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ANTIBACTERIAL, ANTIFUNGAL AND ANTHELMINTIC ACTIVITY OF CURRY LEAVES *MURRAYA KOENIGII* (L.) SPRENG

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ABSTRACT

Antibacterial activity of different solvent extracts of leaves and water extract of stem (branch) and leaves of *Murraya koenigii* were found effective against bacterial species strains such as *Bacillus licheniformis*, *Bacillus aureus*, *Eschericia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus subtilis* . The results confirmed the antimicrobial effect of *Murrayakoenigii* extract against human pathogenic bacteria. Antifungal activity was also investigated against *Fusarium moniliforme*, *Fusarium oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia solanii*. The extract inhibited the radial growth of test fungi. Anthelminthic activity of *Murraya koenigii* was also detected in the different solvent extracts of leaves. Phytochemical screening of *Murraya* leaf extract was also performed which confirmed the presence of various proteins,saponins, phenolics, alkaloids and terpenes, Additionally, anthocyanins, flavonoids and soluble phenols were estimated to be in ample amounts.. The antibacterial activity is possibly due to antioxidant protein of curry leaf. Antfungal and anthemintic activities are presumably due to phenolics , alkaloids or terpenes.

Keywords: *Murraya koenigii*, antibacterial, antifungal, anthelmintic, phytochemial analysis, soluble phenols, anthocyanins, flavonoids

INTRODUCTION

Murraya koenigii (curry leaf) is an important medicinal plant used in Asian countries especially in India and Pakistan in medicines as well as a spice in curry. The plant *Murraya koenigii* (L.) Spreng. belongs to the family Rutaceae and the genus is named after Professor John Adam Murray of Gottingen University, Germany.

This member of the family Rutaceae is an unspined, semi-deciduous, aromatic, pubescent shrub or a small tree, 3-5 m high. The stem is woody, closely crowded and with a shady and crown. Leaves of this plant are alternate 15-20 cm long, glabrous, sometimes slightly pubescent when young and very strongly aromatic (Siriseree, 2010). The leaves of this plant are well-known as curry leaves and which contain compounds that are effective in increasing digestive secretions and relieve nausea, headache, indigestion, high blood pressure and vomiting (Ghani, 2003).

The antimicrobial activity is due to the presence of APC (Antioxidant protein of curry leaf) has been demonstrated to possess potent antibacterial activity against all human pathogenic strains as reported by Mylarappa *et al*., (2009). Numerous compounds with broad spectrum of inhibitory activity against pathogenic bacteria and fungi have been isolated and their mechanism of action demonstrated (Ng, 2004; Das and Biswas, 2012). Hexane and methanolic extract of leaves of *Murraya koenigii* contain steroids, alkaloids, saponin, glycosides and flavonoids which show activity against a variety of bacterial strains (Trease and Evans,1989). Ethanolic extract of leaves of *Murraya koenigii* were reported as more effective against bacteria rather than fungus by Gupta *et al*., (2011)**.**

Antifungal activity is investigated, in this paper, on several fungal strains. Many fungi destroy the foodstuffs and stored grain that becomes unfit for human consumption. Alternate pesticides that do not affect the quality of the environment are usually derived from biologically active plants and play increasingly important role in crop protection strategies. The leaves of *Murraya koenogii* have been reported to contain mono-terpenoids and ses-quiterpenoids which exhibit antifungal activity (Malwal and Sarin ,2011). A series of substituted carbazoles, termed N-alkylated 3,6-dihalogenocarbazoles, that exhibit fungicidal activity (Thevissen *et al*., 2009). The differences observed in their alkaloid composition suggested probable influence of geographical location on the elaboration of carbazole alkaloids (Fiebig, 1985) in the plant and differences in the localization of carbazole alkaloids in the plant parts (Chakrabarty *et al*., 1997; Adebajo *et al*., 2005). The structure of a number of alkaloids of *M. koenigii* has been reported by Narasimhan *et al*., (1970,1975). Stidies on anthelminthic activity were carried out (Qureshi *et al*., 2009) with some modifications in which earthworms (*Pheretima posthuma*) were used to determine the time of paralysis and death in seven solvent extracts which contained phenols, tannins and flavononoids. It has been reported that the methanolic extract of leaves *Murraya koenigii* shows anthelmintic activity using *Pheretima posthuma* worms (Akhtar *et al*., 2000).

The World Health Organization (WHO) estimated that about two billion people harbour parasitic worm infections. In addition to humans, these organisms infect livestock and crops, thereby hampering food production.. Among these most common infections in humans are Helminth infections that are human intestinal parasitic worms vectored through air, food and water. They secrete toxins and steal food and nutrients (Aswar *et al.*, 2009; Kumar *et al*., 2011). The objective of the present investigation was to test and confirm the antibacterial, antifungal and anthemintic activity of *Murraya koenigii* leaves and other plant parts using a variety of organisms.

