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Preliminary phytochemical screening and anthelmintic activity of Leucas indica var.Martinicensis

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ABSTRACT

Medicinal plants and their secondary metabolites have been used since last few centuries as remedies to treat diseases and disorders. As per WHO report (1993), 80% of world population continues to depend on medicine isolated from medicinal plants. Presently there is an increasing interest in herbal medicine related to isolation, characterization and pharmacological screening of extracts obtained from medicinal plants. The ethanolic extract of Leucas indica var. martinicensis was studied for preliminary phytochemical screening and anthelmintic activity at various concentrations (i.e., 10mg/ml, 25mg/ml, 50mg/ml, 100mg/ml) by using adult Indian earth worm, pheretima posthuma from in nalgonda region. Phytochemical Screening revealed the presence of Alkaloids, carbohydrates, and saponins. The mean paralysis time and mean death time for each sample was calculated and compared with the Albendazole which is taken as standard. The result was found that Leucas indica var. martinicensis had an anthelmintic activity which was greater than standard Albendazole.

Keywords: Leucas indica var. martinicensis, standard Albendazole, preliminary phytochemical screening, anthelmintic activity, pheretima posthuma.

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INTRODUCTION

Herbal medicines:

Helminth infections are among the most widespread infections in humans, distressing huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia. Paracytic diseases cause ruthless morbidity affecting principally population in endemic areas. The gastro intestinal helminthes becomes resistant to currently available anti helminthic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural helminthics.[1]

In most developing countries, helminth infections are considered a major health concern because the factors that predispose humans to these infections abound in these regions. Poor sanitation, poverty, unsafe water, malnutrition and ignorance are the factors that sustain the parasite life cycle and favour the proliferation of the disease vectors. Globally, a number of medicinal plants have been used for the treatment of helminth infections by the local people. Although the plant kingdom has remained poorly explored, natural products isolated from plants have been postulated to remain an essential part of the search for novel medicines against human diseases. There is a higher potential of discovery and development of more potent and efficient drugs from plants because a large number of medicinal plants have remained understudied for their pharmacological and phytochemical properties.[2]

Although helminth infections do not always cause obvious diseases, the host's health integrity could be compromised due to the effect of metabolic activities of the parasites on the nutrition as well as the toxic products of excretion. This probably accounts for the increased susceptibility and enhances the progression of bacterial diseases such as tuberculosis and other opportunistic infections among helminth hosts. Globally, the increase in helminth infection and their growing resistance to most chemotherapeutics is a major problem facing human health.[2]

Development of anthelmintic resistance in helminths reported in a number of countries. Medicinal plants have served through ages, as a constant source of medicaments for the exposure of a variety of diseases. The history of herbal medicine is almost as old as human civilization. The plants are known to provide a rich source of botanical anthelmintics, antibacterials and insecticides. A number of medicinal plants have been used to treat parasitic infections in man and animals.[3]

Over View of Helminthiasis:

Worms pathogenic for human beings are metazoans, classified into round worms (nematodes). These biologically diverse eukaryotes vary with respect to life cycle, bodily structure, development, physiology, localization within the host, and susceptibility to chemotherapy. Immature forms invade human beings via skin or gastrointestinal tract and evolve into well differentiated adult worms that have characteristic tissue distributions with few exceptions such as strongyloides and echinococcus these organisms cannot complete their life cycles i.e.. Replicate them, with in the human host. Therefore the extent of exposure to those parasites dictates the severity of infection and reduction in the no. of adult organisms by chemotherapy is sustained unless reinfection occurs.[4]

Anthelmintic resistance:

The ability of worms to survive treatments that are generally effective at the recommended dose rate is considered a major threat to future control of worm parasites of small ruminants and horses. The clinical definition of resistance is a 95% or less reduction in a "faecal egg count" test.Treatment with an anthelmintic drug kills worms whose genotype renders them susceptible to the drug. Worms that are resistant survive and pass on their "resistance" genes. Resistant worms accumulate and finally treatment failure occurs.Due to the development of resistance in helminthes, the need for the new anthelmintic drugs is increased.[5]

Aim & objectives:

To evaluate the phytochemical studies and anthelimentic activity of *Leucas indica var. martinicensis.*

Plant collection:

Leucas indica var. martinicensis commonly called as pedda tummi. It is a tall stout erect herbs or under –shrubs. It is distributed in endemic to chittor district. Occasional on hill slopes among grasses, srikalahasti, nagalapuram. The plant material was identified and authentified by Dr.k.MADHAVA CHETTY, assistant professor, department of botany, Sri Venkateshwara University, Tirupathi, AP, India.[6] The specimen was prepared and submitted in the department of botany under the voucher number: *Leucas indica var. martinicensis* family*: Lamiaceae*, voucher number:1079

Classification:

