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ANTIMICROBIAL POTENTIAL OF SELECTED PLANT EXTRACTS AGAINST IMPORTANT PLANT PATHOGENIC MICROORGANISMS

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ABSTRACT

Evaluation of natural products as safe and effective antimicrobial agents is one of the scientific strategies to combat the menace of drug-resistance and pesticide pollution. Aqueous extract and different solvent extracts of seeds of *Trachyspermum ammi,* leaves of *Murraya koenigii* (Linn.) Spreng., peels of *Cucumis sativus* Linn. and *Citrullus lanatus* Thunb., were screened *in vitro* for antimicrobial activity against phytopathogenic microbes namely, *Xanthomonas axonopodis* pv. *vesicatoria*, *Xanthomonas campestris* pv. *campestris, Pseudomonas syringae, Aspergillus flavus* and *Fusarium verticelloides* by agar cup diffusion method. Among the aqueous and different solvent extracts, methanol extract of *M. koenigii* showed significant activity against all the tested bacteria and *F. verticelloides* (zone of inhibition ranged from 8 to 53 mm).Methanol extracts of *C. sativus* recorded an inhibition zone of 15 mm against *X. a.* pv. *vesicatoria* and13mm against *X. c.* pv. *campestris.* Chloroform, ethyl acetate and methanol extracts of *T. ammi* recorded zone of inhibition ranging from 8 to 32mm against all the test bacteria and *F. verticelloides.* The antimicrobial activity of these promising plant extracts when compared with standard drugs streptocycline, bacteromycin and bavistin revealed significant inhibitory potential. Preliminary phytochemical analysis of the active extracts revealed the presence of tannin in all the tested extracts. The positive results of the present evaluation provide primary platform for further pharmacological studies.

KEYWORDS: *Antimicrobial activity, Murraya koenigii, Trachyspermum ammi and Tannins*

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INTRODUCTION

Infection of plants by various phytopathogenic bacteria and fungi leading to several diseases could be controlled by using different pesticides. However, World Health Organization has banned many agriculturally important pesticides due to soil, air and water pollution and wide range of toxicity against non-target pathogen and human beings[1]. An intensive application of relatively high levels of Hexachlorocyclohexane (HCH) and Dichlorodiphenyltrichloroethane (DDT) were observed in Indian environment and biota and the persistence of organochlorine residues is found to be present in human breast milk[2-3]. Knowledge of the deleterious effect because of extensive use of pesticides and increased antibiotic resistance in pathogenic microbes has triggered interest in the search of new antimicrobial substances/drugs of plant origin. The use of plant derived alternative pesticides seems to be regaining popularity and could play a vital role in combating the diseases. Plants are the natural resource of great variety of bioactive chemical constituents[23] and are rich in secondary metabolites, A single plant may contain several active compounds of biological significance. In this context, the present investigation was done to evaluate the antimicrobial potential and phytochemical constituents of *Murraya koenigii* (veracular name: Curry)*, Trachyspermum ammi* (veracular name: Ajwain)*, Cucumis sativus* (veracular name: Cucumber) and *Citrullus lunata* (veracular name: Water melon) which forms an integral part of Indian diet and medicine. *Murraya koenigii* Linn. belongs to the family Rutaceae, is commonly used as condiment and spice in India. The leaves are acrid, bitter, cooling, analgesic, anti-inflammatory and anthelmintic. It is used in the treatment of piles, vomiting and blood disorders. Application of crushed leaves on skin will cure skin eruption and skin burn[4].*Trachyspermumammi* belongs to the family Apiaceae, seeds of the plant are used for their characteristic aroma and pungent taste, and are mainly used for flavoring numerous foods. It is used to cure stomach disorders, applied externally for relieving colic pain. The seeds are reported to possess anti-aggregatory, anthelmintic, antihyperlipidaemic, antimalarial, insecticideal and kidney stone inhibitory activity[5]. The fruits of *Cucumis sativus (*belonging to the family Cucurbitaceae) are sweet, refrigerant, haemostatic, diuretic, tonic and useful in, thermoplegia, fever, insomnia, haemorrhages, stragurt and general debility. It is useful against burning sensation, bronchitis, cephalalgia, general debility, fever, haemorrhages, hyperdisipia, insomnia, jaundice, strangury and thermoplegia[6]. *Citrullus lanatus* Thunb. (cucurbitaceae)fruits are eaten as a febrifuge when fully ripe. The fruit is diuretic, being effective in the treatment of dropsy and renal stones. The rind of the fruit is prescribed in case of alcoholic poisoning and diabetes[7].

