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ICJPIR |Volume 3 | Issue 2 | April – June- 2016 **Research Article**

Anti microbial activity of aqueous and ethanolic extracts of roots and leaves of murraya koenigii

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ABSTRACT

Murraya koenigii, family Rutaceae, commonly known as Curry leaf plant is a highly valued plant for its medicinal value and characteristic aroma.The plant shows varied pharmacological activities like antimicrobial, antifungal,hypoglycemic,antiobese,antipyretic,hepatoprotective etc., The plant is a rich source of carbazole alkaloids containing mahanimbine as a major alkaloidal constituent in its major proportion which was proved by mayer's alkaloidal test. The aqueous and ethanolic extracts of roots and leaves of the plant were screened for antimicrobial activity for Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli.The antimicrobial activity was tested by diffusion assay method in which cup plate method was chosen. The study shows that aqueous and ethanolic root and leaf extracts possess remarkable antimicrobial activity when compared with standard cephalosporin. Thus,Murraya Koenigii shows tremendous antimicrobial activity with root and leaf extracts.

Keywords: Murraya koenigii, Antimicrobial Activity, Mahanimbine, Diffusion Assay Method,Cephalosporin.

INTRODUCTION

The ethnomedicinal plant Murrya koenigii (Curry-leaf tree) which is native to India exhibits diverse biological activities. Murrya koenigii has been used for centuries in the Ayurvedic system of medicine. The present review gives a detailed description of the pharmacological works carried out on this medicinal herb and also throws light on its therapeutic potential for the treatment and management of various ailments frequently affecting humans. Murraya Koenigii, because of its aromatic characteristic properties, finds use and application in soap making ingredient, body lotions, diffusers, potpourri, scent, air fresheners, body fragrance, perfume, bath and massage oils, aromatherapy, towel scenting, spas and health clinics, incense, facial steams, hair treatments etc..

Murraya Koenigii, commonly known as curry leaf or karipatta in Indian dialects, belonging to Family Rutaceae which represents more than 150 genera and 1600 species[1]. Murraya koenigii is a high value plant for its characteristic aroma and medicinal value. It is an importantexport commodity from India as it fetches good foreign revenue. A number of chemical constituents from every part of the plant have been extracted. The most important chemical constituents responsible for its intense characteristic aroma are P-

gurjunene, P-caryophyllene, Pelemeneand O-phellandrene[2].

The plant is credited with tonic and stomachic properties. Bark and roots are used as stimulant and externally to cure eruptions and bites of poisonous animals. Green leaves are eaten raw for cure of dysentery, diarrhea and for checking vomiting. Leaves and roots are also used traditionally as bitter, anthelmintic, analgesic, curing piles, inflammation and are useful in leucoderma and blood disorders[3].

The plant is known for its many medicinal values such as tonic, stomachic, cure of dysentery, diarrhoea, bitter anthelmintic, analgesic, curing piles, inflammation, itching, anti-oxidative, cytotoxic, anti-microbial, anti-bacterial, anti-ulcer, positive ionotropic, and cholesterol reducing activities[4].

Murraya koenigii contains a number of chemical constituents that interact in a complex way to elicit their pharmacodynamic response. A number of active constituents responsible for the medicinal properties have been isolated and characterized. This plant has been reported to have anti-oxidative, cytotoxic, antimicrobial, antibacterial, anti-ulcer, positive inotropic and cholesterol reducing activities[5].

PLANT REVIEW

TAXONOMIC CLASSIFICATION [6]

Kingdom-Plantae
Sub-kingdom-Tracheobionta
Superdivision-Spermatophyta
Division-Magnoliophyta
Class-Magnoliopsida
Sub-class-Rosidae
Family-Rutaceae
Genus-Murraya J.Koenig ex L.
Species-Murraya Koenigii (L.)Spreng.