Kingdom: *Plantae*

Subkingdom: *Tracheobionta*

Super division: *Spermatophyta*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Subclass: *Asteridae*

Order: *Lamiales*

Family: *Lamiaceae*

Genus: *Leucas*

Species: *indica var.martinicensis.*

Figuer 1. Leucas indica var.martinicensis

Plant profile:

Leucas indica var. martinicensis commonly called as pedda thummi in Telugu. Tall stout erect herbs are under shrubs, branch lets with deflexed hairs. Leaves oblong to lanceolate, irregularly serrate, acuminate, base acute, chartaceous.Flowers white, in axillary whorls. Calyx tubular, sigmoidly curved, ten toothed, posterior tooth twice longer than the rest. Corolla upper lip as long as the lower lip. Stamens 4, Nut let 4, small.[6]

Distribution:

Rare weed in waste lands, fallow felids and on hills.Gogarbham dam area in Tirumala, horsely hills.[6]

Flowering & Fruiting Time:

October – February[6]

Uses:

Leaf extract is used in gastro-intestinal disorders, and as Anti-malarial drug.[6]

Literature Review:

i. Alex Asase (2005) et al Reported that "Whole plant of Leucas indica var. martinicensis is used to treat malaria in the Wechiau Community Hippopotamus Sanctuary area in Ghana.[7]

ii. Cailean Clarkson (2004) et al reported that methanolic extract of entire plant of Leucas indica var. martinicensis showed the antiplasmodial activity against Plasmodium falciparum strain and IC_{50} Value is 13.5micro grams.[8]

iii. F.A. Hamill (2003) et al reported that the entire plant is traditionally used as antimicrobial agent in southern Uganda.[9]

iv. G.L. Pachkore (2010) et al reported that Theomine, Glycine, Leucine and Cineole were Isolated from Leucas indica var. martinicensis.[10]

v. L.C. Di Stasi (2002) et al reported that Leucas indica var. martinicensis is traditionally used externally to treat muscular pain, rheumatism and internally to treat cold and cough in the cities of the Tropical Atlantic Forest, Region of Vale do Ribeira, State of S˜ao Paulo, Brazil.[11]

vi. Maud Kamatenesi-Mugisha (2007) et al report that the water extract of Leucas indica var. martinicensis used orally to reduce the labour pain during child birth in western Uganda population.[12]

vii. Sekou Bah (2006) et al Reported that the whole plant of Leucas indica var. martinicensis is used to treat urinary or schistosomiasis in the Office du Niger area of the Niono District.[13]

viii. Tadesse Eguale (2011) et al Reported that the aqueous and hydro alcoholic extract of the areal part of Leucas indica var. martinicensis inhibits the larval development checked against Haemonchus contortus.[14]

MATERIALS AND METHOD

Extraction:

100mg of dried powder of Leucas indica var. martinicensis was taken in 250ml of round bottom flask and defatted with petroleum ether (60^0-80^0C). After 48 hours, petroleum ether was decanted and remained material was dried in the air. Defatted Leucas indica var. martinicensis powder was taken in 250ml round bottom flask and macerated with aqueous ethanol at room temperature for 72 hours. After 72 hours, ethanolic layer was collected and

evaporated to dryness under water bath. The resultant residue was collected, weighed and stored in the refrigerator until further use.

Preliminary phytochemical screening:

Test for alkaloids:

residue + dilute hydrochloric acid. Shake well, filter. With filtrate, perform Mayer's test, Hager's test and Wagner's test.

***Dragendorff's test*:** to 2-3 ml filtrate, add few drops dragendorff's reagent. Orange brown ppt is formed.

***Mayer's test*:** 2-3 ml filtrates with few drops of Mayer's reagent gives ppt.

Hagers test: - 2-3ml. filtrate with hager's reagent gives yellow ppt.

Wagner's test: - 2-3ml. filtrate with Wagner's reagent gives reddish brown ppt.[15]

II. Test for carbohydrates:

a. ***Molischs test*:**2-3 ml aqueous extract + few drops of alpha naphthol solution + alcohol. Shake and add conc. sulphuric acid from sides of the test tube, formation of violet ring at the junction of two layers.

b. ***Test for reducing sugars*:** Fehling's test: mix Fehling's A and B of 1ml, boil for 1 minute. Add equal volume of test solution. Heat in boiling water bath for 5-10 minutes, brick red colour appears.

c. ***Test for non reducing sugars*:**

i. Test solution does not give response to Fehling's and Benedict's test.

ii. Hydrolyse test solution. Fehling's and Benedict's tests are positive.

III. Test for non-reducing polysaccharides(starch):

IV. Iodine test: Mix 3ml test solution and few drops of dilute iodine solution. Blue colour appears, it disappears on boiling and reappears on cooling.