MATERIALS AND METHODS

Collection of plant materials

Peels of *Cucumis sativus* (Cucumber) and *Citrullus lanatus* (water mellon) were collected from cucumber and water mellon sellers of the city Mysuru. Leaves of *Murraya koenigii* (curry) and seeds of *Trachyspermum ammi* (Ajwain) were collected from the local market of Mysuru, Karnataka.

Extract Preparation

Aqueous extract

Sample (50g) of fresh plant material was ground with 100 ml sterile distilled water in a waring blender for 10 min. Macerate was filtered through double-layered muslin cloth and then centrifuged at 5000 rpm for 20 min. Supernatant was filtered through filter paper and sterilized at 120˚C for 10 min. Extract was preserved at 4^0C until further use[8].

Solvent extract

Thoroughly washed plant material of all the test plants were shade dried and powdered. Twenty grams of powder of different parts of test plants were extracted successively with petroleum ether, chloroforom, ethyl acetate and methanol by cold extraction method. All the extracts were concentrated by evaporation. Extracts were reconstituted in respective solvents in 1:10 (plant extract:solvent) ratio, preserved at 4^0C until subjected to antimicrobial activity assay[8].

Test bacteria

Authentic pure cultures of phytopathogenic bacteria *Xanthomonas axonopod*pv*. vesicatoria* causal organism of bacterial spot of tomato was obtained from the culture collection of Centre for Innovative Studies in Herbal Drug Technology, DOS in Botany, University of Mysore, Mysuru. *Xanthomonas campestris* pv. *campestris* (MTCC 2286), causal agent of black rot of cabbage and *Pseudomonas syringae* (NCIM 5102) causal agent of bacterial canker and dieback of stone fruits were obtained from National Chemical Laboratory, Pune. All the test bacteria were sub-cultured on nutrient agar. A 48 h. old nutrient broth culture of the test bacteria was used for assay.

Test fungi

Authentic pure cultures ofplant pathogenic fungi viz., *Aspergillus flavus* and *Fusarium verticelloides* were obtained from the culture collection of Centre for Innovative Studies in Herbal Drug Technology, Department of Studies in Botany, University of Mysore, Mysuru. Both the test fungi were sub-cultured on Czapeck Dox Agar (CDA) for further use. Suspension culture was prepared from sub-cultures and used for antifungal assay.

Antibacterial activity assay

Antibacterial activity of aqueous as well as solvent extracts was determined by cup diffusion method[9]on nutrient agar medium. Cups were made in nutrient agar plates using sterile cork borer (6mm) and 50 µl of inoculum of bacteria was spread on the solid plates with a sterile swab. Later 100 µl each of the aqueous and solvent extracts was placed in the cups. The treatment also included negative controls viz., sterile water, petroleum ether, chloroform, ethyl acetate and methanol for the respective extracts. The plates were incubated for 24 hours at 37^0C and zone of inhibition if any around the wells were measured in mm[10]. Three replicates were maintained.

Antifungal activity assay
Antifungal activity of plant extracts which has shown potential antibacterial activity was determined by well diffusion method[11] on CDA medium. The plates were incubated for a week at room temperature, zone of inhibition if any around the wells were measured in mm.

Data analysis
Calculation of mean and standard error for every test was done. Significance of difference among different treatment was determined by Analysis of Variance (ANOVA) using Statistical software IBM SPSS statistical for windows, version 2.

Preliminary phytochemical analysis of the Active plant extracts
Plant extracts which exhibited anti-microbial potential (Chloroform, ethyl acetate and methanol extracts of seeds of *Trachyspermum ammi,* peel of *Cucumis sativus* and leaves of *Murraya koenigii*) were subjected to phytochemical analysis following the methods of Trease and Evans[12] and Sofowara[13]. Presence or absence of alkaloids, flavanoids, tannins, saponins, steroids, terpenoids, resins, glycosides and phenols was recorded.

