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PHYTOCHEMICAL SCREENING AND *IN-VITRO* PHARMACOLOGICAL EVALUATION OF *Leucas aspera* LEAVES

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ABSTRACT:

Aromatic herbs and herbal treatments have been essential components of traditional medical practices. These plants are valued for their natural substances, called phytochemicals or secondary metabolites, which are less expensive, more effective, and have less adverse effects. Plants consist of different organelles, tissues, and cells, each of which produces a different medicine. *Leucas aspera*, a member of the Lamiaceae family, is often known as Thumbai. Its leaves have insecticidal properties and are used as an anti-arthritic. Based on the studies that are currently accessible, the current study describes the phytochemistry and biological properties of *Leucas aspera*. *L.aspera* leaves aqueous extract contains alkaloids, flavonoids, tannins, steroids, proteins, phenols and carbohydrates. These phytoconstituents are responsible for the antibacterial, antioxidant, anti inflammatory and anticancer activity of the *L.aspera* aqueous extract.

KEYWORDS: Anticancer activity, Anti-arthritic, Pharmacological properties, Phytochemicals, *Leucas aspera*, Thumbai.

I. INTRODUCTION

Herbal plants are essential for traditional medicine, and even today, the majority of people living in both rural and urban areas use these plants for a varieties of purpose .Because of their advantages for the environment, economy, and health, natural chemicals are currently the subject of more research than manufactured ones. Phytochemicals are the various chemical substances that plants manufacture for their biological needs, such as defence mechanisms against insects, bacteria, and herbivorous animals. Herbal plants are widely employed in the cosmetic and pharmaceutical industries and are a natural source of numerous significant phytochemicals (Khare *et al*., 2007).

Leucas aspera is a member of the Lamiaceae family is found all over India, which is also referred to as "Thumbai" in Tamil (Rai *et al*., 2005). *L. aspera* is distributed throughout India, primarily in the Himalayan region and it is an aromatic herb found in temperate and tropical regions of Asia and Africa. It grows in fallow areas, highland agriculture fields, and other places as an annual herb and competitive weed. *L.aspera* has also been shown to contain steroids, triterpenes, phenols, flavonoids, tannins, and long-chain aliphatic compounds (Mominul Islam *et al.*, 2014) and (Prajapati *et al.*, 2010). The leaf juices are thought to be a treatment for chronic rheumatism, psoriasis, and other chronic skin eruptions (Rai *et al*., 2005). In snake bite cases, bruised leaves are applied externally. The leaves are also utilized as insecticides and mosquito repellents (Reddy *et al*., 1993). The plant extract was administered with honey to treat dyspepsia and stomach pain (Das *et al*.,

2011). The entire plant is traditionally consumed orally for analgesic, antipyretic, and anti-rheumatic effects. Hence, the present study aims to show the potential antibacterial, antioxidant, anti-inflammatory and anticancer activity of *Leucas aspera* aqueous extract.

II. MATERIALS AND METHODS

2.1. Collection of plant material and preparation of *Leucas aspera* aqueous leaf extract:

The *Leucas aspera* leaves were collected from different localities of Coimbatore District, Tamil Nadu. The collected leaves were shade dried and powdered. Then, an aqueous extract was made by boiling 10g of leaf powder in 200ml of distilled water at 60ºC for 1hour in a water bath. Then, the extract was filtered through Whatmann No.1 filter paper and it was used for further analysis.

2.2. Phytochemical screening of *Leucas aspera* aqueous extract:

Qualitative phytochemical screening of aqueous extract of *Leucas aspera* was carried out to identify the presence of secondary metabolites including alkaloids, steroids, glycosides, phenols, tannins, carbohydrate, flavonoids, amino-acids, terpenoids and proteins as per the standard methods (Prashant *et al*.,2011).

