



Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Pharmacognostic effect of leaves extract of *Murraya koenigii* Linn

N. Uma Maheswari and N. Cholarani*

P.G. and Research Department of Microbiology and a Division of Biotechnology, S.T.E.T. Women's College, Sundarakkottai, Mannargudi, Thiruvarur (Dt), Tamil Nadu, India

ABSTRACT

The investigation was conducted with methanolic extract of *Murraya koenigii* L for its anti bacterial, phytochemical screening and antioxidant capacities. Antibacterial activity of the extract was evaluated against various Gram-positive and Gram-negative bacteria using agar well diffusion technique. For phytochemical screening such as flavonoids, alkaloids, resins, glycosides, tannins, saponins, amino acids, steroids and analyse the antioxidant capacity. The methanolic leaf extract showed better antibacterial activity against *pseudomonas aeruginosa* (Gram-negative) *staphylococcus aureus* (Gram-positive) followed by *Bacillus subtilis* (Gram positive) *E.coli* (Gram negative) *Proteus vulgaris* (Gram positive). Qualitative screening for the presence of various phytochemical compound was highly present for amino acids. The high antioxidant capacity was noticed in *proteus vulgaris* (Gram-negative).

Key words: *Murraya koenigii* L, Rutaceae, Antibacterial activity, Phytochemical screening, Antioxidant capacity.

INTRODUCTION

Murraya koenigii Linn medicinal plants^[1] belongs to the family –Rutaceae is commonly called as carry leaf tree, limblee tree. It is an evergreen shrub about 2.5-6 meters in height . the species is native to India. It commonly occurs in the Himalayas, Assam, Sikkim, Kerala, Tamilnadu, A.P, Maharashtra. The leaves of *Murraya koenigii* L are also used in Ayurvedic medicine. It is reported to possess anti-diabetic , antioxidant, anti-inflammatory , hepatoprotective and hypolipidemic activities. Recently reported that girinimbine, a carbazole alkaloid isolated from this plant, inhibited the growth and induced apoptosis in human hepatocellular carcinoma, HepG2 cells. . In traditional system of Medicine, it is used as antiemetic, antidiarrhoeal, dysentery, febrifuge, blood purifier, tonic, stomachic, flavoring agent in curries and chutneys. The oil is used externally for bruises, eruption, in soap and perfume industry. *Murraya koenigii* Linn is being used as stimulant, anti dysentric and for the management of diabetes mellitus. The leaves being bitter, acrid and cooling have been shown to have cooling, anthelmintic and analgesic action. It is known to cure piles, reduce body heat, thirst, inflammation and itching). Even leucoderma and blood disorders have been controlled.

Medicinal plants represent a rich source of antimicrobial agents. The most common bacteria used in the pharmacognostic analysis . *E.coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Bacillus subtilis*, *pseudomonas aeruginosa*. A large number of plants in different location around the world have been extracted and semi-purified to investigate individually their antimicrobial activity. The phytoconstituents isolated so far from the leaves are alkaloids viz., Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. *Murraya koenigii* (Linn.) Spreng., a member of the family Rutaceae, is a deciduous to semi evergreen aromatic tree found throughout India. Traditionally, it is used as an analgesic, febrifuge, stomachic, carminative and for the treatment of dysentery skin eruption.

EXPERIMENTAL SECTION

Plant collection:

The medicinal plant *Murraya koenigii* Linn was collected by hand plucking from follow land in and around orathanadu, Thanjavur (Dt),Tamil nadu, South India brought into the laboratory for further processes. The collected samples were carefully stored in sterile polythene bags and used for the further study.

Preparation of Plant Extract:

The leaves of the plant *Murraya koenigii* Linn leaves were carefully removed and thoroughly washed with the distilled water to remove dust particles^[2]. They were air dried in the shade at room temperature for five days and grounded into powder using pestle and mortar. The powdered plant leaves were stored in an air tight container prior to extract. The solvent used in the extract are methanol. 200 gram of the powdered leaves were subjected to maceration in methanol (200 g/1.5 L).The extract was then concentrated to dryness under pressure giving a dark green solvent.

Screening for Antibacterial Activity assay:**Agar-well diffusion method:**

The antibacterial activities of the leaves were tested against the selected bacterial strains. The petriplates were washed and placed in an hot air oven for sterilization^[3].After sterilization, nutrient agar medium was poured into each sterile petriplates and allowed to solidify in a laminar air flow chamber. After solidification, using a sterile cotton swabs, fresh bacterial culture with known population count was spread over the plate by spread plate technique. One well of 5mm size made in the agar plates with the help of sterile cork borer, the wells were loaded with 200µl of solvent (methanol) extract of leaves extracts. All the plates were incubated at 37°C for 24-48 hrs. After incubation, the plates were observed for formation of clear zone around the well indicated the presence of antibacterial activity. The zone of inhibition was calculated by measuring the diameters of the zone around the well.

