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Research Article

In vitro Evaluation of Antimicrobial Activity of Methanolic Extract of *Murraya Koenigii* Leaves (Curry Leaves)

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Abstract

The microorganisms have developed resistance to many commercial antibiotics due to the indiscriminate use of antibacterial drugs. Therefore, investigation of new molecules has become necessity. As medicinal plants have chemical compounds with antimicrobial action in abundance, vast research is going on those plants. One of such plant is *Murraya Koenigii. Murraya Koenigii* also known as sweet neem is used in traditional medicine to treat various diseases ascardio protective, antimicrobial, antifungal, and antiulcer, antioxidant, antifungal, and antiulcer. The Methanolic Extract of *Murraya Koenigii* Leaves (curry leaves), were investigated against different spices of Gram –ve bacteria i.e. *Pseudomonas aeruginosa*, *Escherichia Coli*, Klebsiella and Gram +ve *staphylococcus aureus* and fungus *Candida albicans* in our study. The screening was performed by standard disc diffusion method. It demonstrated moderate antibacterial activity against *staphylococcus aureus* having the diameter of zone of inhibition of 15 mm and good antibacterial activity against *E.coli* with zone of inhibition of 20 mm and pseudomonas aeruginosa with zone of inhibition of 10 mm and no zone of inhibition on Klebsiella, The leaf extract has shown anti fungal activity on *Candida albicans* with16mm zone of inhibition.

1. INTRODUCTION

Various antimicrobial peptides, proteins, and small molecular weight organic substances are present in Plants acting as host defense mechanisms[1, 2]. Medicinal herbs and plant products were used in treating a wide spectrum of infections and other diseases in traditional medicine like Ayurveda and Unani [3,4].As many microorganisms developed resistance due to the indiscriminate use of antibacterial drugs, there is a need to develop new molecules with minimum side effects. Therefore investigation of the chemical compounds within medicinal plants has become desirable[5]. *Murraya Koenigii* is a medically important herb of Indian origin (Family-Rutaceae). Meethineem (Hindi), karivepaaku (Telugu)its extract is known to show antidiabetic[6],cardio protective, antimicrobial, antifungal, and antiulcer, antioxidant[7, 8]anti-inflammatory[9], hypolipidemic activities[10], anticancer[11], analgesic properties and indigenous medicine as tonic, stomach stimulant and carminative.This plant has been used in poly herbal formations like Siddha medicine The extracts were also proved to be effective in gastric ulceration and was suggested as protective as ranitidine.[12] Biologically active carbazole alkaloids are reported to have antimicrobial properties.[13] Curry leaves have been reported to contain tocopherol, b-carotene, lutein and alkaloids.[14] The aim and Purposeof this study was to evaluate antimicrobial activity of *Murraya Koenigii* (curry leaves) against different bacteria and fungi--*staphylococcus aureus , E.coli*, Klebsiella species and *Candida albicans.*

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2. MATERIALS AND METHODS

2.1 Plant material

Murray Koenigii curry leaves were procured in bulk from a farm and the leaves were identified for the strain and the species by a botanist[5, 7]. The leaves were washed to remove dirt. After washing leaves were shade and dried, followed by hot air oven drying at 40^{o} C for 7 to 8 hours. Powder was made from dried leaves and stored in air tight containers for further analysis. Antimicrobial analysis (0-6 months) were done in the leaf powder *Murry Koenigii* 20 %, were taken for the study.

2.2 Extract Preparation

The extraction of *Murray Koenigii* curry leaves was done with 95% ethanol by Soxhelts apparatus in department of Pharmacology. The extract were dried under vacuum, stored at room temperature and protected from direct sunlight and stored in air tight containers for further analysis.

2.3 Phytochemical Analysis

Preliminary photochemical analysis was made for presence of alkaloid, flavonoids, carbohydrates, glycosides, proteins and amino acids, steroids, vitamin C, fat and fixed oil. Briefly, following tests has been performed for identifying the class of compounds.

a) Test for alkaloids

Two ml of extract was acidified with a few drops of dilute hydrochloric acid and then 1 ml of Dragendorffs reagent was added. The appearance of orange to red precipitate indicates the presence of alkaloids.

b) Test for tannins

To 2 ml of extract, a few drops of 10% lead acetate were added. The appearance of white precipitate indicates the presence of tannins.

c) Test for saponins

To 1 ml ofextract taken in a measuring jar, 9 ml of distilled water was added and shaken vigorously for 15 s and the extract was

allowed to stand for 10 min. Formation of stable foam (1 cm) indicates the presence of saponins.

d) Test for steroids

Chloroform 10 ml was added to 2 ml of extract. To this extract, 1 ml of acetic anhydride was added: then, 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Colour formation at the junction is noted. The appearance of blue-green colour indicates the presence of steroids.

e) Test for triterpenoids

Presence of triterpenoids istested same as that of steroids. The appearance of red, pink or violet colour at the junction indicates the presence of triterpenoids.

f) Test for cardiac glycosides

To 1 ml of extract, a few drops of glacial acetic acid and ferric chloride, and 3-4 drops of concentrated sulphuric acid were added. The appearance of blue-green colour indicates the presence of glycosides.