MEDICINAL USES

M. koenigii is one of the medicinally beneficial plants which have been used century ago by our ancestors. Pharmacologically this plant shows anti-microbial activity, anti-pyretic activity, hypoglycemic property, hepatoprotective activity, anti-inflammatory, cytotoxic, anti-obese activity, chemoprotective activity, anti helmintic activity, inotropic activity, nephroprotective, anti-oxidant, anti-fungal, anti-cancer. It is also used as cardio protective activity, antiamnesic,anti-ulcer, radiation protection activity, anti trichomal

activity, anti diarrhoel, and is also used to treat bronchial disorders, wound healing, dental caries, is also used in cosmetics.

PHYTOCHEMISTRY

The fruit of the Murraya koenigii consist of mahanimbine and koenimbine upon extracted by petroleum ether. Furthermore, isomahanine and murrayanol were isolated together with mahanimbine, murrayazolidine, girinimbine, koenimbine and mahanine[7].

The roots of M.koenigii consist a bioactive compound which is named as murrayanol, murrayagetin, and marmesin-1"-O-rutinoside.The benzene extract of roots consist of mukoline, mukolidine. The root were also found to have girinimbine and the root bark consist of koenoline[8].

The matured curry leaves consist 63.2% of moisture, protein which is of about 1.15% of nitrogen, carbohydrate 14.6% which is of total sugars and total ash 13.06%. Leaf of Murraya koenigii consist of koenimbine, O-methyl murrayamine, Omethyl mahanine, isomahanine, bismahanine and bispyrayafoline, koenigine, koenine, koenidine,mahanimbine, isomahanimbine, koenimbidine and murrayacine, isomahanimbicine[7,8] which has significant pharmacological activities and the major portion of volatile oil consist of bicyclomahanimbicine, mahanimbicine[9].

Bark and roots are used as stimulant and externally to cure eruptions and bites of poisonous animals. Green leaves are eaten raw for cure of dysentery, diarrhoea and vomiting. Leaves and roots are also used traditionally as bitter, anthelmintic, analgesic, curing piles, inflammation, itching and are useful in leucoderma and blood disorders [10, 11].

ANTI-MICROBIAL ACTIVITY

An antimicrobial is any substance of natural, semi synthetic or synthetic origin that kills or inhibits the growth of microorganisms but causes little or no damage to the host. All antibiotics are antimicrobials, but not all antimicrobials are antibiotics.

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungal are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis[12].

AIM AND OBJECTIVE OF WORK

From the literature review Murraya Koenigii showed varied activities against various extracts. Leaf extracts showed antipyretic, anti-inflammatory and hepatoprotective activities with different organic solvents.

Stem extracts showed hypoglycemic activity and anti-obese activity with methanol.,Seed extracts showed anti-depressant activity with hydro alcohol.Root extracts showed anti-microbial, anti-diarrheal, anthelmintic, anti-cancer and antiasthmatic with different organic solvents.

As per the present work, Murraya Koenigii is showing anti-microbial activity using both aqueous and ethanolic extracts of roots and leaves which seems and reports to be in its novel form as only ethanolic extract of Murraya Koenigii roots showed anti-microbial activity.Despite of difference in plant parts, even effects with water gave a huge chance for novelty in the plant. Thus, improving the chance for exploring its effect.

MATERIALS AND METHODS

Collection of plant material

Leaves

The leaf was collected from local regions like bogaram, bhongir and was washed properly with distilled water. It was then shade dried completely and blended finely using a blender.

Roots

The roots were collected from Karimnagar district and was washed clearly with distilled

Water

It was then shade dried completely and blended finely using a blender.

PREPARATION OF EXTRACTS (MACERATION)

Aqueous leaf extract

The powdered leaves were extracted by maceration using distilled water.About 50g of powedered leaves were macerated with 1000ml of water for a week. It was then filtered through a muslin cloth and was evaporated to dryness on a hot water bath to obtain the residue.

Ethanolic leaf extract

The powdered leaves were extracted by maceration using ethanol.About 50g of powdered leaves were macerated with 1000ml of ethanol for 4 days. It was then filtered through a muslin cloth and was evaporated to dryness in a china-dish to obtain the residue.

Aqueous root extract

The powdered roots were extracted by maceration using distilled water.About 25g of powdered roots were macerated with 500ml of water for a week. It was then filtered through a muslin cloth and was evaporated to dryness on a hot water bath to obtain the residue.

