IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 2, February 2024

In-Vitro Antifungal Activity of Leaf extract of Leucas Aspera and there Uses Pharmaceutical Preparation

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Abstract: *The aim of the present study is to probe the antimicrobial activities of methanol leaf extract of Leucas sp. Antifungal susceptibilities of clinically isolated dermatophytes to methanol extracts of Leucas Aspera and Leucas zeylanica leaves were performed using agar well Diffusion method. The result obtained shows that all the extracts expressed Remarkable antifungal activity with zone of inhibition ranging from 5 to 10mm. Maximum inhibition zone was recorded with Penicillium sp. (10 mm) while minimum inhibition zone of was recorded for Candida tropicalis (3 mm). From the study it is evident that Leucas aspera and Leucas Zeylanica possess potential antidermatophytic activity and further study on these plants may lead to explore novel bioactive compounds.In this study, we investigated and compared the total phenolic, alkaloid Content and in-vitro antioxidant activity of Leucas aspera collected from four Different regions; Tirupathi (Southern zone), Lam (Krishna river region), Jagityala (Northern Telangana) and Hyderabad (Southern Telangana) of Andhra Pradesh, India. Quantitative regional variation was observed in total Phenolic content, and alkaloid content in methanolic extracts of Leucas Aspera from above four regions of Andhra Pradesh. Concentration Dependent antioxidant activity was observed for all these extracts and also Observed regional variation for scavenging of Superoxide, Hydroxyl and DPPH Radicals. Among the four regions, Leucas aspera from Jagityala region Contains more phenolic content (48.06±0.4µg/100 µg), Tirupathi region Contains good alkaloid content (58.6±0.1µg/mg) and Hyderabad zone Showed better free radical scavenging activity (IC50 value for superoxide Radical 156.34µg, Hydroxyl radical 122.34µg and DPPH radical 57.12µg Respectively).*

Keywords: Leucas zeylanica

I. INTRODUCTION

In recent years some microorganisms such as bacteria virus and fungi was shows resistance to many types of antibiotics due to viable use of antibiotics can creates obstacles in treatment of many disease.To overcome resistance scientist was started search for new Antimicrobial agent from plant source.

Leucas aspera (Willd.) Linn.

(Family: Lamiaceae) commonly known as 'Thumbai'

[1]Is distributed throughout India from the Himalayas down to Ceylon.

[2] The plant is used Traditionally as an antipyretic and insecticide.

[3]Flowers are valued as stimulant, expectorant, Aperient, diaphoretic, insecticide and Emmenagogue.

[4]Leaves are considered useful In chronic rheumatism, psoriasis and other Chronic skin eruptions. Bruised leaves are Applied locally in snake bites.

VERNACULAR NAMES :

Sanskrit: Dronapushpi, Chitrapathrika, Chitrakshup

Punjabi: Guldor

Bengali: Arunachala, Hulkasha

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Gujarati: Kulnnphul
Hindi: Goma madhupati