2.4 Organisms Used

The test organisms included four clinical isolates of *staphylococcus aureus* which is gram positive and *pseudomonas aeruginosa, E.coli* and *Klebsiella* which are gram negative organisms and *Candida albicans* a fungus. An attempt has been made to test the in vitro antibacterial activity of above leaf extract against above said pathogens. Clinical isolates of these organisms isolated during the study period from pus for *staphylococcus aureus* and from urine for *Escherichia coli* and Klebsiella and pseudomonas sp. were utilized for this study.

2.5 Micro Dilution Assay

The minimum inhibitory concentration was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. The minimum inhibitory concentration values were determined by broth dilution assay of micro dilution assay. Varying Concentrations of the extracts (500mg/ml, 400mg/ml, 300mg/ml, 200mg/ml, 100mg/ml and 50mg/ml) were prepared. 0.1ml of standardized test organism of controls was equally set up by using solvents and test organisms without extract. The inoculum was prepared from fresh overnight broth culture in nutrient broth. Plates were incubated for 24 h at 37C. MIC was recorded as the lowest extract concentration demonstrating no visible growth in the broth [15].

2.6 Test Micro-organisms

Antimicrobial activity of plant extract in different concentrations — 10000,30000,50000 µg/ml is tested against micro organisms *staphylococcus aureus*, *E. coli*, *Klebsiella pneumonia*, *pseudomonas aeruginosa* and *Candida albicans* by kirbybauer's disc diffusion method with Amikacin /ketoconazole as positive control and normal saline as negative control.

2.7 Antimicrobial Screening

Antimicrobial Assay / Antimicrobial activity was evaluated by the Disc diffusion method on nutrient agar medium (NAM) for bacteria and sabouraud's dextrose agar medium (SDA) for fungi. The Nutrient Agar Medium was prepared by dissolving Beef extract-0.3%,Yeast extract- 0.3%,Peptone-0.5%Nacl-0.5%,Agar medium-1.0gram, Distilled water-1000ml and maintained PH 7.0. The sabouraud's Dextrose Agar Medium was prepared by dissolving Peptone 10 grams, Dextrose- 20.0grams, Agar-15.0 grams, cycloheximide and Chloramphenicol in 1000ml water and pH maintained at 5.4 and autoclaved at 115^0c for 15 mints. The sterile medium (20ml) was uniformly inoculated by using sterile cotton swabs with test pure cultures of bacteria *Escherichia Coli*, *Pseudomonas aeruginosa, Klebsiella, Staphylococcus aureus* and fungus *Candida albicans*. The discs (5mm in diameter) were impregnated with10000µg/ml,30000µg/ml and 50000µg/ml extract of *Murray Koenigii* curry leaves (MEKML) followed by air drying and were placed on seeded agar plates. A disc with normal saline kept as negative control. Another disc with Amikacin kept as Positive Control and Ketoconazole was kept as positive control for *Candida albicans*. For each treatment two replicates were prepared. The plates were incubated for bacteria at 37^0c for 24 hours and for fungi 28^0C for 48 hours. After incubation the resulting zone of inhibition were measured.

3. RESULTS AND DISCUSSION

The antimicrobial and antifungal action of extract of *Murray Koenigii* curry leaves (MEMKL) was confirmed in our study. The extract showed inhibition of bacterial growth. The zones of inhibition for bacteria i.e., *Escherichia Coli* is 20 mm for 50000µg/ml; showing 83.33% inhibition zone. For Pseudomonas the inhibition zone was10 mm with 50000µg/ml exhibiting 45.5 % inhibition. There was no inhibition zone with *Klebsiella pneumonia* with 50000µg/ml. *Staphylococcus aureus* exhibited 15 mm inhibitory zone with 65.21% of inhibition when compared with positive controls Amikacin for gram negative and Augmentin (Amoxicillin / Cloxacillin combination) for gram positive. Whereas *Candida albicans* has shown 15mm inhibitory zone with 50000µg/ml extract, the percentage of inhibition being 65.21 when compared to Positive Control ketoconazole as shown in Table 1.

Table 1: Minimum Inhibitory Concentration of Methanolic Extract of *Murraya Koinigii Leaves (MEMKL)* and Amikacin / Ketoconazole in Serial Dilution Method

Organism	Negative Control (Normal Saline)	Positive Control	*MEMKL* 10000 µg/ml	*MEMKL* 30000µg/ml	*MEMKL* 50000 µg/ml	% of Inhibition
Staph. aureus	Nil	Augmentin 23 mm	Nil	Nil	15 mm	65.21%
E. coli	Nil	(Amikacin) 24mm	Nil	Nil	20mm	83.33%
Pseudomonas aeruginosa	Nil	(Amikacin) 28mm	Nil	Nil	10mm	45.5%
Klebsiella pneumonia	Nil	(Amikacin) 22mm	Nil	Nil	Nil	Nil
Candida albicans	Nil	ketoconazole22 mm	Nil	Nil	16mm	72.72%

MEMKL- Methanolic Extract of Murraya Koinigii Leaves, Positive—Control Discs = Amikacin for E. coli, Klebsiella, pseudomonas aeruginosa), Augmentin- (Amoxicillin/Cloxacillin combination) for Staphylococcus aureus, Ketoconazole for Candida albicans

5. CONCLUSION

In conclusion, this study confirms that *Murray Koenigii curry leaves* possesses in-vitro antimicrobial activity. This obviously justifies the use of *Murray Koenigii curry leaves* intraditional medicine. Further research has to be carried out to elucidate basic mechanism of antimicrobial action of above plant *Murray Koenigii*.

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