Antibacterial Activity of *Murraya koenigii* Linn Leaves

Sumit Gupta[1], Padmaa. M. Paarakh[1,*] and Usha Gavani[1]

[*]Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore 560078, Karnataka, India

Summary

Extracts of *Murraya koenigii* Linn (Rutaceae) were screened for their *in vitro* antibacterial activity by agar diffusion method in comparison with standard antibiotic penicillin. The antibacterial activity of petroleum ether, chloroform, acetone, methanol and aqueous extract of leaves of the plant were studied using *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* as test organism. Out of all extracts tested, chloroform, acetone and methanol extracts were effective against all the four microorganisms. Aqueous extract was more effective against *Bacillus subtilis* and *Staphylococcus aureus.* Petroleum ether extract was more effective against *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli*.

Key words: *Murraya koenigii* Linn, *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, in vitro* antibacterial activity.

*** Correspondence Author:**
Dr. Padmaa M Paarakh
Department of Pharmacognosy
The Oxford College of Pharmacy
J.P.Nagar, I. Phase
Bangalore 560078
padmaparas@hotmail.com
Mobile: 09880681532

Introduction

Murraya koenigii Linn (Rutaceae)commonly known as Meethi neem, is an aromatic more or less deciduous shrub or a small tree up to 6 m in height found throughout India up to an altitude of 1500 m and are cultivated for ite aromatic leaves[1]. In traditional system of Medicine, it is used as antiemetic, antidiarrhoeal, dysentery, febrifuge, blood purifier, tonic, stomachic, flavoring agent in curries and chetneys. The oil is used externally for bruises, eruption, in soap and perfume industry[2]. The phytoconstituents isolated so far from the leaves are alkaloids viz., mahanine[3], koenine, koenigine, koenidine[4], girinimbiol, girinimibine[5], koenimbine, O-methyl murrayamine A, O-methyl mahanine, isomahanine, bismahanine, bispyrayafoline[6] and other phytoconstituents such as coumarin glycoside viz., scopotin, murrayanine[7], calcium, phosphorus, iron, thiamine, riboflavin, niacin, vitamin C, carotene and oxalic acid. The essential oil from leaves yielded di-α-phellandrene, D-sabinene, D-α-pinene, dipentene, D-α-terpinol and caryophyllene[8]. It is reported to possess antioxiant, antibacterial, antifungal, larvicidal, anticarcinogenic, hypoglycemic, anti-lipid peroxidative, hypolipidemic and anti-hypertensive activity[9].

Since there is no report on antibacterial activity of leaves of *Murraya koenigii* against these four microorganisms, an attempt was made to evaluate the antibacterial activity of petroleum ether, chloroform, acetone, methanol and aqueous extract of the plant by agar diffusion method using *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* as test organism.

Materials and methods

Plant material:

Murraya koenigii Linn leaves were collected and authenticated by Central Council for Research in Ayurveda and Siddha, Bangalore. A voucher specimen (RRI/BNG/SMP/DrugAuthentication/2008-09/267) has been preserved in our Department for the future reference.

Extraction procedure

Shade dried leaves (470 g) were coarsely powdered and subjected to successive solvent extraction by continuous hot extraction (soxhlet). The extraction was done with different solvents in their increasing order of polarity such as petroleum ether (60-80^{0}C), chloroform, acetone, methanol and water. Each time the marc was air dried and later extracted with other solvents. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The yield was found to be 3.01, 1.77, 3.37, 3.26 and 15.5 % w/w with reference to the air dried plant. The dried extracts were dissolved in dimethyl sulphoxide (DMSO) and subjected to antibacterial activity.

Preliminary phytochemical screening

The coarse powder of leaves of *Murraya koenigii* (25g) was subjected to successive extraction with different solvents in their increasing order of polarity from petroleum ether (60^0-80^{0}C), chloroform, acetone, methanol and water. The extracts were concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents[10].

Microorganisms and media:

Gram Positive Bacteria: *Staphylococcus aureus, Bacillus subtilis*

Gram Negative Bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*

Bacteria's were obtained from the Department of Microbiology, The Oxford College of Science, Bangalore. The bacterial stock cultures were maintained on Muller Hinton agar and stored at 4^{0}C.

Antibacterial activity:

The extracts obtained above were screened for their antibacterial activity in comparison with standard antibiotic Penicillin (10 µg/ml) *in-vitro* by disc diffusion method[11] using *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* as test organism. Each extract were individually loaded on the 3 mm sterile disc at the concentration of 10, 25, 50, 100, 250, 500 and 1000 µg/ml and subjected to antibacterial activity. The results were recorded by measuring the zone of growth inhibition surrounding the disc. The experiments were done in triplicate.

Results and Discussion

The results of antibacterial activity are given in the Table 1 and 2. From the tables, it is clear that all the extract at various concentrations have shown antibacterial activity equivalent to that of standard against all the tested organism. Chloroform, acetone and methanol extracts have shown better activity than the standard against all the four microorganisms. Aqueous extract was more effective against *Bacillus subtilis* and *Staphylococcus aureus.* Petroleum ether extract was more effective against *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli*.