Table 1: Antibacterial activity of *Murraya koenigii* Linn

S.no	Bacterial pathogens	Zone of inhibition(mm)
1	<i>E.coli</i>	8.0± 0.04
2	<i>Bacillus subtilis</i>	10.2± 0.02
3	<i>Pseudomonas aeruginosa</i>	16.0± 0.04
4	<i>Staphylococcus aureus</i>	12.0± 0.06
5	<i>Proteus vulgaris</i>	7.2± 0.02

Phytochemical screening of the leaves extract:

Qualitative screenings for the presence of various phytochemical compounds were performed using the methanolic extract^[4]. 200 gram of the powdered leaves were subjected to maceration in methanol (200 g/1.5 L).The extract was then concentrated to dryness under pressure giving a dark green solvent.

Test for flavonoids:

1 g of the powdered dried leaves of the specimen was boiled with 10 ml of distilled water for 5 minutes and filtered while hot. Few drops of 20 % sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow colour which on addition of acid changed to colorless solution depicted the presence of flavonoids^[5].

Test for tannins:

1 g of each powdered sample was separately boiled with 20 ml distilled water for five minutes in a water bath and was filtered while hot 1 ml of cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10 % ferric chloride were observed for any formation of precipitates and any color change. A bluish-black or brownish green precipitate indicated the presence of tannins^[6].

Test for saponins:

1 g of each powdered dried stain was separately boiled with 10ml of distilled water for 10minutes. The mixture was filtered while hot and allowed to cool^[7]. The following tests were then carried out. Demonstration of frothing: 2.5 ml of filtrate was diluted to 10ml with distilled water and shaken vigorously for 2minutes (frothing indicated the presence of saponin in the filtrate).

Test for alkaloids:

1 g of powdered sample of each specimen was separately boiled with distilled water and 10 ml hydrochloric acid on a water bath and filtered. The pH of the filtrate was adjusted with ammonia to about 6-7. A very small quantity of the following reagents was added separately (Picric acid solution, 10% tannic solution, Meyer's reagent , potassium

mercuric iodide solution) to about 0.5 ml of the filtrate in a different test tube and observed. The test tubes were observed for colored precipitates or turbidity^[8].

Test for amino acids:

2ml of sample added 2ml of ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids the sample^[9].

Test for resins:

0.5g of sample was added 5ml of boiling ethanol^[10]. This was filtered through what man no.1 filter paper and the filtrate diluted with 4ml of 15 aqueous HCl. The formation of a heavy resinous precipitate indicated the presence of resins.

Test for glycosides:

0.5g of sample was stirred with 10ml of boiling distilled water. This was filtered and 2ml of the filtrate hydrolyzed with a few drops of concentrated HCl and the solution rendered alkaline with a few drops of ammonia solution. 5 drops of this solution was added to 2ml of Benedict's qualitative reagent and boiled. Appearance of reddish brown precipitate showed the presence of glycosides^[11].

Test for steroids:

2ml of acetic anhydride was added to 0.5g ethanolic extract with 2ml of sulphuric acid. The colour changed from violet to blue it indicates presence of steroid^[12].

Table 2: Phytochemical analysis of *Murraya koenigii*

S.no	Phytochemical compound	Presence or absence
1	Flavonoids	+
2	Resins	-
3	Glycosides	+
4	Steroids	+
5	Saponins	+
6	Aminoacids	+
7	Alkaloids	-
8	Tannins	+

Test for antioxidant capacity:

(i) Reaction Buffer:

Dilute the Reaction Buffer 1:100 with phosphate buffer saline (hydrophilic) or with methanol (lipophilic)^[12]. Mix to homogeneity. Store the Reaction Buffer at 4°C up to three months.

(ii) Copper Ion Reagent:

Stock standard:

Dilute the Copper Ion Reagent 1:100 with deionized water (hydrophilic) or with methanol (lipophilic). Mix to homogeneity. Store the Copper Ion Reagent at 4°C up to three months.

(iii) Working standard:

Dilute the copper ion solution 1:10 with deionized water (hydrophilic) or with methanol (lipophilic). Mix to homogeneity. Store the working standard Solution at 4°C up to three months.

(iv) Preparation of Uric Acid Standard Curve:

Prepare fresh uric acid standards by weighing out the uric acid powder for a 10 mg/mL solution in 1N NaOH. This 10 mg/mL is equivalent to a concentration of 60 mM. use the 60 mM uric acid solution to prepare a 2 mM solution of uric acid (eg. add 100 µL of the 60 mM uric acid standard to 2.900 mL of deionized water).

