PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTHELMINTIC ACTIVITY OF LEUCAS INDICA VAR. NAGALAPURAMIANA

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ABSTRACT
The herbal medicines are the major remedy in traditional medicinal systems which have been used in medicinal practice for 100 years. These has made a great contribution in maintaining human health. A majority of the world’s population still relies on health needs. The practice continues today because of its biomedical benefits and its place in cultural beliefs in many parts of the world. Presently there is an increasing interest in herbal medicine related to isolation, characterization and pharmacological screening of extracts obtained from medicinal plants. In the present study, ethanolic extract of Leucasindicavar.nagalopuramiana 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 earthworm, pheretimaposthuma 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 result was found that Leucasindicavar.nagalopuramiana had an anthelmintic activity which was greater than standard Albendazole.

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INTRODUCTION

Herbal medicines

India has produced a respectable health care system, Ayurveda which encompasses the entire spectrum of human health and contributes to the positive health of an individual, but to certain inimical interferences with the system over the ages, a need has arisen to unify the entire system and codify it.

Indian material medica includes 2000 natural products of therapeutic importance of which 400 are of animal, mineral origin rest are vegetable origin. There are approximately 1600 Indian medicinal plants, which are used in formulating therapeutic origin according to ayurveda and other traditional system of medicine. Plant medicine can also be useful as starting material for the synthetic preparation of the drugs. Although interest in natural products as a source of new biologically active compounds decrease in the last few decades as synthetic chemistry programs expanded, but now 50% of lead compounds are from natural products or derivatives of natural products.

Natural products have traditionally provided most of the drugs in use. Despite the achievements of synthetic chemistry and advances towards rational drug design, natural products continue to be essential in providing, medicinal compounds and as starting point for the development of synthetic analogues. With the increasing power of screening programs and increasing interest in the reservoir of untested natural products, many future drug developments will be based at least in part, on natural products.

The plant is a biosynthetic laboratory, not only for chemical compounds but also for multitude of compounds like glycoside, alkaloids, etc. These exert physiological and therapeutic effect. The compounds that are responsible for the medicinal property is usually secondary metabolites, systemic study of crude drug embraces through consideration of primary and secondary metabolites derived as a result of plant metabolism. The plant materials subjected for detection of various plant constituents. There are so many plants which are used in treatment of Helminthiasis, but most of them are showing resistance to the drugs. So, this one attempt to treat the disease.

According to the prevalence and incidence statistics i.e., the term ‘prevalence’ of Helminthiasis usually refers to the estimated population of people who are managing Helminthiasis at any given time. The term ‘incidence’ of Helminthiasis refers to the annual diagnosis rate, or the number of new cases of Helminthiasis diagnosed each year. Hence, these two statistics types can differ. A short-lived disease like flu can have high annual incidence but high prevalence. [1]

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 “fecal egg count” test. Treatment with an anthelmintic drugs 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. [2]

AIM & OBJECTIVE:

To evaluate the phytochemical studies and anthelmintic activity of Leucasindica var. nagalapuramiana

PLANT COLLECTION:
Leucasindica var. nagalapuramiana commonly called as China PollaTummi. It is an annual, erect, branched herb.

- It is distributed in endemic to chittoor District
- Occasional on hill slopes among grasses, srikalahasti, Nagalapuram.
- The plant material was identified and authenticated by Dr. K. MADHA CHETTY, Assistant professor, Department of Botany, Sri. Venkateshwara University Tirupathi, AP, India
- The Specimen was prepared and submitted in the Dept. of Botany under the Voucher No: Leucasindicavar.nagalapuramiana, family-Lamiaceae, Voucher No: 239.

PLANT PROFILE:

i. Synonym: Leonurasnagalapuram, Eiatpriumodoratum (L.), Moringaoleifera
ii. Biological source: It is obtained from fresh leaves, stem and roots of Leucasindica var. nagalapuramiana
iii. Family: Labiatae / Lamiaceae

The plant is an erect herb, branches slender, puberulous. Leaves are linear, linear, linear to lanceolate, entire slightly unbulated or serrulate, puberulous on both sides. Flowers are white, in whorls at the end of the branches. Corolla upper lip is as lower as the lower lip. Calyx tubular, sigmoidaly curved, 10 posterior tooth twice lower than the rest. [3]

iv. Plant classification:
   - Kingdom: Plantae
   - Subkingdom: Viridaeplantae
   - Phylum: Tracheophyta
   - Class: Magnoliopsida
   - Subclass: Lamidae
   - Super Order: Lamianae
   - Order: Lamiaceae
Family: Lamiaceae
Genus: Leucas
Species: Indica var. Nagalapuramiana

v. Botanical names:
Leucasindica var. nagalapurumiana

vi. Regional names:
It is called as Chinnapoolatummi in Telugu, Gummi in English, Julphijjar in Hindi, Guma in Sanskrit, Durup in Tamil.

vii. Geographical distribution:
- Endemic to chittoor district.
- Occasional on hill slopes among grasses. Srikalahasti, Nagalapuram.
- Propagation: Seeds.
- Flowers & Fruits: September- March.

viii. Pharmacological and therapeutic uses:
- Leaves used as Stomachic, dermatitis, sores, swellings, skin diseases, cold, cough, fever, snake bite, scorpion sting.\(^3\)
- The aerial parts are used as sedative, Laxative, inflammation, antihelmintic.
- Used to treat anti-diabetic, anti-Viral, Anti-diarrheal activities\(^3, 4\).

