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Phytochemical Analysis and Antibacterial activity of *leucas aspera* against Methicillin resistant *staphylococcus aureus*

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Abstract

Phytochemical investigations and Antibacerial activity studies for leaf extracts of *Leucas aspera* were carried out. Antibaceria activity carried out against certain drug-resistant bacteria (Methicillin Resistant *Staphylococcus aureus.* MRSA).Three common solvents i.e ethanol, methanol and chloroform were used successively for extraction of active principles from the dried powdered leaves. Qualitative phytochemical tests, demonstrated the presence of common phytocompounds in the plant extracts including Alkaloids, Flavonoids, Tannins, and Glycosideas major active constituents. Among the different solvents, methanol extract showed greater antibacterial activity against Methicillin Resistant *Staphylococcus aureus* (19 mm). So the future investigation was carried out for leaf extract using methanol as solvent to find out the minimum inhibitory concentration for selective Methicillin Resistant *Staphylococcus aureus.*

Keywords: *Leucas aspera, Phytochemical analysis, Methicillin Resistant Staphylococcus aureus, antibactrial activity*.

1. INTRODUCTION

Plants have been an important source of medicine for thousands of years. A large number of medicinal plants have been used for years in daily life to combat diseases. The use of antimicrobial drugs day by day increases due to emergence of the new diseases caused by the pathogenic microorganisms and development of microbial resistance towards different antibiotics, the advantages of effective antimicrobial chemotherapy are self-evident, but this has led to a significant problem in ensuring that they are always appropriately used. Surveys of antibiotic use have demonstrated that more than 50% of antibiotic prescribing can be inappropriate; this may reflect prescribing in situations where antibiotics are either ineffective, such as bacterial and viral infections, or that the selected agent, its dose, route of administration or duration of use are inappropriate **(Hugo and Russell's 2004)**. Bacterial drug resistance has many consequences. These include increased mortality and morbidity among patients and a reduction in the number of useful drugs for future generations of patients. Of additional concern is the economic impact of infections caused by antibiotic resistant bacteria **(George and Wistreich 2006)**. In recent years, there has been a dramatic increase in the incidence of hospital-associated (*nosocomial*) infections caused by strains of *Staphylococcus aureus* that are resistant to multiple antibiotics. Presently, more emphasis is laid on use of herbal drugs because of their easy availability and cost effectiveness comparing to the synthetic drugs which are not always affordable by the common people and also the synthetic drugs are having unwanted side effects. It is therefore, essential to search for the efficacious plants of medicinal value for better manifestations **(Ahmed *et al.*, 2006)**. Phytochemical extracts from various species of medicinal plants were isolated and showed greater positive effects towards microorganisms in laboratory. The phytochemical products from medicinal plants have been part of phytomedicine for long time ago and today. The main products are derived from seeds, fruits, leaves, tubers, and barks **(Haller *et al.*, 2002).**

2. MATERIALS AND METHODS

Bacterial isolate

Clinical Pus Specimen was collected from Government Hospital, Rasipuram, Namakkal District. Nutrient broth was prepared and inoculated with the clinical samples by the help of sterile inoculation loop and incubated at 37˚C for 24 hours. Nutrient agar was used to maintain culture for proper viability and further uses. MSA (Mannitol Salt Agar) medium was chosen as selective medium for the growth of *Staphylococcus aureus*. Colonies from Mannitol Salt Agar plates were collected and normal saline was used for making bacterial suspension which helped to inoculate Muller Hinton Agar sterile medium for antibiotic disc sensitivity where Methicillin along with other antibiotic discs was used

to check whether our microorganism was resistant to it or not. After incubation period of 24 hours at suitable temperature 37°C the observation of the inoculated MHA plate was brought out and the result that the zones of inhibition surrounding the antibiotic discs were clear present while that one of Methicillin disc was absent hence the confirmation of MRSA **(Newall *et al*., 1996).**

Collection of Plant materials

Fresh leaves of *Leucas aspera* free from any contamination were collected from green house of Muthayammal College of Arts and Science, Rasipuram, Namakkal. The plant was sent to Botanical Survey (BSI-Southern Circle) Government of India, Coimbatore, Tamil Nadu, for authentication. The leaves were washed thoroughly 3-4 times with running tap water and once with distilled water. The leaf of the plant were blotted and air-dried under room temperature for two weeks. The leaves of *Leucas aspera* were made into powder with the help of mixer **(Xavier and Vijayalakshmi 2007).**

Preparation of the leaf extracts

The leaf extracts were prepared by the organic solvents such as ethanol, methanol and chloroform. 10 grams of ground dry leaf sample of the *Leucas aspera* plant were soaked in 100 ml of ethanol, methanol and chloroform respectively contained in 500 ml capacity beakers. The beakers were covered with aluminium foil, shaken vigorously and allowed to stand in incubator shaker for 24 hours. The crude leaf extracts were obtained and stored in refrigerator. Consecutively each extract was tested for growth/contamination by plating them on nutrient agar at 37ºC for 24 hours. No growth was observed visually and the extract was subsequently used to assay for antimicrobial activity using the agar well diffusion method **(Alade and Irobi 1993)**