MATERIALS AND METHODS

Curry leaves [*Murraya koenigii* (L.) Spreng] were collected from Institute of Environmental Studies plantation at the University of Karachi. The curry leaves were washed and then dried in a hot air oven for three days at a temp of a 40ºC. After complete drying, the leaves were ground to a fine powder to enhance the extraction potential using a domestic electric mixer grinder and the powder weighed. Extracts of Acetone, chloroform, ethanol, hexane, and methanol were investigated against different organisms including *Fusarium. moniliforme , Fusarium oxysporum , Macrophomina phaseolina, Rhizoctonia solanii , Bacillus licheniformis , Bacillus aureus , Eschericia coli, Salmonella typhmurium, , Staphylococcus aureus*, *Bacillus subtilis* which were obtained from the laboratory of Dr. Javed Zaki, University of Karachi and Department of Microbiology, Federal Urdu University, Karachi.

Extraction

The *Murraya* leaves were extracted by using Soxhlet extraction curry leaf powder (10g) was extracted in a Soxhlet apparatus with each solvent (450ml) acetone, hexane, chloroform, ethanol, methanol or distilled water. The solvents were removed through water bath and the extracts were kept at 4ºC until used for antioxidant assays. The efficacy of the extracts reported here was quantified based on the dry weight of the whole extract per volume of assay solution. The extract with concentration 750 mg /ml was used.

Dried powder of curry leaf and stem (branch) (10g) were taken in the conical flask and 100ml distilled water was added and the flask covered with cotton plug and left for 24 h and then the extract was filtered with Whatman No. 1 filter paper and the filtrate dried in a water bath at 45ºC to viscous form.

The extracts were reconstituted according to the solvent that was used to extract them with the concentration of 750 mg/ml. Subsequently, the samples were mixed by shaking.

Determination of Antibacterial activity of *Murraya koenigii* with different solvent extracts

Pure cultures of bacteria were obtained from the Department of Microbiology, University of Karachi. Nutrient broth were inoculated. The disc diffusion method was used to evaluate the antibacterial activity.

Nutrient agar (Oxoid, England) was prepared in plates as the media for test bacteria. Clean glass Petri plates were autoclaved. Melted nutrient agar was poured into the Petri plates and solidified and pure culture of bacteria was inoculated by sterilized blue pipettes. The culture was spread by the spread plate technique. Sterilized filter paper discs (Whatman No. 1) with 4mm diameter were impregnated with the extracts (750 mg/ml). Discs injected with 20 µl of solvent and sterilized water served as negative controls. Each Petri plate was divided into two halves and sterilized discs of filter paper with extract placed on each Petri plates. The culture name with particular solvent extract of *Murraya koenigi* was marked. The plates were covered and incubated at 25° C for 24 h. The antibacterial activity was interpreted from the size of the diameter of zone inhibition measured to the nearest millimeter (mm) as observed from the clear zones surrounding the discs. The plates were observed after 24 h.

Determination of Antifungal activity of *Murraya koenigii*

Each 1.5g of solvent dried extract of *Murraya koenigii* was dissolved in 1ml of the solvent. Clean glass Petri plates alone with filter paper were autoclaved. Melted nutrient agar was poured into the Petri plates and solidified. Pure cultures were added by sterilized blue pipettes which were sterilized and cultures were introduced by spread plate technique. Each Petri plate was divided into two halves and sterilized discs of filter paper dipped in extract were placed into each Petri plate separated by a line. The plates were incubated at 28ºC for 24 h. The Petri plates were observed after 24 h. The fungitoxicity of the extracts in terms of percentage inhibition of mycelial growth was calculated by using the formula:

% inhibition = [(dc – dt) /dc] x 100

Where dc = Average increase in mycelial growth in control, dt = Average increase in mycelial growth in treatment (Kumar *et al*., 2008).

Determination of Anthelminthic activity of *Murraya koenigii* with different solvent extracts:

Adult earthworms *Pheretima posthuma* were collected from moist soil and washed with normal saline to remove all soil and faecal matter and then used for the antihelmintic activity. All earthworms were of approximately equal in size. The earthworms of 0.3-0.5cm in width and 6-7cm in length were used for anthelminthic activity. Due to their physiological and anatomical resemblance with intestinal roundworm parasite of human beings and because they are easily available they are used as a suitable model for screening of anthelminthic activity of *Murraya koenigii* (Chatterjee, 1967; Kumar *et al*., 2011).

