Extraction of phytochemicals from *leucas indica* and analysing the antimicrobial activty

Babu R[1], Kamalakannan S[1] and Jayabarath J[2*]

[1]Department of Biotechnology, [1]Department of Pharmaceutical Technology, Bharathidasan Institute of Technology, Anna University, Tiruchirappalli, Tamilnadu-600024.

[2]Department of Biotechnology, Pavendhar Bharathidasan College of Engineering and Technology, Tiruchirappalli, Tamilnadu - 600024.

***Corresponding author: E.Mail: barath_bio@yahoo.co.in Phone: 09894811886**

ABSTRACT

Phytochemicals are active components found in plants that act against many diseases. A plant of genus *Leucas* belongs to family *Lamiacae* has been widely used by the traditional healers to cure many diseases. The whole plant of *Leucas indica* was used for study the Phytochemicals. The extractions of Phytochemicals from *Leucas indica* were carried out with different solvent such as Water, Ethanol, Chloroform, Acetone, and Petroleum Ether. The preliminary Screening of *Leucas indica* leafs shows the Presence of Alkaloids, Flavonoids, Carbohydrates, Glycosides, Steroids, Saponins, Fixed oils, tannins, Phenolic Compounds, Proteins and Amino acids. The antimicrobial effect of the parts of *Leucas indica* was evaluated on bacterial strains like *Escherichia Coli*, *Pseudomonas Sp*., *Staphylococcus aureus* and fungal strains like *Aspergillus Niger*, *Aspergillus flavus* , *Candida Spp*. The *in vitro* antimicrobial activity was performed by Kirby-Baurer method. The ethanolic extract of *Leucas indica* was analyzed with HPLC. The most susceptible gram negative bacteria were *Staphylococcus aureus* than *Escherichia Coli and Pseudomonas Spp.* The most susceptible fungal species were *Candida spp.* than *Aspergillus niger and Aspergillus flavus*. The results revealed that the crude extract of the leaf parts of *Leucas indica* has a wide range of antimicrobial activity and it's suggested that it is useful in the treatment of infections caused by some of the bacteria.

Key words: *Phytochemicals, Antimicrobial activity, HPLC analysis.*

1. INTRODUCTION

Plants have healing powers on man and animals. Medicinal herbs as a potential source of therapeutic aids have attained a significant position in health systems all over the world. Phytochemicals are active components found in plants that act against diseases. They are non-nutritive compounds that contribute to flavor color. Many Phytochemicals have antimicrobial activity and reduce the risk of many diseases. Plants derived products has been used for their Phytochemicals with various bioactivities including antimicrobial, antioxidant, anti-inflammatory and anticancer activities. Alkaloids, Flavonoids, Phenolic Compounds, Tannins, and Saponins are responsible for the function of antimicrobial activity.

Leucas indica is commonly called Thumbaa in India. It is perenniel herb found in wasted lands and road sides (Chandrashekar et al 2013). It has the many biological activities include antioxidant and antidiabetic activity. Many of species from *Leucas* shows antimicrobial, anti-inflammatory, antioxidant, CNS depressant and wound healing activity (Ramaligam et al 2012). These species shows presence of alkaloids, tannins, Saponins, flavonoids, phenolic compounds, glycosides. The selection of this plant based on its traditional wages. Although very few works have been done on the antibacterial activity of this medicinal plant it needs further study for verification on its extract from different parts and activity against disease causing microorganisms. It is well known that plant produce these chemicals to protect them but recent research demonstrate that they can also protect humans against diseases.

2. MATERIALS AND METHODS

2.1 Plant collection: The Plant *Leucas indica* was collected in the month of January 2013 from the rural areas of Tiruchirappalli, Tamilnadu, India. The plant was authenticated by Dr. Jhon Britto, The Rapinat Herbarium and Center for Molecular Systematic, St. Joseph College, Tiruchirappalli where the voucher Specimen (RB001) has been preserved.

