klebsiella pneumoniae* and *Proteus mirabilis*. Maximum result was observed in high concentration such as 1000mcg. The highest inhibitory effect was observed against *K. pneumoniae* (14mm) in 1000mcg. The extract did not show visible zone of inhibition against Streptococcus mutans, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas fluorescence and Vibrio cholerae. In this study itself it was observed that the zone of inhibition varied with various concentration of extract. The extract showed inhibitory effect against *Bacillus subtilis* from 800mcg. Hence the visible zone of inhibition may obtain in *E. coli*, *P. aeruginosa*, *P. fluorescence* and *V. cholera* while using more concentration of extract than the quantity used in this study (Table 2).

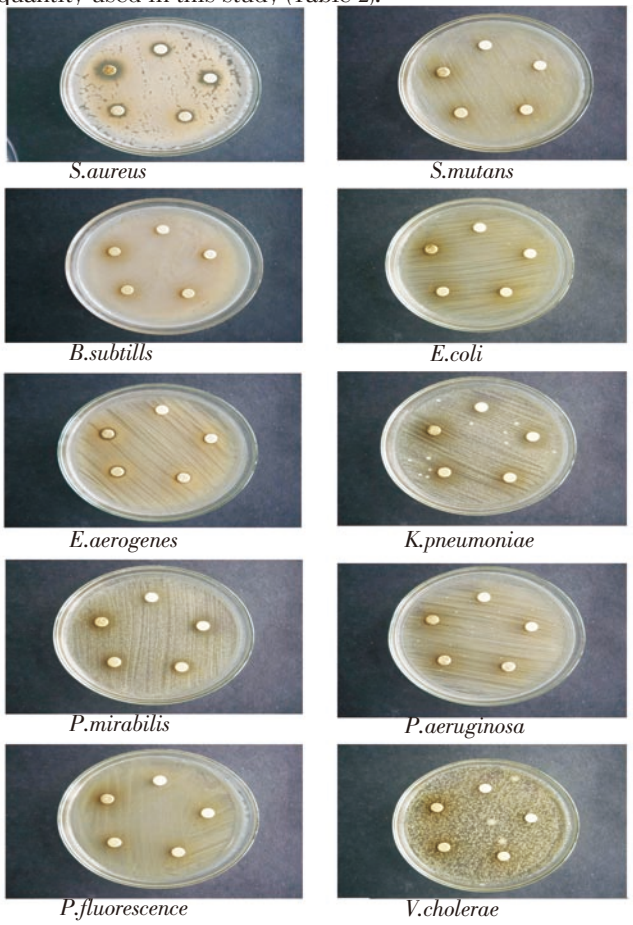

Figure 1. Antibacrteial activity of ethanolic estract of *Leucas vestita* Wall ex Benth

4. Discussion

The Phytochemical compounds present in the extract are known to be biologically active and therefore aid the antimicrobial activities of *L. vestita*. These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins have been found to form irreversible complexes with proline rich protein [7] resulting in the inhibition of cell protein synthesis. It is also reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues[8]. Hence it may help in the healing of wounds. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea, dysentery and other ailments[9, 10]. These observations therefore support the use of *L. vestita* in herbal cure remedies. Another secondary metabolite observed in the plant extract of *L. vestita* was alkaloid. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. It prevents the synthesis of DNA through the inhibition of topoisomerase. Hence it can acts as an anti bacterial and antiviral substance[11]. Alkaloids are one of the largest groups of phyto-chemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications[12]. It is also used as an antimicrobial agent[13]. Saponin was found to be present in *L. vestita* extracts and it is having inhibitory effect on inflamed cells[14]. It is supporting the usefulness of this plant in managing inflammation. Steroidal compounds present in *L. vestita* extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones. Steroidal extracts from some medicinal plants which exhibited antiviral[15] and antibacterial[16] activities on some isolates. Flavonoids, another constituent of *L. vestita* plant extract exhibited a wide range of biological activities like antimicrobial[17], anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties[18,19]. These metabolites

have varying pharmacological effect on animals. Scientists have shown that these metabolites play defensive roles in the plants which producing them. They may also used as an effective anti bacterial and anti fungal agents[20]. This study concludes that the antibacterial efficacy of the ethanolic extract of *L. vestita* was due to the phytochemicals present in it and the inhibitory effect of the extract of against some pathogenic bacterial strains suggests that this extract can be used as a medicine to control these pathogenic bacteria.

Conflict of interest statement

We declare that we have no conflict of interest.

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