All extracts were of concentration 750mg/ml that were used in the bioassay which involved the determination of paralysis and death time of the earthworm. Normal saline water (NSW) was taken as control. Anthelminthic activity were carried following Kumar *et al.*, (2011) with minor modifications. For each solvent extract two earthworms were taken and released in saline water in which no paralysis and death occurred and then one by one each earthworm released into each extract and the time of paralysis and death were noted.

Screening of Phytochemicals in *Murraya koenigii*

Various tests were performed to detect different phytochemicals including tannins, alkaloids, steroid, triterpenoid, carbohydrate, and flavonoids were found in the extracts of *M. koenigii* leaves through Wagner's test which indicates the presence of alkaloids, Molisch's test which indicates the presence of carbohydrate, Froth test which indicates the presence of saponins, Salkowski's test that shows the presence of triterpenes, Ferric chloride test which indicates the presence of phenols, Gelatin test shows the presence of tannins, Alkaline Reagent test, indicates the presence of flavonoids, xanthoproteic test was used to indicate the presence of proteins (Tiwari, 2011, Harborne, 1993).

Estimation of Anthocyanins and Flavonoids:

UV-absorbing pigments were measured spectrophotometrically. Anthocyanins were extracted and determined in accordance with the method of Mancinelli *et al*., (1975). Fresh leaves or stem (branches) were ground in acidified methanol (1:99 HCl : methanol v/v). After keeping the extract at 0ºC for 24h the absorbance was recorded at 530nm. Flavonoids were extracted and measured from *Murraya koenigii* leaves and stems (branches) using the method of Mirecki and Teramura (1984). Extraction mixture consisted of acidified methanol (methanol: water: HCl, 80: 20: 1,v/v) + 1g of fine powder of plant tissue, incubated for 24h at 4ºC. The filtered extract was then used for measuring the absorbance at 315nm, which is indicative of relative concentration of UVB absorbing pigments. Flavonoid contents were expressed as absorbance g^{-1} fresh weight of tissue at 315nm.

Soluble phenol estimation

Soluble phenol contents in leaves of *Murraya koenigii* were determined in accordance with the method of Gonzalez *et al*., (2003). 500 mg powder of curry leaves was taken and separately homogenized with 2 ml 80% ethanol v/v. The homogenate was centrifuged three times at 6000 g for 3 min. 100 µl of the supernatant was added to 0.5 ml Folin-Ciocalteau reagent and 1 ml of 20% Sodium carbonate. Distilled water was added until a final volume of 10 ml was attained. The mixture was incubated at 40°C for 30 min. and the absorbance of the developed blue colour was read at 750nm. Catechol was used as standard. Soluble phenols was estimated as µg mg^{-1} fresh weight.

Statistical analysis

Unless stated otherwise, three replicates were used and the data were subjected to analysis of variance (ANOVA) following Zar (2010). As a post-hoc test Fisher's least significant difference test $LSD_{0.05}$ was used.

RESULT AND DISCUSSION

The statistical analysis of growth inhibition in groups of bacterial strains using analysis of variance (ANOVA) showed significant differences (P<0.01). The results of antibacterial activity of curry leaves (*Murraya koenigii*) against various bacteria and actinomycetes is shown in Table 1 while the respective control values appear in Table 2. It is apparent that high activity was shown by *Murraya koenigii* against 18 bacterial strains. Moderate activity was shown against eleven strains of bacteria. Whereas other bacteria (strains) responded inadequately to *Myrraya koenigii* extracts.

Table 1. Antibacterial activity against different bacterial species by *Murraya koenigii* extracted using different solvents. For diameter each value is mean of two replicates ± standard error.