Ethanolic root extract

The powdered roots were extracted by maceration using ethanol. About 25g of powdered leaves were macerated with 500ml of ethanol for 4 days. It was then filtered through a muslin cloth and was evaporated to dryness in a china-dish to obtain the residue.

PREPARATION OF EXTRACTS (SOXHOLATION)

In the present study,the extracts were prepared by soxholation,a process of continuous extraction in which the same solvent is circulated through the extractor several times.Soxhlet apparatus consists of a body of extractor attached with a sidetube and siphon tube.The extractor from the lower side can be attached to distillation flask and the mouth of the extractor is fixed to a condenser by the standard joints.The powdered crude plant material is packed in the soxhlet apparatus directly or in a thimble pack. Extraction assembly is set up by fixing condenser and distillation flask.Initially for setting of powder, the solvent is allowed to siphon once before heating.Fresh porcelain pieces are added to flask to avoid bumping of the solvent.

The vapours pass through the sidetube and the condensed liquid gradually increases the level of liquid in extractor and in the siphon tube.A siphon is set up and the liquid reaches the point of return and the contents of the extraction chamber are transferred to the flask. The cycle of solvent evaporation and siphoning back can be continued many times without changing solvent, to get efficient extraction.

In the present investigation, dried leaves and dried roots of Murraya Koenigii was extracted by soxholation using solvent like ethanol for ethanolic extracts of roots and leaves and water for aqueous extracts of roots and leaves.Finally, the extracts were evaporated to dryness at room temperature.The extracts thus obtained were weighed and percentage yields were calculated.Color and consistency of each extract were also recorded.The obtained extracts were used for preliminary phytochemical screening by performing various chemical tests to detect the presence of Alkaloids,Carbohydrates,Tannins,Glycosides,Steroids,Terpenoids,Proteins,Amino acids,Flavanoids etc.,

ANTI MICROBIAL EVALUATION

The anti-microbial activity of Murraya koenigii was tested by diffusion assay method**.** In diffusion assay, cup plate method was chosen; cups or cylinders are made on the solidified and seeded agar medium. These cylinders are filled with the appropriate dilutions of the solutions to be assayed or with solutions containing known concentrations of the reference compound and the plates are incubated for a specific period of time kept at constant temperature. The diameters of the zones

are measured in millimeters and the concentrations in the solutions under assays are determined by comparison with standard[15].

EXPERIMENTAL WORK

In the present experiment the antimicrobial activity was tested by cup plate assay method. The antimicrobial activity of Murraya Koenigii's root and leaf extracts were tested and compared with the standard cephalosproin solution at two different concentrations i.e., 250mg/ml and 500mg/ml.DMSO is used as a solvent[13,14].

Test organisms

Staphylococcus aureus MTCC 3160
Pseudomonas aeruginosa MTCC 400
Escherichia coli MTCC 1652

Composition of nutrient agar medium

Nutrient Agar mediu

Per 250ml
Beef Extract Powder-2.5 g
Nutrient Agar-6 g
Sodium Chloride-1.5 g
Peptone-2.5 g
Final pH 7.3 ± 0.1 at 25°C

Preparation of the medium

1. The above required quantities for preparation of medium were taken and suspended in 250ml
   Of purified water.
2. It was agitated properly in one direction inorder to completely dissolve the medium.
3. The medium was finally adjusted to required pH.
4. Autoclaved at 121˚C for about 15 minutes.
5. It is then cooled at room temperature.

Procedure

The above medium was inoculated at 1% level with 18hrs old cultures of the above mentioned test organisms and were transferred into sterile petri dishes. The medium in the plates was allowed to set at room temperature for about 10min and they were set to solidify in a refrigerator for 30min.

After that cylinders were made in the medium. These cylinders are filled with the appropriate dilutions of the solutions to be assayed or with solutions containing known concentrations of the reference compound and the plates are incubated for a specific period of time kept at constant temperature.

The diameters of the zones are measured in millimeters and the concentrations in the solutions under assays are determined by comparison with standard the test solutions which were prepared in DMSO along with the standard solution of cephalosporin were placed in their respective cylinders.