2.3. GC-MS analysis:

AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) device comprised the GC Clarus 500 Perkin Elmer system, which was used for the GC-MS analysis of the aqueous extract of *L.aspera* under the following circumstances: A split ratio of 10:1 was used to inject 0.5 µl of helium (99.999%) at a continuous flow rate of 1 ml/min onto a Column Elite-1 fused silica capillary column (30 mm ×0.25 mm I.D ×1 µ M df, 100% Dimethyl poly siloxane), which was running in electron impact mode at 70 eV. 250 °C for the injector and 280 °C for the ion source. Starting at 110 °C (isothermal for 2 minutes), the oven temperature was designed to increase by 10 °C/min to 200 °C, then by 5 °C/min to 280 °C, culminating in a 9-minute isothermal at 280 °C.

2.4. Antibacterial activity of aqueous extract of *Leucas aspera*:

Leucas aspera's antibacterial activity was evaluated using the disc diffusion method against *Escherichia coli, Bacillus subtilis, Bacillus cereus* and *Pseudomonas aeruginosa*. On Muller-Hington Agar, the test organisms were dispersed. After punching, blank Whatmann filter paper (No. 1) discs with 6 mm diameter, they were sterilized in a hot air oven at 160°C for an hour. Next, sterile filter paper discs were impregnated with a 10% DMSO-prepared *Leucas aspera* aqueous extract at several concentrations (1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, and 5 mg/ml). The discs were allowed to dry under vacuum to get rid of any leftover solvent that might have affected the outcome of the experiment. Using sterile forceps, the extract-impregnated discs were then positioned on the inoculated agar medium. Tests were run twice using Tetracycline as the standard drug. After that, the plates were incubated for 24 hours at 37°C. Following the incubation time, the average of the inhibition zones in millimeters was used to assess the antibacterial activity.

2.5. Antioxidant activity of aqueous extract of *Leucas aspera*:

100µl of different plant extract concentrations (1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, and 5 mg/ml) were produced in methanol, and each test tube was filled with 1 ml of DPPH and 3 ml of methanol. For twenty minutes, the reaction mixture was incubated at room temperature under dark conditions. The mixture's absorbance was measured at 517 nm after 20 minutes. 1ml DPPH and 3ml methanol in a tube was used as the control. Ascorbic acid was used as the standard.

2.6. Anti-inflammatory activity of aqueous extract of *Leucas aspera*:

The test samples were divided into 50µl of varying concentrations of 0.5 mg/ml, 1 mg/ml, 1.5 mg/ml, 2 mg/ml, and 2.5 mg/ml, while the reference drug, diclofenac sodium, was divided into 50µl of varying concentrations of 0.5 mg/ml, 1 mg/ml, 1.5 mg/ml, 2 mg/ml, and 2.5 mg/ml, respectively. To all the tubes 0.45ml of BAS (0.5% w/V) was added. 50µl of water and 0.45 ml of BSA are used as the control. For 20 minutes, the samples were incubated at 37°C. After that, the temperature was raised to 57°C for three minutes. Add 2.5 ml of phosphate buffer to the solutions mentioned above once they have cooled. At 255 nm, the absorbance was measured with a UV-visible spectrophotometer. Diclofenac was used as positive control.

2.7. Anticancer activity of aqueous extract of *Leucas aspera:*

HeLa viable cells were harvested and counted using hemocytometer diluted in DMEM medium to a density of 1 × 104 cells/ml was seeded in 96 well plates for each well and incubated for 24 h to allow attachment. After that, HeLa cells were treated with control and different concentrations of *Leucas aspera* (10 to 35µg/ml). HeLa cells were incubated at 37°C in a humidified 95% air and 5% CO2 incubator for 24 h. After incubation, the drug-containing cells were washed with fresh culture medium and the MTT (5 mg/ml in PBS) dye was added to each well, followed by incubation for another 4 hrs at 37°C. The purple precipitated formazan was dissolved in 100µl of concentrated DMSO and the cell viability was absorbanced and measured at 540nm using a multi-well plate reader. The results were expressed in the percentage of stable cells with respect to the control. The half maximal inhibitory concentration (IC50) values were calculated and the optimum doses were analysed at different time period.