Test cultures	Solvent extract	Test disc 1 mm	Test disc 2 mm	Diameter (mm) Mean ± S.E	Radius	Gram	Remarks (Inhibition activity)
Bacillus licheniformis	Acetone	14	30	22 ±0.614	11	+ve	High
Bacillus licheniformis	Chloroform	12	5	8.5±0.614	4.75	+ve	Moderate
Bacillus licheniformis	Ethanol	16	13	14.5±0.614	7.25	+ve	High
Bacillus licheniformis	Hexane	12	15	13.5±0.614	6.75	+ve	High
Bacillus licheniformis	Methanol	10	11	10.5±0.614	5.25	+ve	High
Bacillus licheniformis	Water extract of branch	4	2	3±0.614	1.5	+ve	Low
Bacillus licheniformis	Water extract of leaves	1	1	1±0.614	0.5	+ve	Low
Bacillus aureus	Acetone	10	17	13±0.1002	6.5	-ve	High
Bacillus aureus	Chloroform	8	5	6.5±0.1002	3.25	-ve	Moderate
Bacillus aureus	Ethanol	16	12	14±0.1002	7	-ve	High
Bacillus aureus	Hexane	14	13	13.5±0.1002	6.75	-ve	High
Bacillus aureus	Methanol	8	7	7.5±0.1002	3.75	-ve	Moderate
Bacillus aureus	Water extract of branch	8	7	7.5±0.1002	3.75	-ve	Moderate
Bacillus aureus	Water extract of leaves	2	4	3±0.1002	1.5	-ve	Low
Escherichia coli	Acetone	3	4	3.5±0.379	1.75	-ve	Low
Escherichia coli	Chloroform	6	7	6.5±0.379	3.75	-ve	Moderate
Escherichia coli	Ethanol	5	5	5±0.379	2.5	-ve	Low
Escherichia coli	Hexane	11	8	9.5±0.379	4.75	-ve	High
Escherichia coli	Methanol	7	8	7.5±0.379	3.75	-ve	High
Escherichia coli	Water extract of branch	4	5	4.5±0.379	2.25	-ve	Low
Escherichia coli	Water extract of leaves	5	5	5±0.379	2.5	-ve	Low
Salmonella typhimurium	Acetone	6	9	7.5±0.223	3.75	-ve	High
Salmonella typhimurium	Chloroform	7	6	6.5±0.223	3.25	-ve	Moderate
Salmonella typhimurium	Ethanol	5	6	5.5±0.223	2.75	-ve	Moderate
Salmonella typhimurium	Hexane	6	8	7±0.223	3.5	-ve	High
Salmonella typhimurium	Methanol	8	7	7.5±0.223	3.75	-ve	High
Salmonella typhimurium	Water extract of branch	4	4	5.5±0.223	2	-ve	Moderate
Salmonella typhimurium	Water extract of leaves	6	5	4±0.223	2.75	-ve	Low
Bacillus subtilis	Acetone	9	7	8±1.46	4	+ve	High
Bacillus subtilis	Chloroform	4	8	6±1.46	3	+ve	Moderate
Bacillus subtilis	Ethanol	12	5	8.5±1.46	4.25	+ve	High
Bacillus subtilis	Hexane	25	11	18±1.46	9	+ve	High
Bacillus subtilis	Methanol	13	8	10.5±1.46	5.25	+ve	High
Bacillus subtilis	Water extract of branch	5	7	5.5±1.46	2.75	+ve	Low
Bacillus subtilis	Water extract of leaves	6	5	6±1.46	3	+ve	Moderate
Staphyloc-occus aureus	Acetone	5	4	4.5±1.215	2.25	+ve	Low
Staphylococcus aureus	Chloroform	7	5	6±1.215	3	+ve	Moderate
Staphylococcus aureus	Ethanol	8	7	7.5±1.215	3.25	+ve	High
Staphylococcus aureus	Hexane	15	20	17.5±1.215	8.75	+ve	High
Staphylococcus aureus	Methanol	11	10	10.5±1.215	5.25	+ve	High
Staphylococcus aureus	Water extract of branch	6	4	5±1.215	2.5	+ve	Low
Staphylococcus aureus	Water extract of leaves	5	5	5±1.215	2.5	+ve	Low