Leucas aspera Willd. (Family: Lamiaceae), known in Tamil as Thumbai, in Hindi as Chhota halkusa, in Telgu as Ummachettu, and in Kannada as Tumbe guda, is an annual,Branched, herb erecting to a height of 15–60 cm with stout And hispid acutely quadrangular stem and branches. Its Leaves are sub-sessile or shortly petiolate, linear or linearly Lanceolate, obtuse, pubescent up to 8.0 cm long and 1.25 cm broad, with entire or crenate margin; petiole 2.5–6 mm long; flowers are white, sessile small, in dense Terminal or axillary whorls; bracts 6 mm long, linear, acute,Bristle-tipped, ciliate with long slender hairs; calyx variable, Tubular, 8–13 mm long; tube curved, contracted above the Nutlets, the lower half usually glabrous and membranous,The upper half ribbed and hispid; mouth small, very oblique,Not villous, the upper part produced forward; teeth small,Triangular, bristle-tipped, ciliate, the upper tooth being the Largest. Its corolla is 1 cm long; tube is 5 mm long and Pubescent above, annulate in the middle; upper lip 3 mm Long, densely white woolly; lower lip about twice as long, The middle lobe obviate, rounded, the lateral lobes small,Subacute. The fruit nutlets are 2.5 mm long, oblong, brown,Smooth, inner face angular and its outer face is rounded(Kirtikar and Basu 1975; Hooker 1984).L. aspera is a small herbaceous erect plant with a free Blooming nature and flowering in the months of August to September. It is pungently aromatic and commonly used as An antipyretic herb in South India. The juice from the leaves Is used as an external application for psoriasis and painful Swellings. The flowers are given with honey to treat coughs And cold in children. The leaves are useful for the treatment of chronic rheumatism. Bruised leaves are applied to the Bites of serpents, poisonous insects, and scorpion sting. The plant extract with honey is a good remedy for Stomach pain and indigestion. L. aspera leaves are used As an insecticide and mosquito repellent in rural areas. The preliminary chemical examination of L. aspera Revealed the presence of triterpenoids in the entire plant (Kamat and Singh 1994). The whole plant is reported toContain oleanolic acid, ursolic acid, and 3-sitosterol (Chaudhury and Ghosh 1969). Aerial parts are reported to contai Nicotine (Mangathayaru et al. 2006), sterols (Khaleque et al. 1970), two new alkaloids (compound A map. 61-2°, α-Sitosterol, and β-sitosterol) (m.p. 183–4o), reducing sugars (galactose), glucoside (230-1o) (Chatterjee and Majumdar1969), diterpenes [leucasperones A and B, leucasperols A And B, isopimarane glycosides (leucasperosides A, B, and C)] Together with other compounds like asperphenamate, maslinic Acid, (−)-isololiolide, linifolioside (Sadhu et al. 2006),Nectandrin B, meso-dihydroguaiaretic acid, macelignan,Acacetin, apigenin 7-O-[6′-O-(p-columbary)-3-D-glucoside],Chrysoeriol, apigenin, erythro-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)propan-1-ol, myristargenol B, and machilin C, (−)-chicanine, (7R,8R)-, and(75,85)-icariin A (Sadhu et al. 2003). Among the 25 Compounds identified from the leaf volatiles, u-farnesene (26.4%), x-thujene (12.6%), and menthol (11.3%) were the Major constituents. The flower is reported to contain ten Compounds, among them amyl propionate (15.2%) and Isoamyl propionate (14.4%) were dominant (KalachaveeduEt al. 2006)

II. MATERIALS AND METHODS

Drugs and chemicals

The following drugs and chemicals were used in the current study: morphine sulfate, diclofenac sodium (Square Pharmaceuticals Ltd., Bangladesh), naloxone (Hamein Pharmaceuticals GmbH), acetic acid, methanol, formalin, methylene blue, L-glutamic acid, cinnamaldehyde (Merck, Germany), glibenclemide (Square Pharmaceuticals Ltd., Bangladesh). All other chemicals and reagents were of analytical grade and high purity.

Plant material and extraction

Leucas aspera leaves were collected from Padma Garden, Rajshahi, Bangladesh and were authenticated by Principal Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. The voucher specimen (“DACB: 38390”) was deposited to the herbarium for further use. The leave was dried at room temperature for 5 days. The sample was ground into the fine powder and mixed with methanol for 7 days. Then, the solvent was removed by rotary evaporator to collect the extract (9.80g extract; yield 3.92% w/w) to be used for further studies.

Animals

Swiss albino mice (20 - 25g) purchased from Pharmacology Laboratory, Jahangirnagar University, Savar, Dhaka, Bangladesh were kept under standard environmental conditions at 25 ± 2°C, 55 - 65% relative humidity with 12h light/dark cycle. The study protocol was approved by the Institutional Animal, Medical Ethics, Biosafety and

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Biosecurity Committee (SUB/IAEC/17.01) of Stamford University Bangladesh. The animal experiments were conducted following the rules for animal experiment approved by the Institutional Animal Ethical Committee.