III. RESULTS AND DISCUSSION

3.1. Collection of plant material:

The *Leucas aspera* leaves were collected from different localities of Coimbatore District, Tamil Nadu and authenticated by the Botanical Survey of India (BSI) in "Tamil Nadu Agriculture University" Coimbatore, Tamil Nadu, India. A voucher specimen (No.: BSI/SRC/5/23/2023/Tech/828) has been deposited at the Herbarium of the Botany department of "Tamil Nadu Agriculture University" for future reference.

3.2. Preparation of aqueous extract of *Leucas aspera*:

The percentage yield of aqueous extract of *Leucas aspera* was determined, their percentage of crude extracts was calculated as 75% and results are shown in Figure 1.

Leaf powder of *Leucas aspera*

Aqueous extract of *Leucas aspera*

Figure 1: Aqueous extract of *Leucas aspera*

3.3. Preliminary phytochemical screening of *L.aspera* leaves aqueous extract:

Phytochemicals are responsible for the pharmacological activities. Evaluation of phytonutrients of *Leucas aspera*, which in turn provide useful tools to determine their role as therapeutic agents. The qualitative phytochemical screening of the plant extracts was shown in the Table 1. It showed the presence of alkaloids, carbohydrate, steroids, proteins, phenols, flavonoids and tannins, whereas terpenoids, amino acids and cardiac glycosides were absent.

The beneficial effects of plant materials typically result from the combination of secondary metabolites present in the plant. In plants, these compounds are mostly metabolites such as alkaloids, flavonoids, glycosides, phenol, proteins, steroids, saponins, resins, gums and tannins (Prashant *et al*.,2011).

Table 1:Preliminary phytochemical screening of *Leucas aspera* extract

Phytochemical compounds	**Aqueous extract of *Leucas aspera***
Alkaloids	+
Flavonoids	+
Tannins	+
Steroids	+
Proteins	+

Phenols	+
Carbohydrates	+
Amino acid	-
Terpenoids	-
Cardiac glycosides	-

3.4. GC-MS Analysis of *Leucas aspera* leaves aqueous extract:

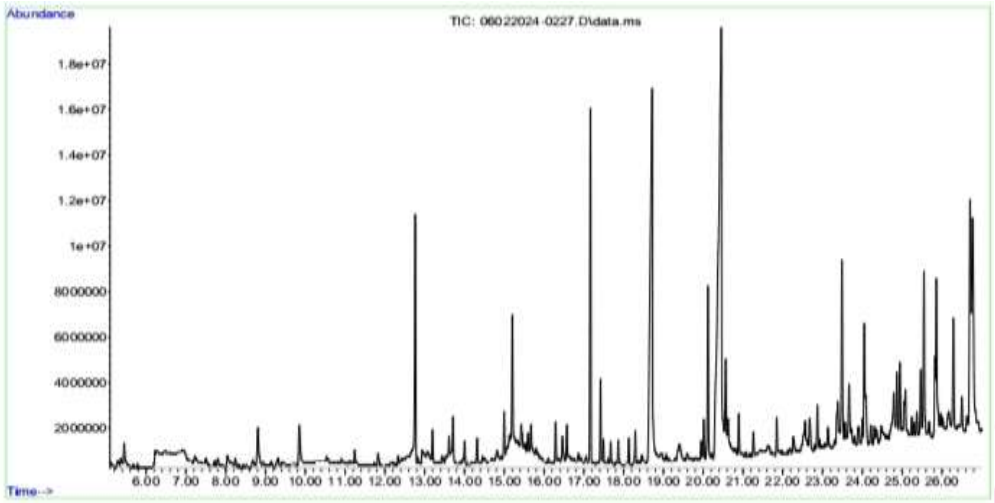

Fig. 2: GC-MS Analysis for the aqueous extract of *Leucas aspera*

The composition and identification of the main components present in the leaves of *Leucas aspera* were found to be 42 compounds which was analysed through GC-MS. The main constituents present in the leaves are n- hexadecenoic acid (14.10%), sitosterol (6.52%) cycloisolongifolene (5.48%), methyl ester, alanine (4.69%), caryophyllene (4.26%), hexadecanoic acid and ethyl ester (3.11%), squalene and supraene (3.01%), phytol (2.85%), 4-(2-Acetoxyethyl) phenyl acetate (2.75%), metacetamol (2.04%) and eicosane, octadecane and eicosane (1.94%). From figure 2, the results showed the presence of many phytochemical constituents with different percentage.

