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Eco- friendly management of plant pathogens by some medicinal plant extracts

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Nine medicinal plants viz., *Acacia nilotica* (L) Del. (Leaf), *Acorus calamus* L. (Rhizome), *Carum copticum* L. (seeds), *Emblica officinalis* Gaert (Leaf), *Eupatorium odaratum* L. (Leaf), *Hyptis suaveolens* Poit. (Leaf), *Millingtonia hortensis* L. (Leaf), *Ocimium gratissium* L. *(Leaf) and Pedalium murex* L. (Leaf and fruits) was screened for antibacterial activity against important phytopathogenic bacteria such as *Xanthomonas campestris* pv. *vesicatoria, Xanthomonas axonapodis* pv. *malvacearum, Xanthomonas oryzae* pv. *oryzae,* and *Erwinia carotovora* (MTCC 1428). Powdered leaves/fruits/seeds/rhizome of all the plants was extracted with different solvents such as petroleum ether, chloroform, methanol and ethanol using cold extraction method. All the extracts were subjected to antibacterial activity against test pathogens. Among different solvent extracts tested, methanol and ethanol extract of *Emblica officinalis, Acacia nilotica,* and *Carum copticum* recorded significant inhibitory activity against all the test pathogens followed by *Pedalium murex, Hyptis suaveolens*, *Millingtonia hortenesis* and *Eupatorium odaratum*. Comparative analysis with antibiotic bacterimycin was also conducted. The result revealed that antibacterial activity of methanol extract of *Emblica officinalis, Acacia nilotica* and *Carum copticum* was highly significant compared to antibiotic. The present study is successful in demonstrating inhibitory activity of important medicinal plants against phytoapthogenic bacteria and proposes the use of these plants in plant disease management after further screening on package and practice.

Key words: Pesticide, phytopathogens, antibacterial activity, phytochemical analysis

Introducton

Economic losses arising from crop diseases caused by phytopathogenic bacteria are principally associated with yield reductions. However, crop quality and safety may also be adversely affected, undermining both consumer confidence and profitability to the producer. Hence protection of plants from agriculture pest and pathogens is the preoccupation of agricultural scientist

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around the world (Agrios, 2005) and it is the unifying goal of plant pathology to control plant disease, and chemicals play a major role in accomplishing that goal in contemporary agricultural production (Epstein and Bassein, 2003; Ragsdale, 2000). Pesticides which are incessantly used on plants to manage these disease cause serious damage to agricultural and natural ecosystems. Thus, there is a need to curtail pesticide use and reduce the environmental impacts of pesticides. In this connection the importance of spices and their derivatives (extracts, essential oils, decoctions, hydrosols) in crop protection is being increasingly recognized under the concept of Integrated Pest and Disease Management (IPDM) (Ragsdale, 2000). Under this concept, all possible modes of plant pests and disease control methods were integrated to minimize the excessive use of synthetic pesticides (Beg and Ahmad, 2002). Exploitation of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would be a more realistic and ecologically sound method for plant protection and will play a prominent role in the development of future commercial pesticides for crop protection strategies, with special reference to the management of bacterial diseases in particular and plant diseases in general (Gottlieb *et al*., 2002). It is known that many plant pathogenic bacteria have acquired resistance to synthetic pesticides (White *et al*., 2002). For instance, pathovars of *Xanthomonas campestris* have developed resistance to some antibiotic such as kanamycin, ampicillin, penicillin and streptomycin (Cooksey, 1987; Bender *et al*., 1990; Rodriguez *et al*., 1997; McManus *et al*., 2002). Other phytopathogenic bacteria which are streptomycin resistance include *Erwinia carotovora* (Fukusawa *et al.,* 1980; McManus *et al*., 2002) and *Xanthomonas dieffenbachiae* (Knauss, 1972). The increasing incidence of pesticides resistance is further fueling the need for new generation of pesticides which are eco-friendly.A green plant represents a reservoir of effective novel chemotherapeutants with different mode of action and can provide valuable sources of natural pesticides against resistance pathogens (Newman *et al*., 2000; Gibbons, 2005).