9.5mm and above= High, 5.5-9mm = Moderate, 1-5mm = Low

Table 2. Control tests of solvents without *Murraya koenigii* extract

Test cultures	Solvent	Test disc (mm)	Diameter Mean	Radius	Gram	Remarks (Inhibitory activity)
Bacillus licheniformis	Acetone	6-4	2mm	1mm	+ve	Low
Bacillus licheniformis	Chloroform	9-4	5mm	2.5mm	+ve	Low
Bacillus licheniformis	Ethanol	5-4	1mm	0.5mm	+ve	Low
Bacillus licheniformis	Hexane	7-4	3mm	1.5mm	+ve	Low
Bacillus licheniformis	Methanol	8-4	4mm	2mm	+ve	Low
Bacillus licheniformis	D.water	4-4	0mm	0mm	+ve	-
Bacillus aureus	Acetone	4-4	0mm	0mm	-ve	-
Bacillus aureus	Chloroform	5-4	1mm	0.5mm	-ve	Low
Bacillus aureus	Ethanol	5-4	1mm	0.5mm	-ve	Low
Bacillus aureus	Hexane	5-4	1mm	0.5mm	-ve	Low
Bacillus aureus	Methanol	7-4	3mm	1.5mm	-ve	Low
Bacillus aureus	D.water	4-4	0mm	0mm	-ve	-
Escherichia coli	Acetone	5-4	1mm	0.5mm	-ve	Low
Escherichia coli	Chloroform	9-4	5mm	2.5mm	-ve	Low
Escherichia coli	Ethanol	6-4	2mm	1mm	-ve	Low
Escherichia coli	Hexane	10-4	6mm	3mm	-ve	Moderate
Escherichia coli	Methanol	7-4	3mm	1.5mm	-ve	Low
Escherichia coli	D.water	4-4	0mm	0mm	-ve	-
Salmonella typhi	Acetone	6.5-4	2.5mm	1.25mm	-ve	Low
Salmonella typhi	Chloroform	4-4	0mm	0mm	-ve	-
Salmonella typhi	Ethanol	7-4	3mm	1.5mm	-ve	Low
Salmonella typhi	Hexane	5-4	1mm	0.5mm	-ve	Low
Salmonella typhi	Methanol	8-4	4mm	2mm	-ve	Low
Salmonella typhi	D.water	6-4	2mm	1mm	-ve	Low
Bacillus subtilis	Acetone	5-4	1mm	0.5mm	+ve	Low
Bacillus subtilis	Chloroform	6-4	2mm	1mm	+ve	Low
Bacillus subtilis	Ethanol	5-4	1mm	0.5mm	+ve	Low
Bacillus subtilis	Hexane	4-4	0mm	0mm	+ve	-
Bacillus subtilis	Methanol	10-4	6mm	3mm	+ve	Low
Bacillus subtilis	D.water	5-4	1mm	0.5mm	+ve	Low
Staphylococcus aureus	Acetone	5-4	1mm	0.5mm	+ve	Low
Staphylococcus aureus	Chloroform	12-4	8mm	4mm	+ve	Moderate
Staphylococcus aureus	Ethanol	4-4	0mm	0mm	+ve	-
Staphylococcus aureus	Hexane	7-4	3mm	1.5mm	+ve	Low
Staphylococcus aureus	Methanol	7-4	3mm	1.5mm	+ve	Low
Staphylococcus aureus	D.water	4-4	0mm	0mm	+ve	-

9.5mm and above= High, 5.5-9mm = Moderate, 1-5mm = Low

Among the 7 solvent extracts hexane, methanol, chloroform, acetone, ethanol and water extract of branch have recorded a significant antibacterial activity. In *Bacillus licheniformis* greater activity was found in acetone with 22mm diameter compared to control (Table 1 and 2), with acetone solvent the activity was low (1mm). Ethanol and hexane also showed greater activity 13.5mm,13.5mm in terms of inhibition zone diameter (Table 1). Leaf extract of water have 1mm zone of inhibition (Table 2), therefore, activity in water extract was low.

Zone of inhibition in *Bacillus aureus* was 13mm in acetone (Table 1) while control was 0 mm (Table 2). It has been reported that antioxidant activity is due to the presence of phenolic compound (Cook and Samman 1996). Acetone extract contain carbazole alkaloids that are responsible for anti microbial activity and ethanolic extract of *Murraya koenigii* also contain flavonoids, alkaloids, tannin, saponins and protein which are responsible for the antibacterial activity (Ibrahim, 2010). In ethanol extract zone of inhibition was 14mm (Table 1) and 0.5mm in control (Table 2). Hexane with 13.5mm (Table 1) and 0.5mm with control (Table 2). Methanol and water extract of branch activity was 7.5mm,7.5mm (Table 1) with 1.5mm, 0mm with control (Table 2). Water extract of leaves contain 3mm (Table 1) and 0mm in control (Table 2). The preliminary phytochemical screening of extracts of *Murraya koenigii* showed the presence of sterols and triterpenoids, alkaloids, phenolic compounds and flavonoids.

The study by Prashant (2011) also disclosed the presence of proteins, mucilage, sterols and triterpenoids, alkaloids, phenolic compounds and flavonoids. Thus the activities of *Murraya koenigii* could be due to one or more of these compounds.

In *Escherichia coli* acetone extract was shown inhibition of 3.5mm (Table1) and 0.5 in control (Table 2), chloroform extract with 6.5mm (Table1) and 2.5mm as control (Table 2). Ethanol with 5mm (Table 1) and 1mm as control (Table 2). Hexane was found to have great activity in *Escherichia coli* is 9.5mm (Table 1) and 3mm as control (Table 2). Methanol was found to have 7.5mm (Table 1) and 1.5mm as control.water extracts of leaves and branch with 4.5mm and 5mm (Table 1) and 0mm with control. Acetone extracts of fresh leaves was found to have mosquitocidal property. A derivative benzisofuranone along with six known carbazole alkaloid and three known steroids were isolated from the stem bark of *M. koenigii*. They were reported for antimicrobial activity (Darvekar, 2011). Remarkable result was found with hexane , methanol, ethanol and acetone that also contain greater amounts of phenol, tannins and flavonoids. Flavonoids and phenols are water soluble therefore they they have antioxidant activity in water extract of *Murraya koenigii* as well (Tiwari *et al*., 2011). In *Salmonella typhimurium* the activity of acetone, chloroform, ethanol, hexane, methanol, water extract of branch (stem) and leaves was moderate to low with 7.5, 6.5, 5.5, 7, 7.5, 5.5 and 4mm clear zones respectively (Table 1) while 6.5, 4, 7, 5, and 6mm in control (Table 2).