3.5. Antibacterial activity of *Leucas aspera* leaves aqueous extract:

The antibacterial activity of the *Leucas aspera* was screened against *Escherichia coli, Bacillus subtilis, Bacillus cereus* and *Pseudomonas aeruginosa* and was shown in Figure 3. The antibacterial activity of aqueous extract of *Leucas aspera* was assessed by their zone of inhibition values which revealed that the plant showed significant antibacterial activity against *Escherichia coli* at 5mg/ml concentration (20mm), whereas *Bacillus subtilis, Bacillus cereus* and *Pseudomonas aeruginosa* were resistant against the aqueous extract of *Leucas aspera*. The tetracycline showed the zone of inhibition of (30mm). Hence, the *Leucas aspera* leaves aqueous extract were found to be as effective as the tetracycline. So, it can be used as an antibacterial agent.

Strong antibacterial action against *E. Coli, Klebsiella, Pseudomonas* and *Staphylococcus aureus* was also demonstrated by methanolic extract of leaves of *L.aspera* (Taharee *et al*.,2016).

Escherichia coli

Fig.3: Antibacterial activity of aqueous extract of *Leucas aspera* against *E.coli*

3.6. Antioxidant activity of *Leucas aspera* leaves aqueous extract:

The percentage radical scavenging activity and the degree of discoloration of free radicals by *Leucas aspera* were determined using DPPH. From the results, the aqueous extract of *Leucas aspera* showed highest radical scavenging activity at 4mg (76.88%. The ascorbic acid showed the percentage of inhibition of 85.35%. Hence, the *Leucas aspera* aqueous extract were found to be effective as the ascorbic acid. So, it can be used as a natural antioxidant activity.

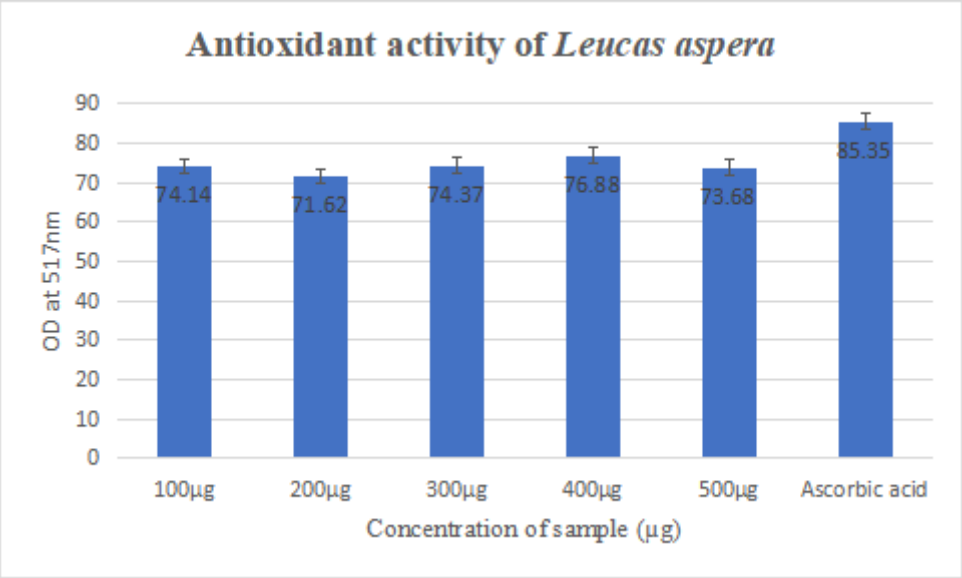

Fig.4: Antioxidant activity of *Leucas aspera* leaves aqueous extract:

3.7. Anti-inflammatory activity of *Leucas aspera* aqueous extract:

The anti-inflammatory activity was performed in aqueous extract of the *Leucas aspera*. From the results, it was observed that 2.5mg/ml concentration of sample showed the significant activity of 86.5%. The reference drug showed the percentage inhibition of 89.91%. Hence, the *Leucas aspera* leaves aqueous extract were found to be as effective as the reference drug. So, it can be used as a natural anti-inflammatory agent.Anti-inflammatory activity of several aerial parts of *L. aspera* has been accounted (Srinivas *et al*.,2000).

