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RESEARCH ARTICLE

Antioxidant and Antifungal Potential of *Murraya Koenigii* Leaves Extracts (Crude) and Essential Oil

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Abstract: The present study was aimed for the evaluation of antioxidant activity and antifungal activity of essential oil and crude extracts (methanol and ethanol) of *Murraya koenigii* leaves. The antioxidant activity of these extracts was determined by DPPH (1, 1- Diphenyl- 2- picrylhydrazyl) assay. It was found that free radical scavenging activity of *Murraya koenigii* essential oil and crude extracts was significantly higher in methanol extract among all the extracts studied. The extracts showed lower antioxidant activity compared to ascorbic acid used as reference. Similarly, the antifungal activity of these extracts was determined by poisoned food technique. On comparing the antifungal activities of three extracts on *Fusarium oxysporum* and *Rhizoctonia solani*, the activity of methanol extract was higher against *Rhizoctonia solani* than *Fusarium oxysporum* in comparison to rest of the extract, while essential oil showed moderate activity. The extracts showed lower antifungal activity compared to acetic acid used as reference. Therefore *Murraya koenigii* can be used as medicinal agents beneficial for enhancing the antioxidant and antifungal activity.

Keywords: DPPH - (1,1- Diphenyl - 2- picrylhydrazyl) assay, Antioxidant activity, Antifungal activity

Introduction

Murraya koenigii (Linn.) spreng. (Family-Rutaceae) commonly called curry leaves, it is basically known for its aroma and medicinal property. Most part of plant is covered with fine down and has a strong peculiar smell. It is more or less deciduous shrub or tree up to 6 m in height and 15-40 cm in diameter with short trunk, thin smooth grey or brown bark and dense shady crown. Leaves are exstipulate, bipinnately compound, 30 cm long, each bearing 24 leaflets, having reticulate venation, leaflets, lanceolate, 4.9 cm long, 1.8 cm broad, having 0.5 cm long petiole[1]**.** People generally use the fresh leaves, dried leaf powder and essential oil for flavouring soups, curries, fish and meat dishes, traditionally curry powder blends *etc*. The aromatherapy industry uses the essential oil in the making of soaps and cosmetics[2]**.** For natural hair tone and hair growth, one can use the blanked residue of boiled curry leaves along with coconut oil. It can be used as antihelmetics, it also acts as febrifuge, blood purifier, antifungal, antidepressant, anti-inflammatory, body aches,

for kidney pain and vomiting[3]. The phytoconstituents isolated so far from the leaves are alkaloids *viz*., mahanine, koenine, koenigine, koenidine, girinimbiol, girinimbine, koenimbine, *o*-methyl murrayanine, *o*-methyl mahanine, isomahanine, bismahanine, bispyrayafoline and other phytoconstituents such as coumarin glucoside *viz*., scoption, murrayanine[4], calcium, phosphorus, iron, thiamine, riboflavin, niacin, vitamin C, carotene and oxalic acid. The essential oil from leaves yielded di-*α*-phillandrene, *D*-sabinene, *D*-*α*-pinene, dipentene, *D*-*α*-terpinol and caryophyllene[5].

During the past decades, it became obvious that most degenerative diseases are associated with reactive oxygen species (ROS) such as superoxide anion radicals, hydroxylradicals, and hydrogen peroxide. Under stress conditions, our bodies produce more ROS which creates homeostatic imbalance and generates oxidative stress and causes cell death and tissue injury[6]. Reactive oxygen species (ROS) and free transition metal ions cause extensive oxidative damage to cellular biomolecules such as DNA, proteins and lipids. Consequently, they contribute to the pathogenesis of oxidative stress-related diseases[7]. Although synthetic antioxidants seem to be promising, their toxicity and unwanted side effects rules out their extensive prescription. Hence, there is great interest in the use of naturally occurring antioxidants for treatment or prophylaxis of various oxidative stress-related diseases[8]**.** Also, the clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens[9]**.** This increase has made the use of broad spectrum antibiotics and immuno-suppressive agents indiscriminately. Synthetic drugs are not only expensive and inadequate for treatment of diseases but are often with serious side-effects[10]. Essential oils, the vast reservoir of secondary metabolites produced by aromatic and officinal plants are of specific interest due to potent biological activities[11]**.** Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drugs because of unmatched availability of chemical diversity[12]**.** Thus, the present study is intended to evaluate the antioxidant and antifungal potential of *Murraya koenigii* leaves crude extracts and essential oil.

Experimental

Different dilutions of the extract and essential oil (200, 400, 600 and 800 µg/mL) were prepared. Then, 1 mL of extract from each dilution was added into test tube containing 2 mL of DPPH solution. Control was prepared by adding 1 mL of methanol to 2 mL of DPPH solution. Ascorbic acid was used as standard. The mixture was shaken vigorously and was left to stand in the dark for 30 min. The absorbance of the resulting solution was measured[13] spectrophotometrically at 517 nm**.** The scavenging activity of each extract on DPPH radical was calculated (Table 1 & Figure 1) using the following equation:

Calculation

$$\% \text{ Scavenging DPPH free radical} = 100 \times (1 - A/B)$$

Where A is absorbance of the solution, containing extracts and B is the absorbance of the DPPH solution without extract (control).