Assay Protocol:

Each uric acid Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed^[13].

1. Add 20 µL of the diluted uric acid Standards or samples to the 96-well microtiter plate.
2. Add 180 µL of the Reaction Buffer to each well using either a multi channel pipette or a plate reader liquid handling system. Mix thoroughly.
3. Obtain an initial absorbance by reading the plate at 490 nm.
4. To initiate the reaction, add 50 µL of the copper ion reagent into each well. Incubate 5 minutes on an orbital shaker.

5. Add 50 μ L of stock solution to each well to terminate the reaction.
6. Read the plate again at 490 nm.

Table 3: Antioxidant capacity of *Murraya koenigii* Linn

S.no	Microorganisms	Initial concentration(μ g/ml)	Final concentration(μ g/ml)
1	<i>E.coli</i>	1.74	1.52
2	<i>Bacillus subtilis</i>	2.21	2.04
3	<i>Pseudomonas aeruginosa</i>	2.52	2.17
4	<i>Staphylococcus aureus</i>	3.25	0.00
5	<i>Proteus vulgaris</i>	3.56	2.82

RESULTS AND DISCUSSION

Murraya koenigii is a deciduous to ever-green aromatic tree found throughout India. It is also called as curry leaf tree is commonly used as spice due to the aromatic nature of leaves. Antibacterial activity of plant leaves methanol extracts were measured by the zone of inhibition. It showed maximum activity against *Pseudomonas aeruginosa* (16 mm) followed by *S.aureus* (12 mm), *B.subtilis* (10.2 mm) and *E.coli* (8 mm). The minimum zone of inhibition was observed at *Proteus vulgaris* (7.2 mm). Among all, the leaves methanol extract was found to be best for the bacterial inhibition. The moisture content of methanolic leaves extract of *Murraya koenigii* plant was found to be 90%, which the least content was found to be a steroid which was only about 0.002 mg/g. The amino acid content was 8.35 mg/g and the glycosides, saponins were observed at 2.096 and 3.94 mg/g. The plant leaves extract showed high antioxidant capacity for the *Proteus vulgaris* (2.82 μ g/ml), *Pseudomaons aeruginosa* (2.17 μ g/ml), *Bacillus subtilis* (2.04 μ g/ml) and *E.coli* showed (1.52 μ g/ml), while the *Staphylococcus aureus* showed no antioxidant capacity respectively by using methanolic extract .Therefore, different plant extracts are being investigated as source of safe, cheap and effective antioxidants The purpose of the present study was to evaluate *Murraya koenigii* L leaves as potential source of natural antibacterial agents. An attempt was made this time to investigate the antibacterial activity of *Murraya koenigii* L against different Gram positive and Gram-negative bacteria. Our results showed the leaves extract of *Murraya koenigii* L have bactericidal effect, presence of phytochemical compounds and antioxidant capacity. The results agree with the previous investigation of evaluate the antimicrobial and phytochemical analysis of *Murraya koenigii* L. In this study, the presence of phytochemical compounds such as tannins, steroids, saponins, amino acids, glycosides, steroids and flavonoids were investigated from the leaves extract of *Murraya koenigii* L.

REFERENCES

- [1]Adebajo, A.C., Ayoola, O.F., Iwalewa, E.O., Akindahunsi, A.A., Omisore, N.O., Adewunmi, C.O. *Phytomedicine.*, **13** : 246-54 (2006).
- [2] Qureshi Md. Shamim , M., shree, P. *Res J. of Pharma. and Phytochem.*, **1** : 78-79 (2007).
- [3]Gupta, A.R. *Indian J .Exp .Biol.*, **44** : 481-4 (2001).
- [4] Mohar Singh Argal. *Phytochem.*, **66**(13) : 1601-6 (2011).
- [5] Evans S, *Plant Foods Hum Nutr* **64** : 39-45 (2002).
- [6]Negbede Gupta, S., George, M., Singhal, M., Sharma, G.N., Garg, V. *J. Adv. Pharm. Technol, Res.*, **1**:68-77 (2008).
- [7] Okwu, N, and Okwu, A. *J. Ethnopharmacol.*, **135** : 88-94 (2004).
- [8] Harper, T., *Biochem. Pharmacol.*, **79** : 361-372 (1981).
- [9] Goodrich, B.S. *Nat. Prod .Commun.*, **7** : 121-124 (1979).
- [10] Moller, R. *Exp. Toxicol. Pathol.*, **63** : 587-591 (1981).
- [11] Sofowara, C.S. *Molecules*, **16** : 9651-9664 (1982).
- [12] Yankuzo. L.,Khan BA, Abraham A, Leelamma S. *Indian J.Med. Res.*, **102** : 184-186 (2011).