The plates thus prepared were left to stand in a refrigerator for about 1hr to allow the test solution for diffusion. Then incubation of the above plates was done for 24hrs at 37℃. The plates were examined for zones of inhibition and the inhibition zone diameters were recorded.

The recorded diameters were compared with all the extracts chosen, thus resultingin anti-microbial efficacy of specimen Murraya Koenigii

RESULTS AND DISCUSSION

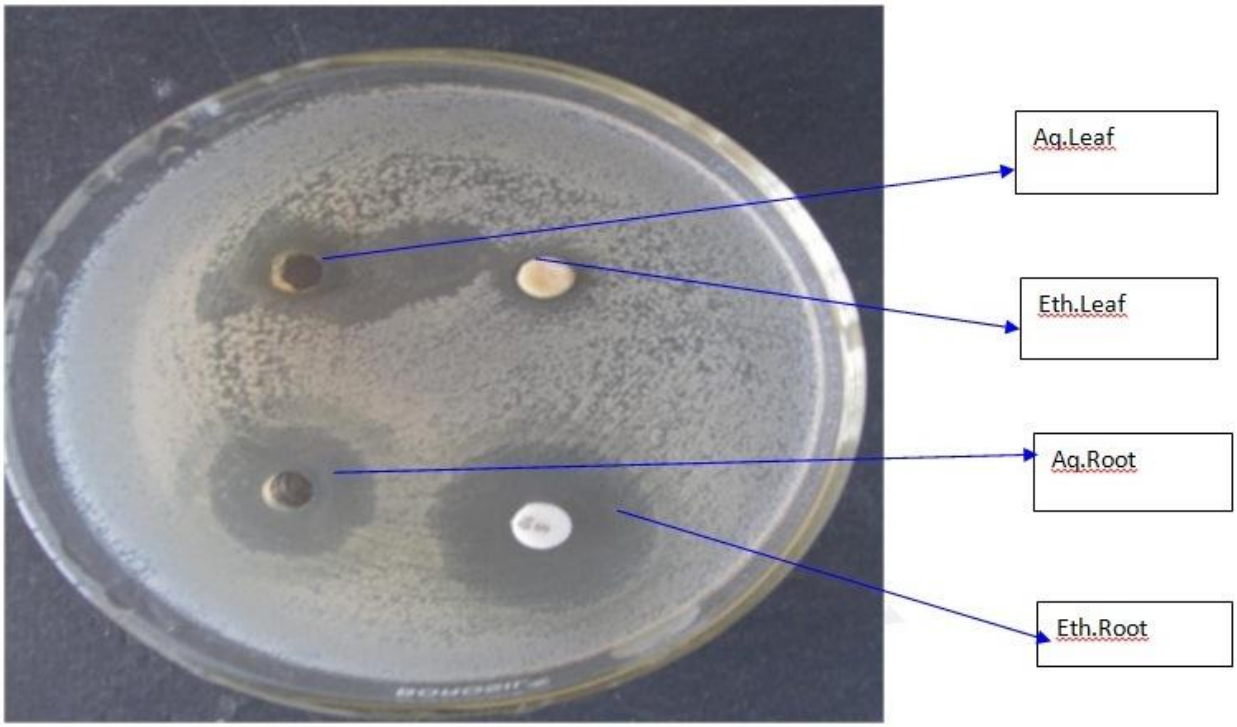
As a result of our studies related to the anti-microbial activity of different extracts of Murraya Koenigii, we report a novel and easy access to activity using aqueous and ethanolic leaf extracts and can be demonstrated over previously reported methods.We acquired the activity using different extracts by using ethanol and distilled water. The formed extracts were analysed by their zone of inhibition.

In the present study we used different species to measure inhibitory potential of Murraya Koenigii.Results of anti-microbial screening of the product have suggested a anti-microbial activity on the species like Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus. Results are tabulated.