It is concluded that the plant extract possess antibacterial activity against test organism used. The zone of inhibition varied among suggesting that the varying degree of efficacy and different phytoconstituents of herb on the target organism. Preliminary phytochemical screening of different extracts showed the presence of alkaloids, tannins, saponin, flavonoids, steroids, coumarins and sugars. The antibacterial activity of the plants may be due to the presence of various active principles in their leaves. Further studies are needed to isolate and characterize the bioactive principles to develop new antibacterial drugs.

Table 1: Antibacterial activity of different extracts of leaves of *Murraya koenigii* against Gram negative organisms

Concentration used [μg/ml]	**Zone of inhibition of extract in mm**									
	Escherichia coli					***Pseudomonas aeruginosa***				
	PEE	**CE**	**AE**	**ME**	**AQE**	**PEE**	**CE**	**AE**	**ME**	**AQE**
10	12	12	13.2	12	-	12	12	12	12	-
25	12.2	12.6	13.4	12.2	12	12.2	12.2	12.6	12.6	-
50	12.4	14	14	12.6	12.6	12.6	12.4	13.2	13.2	12
100	12.6	14.6	15.2	14	13.2	14	12.6	14.6	15.2	12.6
250	13.6	16	16.6	15.2	14	14.6	14	16	16	12.8
500	14.6	17.2	16.8	16	14.6	16	14.6	17.2	17.2	14.6
1000	17.6	18.6	18	17.2	15.2	16.6	16	21.2	18.6	16
Penicillin [10]	16.6					16				

PEE-petroleum ether extract; CE- chloroform extract;AE-acetone extract; ME-methanol extract; AQE- aqueous extract.

Table 2: Antibacterial activity of different extracts of leaves of *Murraya koenigii* against Gram positive organisms

Concentration used [μg/ml]	**Zone of inhibition of extract in mm**									
	Staphylococcus aureus					***Bacillus subtilis***				
	PEE	**CE**	**AE**	**ME**	**AQE**	**PEE**	**CE**	**AE**	**ME**	**AQE**
10	12	12	12.6	12	14	12	12	12.6	14	12.6
25	12	12.6	14.6	14	14.2	12.2	14.6	13.2	14.2	13.2
50	12.6	13.0	15.2	14.2	14.4	12.4	16	14	14.4	13.4
100	13.3	13.6	15.4	14.4	14.6	12.6	17.2	14.4	17.2	13.6
250	14.6	16	16.6	14.6	15.3	13.2	17.6	16.6	18	16
500	16	18	18.6	16	17.3	14.6	18	18	18.6	17.2
1000	16.6	18.2	21.3	18.6	18.6	16.6	19.2	19.2	22	18
Penicillin [10]	17.3					16				

PEE-petroleum ether extract; CE- chloroform extract;AE-acetone extract; ME-methanol extract; AQE- aqueous extract.

Acknowledgement

The authors wish to thank Chairman and Executive Director, Children's Education Society for their facilities and support.

References

1. Anonymous, The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. Publication & Information Directorate, New Delhi : CSIR, 1998:446-448.
2. Prajapati ND, Purohit SS, Sharma AK, Kumar T. A Handbook of Medicinal Plants. Jodhpur: Agrobios, 2003: 352-353.
3. Narasimhan NS, Paradkar MV, Kelkar SL. Alkaloids of *Murraya koenigi*, Structures of mahanine, koenine, koenigine and koenidine. Indian J Chem 1970; 8:473-476.

4. Narasimhan NS, Paradkar MV, Chitguppi VP, Kelkar SL. Alkaloids of *Murraya koenigi*, Structures of mahanimbine, koenimbine, mahanine, koenine, koenigine and koenidine. Indian J Chem 1975; 13: 993-995.
5. Adebajo AC, Avoola OF, Iwalewa EO, Akindahunsi AA, Omisore NO, Cadewunmi CO et al. Anti-trichomonal, biochemical and toxicological activities of methanolic extract and some carbazole alkaloids isolated from the leaves of *Murraya koenigii* growing in Nigeria. Phytomedicine 2006; 13(4): 246-54.
6. Tachibana Y, Kikuzaki H, Lajis NH, Nakatani N. Comparison of antioxidative properties of carbazole alkaloids from *Murraya koenigii* leaves. J Agric Food Chem 2003; 51(22): 6461-7.
7. Adebajo AC, Reisch J: Minor furocoumarins of *Murraya koenigii*. Fitoterpia 2000; 71(3): 334-7.
8. Gopalan C, Rama Shastri BV, Balasubramanian SC. Nutritive value of Indian Foods. New Delhi: ICMR, 1984: 66,117.
9. Iyer D, Uma DP. Phyto-pharmacology of *Murraya koenigii*. Pharmacognosy Reviews 2008; 2: 180-184.
10. Kokate KR. Practical Pharmacognosy. New Delhi : Vallabha Prakashan,1990: 110-117.
11. Greenwood, Slack CB, Peutherer TT. Medical Microbiology, a guide to microbial infections, pathogenesis, immunity, laboratory diagnosis and control. Churchill Livingston, 2002: 225-285.