LITERATURE REVIEW:

1. Somashekar B S. Manju Sharma (2002) studied that (Training module on Diversity of Medicinal Plants of Andhra Pradesh) the presence of each species in the three agroclimatic regions of Andhra Pradesh. List of Endemic species of Peninsular India occurring in Andhra Pradesh. Among these endemic plants, only about 14 are known to possess medicinal properties. In that Leucyasindica var. nagalapurumiana (Lamiaceae), Leucasmollissima var. Sebastiana (Lamiaceae), Leucasmollissima vat. Silvestriana (Lamiaceae), leucasmukherjiana. (Lamiaceae), Leucasnepetaefolia (Lamiaceae)\(^[3]\).

2. Sudhakar Ch. Reddy, K.N. Reddy, P.R.C Prasad and V.S. Raju, (2003) reported that (Threatened Endemic plants from Eastern Ghats, India), The Eastern Ghats are one of the richest floristic and phytogeographical regions of India. On the basis of observation of existing literature and herbarium collections, a short account of threatened endemic plants of Eastern Ghats was presented here. The present article focuses on about 33 endemic taxa are recollected from type locality only, 12 species are included under different IUCN threat categories. Leucasindica (L) R. Br. exVatke var. Nagalapuramiana (Chandr. &Srin.) Moula&Pullaiah (Lamiaceae) Erect herb. Distr.: Endemic to Nagalapuram hills of Chittoor district\(^[6]\).

3. Pullaiah T, (2006) reported that (Biodiversity and conservation with special reference to Eastern Ghats), Eastern Ghats are lying between Mahanandi and Vaigairivers of Peninsular India. The some of endemic and endangered plants of Eastern Ghats include: Andrographisbeddomei, Diclipterabeddomei, Justiciagingiana, Leucasdiffusa, Leucasindica var. nagalapurumiana, L. Fuacidagingiana, L.; mukherjiana, L. nepetifolia. Most of this is dependent on the biotic, edaphic and climatic factors, genetic structure in totality and past history of their populations\(^[7]\).

4. Mostafa M. NilufarNahar, M Moshiuzzaman, TalatMakhrmoor, M Iqbal Choudhary, et al., (2009) reported that (Free radical scavenging phenylethanoid glycosides from Leucasindica Linn), A newphenylethanoid glycoside. 2-(3,4-dihydroxyphenyl) -ethyl-O-alpha-L-rhamnopyranosyl-(13)-O-alpha – L - rhamnopyranosyl- (16) -4 – O – E – feruloyl - beta - D glucopyranoside (3-O-methyl poliu-moside, along with five known phenylethanoid glycosides (2-6) were isolated from the aerial parts of Leucasindica Linn. Compounds 1-6 exhibited significant antioxidant activity in 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical assay method. These compounds were also found to be moderate inhibitors of xanthine oxidase (XO) enzyme.\(^[8]\)

5. Sudhakar Reddy C. (2010) reported that (Gap Analysis for Protected Areas of Andhra Pradesh, India for conserving biodiversity), Gap analysis was carried out to assess the Protected Area (PA) network system in Andhra Pradesh, India. The Deciduous forests of Eastern Ghats of northern Andhra Pradesh were under-represented in Pas. Of the 103 species of endemics, Leucasindicavarnagalapurumiana has included. 64 species were not included in PA system. There is a need to consider for possible ways for effective conservation and to extend the present PA network system in India\(^[9]\).

6. KatraAntriksh, Pradhan Chandan Kumar, Singh Pradeep, (2010) reported that (Leucasischepalatos Roth. Spreng (Lamiaceae), in the Ayurvedic and Modern systems of medicine), Leucaschepalatos Roth. Spreng (Lamiaceae) is the well-known herb in the Ayurvedic and Modern systems of medicine, to cure various disorders. Commonly known as Drupushpi or Guma is mainly a rainy season weed found throughout India, Powered plant material analyzed for the two major attributes, phytochemically rich in Tannins and Flavonoids\(^[10]\).