2.2 Pretreatment of sample: The plant was treated with 0.1 % of $HgCl_2$. The different parts of *Leucas indica* was dried at room temperature and grounded the powder (Chandrashekar et al 2013).

2.3 Preparation of plant extracts: 20g of grounded plant leafs, stem, pseudoverticil, root, were extracted with 100 ml of water, Ethanol, Petroleum Ether, Chloroform and Acetone in a Soxhlet Extractor. The plant extract was concentrated with the removal of solvents.

2.4 Preliminary Phytochemicals Screening: The concentrated extracts of leaf, stem, root, and pseudoverticil were analyzed for the presence of various phytoconstituents by following standard Phytochemicals screening tests. Hager's Test, Wagner's Test was done to confirm the presence of alkaloids. The presence of carbohydrates was idetentified with Fehling's Test and Benedict's test (Himel paudel Chetri et al 2008). Glycosides presence ware confirmed with Keller-Killiani Test and Borntrager's Test. Steroids presence was confirmed with Libermann-Burchard Test and Salkowski Test. Flavonoids and Saponins presence were confirmed with respect to following test NaOH test, NaCl test and foam test. Spot Test and Saponifiaction Test were done to confirm the fixed oils. Lead acetate test and Ferric Chloride Test used to confirm the presence of Tannins and Phenolic compounds. Finally presence of amino acids was confirmed with Ninhydrin Test and Million's Test.

2.5 Antimicrobial Assay

2.5.1 Collection of Microorganisms: The test microorganisms were obtained from the Doctor's Diagnostic Centre and K.A.P Vishwanatham Government Medical College, Tiruchirappalli. The following Bacterial and Fungal species were used to test the antimicrobial activity of plant extracts, *Escherichia coli, Pseudomonas Sp., Staphylococcus Sp. Aspergillus niger, Aspergillus*

*flavous,*and *Candida Sp*. The Muller Hinton Agar Medium was used for the determination of zone of inhibition (Antara sen et al 2012).

2.5.2 Agar diffusion method: The disk diffusion (Kirby-Baurer) technique, which is of the recommended standards of the National Committee for Clinical Laboratory Standards, was used for antimicrobial test. Venire caliber used to measure the zone of inhibition. Positive and negative controls were maintained.

2.5.6 HPLC analysis: The ethanolic extract of Leucas *indica* was analyzed in HPLC. It was done at characterization and measurement laboratory (Shimadzu- promience HPLC analysis), Central Electrochemical Research Institute, karaikudi, Tamilnadu. 20µl of sample was injected in to the Phenomenex C18 column.

3. RESULTS AND DISCUSSION

Plants were the potential source of the biological agents (Mahesh, 2008). Plants of genus *Leucas* is widely used in the traditional medicine to cure many diseases such as cough, cold, diarrhea. It includes around 80 species (Hedge IC, 2005). In this study, we used polar and non-polar solvents to extract the Phytochemicals present in the *Leucas indica*. The preliminary screening of the Phytochemicals reveals the presence of flavonoids, phenolic compounds, tannins, Saponins, carbohydrates and oil components in leafs (Chandrashekar, 2013). In this research work we use various part of the plant separately for the extraction of different types of the Phytochemicals. Leaf, stem and pseudoverticil parts, dry and fresh leaf of the plant contain flavonoids, phenolic compounds, tannins, and Saponins at notable level. (Table 1). The sugar components widely present in the root extracts. The presence of Phytochemicals differs along with the type of plant part and solvent used for the extraction. The solubility of the phytoconstituents depends on the type of the solvent used for the extractions. The presences of flavonoids and phenolic components were the reason of the antimicrobial activity of the plant.

3.1 Detection of Alkaloids: The presence of alkaloids is confirmed by the appearance of yellow color in Hager's Test and reddish colored precipitate in Wager's test. The water, ethanol and acetone solvents were extracting the alkaloids majorly.

