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The efficacy of *Murraya koenigii* leaf extract on some bacterial and a fungal strain by disc diffusion method

Mohar Singh Argal[1], Sanjay Kumar[2], Hotam Singh Choudhary[1], Ravindra M Thakkar[3], Santosh K Verma[4], Chandrabhan Seniya*[1]

[1]Department of Biotechnology, Madhav Institute of Technology & Science, Gwalior, M.P., India
[2]Department of Botany, Nagaland University, Headquarter Lumami, Nagaland, India
[3]Department of Chemical Engineering, I.I.T. Bombay, Mumbai, India
[4]Department of Biotechnology, Shri Rawatpura Sarkar Institutions, Datia, M. P. India

ABSTRACT

In the present study Murraya koenigii commonly called "curry leaf" leaves extracts subjected to a screening study to detect potential antimicrobial activity against Strains of Escherichia coli, Bacillus cereus, Staphylococcus aureus, Bacillus subtilis Klebsiella pneumonia, Staphylococcus epidermidis, Pseudomonas aeruginosa, Staphylococcus faecalis, Vancomycin resistant enterococcus and Candida albicans. The antibacterial activity of the products was evaluated using colonies growing in solid medium, establishing the zone of inhibition in vitro growth (ZOI). Plant (leaf) extract was also used for the phytochemical tests for compounds which include Glycosides, Steroids, Tannins, Alkaloids, Flavonoids, Saponins, Quinone, Protein and Sugar in accordance with the methods. The results showed that most of the bacterial strains (except E. coli, B. cereus and S. faecalis) had intermediate effect at low concentration leaf extract (10 and 15%) of Murraya koenigii but the efficacy of the leaf extract could be increased by increasing the concentration of the extract.

Key words: *Murraya koenigii*, leaves, Antibacterial, Phytochemical.

INTRODUCTION

The different systems of medicine practiced in India such as Ayurveda, Siddha, Unani, Amchi and local health traditions which utilizes a large number of plants for the treatment of human beings as well as animal ailments. The plant *Murraya koenigii* belongs to family Rutaceae, commonly called "curry leaf" in English and locally known as meetha neem. The plant species is native to India and found everywhere in its territory but very common in foothills of Himalaya, Assam, Sikkim, Kerala, Tamil Naidu, Andra Pradesh, Maharastra, and Madhya Pradesh. The

plant is cultivated for its aromatic leaves from Sanjivini Ayurveda City Centre (Gwalior). The leaves are pinnate, with 11-21cm broad, and flowers are small white with pleasant fragrance. The local health practitioners used whole plant as tonic and stomachic. Also, there were reports that the plant, traditionally, is used as tonic, anthelmintic, analgesic, piles, reduces inflammation, itching, carminative, stimulant, stomachic, febrifuge, analgesic, diarrohea, dysentery and insect bites [1, 2]. The leaves are used extensively as a flavoring agent in curries and chutneys [3, 4]. The green leaves were chewed raw for the cure of dysentery [3, 4]. The pastes of leaves were applied externally to treat the bites of poisonous animals [5]. Steam distillate of the leaves could be used as stomachic, purgative, febrifuge and anti emetic [6]. Leaves were applied externally to bruises and eruption [7]. The plant had been reported to possess positive inotropic effect [8] antidiabetic, cholesterol reducing property [9, 5] antibacterial or microbiological activity [10, 11], antiulcer activity [12] antioxidative property, and cytotoxic activity [13]. Fresh juice of the root was taken to relieve pain associated with kidney. Root and bark was found to be stimulant and applied externally for skin eruptions and poisonous bites. Barik [14] reported the presence of acytotoxic coumarin murrayatin compound. The plants containing flavanoids are constantly being screened for antitumour activity [15]. The main constituents reported were alkaloids, volatile oil, Gycozoline, Xanthotoxine and sesquiterpione sterols, aminoacids, glycosides, proteins and flavanoids.

In the light of the above information the present investigation was under taken which deals with the studies of the bioactive compounds analysis and antibacterial efficacy against bacteria (Gram-positive and negative) and a fungus (*Candida albicans*).