7. Yeung Ming Fai, Safacura, (2010) reported that (An update of review on the presence of Oleanolic acid in Natural Products),update of review on the presence of oleanolic acid in natural products based on scientific findings reports 158 families, 767 genera and 1710 species of natural products isolated Oleanolic acid. Leucasaspera (Willd.) Spreng. – Whole plants has Oleanolic acid and Leucaschepalatos(Roxb). Spreng-Whole plant has Oleanolicacid\(^[11]\).

8. R. Preethi, V. VimalDevanathan and M. Loganathan, (2010) reported that (Antioxidant Efficacy of Some Medicinal Plants against Food Borne Pathogens), some medicinal plants are used in traditional treatments to cure variety of diseases for thousands of year. The locally available plants viz., Ficusreligiosa, Leucasaspera. Holarrhenaantidysenterica and Psidiumguajavai were selected which are having efficacy for controlling some food borne pathogen. The high antioxidant activity of 36ig/100mg was observed in Psidiumguajava. The GC-MS study also revealed the various phytic components for H. antidysenterica, which assured that it has high antimicronial, anticancer activity against the food borne pathogens\(^[12]\).
MATERIAL AND METHODS

Extraction:-

100 gm of dried powder of Leucasindicavar.nagalapuramiana was taken in 250ml round bottomed flask and defatted with petroleum ether (60-80°C). After 48 hours, petroleum ether was decanted and remained material was dried in the air. Defatted LeucasindicaVar.nagalapuramina powder was taken in 250ml round bottomed 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. At 100mgml, the paralysis time and death time was found to be 5.3 and 7.6 respectively. These all values was compared with standard albendazole values at the same concentration. In all concentrations the extract value is lesser than albendazole value [paralysis and death time]. [13]

Preliminary phytochemical screening:

a. 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 filtrate with few drops of mayers reagent gives ppt.
   - Hagers test: - 2-3ml filtrate with hagers reagent gives yellow ppt.
   - Wagners test: - 2-3ml filtrate with wagners reagent gives reddish brown ppt.

b. Test for carbohydrates:
   - 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.
   - 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.
   - Test for non-reducing sugars:
     - Test solution does not give response to fehling’s and benedicts test.
     - Hydrolyse test solution. Fehlings and benedicts tests are positive.
   - Test for non-reducing polysaccharides(starch):
     - Iodine test: Mix 3ml test solution and few drops of dilute iodine solution. Blue color appears, it disappears on boiling and reappears on cooling.
     - Tannic acid test for starch: With 20% tannic acid, test solution gives ppt.

Test for proteins and amino acids:-

- Biuret test: To 3ml of test solution add 4% NaOH and few drops of 1%CuSO₄ solution. Violet or pink color appears.
- Millions test: - Mix 3ml of test solution with 5ml of millions reagent. White precipitate. Warm ppt turns brick red or the precipitate dissolves giving red color 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 color appears.

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.

Detection of phytosterols:

- Salkowski reaction: - To 2ml of extract, add 2ml chloroform and 2ml concentrated H₂SO₄. 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.
- Borntragers test: -3ml extract+ dilute HCl, boil, filter, cooled+ equal volume of benzene, shake well, separate organic layer, add ammonia. Ammonial layer turns pink.

Test for flavonoids:

- Lead acetate test: - Residue+ lead acetate solution. Yellow precipitate appears
- Test for tannins and phenolic compounds: - 2-3 ml extract+ add few drops of 5% ferric chloride solution to the extract. Blue colour appears. [4]

ANTHELMINTIC ACTIVITY:

The plant extracts were tested for anthelmintic activity in pheretimaposthuma of nearly equal size (6 cm ± 1 cm). The worms were acclimatized to the laboratory conditions before experimentation. The earth worms were divided into 5 groups of 3 earthworms in each. Test solution control and standard solution were taken in petridish. Earth worms of nearly equal size were taken for each concentration and placed in petridish at room temperature and time taken for complete paralysis and death was recorded.
RESULT & DISCUSSIONS:
The preliminary phytochemical studies of Leucasindicavarnagalopuramiana was performed and the results were shown in Table I.

TABLE I: Preliminary phytochemical screening of Leucasindicavarnagalopuramiana:

<table>
<thead>
<tr>
<th>Identification tests</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Positive</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Negative</td>
</tr>
<tr>
<td>Proteins &amp; amino acids</td>
<td>Negative</td>
</tr>
<tr>
<td>Saponins</td>
<td>Positive [foam length=1cm]</td>
</tr>
<tr>
<td>Phyto sterols</td>
<td>Negative</td>
</tr>
<tr>
<td>Flavinoids</td>
<td>Positive</td>
</tr>
<tr>
<td>Phenolics</td>
<td>Positive</td>
</tr>
</tbody>
</table>

I. The standard Albendazole was screened at various concentrations (10mg/ml, 25mg/ml, 50mg/ml and 100mg/ml) for its anthelmintic activity. The paralysis and death time was decreased with increasing concentration which is shown in table II.