Phytochemical analysis of plant extracts

Qualitative chemical tests were carried out using extract from plant to identify the phytochemicals. Preliminary phytochemical screening was carried out to find the presence of the active chemical constituents in extracts such as alkaloids, flavonoids, tannins, carbohydrates, phenolic compounds, terpenoids, glycosides, steroids, fixed oils and fats. In general, tests for the presence of phytochemical compounds involved the addition of appropriate chemical reagent(s) to the extract in test tubes. The alkaloid was tested by using Mayer's test; the flavonoids were tested by lead acetate test. The tannins were tested by ferric chloride test. The total phenolic content in extract was determined by ferric chloride test. Steroids tested by salkowski test. The terpenoids tested by chloroform and con.sulphuric acid. The Carbohydrate was tested by benedict's test. The glycosides were tested by aqueous NaOH **(Springbob *et al*., 2009).**

Antibacterial activity of *Leucas aspera* against isolated MRSA

Sterile MHA plate was prepared and isolated MRSA were spread over the Muller Hinton Agar plate by the help of sterile cotton swab which was soaked into prepared MRSA suspension for inoculation. The sterile gel puncher was used to make wells of 1cm diameter by which the crude leaf extracts were applied. 500µl micropipette was used to pour the extracted liquids into their specific wells. Plate was incubated at 37°C and within 24 hours the zone of inhibition was observed on the surrounding areas of organic solvents; ethanol and methanol extracts while chloroform has never showed clear zone nearby it and the clear zone was measured in mm.

3. RESULT AND DISCUSSION

The current study carried out on the *Leucas aspera* proved the presence of pharmacological active chemical products. The pharmacological active chemical components from *Leucas aspera* were tested with the results such as are shown in the table1. In analysis of *Leucas aspera* compounds extracted by the help of organic solvents such as Methanol, Ethanol, and Chloroform**;** Alkaloids, Flavonoids, Tannins, and Glycosides were present in Methanol and Ethanol solvents respectively and absent in Chloroform, Carbohydrates and Terpenoids were present only in Methanol and absent in Ethanol and Chloroform.

The antibacterial activity of *Leucas aspera* leaf were investigated using agar well diffusion method against Methicillin Resistant *Staphylococcus aureus.* The extracts were prepared using various solvents such as ethanol, methanol and chloroform. All the examined extract showed antibacterial activity against Methicillin Resistant *Staphylococcus aureus.* Antibacterial activity of *Leucas aspera* against Methicillin Resistant *Stapyloccus aureus* was tested. The maximum antibacterial sensitivity was observed in Methanol extract (19mm) and ethanol extract (17mm). Among the three solvent extracts, Methanol was found to be highly active towards Methicillin Resistant *Staphyloccus aureus* compared to that of Ethanol. The results are shown in table 2. The result reveals that methanol based leaf extract was more effective when compared with other extracts and for all solvent used, methanol was best.

Table 1- Phytochemical analysis of crude extracts from *Leucas aspera* leaves

S.No	Chemical Constituents	Methanol	Ethanol	Chloroform
1	Alkaloids	+	+	-
2	Flavonoids	+	+	-
3	Tannins	+	+	-
4	Carbohydrates	+	-	-
5	Terpenoids	+	-	-
6	Glycosides	+	+	-
7	Steroids	-	-	+
8	Phenols	+	+	-

Note:- **+: Present** **- : Absent**

Table 2- Antibacterial activity of *Leucus aspera* against MRSA

Test organisms	Zone of Inhibition (mm)		
Methicillin Resistance	Ethanol extract	Methanol extract	Chloroform extract
Staphylococcus aureus	17	19	-

4. CONCLUSION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasing problem in human medicine that may have catastrophic effects. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global public health problem, associated with considerable morbidity and mortality. Therefore, there is an urgent need to develop new drugs. It has been proved that in our surrounding environment different natural medicinal plants have secondary metabolites with the ability to cure and save lives of so many. The various infections for instance *Staphylococcus aureus, Vibrio cholerae, Salmonella typhi, Klebsiella aerogenes, Escherichia coli, Proteus vulgaris, Pseudomonas pyocyanea* and *Dys. Flexneri* should be treated **(Rao and Narasimha 1971).** Hepatoprotective activity of *Leucas aspera* attributed to the confirmed presence of flavonoids **(Mangathayaru *et al.*, 2005),** Terpenoids isolated from a methanolic leaf extract, was studied for anti snake venom activity (**Venkatesan *et al.*, 2013).** The discovery of new potent drug from the bioactive compounds of *Leucas aspera* against MRSA will be based on the results of this, research work.

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