3.1.2 Detection of Carbohydrates: The presences of Carbohydrates were confirmed by the formation of brick red precipitate in Fehling's test and Benedict's test indicates the presence of Carbohydrates. Water and ethanol are the efficient solvents for the extraction of carbohydrates.

3.1.3 Detection of Glycosides: Keller-Killiani and Borntrager's tests were used to analyze the presence of Glycosides. The formation of reddish brown color indicates the presence of Glycosides. Water and ethanol solvents are widely extracting the glycosidic components.

3.1.4 Detection of Steroids: The solvents chloroform, acetone, petroleum ether extracts the steroid components present in the plant. Their presence of steroid was confirmed by Libermann-Burchard and Salkowski Tests with the formation of bluish brown color and conversion of bluish red to cherry red colour respectively. The steroid presents only in the stem and leaf parts.

3.1.5 Detection of Flavonoids: The flavonoids components present in the plants were extracted by both polar and non polar solvents their presence was confirmed with the NaOH and NaCl Tests. The formation of yellow color indicates the presence of flavonoids.

3.1.6 Detection of Saponins: The presence of Saponins was identified by the foam test with the formation of stable foam layer. Acetone was not suitable for the extraction of Saponins present in the plant. From this it is identified that all parts of the *Leucas indica* contains the Saponins.

3.1.7 Detection of Fixed oils: During the screening of Phytochemicals with spot test and saponifiaction test, the oil stains and soap indicates the presence of fixed oils. The maximum amount of fixed oils is observed in root of *Leucas indica.*

3.1.8 Detection of Tannins and Phenolic compounds: The formation of white precipitates and greenish color indicates the presence of tannins and phenolic compounds by the Lead acetate and Ferric chloride tests respectively. The leaf part of *Leucas indica* has the tannins and phenolic components. But chloroform is not a suitable solvent for extracting phenolic compounds.

3.1.9 Detection of Proteins and Amino acids: The presence of proteins were identified by the Ninhydrin and Millions tests. In the Ninhydrin and Millions test blue color and yellow color precipitate indicates the presence of proteins respectively. Water and ethanol are the suitable solvents extracting proteins from *Leucas indica.*

3.2 Antimicrobial activity of Phytochemicals: Alkaloids, Flavonoids, Phenolic Compounds, Tannins, Saponins are present in the plant of *Leucas indica* was responsible for the function of antimicrobial activity.

3.2.1 Activity of Phytochemicals against *Escherichia coli*: The antibacterial activities of Phytochemicals were carried out by using Kirby-Baurer method. The inhibition zones were observed and measured by using vernier caliper. The stem, dry leaf and pseudoverticil extracts of *Leucas indica* had high antibacterial activity than its root and fresh leaf extracts. Here the *Escherichia coli* (22 mm) had sensitive to ethanolic extracts of pseudoverticil. (Fig 2.1)

3.2.2 Activity of Phytochemicals against *Pseudomonas Sp.*: *Pseudomonas Sp.* was more sensitive to the root and fresh leaf extract of *leucas indica.* The dry leaf and stem extract has moderate activity (10±2 mm) and the pseudoverticil part has low activity against the Pseudomonas Species (Fig 2.2)

3.2.3 Activity of Phytochemicals against *Staphylococcus aureus*: The pseudoverticil extract has high activity (25±2) against *Staphylococcus aureus* than any other parts of the *Leucas indica*. The stem and fresh leaf extract has moderate (17±2) effect to that *Staphylococcus aureus*. The root extracts show no activity against *Staphylococcus aureus.* (Fig 2.3)

3.2.4 Activity of Phytochemicals against fungal species: The fungal strain *Aspergillus niger* and *Aspergillus flavus* was resistant to the plant extracts. The dry leaf and pseudoverticil extract has moderate activity against *Candida Sp.* The presence

fixed oil components in the plant act as the antifungal agent (Misra et al 1994). The stem and root extract has less activity. Since fresh leaf has no activity against *Candida Sp*. Because the fresh leaf extract does not contains oils. (Fig 2.4)

