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BIOEFFICACY AND PHYTOCHEMICAL ANALYSIS OF *LEPIDAGATHIS KERALENSIS* OF ACANTHACEAE

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INTRODUCTION

Naturally occurring substances are plants, animals and mineral origin. They are organic substances and obtained in both prelimary and secondary metabolic process, they also provide a source of medicinal since the earliest time. The plant kingdom has proven to be the most useful in the treatment of disease and they provide an important source of all the worlds pharmaceuticals. Plants in all facets of life served a valuable starting material for drug development, industrial production of household products which include fashion products, insecticides etc. Phytochemicals therefore are used as templates for achieving standard programs, which are intended to make safe and effective drugs.

Lepidagathis keralensis a medicinal plant vernacularly called Paaramullu belongs to the family Acanthaceae is a perennial, prostrate, much branched herbs with woody rootstock, attached to hard lateritic soil and is first reported by PV Madhusoodanan *et al* (1992) and is endemic to Kerala. Leena *et al* (2016) carried out the invitro antimicrobial efficacy and GC-MS analysis of Bioactive components from *Lepidagathis keralencis.* The fresh roots of this plant is used for treating bronchial Asthma in children by the Paniya tribes (Divakar *et al*, 2010). *Lepidagathis cristata* widely studied for its phytochemical and pharmacological activities and proved its anti inflammatory and wound healing properties. (Reddy and Rao, 2013). Phytochemicals of the plants are play an important role in biological activities (Chandrashekhar *et al* 2012). The chief of which include alkaloids, tannins, flavonoids, phenolic compounds saponins etc. Phytochemical screening of leaves of *Annona muricata* reveals that has saponins,condenced tannins, glycosides and flavonoids (FKN, Arthur *e t al* 2011).Subhan murugesan *et al* (2011)worked out the Phytochemical screening and antimicrobial activity of the leaves of *Memycylon umbellatum.* MO, Ogunkoya *et al* (2010) carried out the Phytochemical screening of leaf and stem of *Anacardium occidentale* in both water and ethanol extract.

The aim of the present study was to carry out preliminary phytochemical screening and antifungal activity of aqueous leaf extract of *Lepidagathis keralensis* Madhu *and* Singh against three species of *Aspergillus.*

MATERIALS AND METRHODS

Collection of plant material

The fresh leaves of *lepidagathis keralensis* used for this analysis were collected from the midland laterite terraces of Kasaragod district of Kerala. Authentic specimen (H3-2015) is deposited in the Dept. of PG studies and Research in Botany Sir Syed College Taliparamba, Kannur, Kerala.

Phytochemical screening of leaf

The leaves were washed with distilled and sterilized water,oven dried at 80^0c for 2h to obtain a constant weight. The samples were then freely ground and stored in plastic container at ambient temperature. 5g sample was placed in the conical flask containing 100 ml water. The mixture were covered and allowed to soak for 3 hr after which they are filtered. The preparation covered and labeled. Phytochemical screening done by standard methods (Harbone 1998, khandelueal 2001). The procedure as follows.

Test for Carbohydrates

Molisch Test:- To 2ml of each extract in test tubes, a few drops of Molisch reagent was added, followed by 1ml of conc. H_2SO4 slowly down the size of the tube so that the acid forms an immiscible reddish brown layer with the extract solution (light brown layer).

Test for Alkaloids

To 3 ml of each extract mixture in a test tube, 1mlof 1% HCl was added and to 2 ml of extract mixture2 drops of Mayer's, Wagner's and Dragendroff reagents were added separately. A creamy white (Mayer), a reddish brown

(Wagner) and an orange brown (Dragendroff) precipitates in the ethanol and water extracts was taken as evidence of the presence of alkaloids.

Test for Tannins
Two (2) drops of 5% $FeCl_3$was added to 1ml of each extract in separate test tubes and the appearance of a dirty-green precipitate was considered as indication for the presence of tannins.

Test for Glycosides
Ten milliliters (10 ml) of 50% $H_2SO4$was added to 1ml of each extract in separate t est-tubes. The mixture was heated in boiling water for 15 min and 10 ml of Fehling's solution (5 ml each of solutions A and B) was added to the mixture and boiled. The presence of a brick-red precipitate indicated the presence of glycosides.

Test for saponins
Two milliliters (2 ml) of each extract in a test tube was vigorously shaken for 2 min and the presence of frothing indicated the presence of saponins.