EXPERIMENTAL SECTION

The plant leaves were collected from Sanjivini Ayurveda City Centre (Gwalior) and identified the same for physical characteristics on morphology of *Murraya koenigii* in Department of Botany, Jiwaji University, Gwalior (India).

Murraya koenigii leaves extract preparation

The collected plant leaves were washed thoroughly 2-3 times with running water and with distilled water. The leaves were air-dried under shade. The leaves were crushed to make possible fine powder with the help of mortar and pestle and stored for further analysis.

5gm, 7.5gm and 10 gm of the leaves powder was dissolved in 50 ml, 200 ml and 200 ml of solvent (70% Ethyl alcohol, 90% Ethyl alcohol, Methanol and Chloroform) to prepare 10%, 15% and 20% extract in a 100 ml and 300 ml of flask respectively. The flasks were covered with the aluminum foil and kept on rotating shaker (500 rpm) for 48 h. The solution was filtered twice, firstly with cheese cloth (four fold) and then with Whatman's filters paper. The filtrates were collected in Falcon tubes and were concentrated upto dryness by keeping it in incubator at 35^{o}C. The stock solution of each extract was prepared in Dimethylesulfoxide (DMSO).

Phytochemical screening

Test for Glycosides

A 25ml of dilute H_2SO_4 was added to 5ml of plant extract in a 100 ml flask. It was boiled (15 min), cooled and neutralized with 10% NaOH. The fehling solution A and B (5 ml) was added to the neutralized solution and a brick red precipitate of reducing sugars indicates the presence of glycosides.

Test for steroids
One gram of the test substance (plant extracts) was dissolved in a few drops of acetic acid. It was gently warmed and cooled under the tap water and a drop of concentrated sulphuric acid was added along the sides of the test tube. Appearance of green colour indicates the presence of Steroids.

Test for tannins
The substance (extracts) mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of Tannins.

Test for alkaloids
Test substance (plant extracts powder) was shaken with few drops of 2N HCL. An aqueous layer formed which was decanted and one or two drops of Mayer's reagent added. Formation of white turbidity or precipitate indicates the presence of alkaloids.

Test for flavonoids
Shinado's test: To the substance (extracts) in alcohol, few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes. Red coloration shows the presence of Flavonoids.

Test for saponins
The substance (extracts) shaken with water, foamy lather formation indicates the presence of saponins.

Test for quinones
To the test substance, sodium hydroxide was added. Blue green or red color indicates the presence of Quinone.

Test for protein
To the test solution the Biuret Reagent is added. The blue reagent turns violet in the presence of proteins.

Test for sugars
The substance was mixed with equal volume of Fehling's A and B solutions, heated in water bath. Formation of red colour indicates of the presence of sugar.

Bacterial culture preparation and determination of Zone of inhibition (ZOI)
Strains of *Escherichia coli* (*E. coli*), *Bacillus cereus* (*B. cereus), Staphylococcus aureus (S. aureus*), *Bacillus subtilis* (*B. subtilis*) *Klebsiella pneumoniae* (*K. pneumoniae*) *Staphylococcus epidermidis* (*S. epidermidis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus faecalis (S. facecalis*), *Vancomycin resistant enterococcus (VRE) and Candida albicans (C. albicans)* were collected from (Defence Research & Development Establishment) (DRDE), Gwalior.

Muller Hinton (MH) Agar media (200ml) was prepared for each bacterium (five petri plates) and poured into sterile petri-plates for collected bacterial culture. Each petri-plate was inoculated with collected bacterial cultures (for fresh growth of each bacterium) by sterile streaking loop method. The circular wells were prepared in each plate with the help of micro tip (diameter of 6mm) and 50 µl of each plant parts extract was added. The petri-plates were inoculated and incubated at 37^0C and observed for possible results after 24 h. All disc diffusion tests were performed in triplicate (with five petriplates) for all bacterial strains and the antibacterial activity

was expressed as mean value of inhibition diameter (mm) produced by various prepared extract.