The popularity of botanical pesticides is once again increasing and some plant products are being used globally as green pesticides. The body of scientific literature documenting bioactivity of plant derivatives to different pests continues to expand, yet only a handful of botanicals are currently used in agriculture (Dubey *et al.,* 2008). There are a lot of reports on the use of several plant byproducts on several human pathogenic bacteria and fungi, but reports on management of phytopathogenic bacteria are less. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant extracts for their anti-microbial activity may provide new antimicrobial substances In search of better alternatives natural products are considered to be environmentally safe for management of plant diseases and hence the present study was carried out.

Material and methods

Different parts of nine medicinal plants belonging to eight different families of plant kingdom viz., *Acacia nilotica* (L) Del. (Leaf), *Acorus calamus* L. (Rhizome), *Carum copticum* L. (seeds) *Emblica officinalis* Gaert. (Leaf), *Eupatorium odaratum* L. (Leaf), *Hyptis suaveolens* Poit. (Leaf), *Millingtonia hortensis* L. (Leaf), *Ocimium gratissium L. (Leaf)* and *Pedalium murex* L. (Leaf and fruits) was collected from Mysore, Karnataka, India (Table 1). A voucher specimen of the plants has been deposited in the herbarium of Department of Studies in Botany, University of Mysore, Mysore, Karnataka, India.

Table 1. Test plants used for antibacterial activity assay

Name	Family	Plant part used
Acacia nilotica (L) Del.,	Mimosaceae	Leaf
Acorus calamus L.	Aracaceae	Rhizome
Carum capticum L.	Umbelliferae	Seed
Emblica officinalis Gaert.	Euphorbiaceae	Leaf
Eupatorium odaratum L.	Asteraceae	Leaf
Hyptis suaveolens Poit	Lamiaceae	Leaf
Millingtonia hortensis L.	Bignoniaceae	Leaf
Ocimum gratissimum L.	Lamiaceae	Leaf
Pedalium murex L.	Pedaliaceae	Leaf and fruits

Preparation of aqueous extracts

Sample (50 g) of fresh plant material was macerated with 100 ml sterile distilled water in a waring blender (Waring International, new Hart ford, CT, USA) for 10 min. The macerate was first filtered through double-layered muslin cloth and then centrifuged at 4000 *g* for 10 min. The supernatant was filtered through Whatman No.1 filter paper and sterilized at 120 °C for 30 min. The extract was preserved aseptically in a brown bottle at 5 ºC until further use. The extract was subjected to antibacterial activity assay.

Preparation of solvent extracts

Thoroughly washed plant material of all the test plants were shade dried and powdered with the help of waring blender. Twenty-five grams of the powder of different parts of medicinal plants was added to different conical flasks containing 100 ml of different solvents viz., petroleum ether, chloroform, methanol and ethanol and kept in rotary shaker for 48 hrs. Later the extract was filtered using whatmann filter paper No 1. All the extracts were concentrated using rotary flash evaporator and preserved at 4 ºC in air tight brown bottle until further use. All the extracts were subjected to antibacterial activity against test phytopathogenic bacteria.

Plant pathogenic bacterial cultures

Authentic pure cultures of phytopathogenic *Xanthomonas axonopodis* pv *malvacearum.* (*X. a*. pv. *m.*) isolated from cotton (*Gossypium herbaceum* L.) *Xanthomonas oryzae* pv. *oryzae. (X. o.* pv. *o)* isolated from paddy (*Oryza sativa* L.) and *Xanthomonas campestris* pv. *vasicatoria.* (*X. c*. pv. *v.*) isolated from tomato (*Lycopersicon esculentum* Mill.) were obtained from DANIDA lab, University of Mysore, India and standard culture of *Erwinia carotovora* (MTCC 1428) were obtained from MTCC Chandighar, India

Antibacterial activity assay

Antibacterial activity of solvent extracts was determined by cup diffusion method on nutrient agar medium (Anon, 1996). Cups were made in nutrient agar plate using sterile cork borer (5 mm) and inoculum containing 10^{-6} CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 µl each of all aqueous and solvent extracts (150 µg ml $^{-1}$) were placed in the cups made in inoculated plates. The treatments also included 50 µl of sterilized distilled water and methanol separately which served as control. Antibiotic bacterimycin 2000 at recommended dosage of 3µg ml $^{-1}$ were also tested for comparative efficacy studies. The plates were incubated for 24 hours at room temperature and zone of inhibition if any around the wells were measured in mm (millimeter). For each treatment three replicates were maintained. The data was subjected to statistical analysis using SPSS for windows software.