Test for Phlobatannins
This was carried out by boiling 0.5 ml of the extract mixture with 5 ml of water and 1% HCl in a test tube for 2 min. The colour change was regarded as (g) Test for Sterols: The Lieberman-Burchard reaction was used for this purpose. Briefly, 1 ml of conc.H_2SO4 was added to 1 ml of each extract in a test tube and observed for the appearance of a red colour indicating the presence of sterols.

Test for flavonoids
2 cm^3 of extract was heated with 10 cm^3 of ethyl acetate on a water bath and cooled. The layers were allowed to separate and the color of the NH_3 layer noted red coloration formed.

Test for resins
5 cm^3 of copper was added to 5 cm^3 of each extract. The resulting solution was shaken vigorously and allowed to separate. A green colored precipitate indicating presence of resin was noticed.

Test for sterols
Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride then boiled and cooled. Conc. Sulphuric acid added. Appearance of brown ring at the junction indicates the presence of sterols.

Preparation of aqueous phytoleaf extract and inoculation of test fungi
The bioefficacy of leaf extract determined by testing *Aspergillus flavus* LINK; FR, *Aspergillus fumigates* FRESEN and *Aspergillus niger* VANTIEGHEM that are isolated from different seeds invitro.

The leaves were cut into small pieces washed with tap water, distilled and sterilized water and surface sterilized with 0.5% of sodium hypochlorite solution in a conical flask for 1-3 minutes.40g of leaves are crushed in mortar and pestles with 40 ml of distilled and sterilized water(w/v). The extract then filtered in a sterilized cloth and added to the potato dextrose agar medium. 20ml of sterilized and melted PDA was poured in to 9cm of sterilized petridishes. After solidification the plates were inoculated with test fungus using 2mm dm disc of actively growing colony.

The plates were incubated at a 25 ± 2^0c for seven days and the observation was recorded. Each treatment was replicated thrice. At the end of the incubation period radial growth of the pathogen and test fungus was measured both towards the interaction side and side of the petridish. Percentage inhibition of fungi was determined by applying the formula of Skidmore (1976),

$$\frac{C-C_1}{C} \times 100$$

Where 'C' represent the distance in mm of fungal growth from the point of inoculation towards the colony margin and 'C_1' is the fungal growth of fungus towards antagonistic.

RESULTS AND DISCUSSION

Preliminary phytochemical screening
The results of Preliminary phytochemical screening were done in table 1.

Tannins, saponins, resins, flavonoids, alkaloids were present in water extract. carbohydrate,glycocides, phlobatannins, sterol were absent in this extract.

The in vitro studies of aqueous leaf extract of *Lepidagathis keralensis* Madhu *and* Singh showed significant antifungal activity showed 94.4% of inhibition of *Aspergillus flavus* (Fig.1A,table.1),97.21% inhibition of *Aspergillus fumigatus* (Fig.1B,table.1) and 97.2% inhibition of *Aspergillus niger* (Fig.1c,table.1)

Fungi	% of inhibition	% of mycelial growth on control
Aspergillus flavus LINK;FR	94.4	100
Aspergillus fumigatus FRESEN	97.2	90
Aspergillus niger VANTIEGHEM	97.2	100

Table 1: Percent inhibitory action of *Lepidagathis keralensis* Madhu *and* Singh against fungi under in- vitro condition. GC-MS analysis of the leaf from *Lepidagathis keralencis* revealed the presence of 38 phytochemicals. In these major components were n-hexadecanoic acid,2-decanynoic acid,1,6-octadiene,3,7-dimethyl and 1,5-hepta diene and 3,3-dimethyl (Ref;Leena *et al*,2016).

The antifungal activity due to the presence of these phytocomponents.

From the results of phytochemical screening and antifungal activity against *Aspergillus niger,A.fumigatus, A.flavus* reveals that this plant can act against fungal disease. *A.flavus* is the main causative agent of aspergillosis –a respiratory disease. There is an indegenous knowledge that boiled water prepared by using this plant leaf can act against skin disease of new born babies and it is due to the presence of saponins. Tannin, resins can reduce the microbial activity.

CONCLUSION

Lepidagathis kerelensis madhu and singh is endemic to lateritic rock of Kerala. phytochemical screening revealed that it has phytochemicals and have antifungal activity. Soil mining,urbanization and laterite stone quarries leads to the destruction of these ethnobotanically important plant. This plant will have a pharmacognostic future due to the presence of phytoconstituents. From the present study it is suggested that this plant must protected for the future generations by the preservation of lateritic hills.

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