Tannic acid test for starch: With 20% tannic acid, test solution gives precipitate.[15]

V. Test for proteins and amino acids:

Biuret test: - To 3ml of test solution add 4% NaOH and few drops of 1%$CuSO_4$ solution. Violet or pink colour appears.

Millons test: - Mix 3ml of test solution with 5ml of millons reagent. White precipitate. Warm precipitate turns brick red or the precipitate dissolves giving red colour solution

Nin-hydrin test: - Heat 3ml of test solution and 3 drops of 5% nin-hydrin solution in boiling water bath 10 minutes. Purple or bluish colour appears.[15]

VI. **Detection of saponins:-**Dilute 1ml of alcoholic and aqueous extracts separately with distilled water to 20ml and shake in graduated cylinder for 15 minutes. A 1cm layer of foam indicates the presence of saponins.[16]

VII. **Detection of phytosterols:-**

Salkowski reaction: - To 2ml of extract, add 2ml chloroform and 2ml concentrated H_2SO_4. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

Liebermann-Buchard reaction: - Mix 2ml extract with chloroform. Add 1-2ml acetic anhydride and 2 drops of concentrated sulphuric acid from the sides of the test tube. First red, then blue and finally green colour appears.[16]

VIII. **Test for flavonoids:-**

IX. **Lead acetate test: -**Residue+ lead acetate solution. Yellow precipitate appears[16]

X. **Test for tannins and phenolic compounds:-**2-3 ml extract+ add few drops of 5% ferric chloride solution to the extract. Blue colour appears.[16]

Anthelmintic activity:

The plant extracts were tested for anti helminthic activity in pheritima posthuma of nearly equal size (6cm+/-1cm). The worms were acclimatized to the laboratory conditions before experimentation. The earth worms were divided in 5 groups of 3 earth worms in each. Test solution, control and standard solution were taken in petri dish. Earth worms nearly equal size were taken for each concentration and placed in petri dish at room temperature and the time taken for complete paralysis and death are recorded.[17]

RESULTS AND DISCUSSION:

Ethanolic extract of *Leucas indica var. Martinicensis* was screened for phytochemical constituents.

Table I:Preliminary phytochemical screening of *Leucas indica var.martinicensis*:-

Test	**Aqueous ethanolic extract of Leucas indica var.martinicensis**
Carbohydrates	Present
Saponins	Present
Phenolics	Present
Flavonoids	Present
Alkaloids	Absent
Protiens	Absent
Amino Acids	Absent
Phytosterols	Absent

Ethanolic extract of *Leucasindica var. martinicensis*was screened for anthelmintic activity using pheretima posthuma earthworms.

The standard Albendazole was screened at various concentrations (10mg/ml, 25mg/ml, 50mg/ml and 100mg/ml) for its anthelmintic activity.

Table II: - Anthelmintic activity of standard Albendazole.

Albendazole(mg/ml)	Paralysis time(min)	Standard deviation	Death time (min)	Standard deviation.
10 mg/ml	25.15	±1.779	33.66	±2.081
25 mg/ml	20.716	±1.11	26.75	±1.299
50 mg/ml	17.95	±1.653	22.1	±1.852
100 mg/ml	15.466	±0.472	18	±1

The ethanolic extract of *Leucas indica var. martinicensis* was screened at various concentrations (10mg/ml, 25mg/ml, 50mg/ml and 100mg/ml) for its anthelmintic activity.

Table III: Anthelmintic activity of ethanolic extract of *Leucas indica var.martinicensis*

Leucas indica var. martinicensis(mg/ml)	Paralysis time(min)	Standard deviation	Death time (min)	Standard deviation.
10 mg/ml	18	±0	22	±1
25 mg/ml	15	±1.732	19	±1.732
50 mg/ml	10	±0	15	±1
100 mg/ml	6.333	±1.527	9	±1

The paralysis and death time was decreased with increasing concentration which is shown in the following table.

Table IV: Comparative study of anthelmintic activity of the standard and the plant extract:

Drug	Paralysis time (mins)	Death time (mins)
Albendazole(10mg/ml)	25.15	33.66
Leucas indica var.Martinicensis(10mg/ml)	18	22
Albendazole(25mg/ml)	20.716	26.75
Leucas indica var.Martinicensis (25mg/ml)	15	19
Albendazole(50mg/ml)	17.95	22.1
Leucas indica var.Martinicensis (50mg/ml)	10	15
Albendazole(100mg/ml)	15.466	18
Leucas indica var.Martinicensis (100mg/ml)	6.333	9

These all values of the ethanolic extract of *Leucas indica var. martinicensis* are compared with standard Albendazole values at the same concentration. At all concentrations the extract of *Leucas indica var. martinicensis* values are lesser than the Albendazole values (paralysis time and death time).