Phytochemical analysis

Preliminary phytochemical analysis of evaporated methanol extracts was conducted on all the nine species of medicinal plants following procedures of Anon (1985) and Harborne (1998). The presence or absence of metabolites such as Cardiac glycoside, sapnonin, steroids, phenols, gum and mucilage, flavonoids and alkaloids was recorded.

Results

Antibacterial activity

Aqueous extract: Antibacterial activity of aqueous extracts of all the nine plants was presented in Table 2. Significant antibacterial activity was observed in *Acacia nilotica* (leaf) and *Carum copticum* (seeds) against different pathovar of *Xanthomonas* sp and *Erwinia carotovora*. Highly significant inhibitory activity was observed against *Xanthomonas oryzae* pv *oryzae* for *Acacia nilotica* (Leaf) than other Xanthomonas pathovars where as for *Carum copticm* (Seeds) it was against *Xanthomonas campestris* pv *vesicatoria.* Inhibitory activity was not observed in other plants. *Xanthomonas oryzae* pv *oryzae, Erwinia carotovora* were significantly inhibited compared to bacteriamycin than *Xanthomonas axonopodis* pv. *malvacearum, Xanthomonas campestris* pv. *vesicatoria* by aqueous extract of *Acacia nilotica*.

Solvent extract: Antibacterial activity of solvent extracts of all the nine plants were presented in Table 3. The screening revealed that plant extracts were effective in inhibiting the phytopathogenic bacteria by well diffusion method.

The results showed that the methanol extract of all test plants had more inhibitory effect than the other extracts. Where as petroleum ether and chloroform extract of *Carum copticum* and *Ocimium gratissium* and chloroform extract of *Millingtonia hortensis, Emblica officinalis* and *Pedalium murex* were also found inhibitory to the test pathogen. Methanol and ethanol extracts of *Hyptis suaveolens* and *Carum copticum,* methanol extract of *Acacia nilotica, Emblica officinalis, Millingtonia hortensis* and *Pedalium murex* was found highly significant against *X. oryzae* pv. *oryzae* and *X campestris pv vesicatoria.* Methanol extract of *Eupatorium odaratum* showed significant activity against all the test pathogens. Where as *Acorus calamus* showed least inhibition activity. It is interesting to note that solvent recorded significant inhibitory activity compared to aqueous extract. It was also observed that latter did not record activity in seven plants expect *Acacia nilotica* and *Carum copticum*.

Comparative evaluation of bacterimycin with solvent extracts revealed that methanol and ethanol extracts of *Millingtonia hortensis, Carum copticum* and *Eupatorium odaratum,* methanol extract of *Hyptis suaveolens* and ethanol extract of *Pedalium murex* recorded significant inhibitory activity against phytopathogenic bacteria when compared with control.

Phytochemical analysis

Phytochemical analyses of all test plants have revealed the presence of phenol in the extract followed by flavonoids. It was also observed that *Acacia nilotica* methanol extract of leaf was found positive for steroids, phenols, flavonoids and alkaloid, *Acorus calamus* extract showed the presence of cardiac glycoside, steroids, saponins, phenol and flavonoids. Methanol extract of *Carum capticum* of seed was found positive for cardiac glycoside, steroids, phenols and flavonoids and *Emblica officinalis* extract showed the presence of cardiac glycoside, steroids, saponins, phenols, flavonoids and alkaloid. *Eupatorium odaratum* extract was found positive for cardiac glycoside, steroids, phenols, flavonoids and alkaloid. *Hyptis suaveolens* extract showed the presence of steroids, alkaloid and flavonoids, *Millingtonia hortensis* extract was found positive for saponins, phenols and alkaloid. *Ocimum gratissimum* extract was found to be positive for *c*ardiac glycoside, phenols, gum and mucilage, flavonoid and alkaloid. *Pedalium murex* extract of fruits showed the presence of alkaloid, saponins, gum and mucilage and flavonoids, whereas leaf of *Pedalium murex* showed positive for the presence of flavonoids, saponins, gum and mucilage, phenols, alkaloid and steroids.