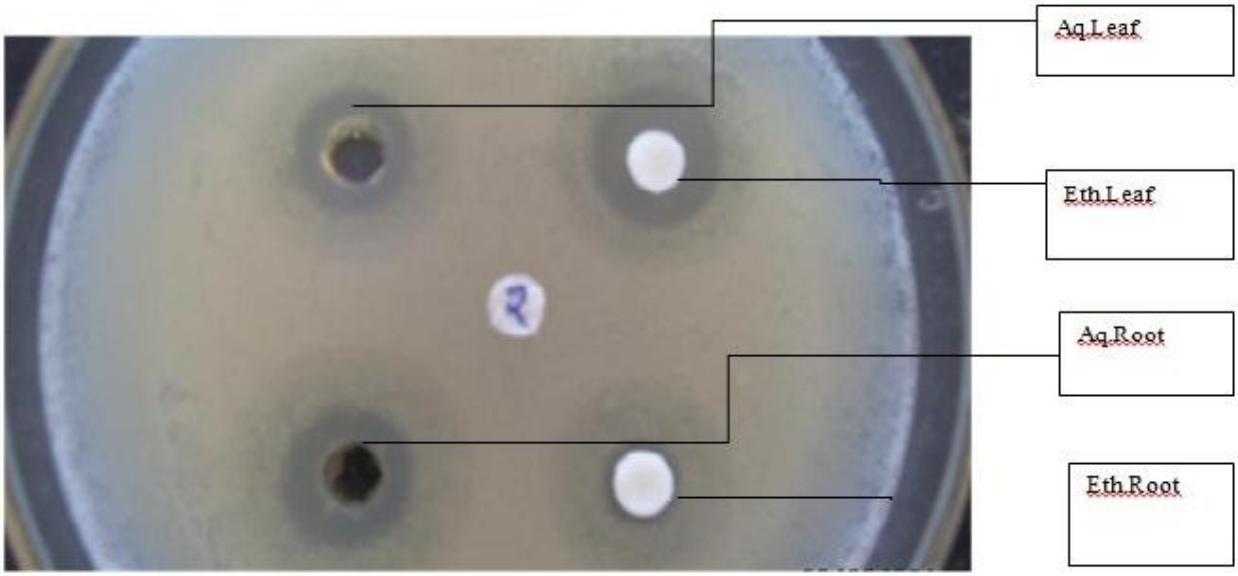
Comparing the effect of the Murraya Koenigii on microbial growth allowed concluding the extracts responsible for effective zone of inhibition. The presence of root & leaf extracts seems to be mandatory for potential inhibition.

Table: antimicrobial activity ofaqueous and ethanolic extracts of roots and leaves of murraya koenigii.

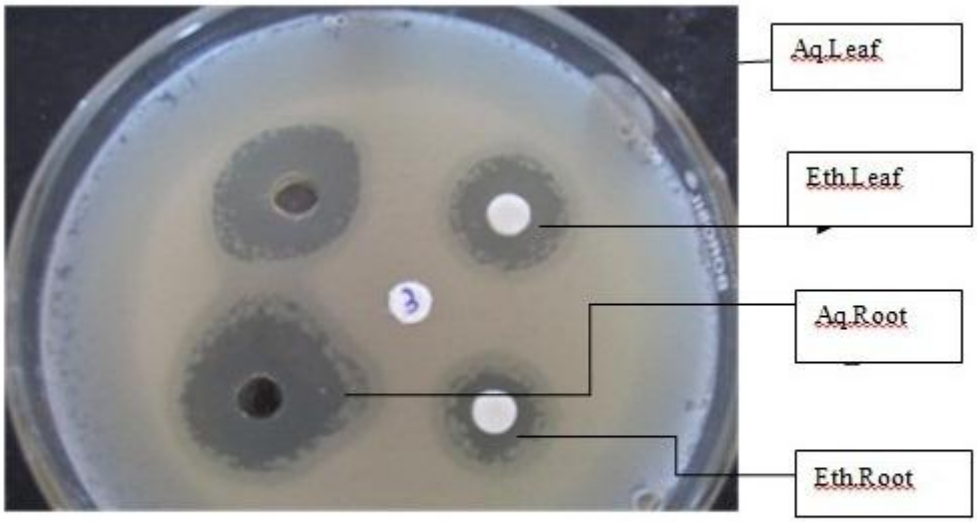
COMPOUND	STAPHYLOCOCCUS AUREUS		PSEUDOMONAS AERUGINOSA		ESCHERICHIA COLI	
	Zone of inhibition (mm)		Zone of inhibition (mm)		Zone of inhibition (mm)	
	250mg/ml	500mg/ml	250mg/ml	500mg/ml	250mg/ml	500mg/ml
Aqueous leaf	12	14	14	20	25	32
Ethanolic leaf	12	16	14	18	18	22
Aqueous root	10	12	10	12	16	22
Ethanolic root	14	16	16	18	18	24
Cephalosporin	16	20	18	22	20	26
Control(DMSO)	-	-	-	-	-	-