Table.1.Phytochemicals Screening

Parts	Solvent	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Root	Water	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+
	Ethanol	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+
	Chloroform	-	-	+	-	-	-	+	+	-	-	+	+	+	-	-	-	+
	Acetone	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-
	Petroleum Ether	-	-	+	-	-	-	-	-	+	-	+	+	-	-	-	-	-
Stem	Water	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+
	Ethanol	+	+	+	+	-	-	-	-	+	+	+	-	-	+	+	-	+
	Chloroform	-	-	+	+	-	-	+	+	-	-	+	+	+	-	-	-	-
	Acetone	+	+	+	-	-	-	+	+	-	-	-	+	-	+	+	-	-
	Petroleum Ether	-	-	+	-	-	-	+	+	+	-	+	-	-	-	-	-	-
Pseudo verticil	Water	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	-
	Ethanol	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+
	Chloroform	-	-	+	+	-	-	+	+	+	-	+	+	+	-	-	-	-
	Acetone	+	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-
	Petroleum Ether	-	-	-	-	-	-	+	-	+	+	+	-	-	+	-	-	-
Dry leaf	Water	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+
	Ethanol	+	+	+	+	+	+	-	+	+	-	+	-	-	+	+	+	+
	Chloroform	-	-	+	+	-	+	+	+	-	-	+	+	+	+	-	-	+
	Acetone	+	+	+	+	-	+	-	+	+	-	-	-	-	+	+	-	-
	Petroleum Ether	-	-	+	-	-	-	+	+	+	-	+	+	+	+	+	-	-
Fresh leaf	Water	+	+	+	+	-	-	-	-	+	+	+	-	-	+	+	+	-
	Ethanol	+	+	+	+	-	-	+	+	+	-	+	-	-	+	+	-	-
	Chloroform	-	-	+	-	-	-	+	+	-	+	+	-	-	+	-	-	-
	Acetone	+	+	+	+	-	-	-	+	+	-	-	+	-	+	+	-	-
	Petroleum Ether	-	-	+	-	+	+	+	-	+	-	+	-	-	+	-	-	+

Alkaloids – A : Hager's Test, B: Wagner's Test; Carbohydrates – C : Fehling's Test, D – Benedict's Test; Glycosides – E : Borntrager's Test, F : Keller-Killiani Test; Steroids – G : Salkowski Test, H – Libermann Burchard; Flavonoids – I : NaOH Test, J – NaCl Test; Saponins – K : Foam Test; Fixed Oils – L : Saponification Test, M : Spot Test; Tannins and Phenolic compounds – N : Lead Acetate Test, O-Ferric chloride Test; Proteins and Amino Acids – P : Ninhydrin Test, Q – Million's Test.