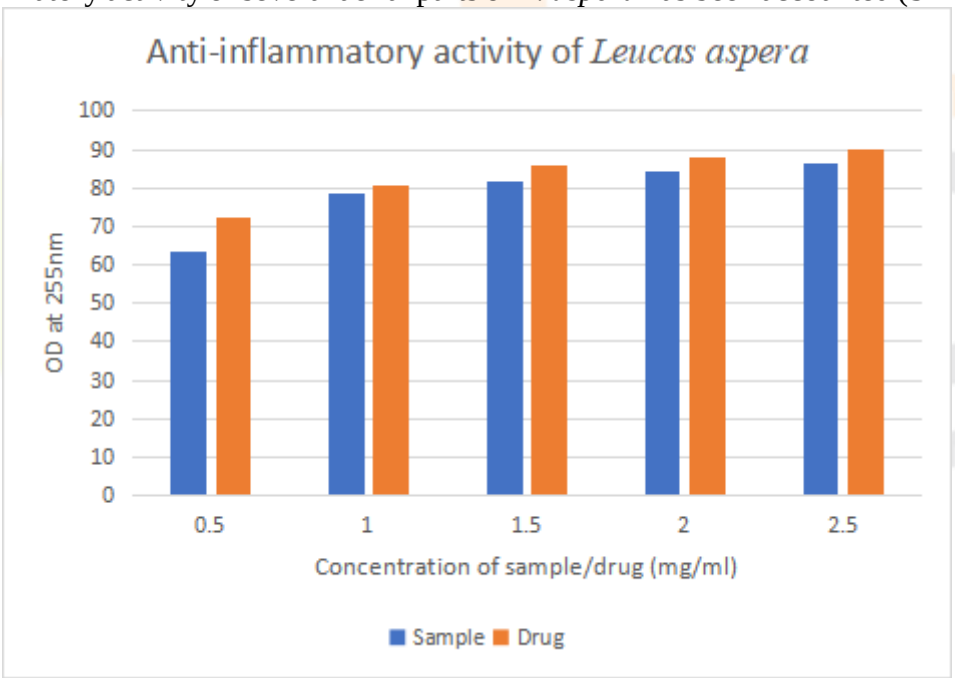

Fig.5: Anti-inflammatory activity of *Leucas aspera* aqueous extract

3.8. Anticancer activity of *Leucas aspera* aqueous extract:

Inhibitory effect of the aqueous extract of *Leucas aspera* leaves have been tested *in vitro* against Human cervical cancer (HeLA)cell line by using colorimetric MTT assay. Figure 4 showed the *in vitro* cytotoxicity activity of compounds against selected cancer cells. After treatment with various concentrations of leaf extract

(10µg, 15µg, 20µg, 25µg, 30 µg and 35µg) the parameters like cell viability, growth and morphological changes of the cell lines were noted. The experimental results demonstrated that the compound has the ability to inhibit cell proliferation in a dose dependent manner. From Figure 8, the IC_{50} values of compounds against cervical cancer cells was calculated as 24µg/ml. From the results, it is observed that when the concentration increases the cell viability decreases. The drug which having lesser IC_{50} value is more potential. The IC50 values were determined from the *Leucas aspera* dose responsive curve where inhibition of 50% cytotoxicity compared to control cells. The anticancer activity against HeLA (Human cervical cancer cell line) cells was analysed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterttrazolium bromide (MTT) assay. Data were collected from triplicate experiments and the percentage of *Leucas aspera* extract induced cell growth inhibition was determined by comparing with DMSO treated control cells. Hence, the *Leucas aspera* leaves aqueous extract were found to be effective. So, it can be used as a natural anticancer agent. In the same way, the maximum inhibition of cell growth was observed at the concentration of 200 µg/ml of *Leucas aspera* extract. The plant extract has potent to decrease the viability of HeLA cells in a dose – dependent manner (Mahimaidoss *et al.,*2014).