Detection of Alkaloids
Dragendroff's test: To 0.5ml of test extract, 2.0ml of HCl was added. To this acidic medium, 1.0ml of reagent was added. An orange red precipitate produced immediately indicates the presence of alkaloids.
Mayers test: (potassium iodide):1.3g of mercuric chloride was dissolved in 60ml distilled water and 5.0g of potassium iodide in 10ml of water. The two solutions were mixed and diluted to 100ml with distilled water. To 1.0ml of extract few drops of reagent was added. Formation of white or pale yellow precipitate indicate the presence of alkaloids.

Detection of flavonoids
Test tubes containing 0.5ml of test extracts, 5-10 drops of dilute Hcl and small piece of zinc or magnesium were added and the solution was boiled for few min. In the presence of flavanoids, reddish pink or dirty brown color is produced.

Detection of tannins
Ferric chloride test: To 1-2ml of test extracts, few drops of 5% aqueous $Fecl_3$ solution were added. A bluish black color, which disappears on addition of a few ml of dilute H_2SO_4 followed by the formation of yellowish brown precipitate indicate the presence of tannins.

Detection of saponins
To the test tube containing about 2.5ml of test extract, a drop of sodium bicarbonate solution was added. The mixture was shaken vigorously and kept for 3min. Formation of honey comb like froth indicate the presence of saponins.

Detection of steroids
Libermann-Burchard's test: To 1.0ml of test extracts, 1.0ml of conc. $H_2SO_4$was added followed by the addition of 2.0ml of acetic anhydride solution. A greenish color developed and turned blue indicate the presence of steroids.

Detection of terpenoids
Salkwoski reaction: 0.5ml of the test extracts was mixed in 0.2ml ofchloroform and conc. H_2SO_4 (0.3ml) was carefully added to form a layer. A reddish brown coloration in the inter phase formed indicate the presence of terpenoids.

Detection of resins
To 2.0ml of test extracts, 5-10ml of acetic anhydride was added and dissolved by gentle heating, and cooled and then 0.5ml of H_2SO_4 was added. A bright purple color rapidly changing into violet indicate the presence of resins.

Detection of glycosides
A small amount of test plant extract was dissolved in 1.0ml of water and then aqueous sodium hydroxide solution was added. Formation of a yellow color indicate the presence of glycosides.

Detection of Phenols
Ferric chloride test: To 1.0ml of test extracts, 2.0ml of distilled water followed by few drops of 10% aqueous $Fecl_3$ solution was added. Formation of blue or green color indicate the presence of phenols.

Lead acetate test
1.0ml of test extracts was diluted to 5.0ml with distilled water and to this few drops of 1% aqueous solution of lead acetate was added. Formation of yellow precipitate indicated the presence of phenols.

RESULTS AND DISCUSSION

Antimicrobial activity assay
Despite the increasing interest of public in phytomedicine, very few drugs from higher plants have application in conventional agriculture practices. In the present investigation, aqueous and different solvent extracts of peels of *Cucumis sativus* and *Citrullus lanatus*, leaves of *M. koenigii* and seeds of *T. ammi* were screened in vitro for antibacterial activity against phytopathogenic bacteria. Aqueous extracts of the test plants did not show activity against test bacteria. Petroleum ether, chloroform, ethyl acetate and methanol extracts of all the three plants tested had variable effect oninhibition of bacterial growth(Table 1). Methanol extract of *C. sativus* and *M. koenigi* has shown considerable zone of Inhibition. Chloroform, ethyl acetate and methanol extracts of *T. ammi* has shown good activity against all the tested bacteria (Figure 1). Reports available on the antibacterial activity of *C. sativas*[14-15] and *C. lanatus*[7,16] have tested the stem, leaves and fruits of the plant have been used but no report was found with the use of peel of *C. lunatus*. Foongand coworkers[17]. have reported antibacterial activity of *C. sativus* peel against human pathogenic bacteria. Results revealed the antibacterial potential of methanol extracts of*C.sativus* peelswith zone of inhibitionranging from 14-15mmagainst *Xanthomonas campestris* pv. c*ampestris*, and *X. axonopodis* pv. *vesicatoria*but no activity was found against