Bacillus subtilis showed greater inhibition in methanol, hexane and ethanol with 10.5mm, 18mm and 8.5mm (Table 1) while 10mm, 4mm and 5mm in control. acetone, chloroform, water extract of branch and leaves show moderate to low activity with 8, 6, 6 and 5.5mm (Table 1). In control 5mm, 6mm, and 5mm in treatment of Bs with *Murraua koenigii* (Table 2). In *Staphylococcus aureus* high zone of inhibition was found in hexane and methanol with 17.5mm and 10.5mm and 7mm, 7mm in control (Table 2) while acetone, chloroform, ethanol, water extract of branch and leaves with 4.5mm, 6mm, 7.5mm, 5mm, 5mm (Table1) shows moderate activity because 5, 12, 4 and 4mm was found in control of *Murraya koenigii* (Table 2).

Murraya koenigii extracts in various solvents showed substantial antifungal activity against a number of fungi and the radial growth of various fungi was inhibited to significantly varying degree ($p<0.01$) (Table 3). The control values of the fungi (without *Murraya koenigii* extract appear in Table 4. All four test species including *Fusarium oxysporum*, *F. moniliforme*, *Macrophomina phaseolina* and *Rhizoctonia solani* were suppressed by curry leaf extracts in various solvents. Among the solvents, acetone and methanol extracts generally showed high antifungal activity.

Table 3. Antifungal activity of *Murraya koenigii* with different solvents. Each value is a mean of 2 replicates. Mean ± standard error.

Test cultures	Solvent extract	Test disc 1-Td* (mm)	Test disc 2-Td (mm)	Diameter Mean ±S.E (mm)	Radius	Gram +/-	%Inhibition= dc-dt/dc*100	Remarks
Fusarium moniliforme	Acetone	11	12	11.5± 0.856	5.75	+ve	-91.66	High
Fusarium moniliforme	Chloroform	8	7	7.5 ±0.856	3.75	+ve	-7.1	Moderate
Fusarium moniliforme	Ethanol	9	10	9.5 ±0.856	4.75	+ve	-850	High
Fusarium moniliforme	Hexane	8	7	7.5 ±0.856	3.75	+ve	-275	Moderate
Fusarium moniliforme	Methanol	16	8	12 ±0.856	6	+ve	-1100	High
Fusarium moniliforme	Water extract of branch	8	6	7 ±0.856	3.5	+ve	-600	Moderate
Fusarium moniliforme	Water extract of leaves	9	7	8.5± 0.856	4.25	+ve	-425	Moderate
Fusarium oxysporum	Acetone	14	12	13 ±0.408	6.5	-ve	-650	High
Fusarium oxysporum	Chloroform	7	6	6.5±0.408	3.25	-ve	-550	Moderate
Fusarium	Ethanol	3	7	5±0.408	2.5	-ve	-250	Low

oxysporum								
Fusarium oxysporum	Hexane	15	10	12.5±0.408	6.25	-ve	-525	High
Fusarium oxysporum	Methanol	12	9	10.5±0.408	5.25	-ve	-1000	High
Fusarium oxysporum	Water extract of branch	7	8	7.5±0.408	3.75	-ve	-375	Moderate
Fusarium oxysporum	Water extract of leaves	2	7	4.5±0.408	2.25	-ve	-225	Low
Macrophomina phaseolina	Acetone	12	11	11.5±0.875	5.75	+ve	-283	High
Macrophomina phaseolina	Chloroform	8	8.5	8.25±0.875	4.125	+ve	-106	Moderate
Macrophomina phaseolina	Ethanol	11	7	9±0.875	4.5	+ve	-200	Moderate
Macrophomina phaseolina	Hexane	10	8	9±0.875	4.5	+ve	-200	Moderate
Macrophomina phaseolina	Methanol	30	13	21.5±0.875	10.75	+ve	-437	High
Macrophomina phaseolina	Water extract of branch	14	17	15.5±0.875	7.75	+ve	-775	High
Macrophomina phaseolina	Water extract of leaves	20	17	18.5±0.875	9.25	+ve	-925	High
Rhizoctonia solanii	Acetone	10	15	12.5±0.458	6	+ve	-600	High
Rhizoctonia solanii	Chloroform	5mm	6mm	5.5mm±0.458	2.75	+ve	8.3	Moderate
Rhizoctonia solanii	Ethanol	6mm	7mm	6.5mm±0.458	3.25	+ve	-62.5	Moderate
Rhizoctonia solanii	Hexane	7mm	7mm	7mm±0.458	3.5	+ve	-40	Moderate
Rhizoctonia solanii	Methanol	10mm	7mm	8.5mm±0.458	4.25	+ve	-41.66	Moderate
Rhizoctonia solanii	Water extract of branch	14mm	15mm	14.5mm±0.458	7.25	+ve	-725	High
Rhizoctonia solanii	Water extract of leaves	13mm	14mm	13.5mm±0.458	6.75	+ve	-675	High