E.Coli



S.aureus



P.aeruginosa

CONCLUSION

From the above results it is evident that Murraya Koenigii showed significant broad spectrum anti-microbial activity at 250mg/ml & 500mg/ml concentration levels when compared with standard drug Cephalosporin. In particulars, aqueous and ethanolic extracts of leaves showed maximal activity.

Thus, it has been proved from the above experiment that not only Murraya Koenigii roots shows antimicrobial activity but also leaves show tremendous antimicrobial activity. Moreover, aqueous extracts of both roots and leaves of Murraya Koenigii shows excellent antimicrobial activity also got cleared. Hence, the above criterion which was taken into consideration regarding antimicrobial activity of root and leaf extacts of Murraya Koenigii has been concluded from the results achieved.

REFERENCES

[1]. Satyavati GV, Gupta AK, Tendon N, Medicinal Plants of India, Vol-2, Indian council of medical research, New Delhi India, 1987,289-299.
[2]. Kumar VS, Sharma A, Tiwari R, Sushil K. Murraya Koenigii-a review, JMAPS 1999;
[3]. Ito C. Studies On Medicinal Resources of Rutaceous Plants and Development To Pharmaceutical Chemistry, Natural Med.21 (4): 2000; 117-122.
[4]. Kirtikar KR, Basu BD. Indian Medicinal Plants, Edition 2, Vol. I, Oriental Enterprises, Uttarchal Pradesh, 1981, 473.
[5]. Shah KJ, Juvekar AR. Positive inotropic effect of Murraya koenigii (Linn.) Spreng extract on an isolated perfused frog heart. Indian Journal of Experimental Biology, 44: 2006; 481- 484.
[6]. Kumar S.R,Loveleena D,Godwin S.,Medicinal property of Murraya Koenigii-a review, International Research Journal of Biological Sciences.,2(9),2013,80-83.
[7]. Gahlawat D.K, Jakhar S, Dahiya P., Murraya koenigii (L.) Spreng: An ethnobotanical, phytochemical and pharmacological review,Journal of Pharmacognosy and Phytochemistry., 3(3), 2014,109-119.
[8]. Jain V, Momin M, Laddha K., Murraya Koenigii: An Updated Review, International Journal of Ayurvedic and Herbal Medicine.,2(4), 2012, 607-627.
[9]. Ganesan P, Phaiphan A, Murugan Y, Baharin B.S., Comparative study of bioactive compounds in curry and coriander leaves: An update, Journal of Chemical and Pharmaceutical Research.,5(11), 2013, 590-594.
[10]. Nadkarni KM, Indian Materia Medica, Edition 3, Vol. I, Popular Prakashan, Mumbai, 1976, 196.
[11]. Kirtikar KR, Basu BD. Indian Medicinal Plants, Edition 2, Vol. I, Oriental Enterprises, Uttarchal Pradesh, 1981, 473.
[12]. Merriam-Webster Online Dictionary. Archived from the original on 24 April 2009. Retrieved 05-02-2009.
[13]. Y. Rajendra prasad, P. Praveen kumar, P. Ravi kumar and A. Srinivasa rao, E-Journal of Chemistry; Vol.No.5: January 2008; Pg.No.144-148.
[14]. Kishor H. Chikhalia, Mayank J. Patel and Dhaval B. Vashi, ARKIVOC; 2008; Pg.No.189-197.
[15]. Amit R Trivedi, Dipti K.Dodiya et.al.ARKIBOC, 2008: Pg.No.131-141.