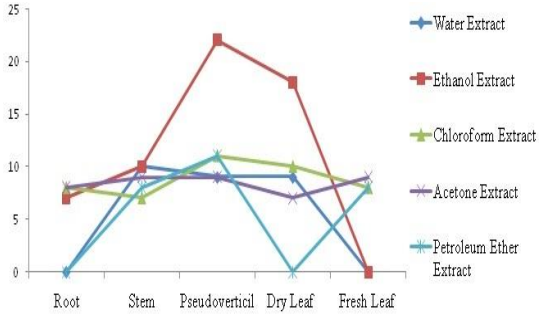

Figure.2.1.Inhibition zone for Escherichia Coli

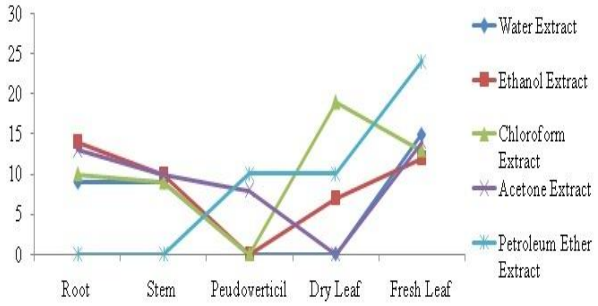

Figure.2.2.Zone of Inhibition for *Pseudomonas Spp*

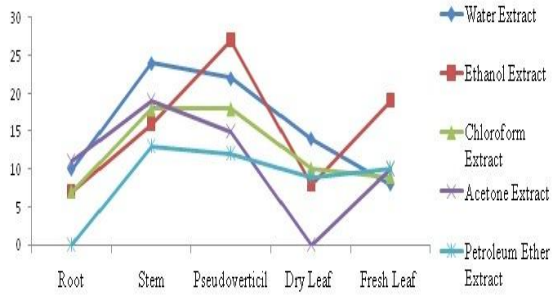

Figure.2.3.Inhibition zone (mm) for *Staphylococcus aureu*

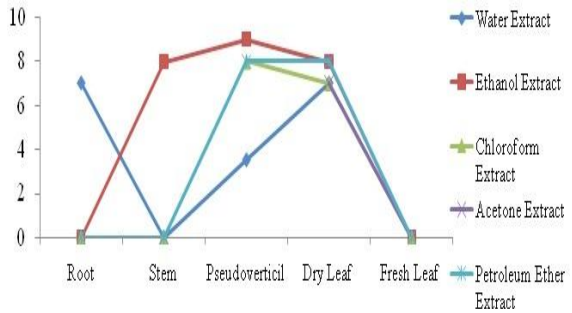

Figure.2.4.Inhibition zone (mm) for *Candida Sp*.

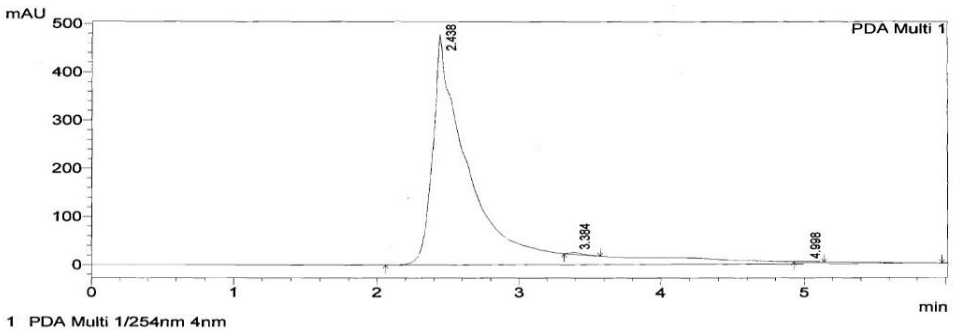

Figure.3.1.HPLC chromatogram of ethanolic extract of *Leucas indica* – Dry leaf

3.3 HPLC analysis

The ethanolic extract of *Leucas indica* was analyzed with HPLC, because the ethanolic extract gives the good zone of inhibition in the antimicrobial assays. From the standard graphs it was found that the peak value of 2.4 associate with the flavonoids. Extract of dry leaf gives the single peak. (Fig 3.1).

4. CONCLUSION

From this work we conclude that ethanol and water are the best solvents for the extractions of Phytochemicals. The HPLC analysis gives the idea that the flavonoids are present in higher concentration and produces the peak at 2.4.The presence of flavonoids, phenolic compounds, tannins, Saponins are responsible for the antimicrobial activity. The pseudoverticil part and dry leaf extract has the high activity against the bacterial strains like *Staphylococcus aureus* and *Pseudomonas Sp. Leucas indica* has a wide range of antimicrobial activity and it's useful in the treatment of infections caused by bacteria. We suggest that further investigation could be done with this plant for the isolation of novel components which may have the potential biological activities.

REFERENCES

Amar jyoti Das, A study of antibacterial activity of ethanolic extracts and aqueous extracts of *Leucas longifolia* Leaves against *Eschreichia coli*, International research journal of pharmacy, 3(1), 2012, 130-131.