Pseudomonas syringae. None of the earlier reports have tested the activity against plant pathogenic bacteria and hence the present study is of first report of its kind. *C. lanatus* peel extracts did not show activity. Some reports available on antimicrobial activity and phytochemistryof *T. ammi*[5,11]and *M. Koenigii*[18-19], but no reports are available about screening of these plants particularly against important plant pathogens viz., *Xanthomonas campestris* pv. c*ampestris*, *X. axonopodis* pv. *vesicatoria* and *Pseudomonas syringae.* In the present investigation, the methanol extract of *M.koenigii* exhibited potential activity against all the tested bacteria with zone of inhibition ranging between 7to 53 mm. The potential antibacterial activity exhibited by ethyl acetate, chloroform and methanol extract of seeds of *T. ammi*, methanol extract of leaves *M. koenigii* and methanol extract of peel of *C. sativus*, encouraged us to screen these extracts for antifungal activity against two important plant pathogens viz., *A. flavus* and *F. verticelloides.* The results revealed the susceptibility of *F. verticelloides* to ethyl acetate, chloroform and methanol extracts of *T. ammi* and methanol extract of *M. koenigii,* in which the zone of inhibition ranged between 14mm to 18mm (Table2).Antimicrobial activity of *C. sativus, M. koenigii* and *T. ammi* has been reported [4,17,20-22]against human pathogenic bacteria and fungi but present study is done against plant pathogenic microbes. Methanol extracts of *C. sativus* and *M. koenigii* and *T. ammi*have shown maximum activity and the chloroform and ethyl acetate extracts of *T. ammi* has shown considerable activity against both plant pathogenic test bacteria and fungi.The extracts which showed potential activity were further investigated by comparing with two standard antibacterial drugs viz., Bacteromycine and Streptocycline, in which the Bacteromycine, Streptocycline exhibited an average of 13 mm and 26 mm zone of inhibition respectively, against all the test bacteria. The present comparative study reveals that the methanol extracts has greater activity than the bacteromycine and nearly equivalent activity to Streptocycline. Antifungal activity of the test extracts was compared with the standard antifungal drug Bavistinwhich showed an average of 20 mm zone of inhibition and the test plant extracts were found to have less activity (ranging from 12 to 17mm) than Bavistin (20 mm). Univarient analysis (ANOVA) results indicated the significant variation ($P \leq 0.05$) in antibacterial (P= 0.01) and antifungal activity (P=0.00) exhibited by the test plants

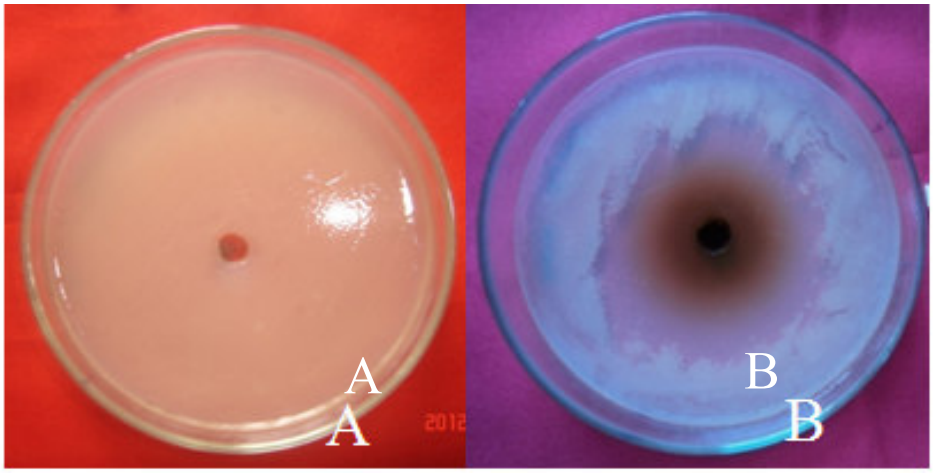

Figure 1

Methanol extract of Murraya koenigii showing activity against X.axonopodis pv. Vesicatoria (53 mm zone of inhibition) A-Control (Methanol); B-methanol extract of Murraya koenigiileaves

Preliminary phytochemical analysis of the active plant extracts

Phytochemical screening of the extracts which showed promising activity viz.,chloroform, ethyl acetate and methanol extracts of *T. ammi* and methanol extracts of *Cucumis sativus* and *Citrullus lanatus* revealed the presence of terpenoids and tannins in chloroform as well as ethyl acetate extract of *T. ammi,* presence of terpenoids, tannis, glycoside, saponins and phenols in methanol extract of *T. ammi.* Only tannin was present in methanol extract of *M. koenigii.* Methanol extract of *C. sativus* contained flavanoids, tannins, glycosides and phenols. The presence of tannins in the test plants indicates that the active compound responsible for inhibiting the growth of phytopathogens could be tannins. The presence of more phytochemicals in an extract correlates with more potential activity exhibited by that extract. Then the results of the investigation suggests that the *T. ammi, M. koenigii* and *C. sativus* are ideal candidate plants for further screening to manage the disease caused by test pathogens at green house and field on respective crops.