TABLE II: Anthelmintic activity of standard Albendazole:

<table>
<thead>
<tr>
<th>Albendazole (mg/ml)</th>
<th>Paralysis time (Min)</th>
<th>SD</th>
<th>Death Time (Min)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/ml</td>
<td>25.15</td>
<td>±1.779</td>
<td>33.66</td>
<td>±2.081</td>
</tr>
<tr>
<td>25 mg/ml</td>
<td>20.716</td>
<td>±1.11</td>
<td>26.75</td>
<td>±1.299</td>
</tr>
<tr>
<td>50 mg/ml</td>
<td>17.95</td>
<td>±1.653</td>
<td>22.1</td>
<td>±1.852</td>
</tr>
<tr>
<td>100 mg/ml</td>
<td>15.466</td>
<td>±0.472</td>
<td>18</td>
<td>±1</td>
</tr>
</tbody>
</table>

II. Ethanolic extract of Leucasindicavarnagalapuramiana was screened for anti-helminthic activity using pheretimaposthuma earthworms. The paralysis and death time was decreased with increasing concentration which is shown in table III

Table III: Anthelmintic activity of ethanolic extract of Leucasindica var.nagalapuramiana

<table>
<thead>
<tr>
<th>Leucasindicavarnagalapuramiana (mg/ml)</th>
<th>Paralysis time (Min)</th>
<th>SD</th>
<th>Death Time (Min)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/ml</td>
<td>17</td>
<td>±1</td>
<td>26.33</td>
<td>±5.131</td>
</tr>
<tr>
<td>25 mg/ml</td>
<td>13.66</td>
<td>±1.527</td>
<td>16.66</td>
<td>±1.154</td>
</tr>
<tr>
<td>50 mg/ml</td>
<td>7.666</td>
<td>±1.527</td>
<td>10.333</td>
<td>±2.08</td>
</tr>
<tr>
<td>100 mg/ml</td>
<td>5.333</td>
<td>±1.527</td>
<td>7.666</td>
<td>±2.081</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>paralysis time (mins)</th>
<th>Death</th>
<th>time (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole(10mg/ml)</td>
<td>25.15</td>
<td>33.66</td>
<td></td>
</tr>
<tr>
<td>Leucasindicavarnagalapuramiana(10mg/ml)</td>
<td>17</td>
<td>26.33</td>
<td></td>
</tr>
<tr>
<td>Albendazole(25mg/ml)</td>
<td>20.716</td>
<td>26.75</td>
<td></td>
</tr>
<tr>
<td>Leucasindicavarnagalapuramiana(25mg/ml)</td>
<td>13.66</td>
<td>16.66</td>
<td></td>
</tr>
<tr>
<td>Albendazole(50mg/ml)</td>
<td>17.95</td>
<td>22.1</td>
<td></td>
</tr>
<tr>
<td>Leucasindicavarnagalapuramiana(50mg/ml)</td>
<td>7.666</td>
<td>10.333</td>
<td></td>
</tr>
<tr>
<td>Albendazole(100mg/ml)</td>
<td>15.466</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Leucasindicavarnagalapuramiana(100mg/ml)</td>
<td>5.333</td>
<td>7.666</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: Comparative study of anthelmintic activity of the standard and the plant extract

Fig.2: Albendazole (10mg/ml):

Fig.3: Albendazole (25mg/ml):

Fig.4: Albendazole (50mg/ml):

Fig.5: Albendazole (100mg/ml):
CONCLUSION

The ethanolic extract of Leucasindicavar.nagalapuramiana was studied for preliminary phytochemical screening and anthelmintic activity at various concentrations (i.e., 10mg/ml, 25mg/ml, 50mg/ml, and 100mg/ml) by using adult Indian earthworm, pheretimaposthuma. 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. Ethanolic extract of Leucasindicavar.nagalapuramiana was screened for anti-helmintic activity using pheretimaposthuma earthworms. The ethanolic extract was screened at various Concentrations (10 mg/ml, 25 mg/ml and 100 mg/ml). At 10 mg/ml, the worms were paralyzed at 17 min and followed by death Occurred at 26 minutes. At 25mg/ml the worms were paralysed at 13.66min and followed by death occurred at 10.33min. At 100mg/ml the worms were paralysed at 7.666 min and followed by death occurred at 7.666min.

The paralysis and death time was decreased with increasing concentration. The result was found that Leucasindicavar.nagalapuramiana had an anthelmintic activity which was greater than standard Albendazole.

REFERENCES:

8. Sudhakar Reddy C., (2010). Gap Analysis for Protected Areas of Andhra Pradesh, India for conserving biodiversity was carried out to assess the Protected Area (PA) network system in Andhra Pradesh, “ Journal of American Science, 6(11), 472-484.