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In vitro evaluation of antifungal activity of *Murraya Koenigii* leaf extract

ABSTRACT

Many traditional plants are found to have therapeutic value. *Murraya koenigii* leaves are used for topical application to treat dandruff by most of the females in the coastal regions of Andhra Pradesh. *Murraya koenigii* leaf extract in dimethyl sulphoxide (DMSO) was used to find out its action on eight test organisms. High inhibition zones were observed with test organisms.

KEY WORDS Fungal species, leaf, inoculation, inhibition

INTRODUCTION

A handsome, aromatic, more or less deciduous shrub or tree up to 6m in height and 15-40cm in diameter with short trunk, thin smooth grey or brown bark and dense shady crown^{[1],[2]}. In India it occurs in foothill of Himalaya, Assam, Sikkim, Kerala, Tamilnadu, Andhra Pradesh and Maharashtra ^{[3], [4]}.

Leaves are aromatic and contain proteins, carbohydrates, fiber, minerals, carotene, nicotinic acid and vitamin C. It is rich in vitamin A. and calcium The leaves contain high amount of oxalic acid, leaves also contains crystalline glycosides,carbazole alkaloids, koenigin, resin, fresh leaves contain yellow color 2.5 % volatile oil.^[4] It also contain girinimbin, iso-mahanimbin, koenine, koenigine, koenidine and koenimbine.^[5] Mahanimbicine and bicyclomahanimbicine, phebalosin, coumarine as Murrayone imperatoxin etc isolated from leaves.^[6] Triterpenoid alkaloids cyclomahanimbicine, tetrahydromahanmbinealso presents in the leaves.^{[7],[8]} Murrayastine, murrayaline, pypayafolinecarbazole alkaloids and many other chemical compounds have been reported in the leaves of *Murraya koenigii*.^[9] Bark mainly contain the carbazole alkaloids as murrayacine, murrayazolidine, murrayazoline, mahanimbicine, girinimbicine, koenioline, xynthyletin. ^[10] The pulp of fruits generally contain 64.9% moisture, 9.76% total sugar, 9.58% reducing sugar, 0.17% non reducing sugar and negligible amount of tannin and acids. It also contains 13.35% of vit. C. The pulp of fruits also contain trace amount of minerals 1.97% phosphorus, 0.082% potassium, 0.811% calcium, 0.166% magnesium, 0.007% iron. It also contain markable amount of protein. ^[2]

Curry leaf tree (*Murraya koenigii* L., Family: Rutaceae) is a plant which has various important uses in the traditional system of medicine in Eastern Asia. ^[11] Based on ethnomedicine, *Murraya koenigii* is used as a stimulant, antidysentric and for the management of diabetes Mellitus . ^{[12],[13]} The plant is highly valued for its leaves an important ingredient in an Indian cuisine to promote appetite and digestion. ^[3] The leaves, root and bark are tonic, stomachic, and carminative. Leaves are used internally in dysentery also checking vomittig.^{[3],[4]} Steam distillate of the leaves can be used as stomachic, purgative, febrifuge and anti emetic. ^[12] Leaves are applied externally to bruises and eruption. ^[14] The leaves and roots are bitter, acrid, cooling, anthelmintic, analgesic, it cures piles, allays heat of the body, thirst, inflammation and itching. It is also useful in leucoderma and blood disorders. An infusion of the toasted leaves in used to stop vomiting.^[1] The juice of the root is good for pain associated with kidney. Fruits are also considered as astringent Indo-China. Crushed leaves are applied externally cures skin eruption and to relieves burn. The pastes of leaves are applied externally to treat the bites of poisonous animals. ^[3] Bark and roots are used as stimulants and externally they are applied to cures skin eruption and the bites of poisonous animals. The plant is credited with tonic and stomachic property. ^[1] The fruits having knowing for its very high nutritional values. These fruits are also having many medicinal properties. The branches of *Murraya koenigii* are very popular for cleaning the teeth as datun. It is also said that the branches of *Murraya koenigii* are used to strengthen gums and teeth's. ^[15] It has also been used as an anti-periodic and many a time the powdered dry leaf, mixed with honey and juice of betel nut, is recommended in the Ayurvedic system of medicine^[16]

MATERIALS AND METHODS

Murraya koenigii leaves were collected from a private firm near ongole and authenticated in the department of botany. Healthy fresh leaves were washed with tap water for several times and dried under shade. Finally powdered leaves were extracted with methanolic extract in a Soxhlet apparatus. The plant extract was further concentrated in rotavapour and residues were weighed. Different concentrations of stock solution (0.2 to 2.5mg) were prepared by dissolving the plant extract in dimethyl sulphoxide(DMSO).