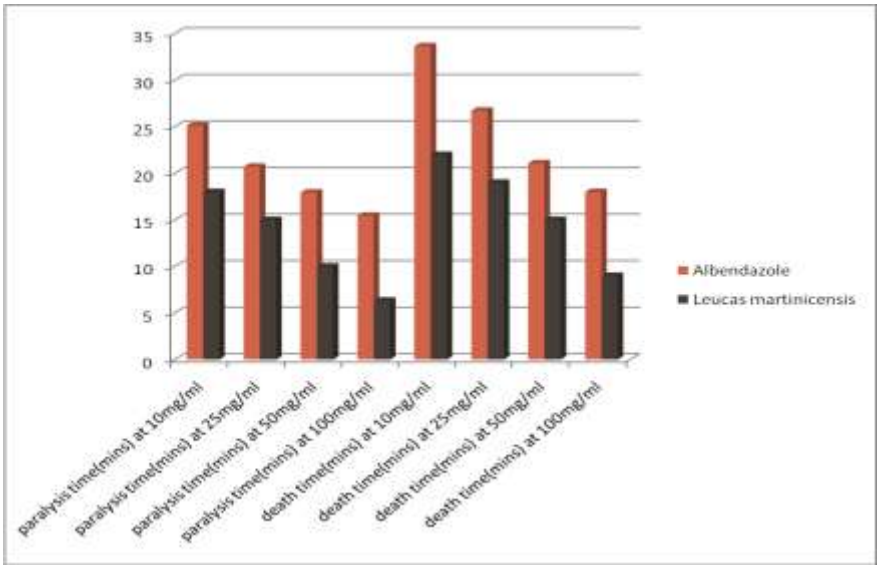

Figure. 2:Comparative histogram of anthelmintic activity of the standard Albendazole and the plant extract of Leucas indica var. martinicensis

Figure.3:Albendazole(10mg/ml):

Figure.4:Albendazole(25mg/ml):

Figure.5:Albendazole(50mg/ml):

Figure.6: Albendazole(100mg/ml):

Figure.7: *Leucas indica var.martinicensis*(10mg/ml)

Figure.8: *Leucas indica var.martinicensis*(25mg/ml):

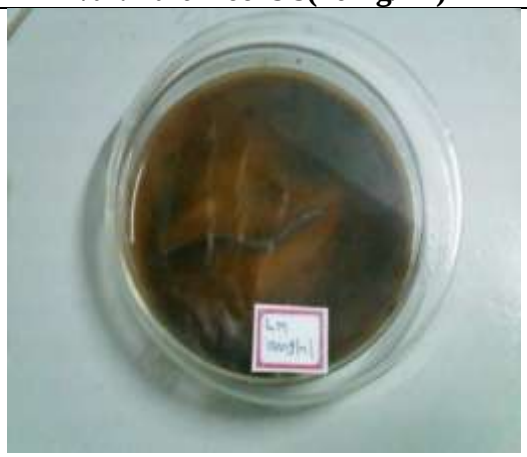

Figure.9: *Leucas indica var.martinicensis*(50mg/ml):

Figure.10: *Leucas indica var.martinicensis*(100mg/ml):

CONCLUSION:

The ethanolic extract of *Leucas indica var.martinicensis* was studied for preliminary phytochemical screening and anthelmintic activity at various concentrations (i.e., 10mg/ml, 25mg/ml, 50mg/ml, 100mg/ml) by using adult Indian earth worm, pheretima posthuma from in nalgonda region. Screening revealed the presence of Alkaloids, carbohydrates, and saponins. The mean paralysis time and mean death time for each sample was calculated and compared with the Albendazole which is taken as standard. The standard Albendazole was screened at various concentrations (10mg/ml, 25mg/ml, 50mg/ml and 100mg/ml) for its anthelmintic activity. At 10mg/ml the worms were paralysed at 25.15min and followed by death occurred at 33.66min. At 25mg/ml the worms were paralysed at 20.716min and followed by death occurred at 26.75min. At 50mg/ml the worms were paralysed at 17.95min and followed by death occurred at 22.1min. At 100mg/ml the worms were paralysed at 15.46min and followed by death occurred at 9min.

The ethanolic extract of *Leucas indica var.martinicensis* was screened at various concentrations (10mg/ml, 25mg/ml, 50mg/ml and 100mg/ml) for its anthelmintic activity. At 10mg/ml the worms were paralysed at 18min and followed by death occurred at 22min. At 25mg/ml the worms were paralysed at 15min and followed by death occurred at 19min. At 50mg/ml the worms were paralysed at 10min and followed by death occurred at 15min. At 100mg/ml the worms were paralysed at 6.33 min and followed by death occurred at 9min.

The paralysis and death time was decreased with increasing concentration. The result was found that *Leucas indica var.martinicensis* had an anthelmintic activity which was greater than standard Albendazole. Further research is required to find the responsible compound for its anthelmintic activity.

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