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Antifungal Potential of Murraya koienigii L. leaf extracts(Crude) and Essential oils

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Abstract:The present study was aimed for the evaluation of phytochemical analysis and antifungal activity of essential oil and crude extracts of *Murraya koenigii* leaves.

The phytochemical analysis done by various tests. The antifungal of these extracts was determined by poisoned food technique. On comparing the antifungal activities of three extracts on fusarium oxysporum the activity of methanolic extract is highest. While essential oil shows moderate activity. The extracts showed lower antifungal activity compared to acetic acid used as control.

Introduction: The Indian plant *Murraya koenigii* belong to the family- Rutaceae, commonly called curry leaf . The species is native of India and is found everywhere in India and is found everywhere in India. It is an aromatic more or less deciduous shrub shrub or a small tree up to 6m. It is cultivated for it's aromatic leaves. The plant has been used in traditional Indian medicine for a range of ailments. The whole plant is considered to be a tonic and stomachic. Roots and bark are stimulant and are applied externally for skin erruptions and poisonous bites. Green leaves are febrifuge and used in dysentery.

The paste of the leaves and juice of roots relives boils and renal pain respectively.

Morphological Description: *Murraya koenigii* is a small shrub with

dark green and brown stem. The leaves are long are seen to be in reticulate veination. Flowers found on the plants are white, funnel-shaped having sweet aromatic, smell and round shaped. Fruits are 1.4 to 1.6 cm in length.

Taxonomic Classification

Kingdom- Plantae

Subkingdom – Tracheophyta

Superdivision—spermatophyta

Division—Magnoliophyta

Class—Magnoliopsida

Subclass—Rosidae

Family- Rutaceae

Genus—Murraya

Species- koenigii

Material and Methods:

Collection of plant material: The fresh leaves are collected from the Botanical garden of KKM College Manwat. The taxonomic identification of the plant was confirmed and processed for further investigations.

Extraction of plant material:

Freshly collected leaves of Murraya koenigii were shade dried and powdered using mechanical grinder. 4 gms of pulverized leaf material were soaked in 100 ml of different solvents like methanol, ethanol, essential oil(L R Grade, Merk, India) at room temperature for 48 hours. Each extract was filtered under vaccum through Whatmann No. 42(125mm) filter paper and the process repeated until the all the soluble compounds have been extracted. The extracts were evaporated to dryness. The percentage yield of extracts ranged from 7-19%(w/w).

Qualitative phytochemical Examination of the Extracts:

To examine different phytochemicals tests were done by following procedure.Ethanol, methanol and essential oil extracts were dissolved individually in 3ml of DMSO and filtered. The filtrates were used for the test.

Alkaloids:

Alkaloids are basic nitrogenous compounds, with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagent was added.

1 ml of Mayer's reagent was taken in a test tube containing 1ml of filtrate. Formation of yellow color precipitate indicates presence of alkaloids.

Phytosterols:

Phytosterols are a group of steroid alcohols, phytochemicals naturally occurring in plants. The presence of phytosterols can be shown by Salkowski's test in which 1 ml of filtrate was taken in a clean test tube and conc. H_2So_4 is added, thoroughly shaken and allowed to stand. Lower layer turning red indicates presence of sterols and turning yellow indicates the presence of terpenes.

Glycosides: Glycosides are compounds which upon hydrolysis give rise to one or more sugars(glycones) and a compound which is not a sugar(aglycone or genine). To the extract in glacial acetic acid, few drops of ferric chloride and conc. H_2So_4 were added and observed for a reddish brown coloration at the junction of two layers. The bluish green color appeared in the upper layer.

Phenolics and Tannins:

Tannin is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. Tannins are incompatible with alkalies, gelatin, heavy metals, iron, lime water, metallic salts, strong oxidizing agents and Zinc sulfate, since they form complexes and precipitate in aqueous solution.

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for Gallic tannins and blackish green for catecholic tannins. The presence of phenolics was indicated by formation of the white precipitate when few drops of $Fecl_3$ solution were added to 1 ml of extract.

Saponins:

Saponins are a class of phytochemical compounds. More specifically, they are amphipathic glycosides grouped in terms of phenomenology by the soap like foam they produce when shaken in solutions and in terms of structure by their composition of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivatives. To the extract 20 ml of distilled water was added and shaken in graduated cylinder for 15 minutes. Formation of foam indicated presence of Saponins.

Flavonoids:

4 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.

Result :

Table-1 **Phytochemical analysis of Murraya kornigi**

Secondary metabolites	Methanol	Ethanol	Essential oil
Alkaloids	+	+	+
Phytosterols	+	+	-
Phenols	+	+	-
Tannins	+	+	+
Flavonoids	+	+	-
Glycosides	+	+	+

Antifungal Assay:

Penicillium digitatum, A.niger, A.flavus and *Fusarium* was obtained from purely cultured slants. The antifungal activity of plant extracts and essential oil was evaluated against selected fungal pathogens by using poisned food technique(Table- 2).

In poisoned food technique, four fungal fruit rot causing fungal pathogens were inoculated colonies of molds.

100 µl of plant extract was mixed with 15 ml of cooled, molten PDA medium and allowed to solidify at room temperature for 30 min.

A mycelial disc 5 mm in diameter, cut out from periphery of 3 to 7 day old cultures was aseptically inoculated on to the agar plates containing the plant extract. PDA plates with 100µl of acetic acid were used as positive control.

The inoculated plates were incubated at 25℃ and colony diameter was measured and recorded after 5 days. Percent mycelial growth inhibition was calculated as given below-

% Mycelial growth inhibition = (I- A/B)× 100

Where, A—diameter of fungal colony in plant extract

B—diameter of fungal colony in control

Table-2 Antifungal activity of Murraya koenigii

Murraya koenigii leaf extract	A.niger	A.flavus	F. oxysporum	P. digitatum
Methanol	58.65	56.44	62.07	52.27
Ethanol	56.20	54.33	55.39	45.07
Essential oil	52.48	50.66	55.69	48.38
Acetic acid (Positive Control)	100	100	100	100

Result:-The methanolic extract of *M. koenigii* was effective in terms of percent mycelial growth inhibition in case of *A. niger* than *P. digitatum*. The ethanolic extract showed more inhibition in *F.oxysporum*(62.07) in comparison to others.

The essential oil showed moderate activity in three *A. niger*, *A.flavus* and *Fusarium*. But in case of *Penicillium* it proves toxic comparatively.

Among all the extracts methanolic extract exhibited highest activity against selected fungal pathogen while Acetic acid control(Positive control) shown the highest antifungal activity in comparison to all the two extracts and essential oil.

Conclusion:

Plants has the defence ability due to the the secondary metabolites or phytochemicals present in it's different parts. It proves that antifungal activity of methanolic extract, ethanolic extract and essential oil of *M.koienigii* leaves and it's active constituents would be helpful in treating various kinds of fruit rot pathogens. The result of phytochemical analysis comprehensively validate the presence of therapeutically important and valuable secondary metabolites. Along with it confirms the ethanobotanical claim of the plant as an efficient traditional medicine for fungal diseases.

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