The well grown bacterial colony from MH agar media was picked and sub-cultured in Nutrient broth media and incubated (24 h) and maintained at 37^0C for further required purpose.

RESULTS AND DISCUSSION

Phytochemical screening of *Murraya koenigii* leaves showed the presence of glycosides, steroids, tannins, alkaloids, flavonoids, saponins, quinone, protein and sugar (Table 1). The presence of alkaloids in *Murraya koenigii* was very prominent in all the extracts (Ethyl alcohol, Methanol and Chloroform). The alkaloids contained nitrogen group and the solvents might break the nitrogen bonds and made some nitrogenous compounds with strong chemical reaction and showed prominent presence of the compound. The steroids, saponins and proteins showed greater intensity of their presence in ethyl alcohol and methanol extraction than chloroform. The color intensity for the presence of flavonoids was maximum in methanol extraction than ethyl alcohol and chloroform. Glycosides showed their presence in ethyl alcohol and methanol extraction while tannins in chloroform, quinone in methanol and sugar in ethyl alcohol only.

The strains *E. coli*, *B. cereus* and *S. faecalis* were susceptible to 10% leaf extraction in 90% ethyl alcohol, Methanol and 70% ethyl alcohol of *Murraya koenigii* with zone of inhibition (≥16 mm) diameter respectively (Table 2). The bacterial strains *B. cereus* (Methanol and Chloroform leaf extraction), *S. aureus* (70% ethyl alcohol extraction) *B. subtilis* (Methanol and Chloroform extraction), *K. pneumoniae* and *S. epidermidis* (Methanol extraction), *P. aeruginosa* (90% and 70% ethyl alcohol extraction), *VRE* (70% ethyl alcohol and Chloroform) and a fungal strain *C. albicans* (Chloroform extraction) were resistant to *Murraya koenigii* leaf extraction in different solvents with ≤10 mm zone of inhibition (diameter) respectively (Table 2). The bacterial strains *E. coli* (70% ethyl alcohol and Chloroform extraction), *B. cereus* (90% ethyl alcohol extraction), *S. aureus* (90% ethyl alcohol, Methanol and Chloform extraction), *B. subtilis* (70% and 90% ethyl alcohol extraction), *K. pneumonia* and *S. epidermidis* (70%, 90% ethyl alcohol and Chloroform extraction), *P. aeruginosa* (Methanol and Chloroform extraction), *S. faecalis* (70% ethyl alcohol, Methanol and Chloroform extraction), *VRE* (90% ethyl alcohol and Methanol extraction) and a fungal strain *C. albicans* (70%, 90% ethyl alcohol and Methanol extraction) showed intermediate effect towards the *Murraya koenigii* leaf extraction in different solvents with ≥11≤15 mm zone of inhibition in diameter respectively (Table 2).

The 90% ethyl alcohol leaf extraction (10%) of *Murraya koenigii* was very effective against *S. faecalis* (18mm), 70% ethyl alcohol (10%) for *B. cereus* (16 mm) while 90% ethyl alcohol and Methanol leaf extraction (10%) for *E. coli* (16 mm) respectively (Table 2).

The bacterial strains *E. coli*, *B. cereus* and *S. faecalis* were susceptible to 15% leaf extraction in 90% ethyl alcohol, Methanol and 70% ethyl alcohol of *Murraya koenigii* with with zone of inhibition (≥16 mm) diameter respectively (Table 3).

The bacterial strains *B. cereus* (Methanol leaf extraction), *S. aureus* (70% ethyl alcohol extraction) *B. subtilis* (Methanol and Chloroform extraction), *VRE* (Chloroform extraction) and a fungal strain *C. albicans* (Chloroform extraction) were resistant to *Murraya koenigii* leaf extraction (15%) in different solvents with ≤10 mm zone of inhibition (diameter) respectively (Table 3).