Table 1
Antibacterial activity of different plant extracts against the test bacteria

Medicinal Plant	Zone of Inhibition (mm) against test bacteria			
	Extract	*Xanthomonas Campestris* pv. *campestris,*	*Xanthomonas axonopodis* pv. *vesicatoria*	*Pseudomonas syringae*
Citrullus lanatus (peel)	Aqueous	0.00	0.00	0.00
	Petroleum ether	0.00	0.00	0.00
	Chloroform	0.00	0.00	0.00
	Ethyl acetate	0.00	0.00	0.00
	Methanol	0.00	0.00	0.00
Cucumis sativus (peel)	Aqueous	0.00	0.00	0.00
	Petroleum ether	0.00	0.00	0.00
	Chloroform	0.00	0.00	0.00
	Ethyl acetate	0.00	0.00	0.00
	Methanol	14.01±0.02	15.02±0.02	0.00
Murraya koenigii (leaves)	Aqueous	0.00	0.00	0.00
	Petroleum ether	0.00	0.00	0.00
	Chloroform	0.00	0.00	0.00
	Ethyl acetate	0.00	0.00	0.00
	Methanol	7.01±0.02	53.04±0.04	20.00±0.00
Trachyspermum ammi(seeds)	Aqueous	0.00	0.00	0.00
	Petroleum ether	0.00	0.00	0.00
	Chloroform	11.01±0.02	9.01±0.01	8.01±0.01
	Ethyl acetate	17.01±0.03	16.00±0.00	14.03±0.02
	Methanol	17.02±0.02	16.01±0.00	32.04±0.04
Standard drugs	Bacteromycine	13.00±0.00	12.00±0.00	13.00±0.00
	Streptocycline	26.00±0.00	24.00±0.00	26.00±0.00

Values are the mean of three replicates ± standard deviation P value=0.01 ≤0.05

Table 2
Antifungal activity of different extracts against the test fungi in mm

Test fungi	Solvent extracts of test plants and Zone of Inhibition in mm					
	Chloroform extract of *T. ammi*	Ethyl acetate extract of *T. ammi*	Methanol extract of *T. ammi*	Methanol extract of *M. koenigii*	Methanol extract of *C. sativus*	Bevistin (Standard drug)
Aspergillus flavus	0.00	0.00	0.00	0.00	0. 00	-
Fusarium verticelloides	14.00±0.00	18.01±0.13	15.06±0.00	17.00±0.47	0.00	20.00±0.00

Values are the mean of three replicates ± standard Error P value=0.00 ≤ 0.05

Table 3
Phytochemical analysis of solvent extracts of test plants

Phytochemicals	Chloroform extract of *T. ammi* (seeds)	Ethyl acetate extract of *T. ammi* (seeds)	Methanol extract of *T. ammi* (seeds)	Methanol extract of *M. koenigii* (leaves)	Methanol extract of *C. sativus* (peel)
Alkaloids	-	-	-	-	-
Flavanoids	-	-	-	-	+
Terpenoids	+	+	+	-	-
Tannins	+	+	+	+	+
Glycosides	-	-	+	-	+
Saponins	-	-	+	-	-
Steroids	-	-	-	-	-
Phenols	-	-	+	-	+
Resines	-	-	-	-	-

- Absent, + Present

CONCLUSION

The findings of the present investigation is an attempt towards crop protection strategies for microbial disease management. Methanol extracts showed significant activity when compared to synthetic antibiotics Bacteromycine and Streptocycline. This tends to express the active ingredients is an effective compound and plant parts may be better extracted for the active principle with methanol than other organic solvents. The results of present investigation are successful in identifying the nature of the bioactive principle and its solubility, which will help in further isolation and characterization of the active principle responsible for the activity.

CONFLICT OF INTEREST

Conflict of interest declared none.

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