Fungal culture

Rhizoctonia solani and *Fusarium oxysporum* from the microbial culture collection bank of department of Microbiology and Fermentation Technology, JSBB, SHIATS.

Antifungal activity assessment

The antifungal activity of plant extracts and essential oil was evaluated against food-associated fungi by using poisoned food technique (Table 2). In poisoned food technique, the two

food-associated fungi were inoculated on Potato dextrose agar (PDA) plates and incubated for 25 ^{0}C for 3 to 7 days, to obtain young, actively growing colonies of molds. 100 µL of plant extract was mixed with 15 mL of cooled (45 ^{0}C) molten PDA medium and allowed to solidify at room temperature for thirty minutes. A mycelial disc 5 mm diameter, cut out from periphery of 3 to 7 day old cultures, was aseptically inoculated onto the agar plates containing the plant extract. PDA plates with 100µl of acetic acid were used as positive control[14,15]. The inoculated plates were incubated at 25 ^{0}C and colony diameter was measured and recorded after 5 days. Percent mycelial growth inhibition was calculated as given below:

Calculation

$$\% \text{ Mycelial growth inhibition} = [1-A/B] \times 100,$$

Where, A is mean diameter of fungal colony in plant extract and B is mean diameter of fungal colony in control.

Table 1. DPPH Free radical scavenging activity

Concentration µg/mL	% of inhibition of Methanol	% of inhibition of Ethanol	% of inhibition of Essential oil	% of inhibition of Ascorbic acid
200	65.21	59.15	10.78	89.98
400	67.75	62.27	11.88	91.62
600	71.37	66.66	12.43	92.16
800	72.10	70.69	17.55	93.26

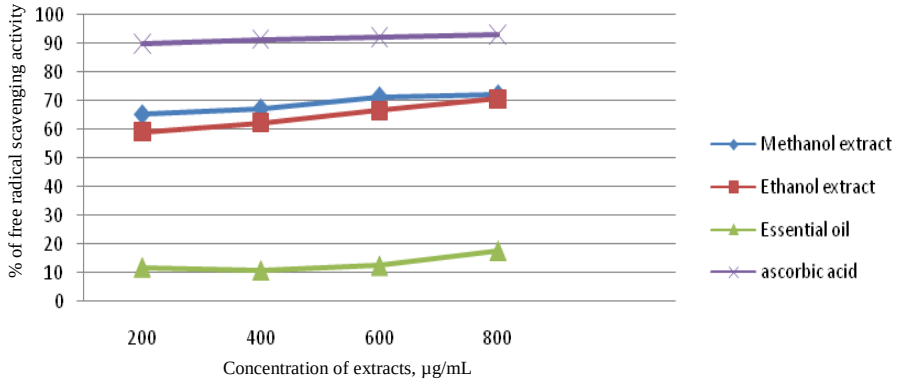

Figure 1. Plot of percent of free radical scavenging assay

Table 2. Antifungal activity of *Murraya koenigii*

Murraya koenigii leaves extracts	*Fusarium oxysporum*	*Rhizoctonia solani*
	% Mycelia growth inhibition	% Mycelial growth inhibition
Methanol	58.39	70.43
Ethanol	61.07	53.77
Essential oil	55.69	48.38
Acetic acid (positive control)	100	100

Results and Discussion

Antioxidant activity

Extracts of *Murraya koenigii* posses antioxidant properties. It inhibits formation of oxygen derived free radicals such as superoxide, hydroxyl radicals, lipid peroxidation and nitric oxide. Decolouration due to reaction of antioxidant in samples with the suitable free DPPH radical was measured spectrophotometrically. Reduction of DPPH radicals can be observed by the decrease in absorbance at 517 nm. Among all the extracts the methanol extract at the concentration of 200 µg/mL exhibited the highest free radical scavenging potential (65.21%), followed by ethanol extract (59.15%) and essential oil (10.78%). Similarly 400 µg/mL methanol extract showed the highest free radical scavenging activity (67.75%) as compared to ethanol extract (62.27%) and essential oil (11.88%). Methanol extract of 600 µg/mL had higher free radical scavenging activity (71.37%) followed by ethanol extract (66.66%) and essential oil (12.43%). Whereas, 800 µg/mL of methanol extract had highest free radical scavenging activity (72.10%) than ethanol extract (70.69%) and essential oil (17.55).

Antifungal activity

The methanolic extract of *Murraya koenigii* was effective in terms of percent mycelial growth inhibition in case of rhizoctonia *solani* (70%) then *Fusarium oxysporum* (58%). The ethanolic extract showed more inhibition in Fusarium oxysporum (61.07%) in comparison to *Rhizoctonia solani* (53.77%). The essential oil showed moderate activity in both *Fusarium oxysporum* (55.69%) and *Rhizoctonia solani* (48.38%). Among all the extracts methanolic extract exhibited highest activity against *Rhizoctonia solani* and ethanolic extract showed higher activity against *Fusarium oxysporum*. While acetic acid (positive control) shown highest antifungal activity in comparison to all the two extract and essential oil.

Conclusion

It is concluded that antioxidant and antifungal activity of methanolic extract, ethanolic extract and essential oil of *Murraya koenigii* leaves and its active constituents would be helpful in treating various kinds of plants and human diseases.

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