Table 1: Phytochemicals screening in the different organic leaf extracts of *Murraya koenigii*

Phytochemical compounds analysed	Organic Solvents		
	Ethyl Alcohol	Methanol	Chloroform
Glycosides	+	+	-
Steroids	++	++	+
Tannins	-	-	+
Alkaloids	++	++	++
Flavonoids	+	++	+
Saponins	++	++	+
Quinone	-	+	-
Protein	++	++	+
Sugar	+	-	-

(+ means Present; ++ means Prominent, - means absent)

Table 2: Antibacterial and antifungal activity of *Murraya koenigii* leaf Extract (10%)

Bacterial and Fungal Strains	Leaf extract in Organic solvents			
	90% Ethyl Alcohol	70% Ethyl Alcohol	Methanol	Chloroform
	Zone of inhibition (mm, diameter)			
E. coli	16mm	12mm	16mm	12mm
B. cereus	12mm	16mm	10mm	10mm
S. aureus	11mm	10mm	14mm	13mm
B. subtilis	12mm	14mm	10mm	09mm
K. pneumoniae	14mm	12mm	09mm	12mm
S. epidermidis	12mm	13mm	08mm	14mm
P. aeruginosa	09mm	10mm	14mm	12mm
S. facecalis	18mm	12mm	15mm	13mm
Vancomycin-resistant Enterococcus	13mm	08mm	13mm	10mm
C. albicans	13 mm	12 mm	13 mm	07 mm

The bacterial strains *E. coli* (70% ethyl alcohol and Chloroform extraction), *B. cereus* (90% ethyl alcohol and Chloroform extraction), *S. aureus* (90% ethyl alcohol, Methanol and Chloform extraction), *B. subtilis* (70% and 90% ethyl alcohol extraction), *K. pneumonia, S. epidermidis* and *P. aeruginosa* (70%, 90% ethyl alcohol, Methanol and Chloroform extraction), *S. faecalis* (70% ethyl alcohol, Methanol and Chloroform extraction), *VRE* (70%, 90% ethyl alcohol and Methanol extraction) and a fungal strain *C. albicans* (70%, 90% ethyl alcohol and Methanol extraction) showed intermediate effect towards the *Murraya koenigii* leaf extraction (15%) in different solvents with ≥11≤15 mm zone of inhibition in diameter respectively (Table 3).

The 90% ethyl alcohol leaf extraction (15%) of *Murraya koenigii* was very effective against *S. faecalis* (18mm), 70% ethyl alcohol (15%) for *B. cereus* (16 mm) while 90% ethyl alcohol and Methanol leaf extraction (15%) for *E. coli* (18 mm and 16 mm) respectively (Table 3).

Table 3: Antibacterial and antifungal activity of *Murraya koenigii* leaf Extract (15%)

Bacterial and Fungal Strains	Leaf extract in Organic solvents			
	90% Ethyl Alcohol	70% Ethyl Alcohol	Methanol	Chloroform
	Zone of inhibition (mm, diameter)			
E. coli	18mm	12mm	16mm	12mm
B. cereus	12mm	16mm	10mm	11mm
S. aureus	13mm	10mm	14mm	13mm
B. subtilis	13mm	14mm	10mm	10mm
K. pneumoniae	11mm	11mm	11mm	11mm
S. epidermidis	13mm	13mm	13mm	12mm
P. aeruginosa	12mm	13mm	11mm	14mm
S. facecalis	18mm	11mm	15mm	11mm
Vancomycin-resistant Enterococcus	14mm	11mm	12mm	8mmm
C. albicans	15mm	13mm	15mm	10mm

Table 4: Antibacterial and antifungal activity of *Murraya koenigii* leaf Extract (20%)

Bacterial and Fungal Strains	Leaf extract in Organic solvents			
	90% Ethyl Alcohol	70% Ethyl Alcohol	Methanol	Chloroform
	Zone of inhibition (mm, diameter)			
E. coli	18mm	17mm	16mm	13mm
B. cereus	12mm	16mm	10mm	15mm
S. aureus	16mm	10mm	14mm	17mm
B. subtilis	15mm	16mm	16mm	11mm
K. pneumoniae	15mm	15mm	16mm	11mm
S. epidermidis	13mm	13mm	11mm	13mm
P. aeruginosa	13mm	14mm	13mm	19mm
S. facecalis	18mm	13mm	15mm	16mm
Vancomycin-resistant Enterococcus	16mm	15mm	13mm	12mm
C. albicans	12mm	13mm	13mm	13mm