Eight test organisms, *Aspergillus niger* (MTCC 281), *Rhizopus oryzae* (MTCC 262) *Aspergillus terreus* (MTCC1281), *Cladosporium species* (MTCC2223), *Collectotricum capsici* (MTCC2071), *Armillaria mellea*(MTCC409) and *Candida albicans* (MTCC 183) were obtained from BA&KR PHARMACY COLLEGE ,ONGOLE.

Agar cup bioassay was employed for testing of antifungal activity of plant extract [6]. The readymade PDA medium was suspended in distilled water and autoclaved at pressure of 15 lb/sq in for 20min. seven day old culture of test organism (0.5ml) was inoculated on to the medium. After inoculation, cups were scooped out from Petri plates with 8mm sterile cork borer. To each cup, different concentrations of test solutions (0.2 to 2.5mg) were added. Controls were maintained with DMSO and Bavistin (5µg) was used as standard. The treated and the controls were kept in an incubator at 26° for 24 h to 78 h and inhibition zones were measured. Three to four replicates were maintained for each treatment.

Effect of different concentrations (0.2-2.5 mg) of *Murraya koenigii* leaf extract against eight different fungi is given in the Table 1. The antifungal activity was observed in all the concentrations tested, but less effective below the 0.2 mg concentration of leaf extract, hence the data was not included. The minimum activity of extract at lower concentrations may be due to the crude nature of the test solution. Among different doses, the diameter of inhibition zones ranged from 8 to 19 mm among different fungal species and increased with the increase in concentration of test solution. Maximum antifungal activity was observed at 2.5mg concentration of leaf extract. A similar study of screening the natural plant extracts against different fungal and bacterial pathogens was well recorded [17], [18]. Since plants have co-evolved with pathogens, it is reasonable to expect a variety of such compounds with specific as well as general antifungal activity. [19], [20].

RESULTS

Different concentrations of the test solution inhibited all the fungal species with varying degree of sensitivity. Among all, high inhibition zones were observed in *Colletotricum crassipes* (19 mm). This was followed by *Cladosporium* (17.5 mm), *Armillaria mellea* (17 mm), *Colletotricum capsici* (17 mm), *Aspergillus niger* (16.5 mm), *Rhizopus oryzae* (16.5 mm) respectively. *Aspergillus terreus* and *Candida albicans* showed less inhibition zones (15.5 mm, 16.0 mm) compared to others organisms.

CONCLUSION

The present study clearly showed that the leaf extract of *Murraya koenigii* exhibited antifungal activity against many fungal strains, which included plant pathogens also. Thus there is a possibility of developing *Murraya koenigii* as an important source of biopesticide and antifungal agent. However further studies are needed for isolation and purification of bioactive constituents and also its usage in antidandruff formulations can be developed.

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S.NO	TEST FUNGI	CONCENTRATION OF LEAF EXTRACT OF <i>Murraya koenigii</i>										
		0.2	0.5	0.7	0.9	1.1	1.3	1.5	1.7	1.9	2.1	2.5
1	<i>Aspergillus terreus</i>	8	9	10	11	12	13	14	14	15	15.5	
2	<i>Cladosporium species</i>	11	11	12	12	13	14	15	16	16.5	17	17.5
3	<i>Colletotricum capsici</i>	10	10	11	12	12.5	13	14	15	15.5	16	16.5
4	<i>Aspergillus niger</i>	8	9	10	10	11	12	12	13.5	14.5	16.5	17
5	<i>Rhizopus oryzae</i>	9	10	11	12	13	14	15	15.5	15.5	16	16.5
6	<i>Armillaria mellea</i>	10	10	11	12.5	13	14	15.5	16	16	17	17
7	<i>Colletotrichium crassipes</i>	9	10	11.5	12.5	13	14	14.5	15	16	17	19
8	<i>Candida albicans</i>	9	9	10	11	12	13	14.5	15	15.5	15.5	16

TABLE 1: ZONE OF INHIBITION OF LEAF EXTRACT OF MURRAYA KOENIGII FOR ANTIFUNGAL ACTIVITY