Antara sen, Amla Batra, Evauation of Antimicrobial activity of different solvent extracts of medicinal plant: *Melia Azedarach L*. International Journal of Current Pharmaceutical Research,4(2), 2011, 67-73.

Basker S, Murugesan S, Narmatha Bai V, Antibacterial efficacy and phytochemical analysis of an Endemic orchid *Coelogyne Stricta*, International journal of Medico biological Research, 1(6), 2012, 305-309.

Chandrashekar R, and Rav S N, Analysis of ethanolic extract of leaves of Leucas indica(EELLI).International Journal of pharma and Bioscience 4(1) 2013 33-38

Darsan B Menon, Sasikumar J M, Latha K, Phytochemical analysis and antioxidant activity of methanolic extract of *Plectranthus hadiensis* (Forssk.) Schweinf. Ex Spreng. Aerial parts. Indian Journal of Natural Products and Resources, 3(3), 2005, 359-365.

Elagayar A, Draughon F A, Antimicrobial activity of essential oils from plants against some selected pathogens and saprophytic microorganisms. Journal of Food Protection, 64(7), 2001, 1019-1024.

Elizabeth K M, Antimicrobial activity of *Terminalia belerica* Indian journal of clinical Biochemistry, 20(2), 2005, 150-153.

Fasoyiro S B, Adegoke G O, Phytochemical characterization and the antimicrobial Property of *Aframomumdanielli*Extract, African Journal of Agricultural Research, 2(3), 2007, 76-79.

Himel paudel Chetri, Nishav Shreshthayogol, Phytochemical and antimicrobial Evaluations of some Medicinal Plants of Nepal, Kathmandu University Journal of Science, Engineering and Technology, 1, 2008, 49-54.

Jaishree Jasutkar , Koche K, Preliminary Phytochemistry and Bacterial activity of *Leucas aspera*, International Journal of pharmacy and biological sciences, 3(1), 2013, 76-78.

James Hamuel Doughari, Phytochemicals:Extraction Methods, Basic Structures and mode of action as potential Chemotherapeutic Agents',Federal University of Technology, 2009, 1-26.
Jigna Parkesh, Sumitra Chanda V, *In vitro* antimicrobial activity and phytochemical analysis of some Indian medical plants. Turk J boil, 31, 2007, 53-58.
Koche DK, Syed Imran, Phytochemical screening of eight ethanomedical plants from Akola District (MS), International Journal of Pharma and Bio science, 4, 2010, 256-260.
Mahesh B, Satish S, Antimicrobial activity of some Important Medicinal Plants against Plant and Human Pathogens, World Journal of Agricultural Sciences, 4, 2008, 839-843.
Mishra AK, Dubey, Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities, Application of Environmental Microbiology, 60, 1977, 1101-1105.
Mogana R, Khoo Teng-Jin, In Vitro antimicrobial, Antioxidant activities and phytochemical Analysis of *Canarium ptentinervium* Miq., From Malaysia, Biotechnology Research international, Article ID 768673, 2011, 1-5.
Pathak P, Saraswathy, *In vitro* Antimicrobial activity and Phytohemical analysis of the leaves of *Annona muricata*. Internatonal Journal of Pharmaceutical Research & Development, 2, 2010, 1-6.
Prashant Tiwari, Bimlesh Kumar, Phytochemical Screening and Extraction: A Review. International Pharmaceutical Sciencia, 1(1), 2011, 98-106.
Saroj Kothari, Vaibhav Mishra, Antimicrobial activity and phytochemical screening of serial extracts from leaves of *Aeglie Marmelos* (linn.) Acta Poloniae, Pharmaceutical Drug Research, 68(5), 2011, 687-692.
Umamahewari, Shreevidhya, In vitro antibacterial activity of *Bouganivillea spectabilis* Leaves Extracts. Advances in Biological Research, 2(1-2), 2008, 1-5.