The bacterial strains *E. coli*, *B. cereus*, *S. aureus*, *B. subtilis*, *K. pneumonia*, *P. aeruginosa*, *S. faecalis* and *VRE* were susceptible to 20% leaf extraction in 90% ethyl alcohol, 70% ethyl alcohol, Methanol and Chloroform of *Murraya koenigii* with zone of inhibition (≥16 mm) diameter respectively (Table 4). The strains *B. aureus* and *S. aureus* showed resistance to methanol and 70% ethyl alcohol leaf extract (20%) with zone of inhibition ≤10 mm (diameter) respectively (Table 4).

The bacterial strains *E. coli* (Chloroform extraction), *B. cereus* (90% ethyl alcohol and Chloroform extraction), *S. aureus* (90% ethyl alcohol and Methanol extraction), *B. subtilis* (90% ethyl alcohol and Chloroform extraction), *K. pneumonia* (70%, 90% ethyl alcohol and Chloroform extraction), *S. epidermidis* (70%, 90% ethyl alcohol, Methanol and Chloroform extraction), *P. aeruginosa* (70%, 90% ethyl alcohol and Methanol extraction), *S. faecalis* (70%

ethyl alcohol and Methanol extraction), *VRE* (70% ethyl alcohol, Methanol and Chloroform extraction) and a fungal strain *C. albicans* (70%, 90% ethyl alcohol, Methanol and Chloroform extraction) showed intermediate effect towards the *Murraya koenigii* leaf extraction (20%) in different solvents with ≥11≤15 mm zone of inhibition in diameter respectively (Table 4).

The 70%, 90% ethyl alcohol and Methanol leaf extraction (20%) of *Murraya koenigii* was very effective against *E. coli* (16-18 mm), 70% ethyl alcohol against *B. cereus* (16 mm), 90% ethyl alcohol and chloroform against *S. aureus* (16-17 mm), 70% ethyl alcohol and Methanol against B. subtilis (16 mm), Methanol against *K. pneumonia* (16 mm), Chloroform against *P. aeruginosa* (19 mm), 90% ethyl alcohol and Chloroform against *S. faecalis* (18 and 16mm) and 90% ethyl alcohol against *VRE* (16 mm) respectively (Table 4).

CONCLUSION

Phytochemical analysis indicated that alkaloids were prominent in the leaf extract (ethyl alcohol, methanol and chloroform) along with other compounds of *Murraya koenigii* which might have an important role in anti-bacterial and antifungal activity.

The bacterial strains *E. coli, B. cereus* and *S. faecalis* were sensitive to leaf extract (10 and 15%) of *Murraya koenigii* in ethyl alcohol (90 and 70%) and methanol but the sensitivity of the bacterial strains extends (E. coli, B. cereus, S. aureus, B. subtilis, K. pneumonia, P. aeruginosa, S. faecalis and VRE) as 20% leaf extract in different solvents (70%, 90% ethyl alcohol, Methanol and Chloroform) were applied to them.

The strains B. cereus and S. aureus showed resistance to leaf extract (10%, 15% and 20%) of *Murraya koenigii* in 70% ethyl alcohol and Methanol with zone of inhibition (10 mm) in diameter. The other bacterial and fungal strain showed mixed response to leaf extract (10%, 15% and 20%) in different solvents (70%, 90% ethyl alcohol, Methanol and Chloroform) with variable zone of inhibition.

The results suggested that most of the bacterial strains (except E. coli, B. cereus and S. faecalis) had intermediate effect at low concentration leaf extract (10 and 15%) of *Murraya koenigii* but the efficacy of the leaf extract could be increased by increasing the concentration of the extract.

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