Acute toxicity test

For acute toxicity test, 30 mice were divided into 6 groups (n = 5). The test groups received L. aspera leaf extract orally at doses of 100, 200, 500, 1,000, 2,000, and 4,000 mg/kg body weight; while the control group received deionized water (0.1 mL/mouse, p.o.). Then, the animals were observed continuously for the first 4h for any behavioral changes and were kept under watching up to 14 days to find out the mortality, if any .

Antinociceptive activity

Hot plate test

The hot-plate test was employed to measure antinociceptive activity as described by Woolfe and McDonald . The response in the form of jumping, paw withdrawal, or licking was defined as hot plate latency. Each animal was placed on hot plate kept at 52 ± 0.5°C.

Tail immersion test

The tail immersion test was performed by Cha., et al. [27] method to evaluate the antinociceptive activity of L. aspera. 25 mice were Divided into 5 groups (n = 5). The two-thirds of animals tail was immersed in hot water at a temperature of 54 ± 1°C. The reaction time Was recorded with a stopwatch at 0, 30, 60, 90 and 120 minutes of extract administration (250, 500, and 750 mg/kg, p.o.), control (0.1 ml/Mouse, p.o.) or morphine sulphate (5 mg/kg, i.p.). To avoid tissue damage, the cut-off time was set at 20 s. The results were calculated as A percentage of the maximal possible effect (%MPE), calculated by the formula:

$$\% \text{ MPE} = [(\text{Post drug latency} - \text{pre drug latency})/(\text{Cut off period} - \text{pre drug latency})] \times 100$$

Acetic acid-induced writhing test

The acetic acid-induced writhing test method was described by Zakaria., et al [28]. 25 mice were treated with deionized water (0.1 ml/Mouse, p.o.), diclofenac sodium (10 mg/kg, i.p.), or MELA (250, 500 and 750 mg/kg body weight, p.o.). Thirty minutes later, all mice were treated with intraperitoneal injection of 0.6% acetic acid to cause a typical stretching response. Five minutes after acetic acid injection, Mice were kept in individual cages and number of writhes for each group was counted for 30 min. The analgesic activity was calculated Using the following formula:

$$\% \text{ Inhibition} = [\text{Mean no of writhes(control)} - \text{mean no of writhes (test)}/\text{Mean no of writhes (control)}] \times 100$$

Formalin-induced paw licking test

The test was performed following the method described by Zakaria., et al [28]. About 20 µL of 2.5% formalin was injected into the Sub-plantar area of the right hind paw. 25 mice were divided into five groups (n = 5), and the control animals were orally administered With deionized water (0.1 mL/mouse, p.o.), or diclofenac sodium (10 mg/kg. i.p.), while the experimental animals with MELA (250, 500 And 750 mg/kg, p.o.) 60 minutes before the formalin injection. After formalin injection, the animals were placed in an observation circle And watched for 30 minutes. The injected paws number of licking was taken in two phases. The early phase (0 – 5 minutes) was recorded During the first 5 minutes, while the late phase (15 – 30 minutes) was recorded during the last 15 minutes.

Glutamate-induced nociception test

The participation of glutamate receptors was evaluated using the method narrated by Bearish., et al [29]. The test animals were treated With MELA (250, 500 and 750 mg/kg, p.o.), while the control group received deionized water (0.1 ml/mouse, p.o.) and the reference group Was treated by diclofenac sodium (10 mg/kg i.p.). Thirty minutes after treatments, 20 µl (10 µmol/paw) of glutamate was injected into ventral surface of the right hind paw of mice. Mice were individually placed in an observation chamber for 15 minutes. Injected paw Licking was observed as an indication of nociception.

Cinnamaldehyde-induced nociception test

Cinnamaldehyde, a TRPA1 receptor agonist, was used to investigate the antinociceptive effect of MELA in mouse paw licking test .The mice were randomly separated into five groups (n = 5) and were treated with MELA at 250, 500 and

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750 mg/kg oral doses, 1h before Cinnamaldehyde injection. The control group received deionized water orally at 0.1 ml/mouse 30 minutes before the experiment; while The standard drug group received diclofenac sodium (10 mg/kg, i.p.)