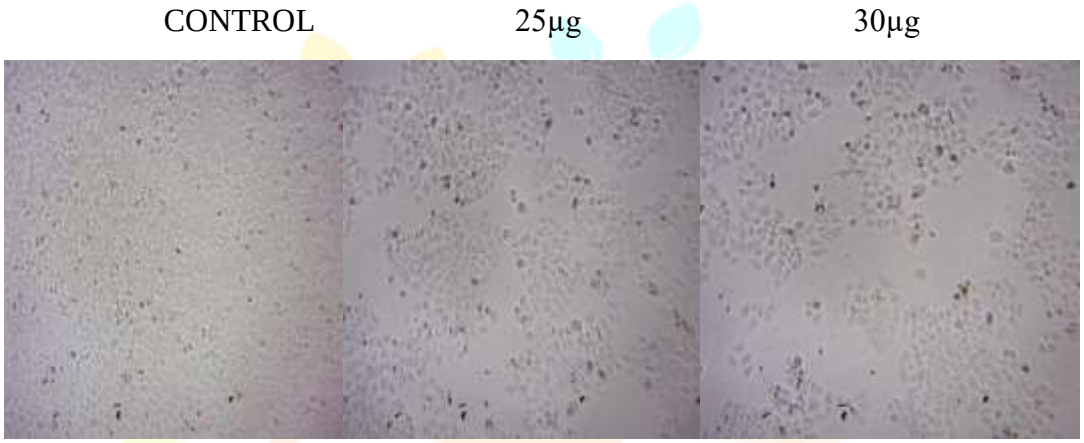

Figure 6: Anticancer activity of aqueous extract of *Leucas aspera* leaves

Table 2: Anticancer activity of aqueous extract of Leucas aspera leaves

La-HeLa cells	Control	10µg	15µg	20µg	25µg	30µg	35µg
	100	95.76732	85.71429	70.37037	48.67725	44.97354	33.33333
	100	94.17989	81.48148	72.48677	51.85185	46.03175	34.92063
	100	97.8836	83.06878	75.66138	49.73545	41.26984	35.97884
Average	100	95.94356	83.42152	72.83951	50.08818	44.09171	34.74427
SD	0	1.858143	2.138338	2.663084	1.616427	2.500433	1.331544

IV. CONCLUSION

Traditional method of treating many diseases through plant extracts plays an important role across the world. *Leucas aspera* consists of many valuable compounds in which it can be treated many diseases. The secondary metabolites such as flavonoids, lignans, glycoside, phenolic compounds, sterols and terpenes exhibit large quantity of therapeutic values. These phytochemicals which were present in the plant *Leucas aspera* play an important role in therapeutic activity. The antibacterial activity of aqueous leaf extracts of *Leucas aspera* were performed against four bacterial species such as *Escherichia coli, Bacillus subtilis, Bacillus cereus* and *Pseudomonas aeruginosa.* Maximum zone of inhibition was observed against *Escherichia coli* at 5mg/ml concentration (20mm). Antioxidant activity of aqueous extract of *Leucas aspera* were evaluated by DPPH assay, 4mg/ml concentration showed 76.88% of radical scavenging activity. Anti-inflammatory activity was determined by inhibition of protein denaturation assay. The protein inhibitory activity of the aqueous extract was estimated at 2.5mg/ml concentration and the results showed 86.5% inhibition. In this study, the effect of *Leucas aspera* response against HeLa cell line by using colorimetric MTT assay was determined. According to all the results obtained from this study, the aqueous extract of

Leucas aspera showed higher biological activities. Therefore, further research is needed to search for bioactive constituents and other health-promoting activities.

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