*Td= Test disc; 9.5mm and above= High, 5.5-9mm = Moderate, 1-5mm = Low

Against *Fusarium moniliforme* greater activity was shown by *Murraya* in acetone, ethanol, methanol, water extract of leaves and hexane with 11.5mm,9.5mm, 12mm, 8mm, 7.5mm inhibition zone diameter (Table 3) and with control 3mm, 0.5mm,0.5mm, 0.5mm and 1mm (Table 4). water extract of branch also showed 7mm inhibition and 0.5mm. Best results were found in acetone, ethanol, hexane and methanol extracts. Chloroform, water extract of branch and leaves also showed noteworthy results. Essential oils are known to be responsible for antifungal activity. In *Fusarium oxysporum* acetone, methanol hexane and water extract of branch zone of inhibition was found 13mm, 12.5mm,10.5mm and 7.5mm (Table 3)while 0mm, 1mm, 0.5mm and 0mm as control (Table 4). In *Macrophomina phaseolina* methanol , water extract of branch and leaves shown more activity with 21.5mm, 15.5mm, 18.5mm (Table 3) and 2mm, 0mm and 0mm as control (Table 4).In *Rhizoctonia solanii* acetone, water extract of branch and leaves, methanol and hexane shows activity with 12mm, 14.5mm,13.5mm, 8.5mm, 7mm (Table 3) compared to controls 0mm, 0mm, 0mm, 3mm and 2.5mm respectively (Table 4). Extract of branch and leaves in methanol are effective in inhibiting the growth of *R. solani* which cause diseases including damping off, root rot, collar rot and wire stem of plants.

Table 4. Control tests of the fungi in various solvents without *Murraya koenigii*

Test cultures	Solvent	Test disc (mm)	Diameter	Radius	Gram	Remarks
Fusarium moniliforme	Acetone	10-4	6mm	3mm	+ve	Moderate
Fusarium moniliforme	Chloroform	11-4	7mm	3.5mm	+ve	Moderate
Fusarium moniliforme	Ethanol	5-4	1mm	0.5mm	+ve	Low
Fusarium moniliforme	Hexane	6-4	2mm	1mm	+ve	Low
Fusarium moniliforme	Methanol	5-4	1mm	0.5mm	+ve	Low
Fusarium moniliforme	D.water	5-4	1mm	0.5mm	+ve	Low
Fusarium oxysporum	Acetone	4-4	0mm	0mm	-ve	-
Fusarium oxysporum	Chloroform	5-4	1mm	0.5mm	-ve	Low
Fusarium oxysporum	Ethanol	4-4	0mm	0mm	-ve	-
Fusarium oxysporum	Hexane	6-4	2mm	1mm	-ve	Low
Fusarium oxysporum	Methanol	5-4	1mm	0.5mm	-ve	Low
Fusarium oxysporum	D.water	4-4	0mm	0mm	-ve	-
Macrophomina phaseolina	Acetone	7-4	3mm	1.5mm	+ve	Low
Macrophomina phaseolina	Chloroform	8-4	4mm	2mm	+ve	Low
Macrophomina phaseolina	Ethanol	7-4	3mm	1.5mm	+ve	Low
Macrophomina phaseolina	Hexane	9-4	5mm	2.5mm	+ve	Low
Macrophomina phaseolina	Methanol	8-4	4mm	2mm	+ve	Low
Macrophomina phaseolina	D.water	4-4	0mm	0mm	+ve	-
Rhizoctonia solanii	Acetone	4-4	0mm	0mm	+ve	-
Rhizoctonia solanii	Chloroform	10-4	6mm	3mm	+ve	Moderate
Rhizoctonia solanii	Ethanol	8-4	4mm	2mm	+ve	Low
Rhizoctonia solanii	Hexane	9-4	5mm	2.5mm	+ve	Low
Rhizoctonia solanii	Methanol	10-4	6mm	3mm	+ve	Moderate
Rhizoctonia solanii	D.water	4-4	0mm	0mm	+ve	-

9.5mm and above= High, 5.5-9mm = Moderate, 1-5mm = Low

Table 5. Anthelmintic activity of *Murraya koenigii* in terms of paralysis and death time of earthworm (*Pheritima posthuma*) in various solvent extracts.