Results

Acute toxicity test

In this test, the highest dose of 4,000 mg/kg did not show any mortality in the test animals. Consequently, the LD50 of L. aspera is Estimated to be more than 4000 mg/kg.

Antinociceptive activity

Hot plate test

MELA appeared a significant antinociceptive effect at 500, and 750 mg/kg doses (*$p < 0.05$) as presented in table 1. Naloxone did not Show any remarkable reduction of antinociceptive effect of L. aspera; while morphine (5 mg/kg) confirmed a significant antinociceptive Effect compared to the control group (*$p < 0.05$).

Tail immersion test

In the tail immersion test, the antinociceptive effect of L. aspera and morphine are shown in table 2. The extract effect was more prominent after 90 min at 250, 500, and 750 mg/kg. Morphine (5 mg/kg, i.p.) showed a notable antinociceptive effect compared to control (*$p < 0.05$), as morphine was antagonized by naloxone in this test for antinociceptive activity.

III. PHARMACOLOGICAL PROPERTIES

All the parts of L. aspera showed various pharmacological Properties. The present review article is dealing with the Pharmacological activities of this plant in different solvents.13,15

Antimicrobial Property

From the previous study, it was observed that the chloroform And petroleum ether extracts of L. aspera had good antifungal Activity against the Trichophyton and Microsporum gypseum. Its root, flower, leaf, and stem exhibits good antibacterial Activity against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium,Salmonella choleraesuis, and Shigella flexneri.16 The earlier Study reported that the ethanolic and methanolic decoctions Contain more active principles than the water and these organic Solvents showed better antimicrobial properties against various Bacterial strains.17 The ethanolic extract of the whole plant Parts showed potent bactericidal activity. Gram-positiveBacterial strains, such as, Bacillus cereus, Bacillus subtilis,Bacillus Megaterium, and S.aureus are found to be more sensitive Than gram-negative bacterial strains, like Salmonella typhi,Salmonella paratyphi, Salmonella dysenteriae, E. coli, Vibrio cholera, and Pseudomonas aeroginosa in the ethanolic Extract.18 80% ethanolic extract of this plant showed good

Antibacterial property against S. aureus and B.subtilis.

The Dichloromethane fraction of the methanolic extract of L. aspera Leaves was observed with profound antibacterial activity And in the case of ethyl acetate extract, it was active against Plasmodium Falciparum,v as well as, against the gram-positive Bacterial strains in the disc diffusion method.21,22 Volatile Oil from L. aspera showed significant antibacterial activity Against P. aeruginosa, Haemophilus influenza, S. aureus, And Candida albicans. However, it did not show any activity Against B.subtilis, Proteus vulgaris, Neisseria gonorrhea, and Trichoderma vibriae.23,24

Anti-Inflammatory Property

In an earlier study, the ethanolic and aqueous decoctions of L. aspera reported having a significant anti-inflammatory Effect. The petroleum ether and ethanolic decoction showed Anti-inflammatory properties with respect to the standard Diclofenac sodium and analgin.25 The extracts are highly Effective against acute and chronic inflammations. L. aspera Showed activity against mast cell degranulation persuaded by Proprancolol and carbachol.26 Petroleum ether, chloroform, Ethanol, and aqueous raw extracts were previously investigated For the anti-inflammatory property

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IV. CONCLUSION

The results of this study support the use of Leucas aspera and Leucas zeylanica in Traditional Indian medicine and show that Methanolic leaf extract of these plants can be used as an easily accessible source of natural antifungal Agent and can be of assistance in some Dermatological problems. This was the first report that Leucas zeylanica showed antifungal activity against these dermatophytes. Further studies might Aim at the isolation and identification of active Substances from the active plant extracts which could also disclose compounds with better Therapeutic value.

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