SOLVENT EXTRACT	TIME OF PARALY-SIS (Sec)	TIME OF DEATH (Sec)	MEAN PARALYSI-S TIME (Sec)	MEAN DEATH TIME(Sec)	CONTROL NSW paralysis & death (Sec)	CONC. (mg/ml)	Remarks
Water extract of leaves 1	60sec	89sec	-	-	Nil	750mg/ml	
Water extract of leaves 2	75sec	90sec	67.5sec	89.5sec	Nil	750mg/ml	Low
Water extract of branch 1	40sec	59 sec	-	-	Nil	750mg/ml	
Water extract of branch 2	38sec	62 sec	39 sec	60.5 sec	Nil	750mg/ml	Moderat e
Acetone 1	15sec	39 sec	45/2=	86/2=	Nil	750mg/ml	
Acetone 2	30sec	47 sec	22.5 sec	43 sec	Nil	750mg/ml	High
Chlorofor-m 1	12sec	30 sec	22/2=	70/2=	Nil	500mg/ml	
Chlorofor-m 2	10sec	40 sec	11 sec	35 sec	Nil	500mg/ml	High
Ethanol 1	12sec	15sec	22/2=	35/2=	Nil	500mg/ml	
Ethanol 2	10sec	20sec	11 sec	17.5 sec	Nil	500mg/ml	High
Hexane 1	4sec	20sec	18/2=	62/2=	Nil	750mg/ml	
Hexane 2	14sec	42sec	9 sec	31 sec	Nil	750mg/ml	High
Methanol 1	36sec	59sec	111/2=	149/2=	Nil	750mg/ml	
Methanol 2	75sec	90sec	55.5 sec	74.5 sec	Nil	750mg/ml	High

9.5mm and above= High, 5.5-9mm = Moderate, 1-5mm = Low

A significant activity of water extract of stem (branch) showed the paralysis and death time of earthworm as compared to the water extract of leaves but greater activity was shown by hexane extract of *Murraya koenigii* with regard to time of paralysis and death (Table 5). In normal saline water no paralysis and death was found which was used as control. A significant activity with low concentration of 500mg/ml was shown by ethanol extract with regard to paralysis 11sec and death 17.5sec while chloroform with the same concentration of 500mg/ml showed 11sec for paralysis and 35sec for death. Hexane with similar concentration showed 9 sec in paralysis and 31 sec in death of earthworms. Overall the results were remarkable in that the extract of *Murraya koenigii* is proven to be anthelminthic activity and can be used as an alternative strategy against gastrointestinal nematodes (Kumar, 2011). Antihelmintic activity of solvents extracts is may be due to the presence of phenols, tannins and flavonoids (Quraishi *et al*., 2009; Maheswari and Cholarani, 2013).

Table 6. Concentration of anthocyanins, flavonoids and total soluble phenols in the leaves and branches (stem) of *Murraya koenigii*. Means ± standard error.

Plant part	Anthocyanins A530 g^{-1}fr. Wt.	Flavonoids A315g^{-1} fr wt	Soluble phenols µg/g
Leaves	0.115 ± 0.027	0.252 ± 0.022	52.3 ± 6.4
Branches (stem)	0.128 ± o.o19	0.368 ± 0.027	43.6 ± 3.7
$LSD_{0.05}$	0.08	0.14	7.9

The level of anthocyanins was greater in the stem (branches) compared to leaves (Table 6). Likewise, flavonoids content was also greater in the stem relative to leaves. However, total soluble phenol content was higher in the leaves compared to stem. These presumably contribute towards its antioxidant activity of *Murraya koenigii* and also provide a defense mechanism to the plant against the pathogens.

CONCLUSIONS

In the light of our research in effectiveness of *Murraya koenigii* which indicated inhibitory activity against bacterial strains and fungi, through solvent extracts, which can be helpful in minimization of certain infectious diseases and several other problems due to the presence of certain phytochemicals. Solvent extracts of leaves and water extract of leaves and branches of *Murraya koenigii* are effective against a number of fungal strains and can be used to inhibit the growth of fungi in order to preserve the stored crops, food and cereals. Leaves of *Murraya koenigii* can also be effective in treatment of helminths in human intestine due to their anthelmintic activity as demonstrated here. Ethanolic and hexane solvent extract exhibited remarkable anthelmintic activity. Antimicrobial activity may be due to the presence of flavonoids and phenols. *Murraya koenigii* was shown to contain ample antioxidants as the total soluble phenols, anthocyanins and flavonoids were estimated to be present in significant amounts in leaves as well as in stem (branches).

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