Discussion

The problems caused by synthetic pesticides and their residues have increased the need for effective biodegradable pesticides with greater selectivity. Alternative strategies have included the search for new types of pesticides which are often effective against a limited number of specific target species, are biodegradable into nontoxic products and are suitable for use in integrated pest management programs. However, the most species of higher plants which are known to produce plethora of secondary metabolites of biological significance used for the management of human disease management have never been described surveyed against phytopathogenic bacteria. Their chemical or biologically active constituent which is potential to be used as new sources of commercially valuable pesticides remain to be discovered. Considering these as a first step, in the present investigation nine plants were

Table 2. Antibacterial activity of aqueous extract of different parts of plants on phytopathogenic bacteria (Zone of inhibition measured in mm) at 50µl (150mg ml^{-1}).

Test plants	Plant part used	Zone of inhibition			
		Plant pathogenic bacteria			
		X. a.* pv. *malvacearum	***X. c.* pv. *vesicatoria***	***X. o.* pv. *oryzae***	***E. carotovora***
Acacia nilotica	**Leaf**	14.00±1.00	13.00±0.54	14.30±0.33	14.00±0.57
Acorus calamus	**Rhizome**	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
Carum capticum	**Seed**	12.23±0.57	13.00±0.57	12.18±0.44	12.10±0.46
Emblica officinalis	**Leaf**	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
Eupatorium odaratum	**Leaf**	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
Hyptis suaveolens	**Leaf**	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
Millingtonia hortensis.	**Leaf**	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
Ocimum gratissimum .	**Leaf**	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
Pedalium murex	**Fruit**	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
Pedalium murex.	**Leaf**	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00

The value means of three replicates ± standard error
X. a. pv. *m.: Xanthomonas axonopodis pv. malvacearum.*
X. c. pv. *v.: Xanthomonas campestris pv. vesicatoria*
X. o. pv. *o.: Xanthomonas oryzae pv oryzae*
E. c: Erwinia carotovora (MTCC 1428)

screened *in vitro* for antibacterial activity against important phytopathogenic bacteria. Despite the increasing interest of public in phytomedicine, very few drugs from higher plants have attained any prominence in conventional agriculture practices. There are few reports on antibacterial activity of *Acacia nilotica* on phytopathogenic bacteria such as *X. axonopodis* pv. *malvacearum* and *X. campestris* pv *vesicatoria (*Raghavendra *et al.,* 2006) but reports on *X. oryzae* pv. *oryzae* and *E. carotovora* were not available. The present study reveals the antibacterial activity of *Acacia nilotica* againsst *X. oryzae* pv. *oryzae* and *E. carotovora* for the first time showing broad spectrum inhibitory activity of the plant. Even though several reports are also available on usage of *Acacia nilotica,* (Kambiz and Afolayan, 2001; Rani and Khullar, 2004; Khan, *et al.,* 2009; Eldeen, *et al.,* 2010), *Acorus calamus* (Grosvenor *et al.* ,1995, MacGaw *et al*., 2002, Rani *et al*., 2003; Aqil and Ahmad 2007; Aqil *et al.,* 2006), *Carum copticum* (Mitra *et al.,* 2000; Patel , *et al.,* 2008), *Emblica officinalis* (Ahmad *et al.,* 1998; Tasduq, *et al.,* 2005; Saeed, 2007; Srikumar, *et al.,* 2007), *Eupatorium odaratum* (Suksamrarn, *et al.,* 2004; Chomnawang, *et al.,* 2005; Owolabi *et.al.*, 2010), *Hyptis suaveolens (*Iwu, *et al.,* 1990; Rojas *et al.,* 1992; Asekun, *et al.,* 1999; Chomnawang, *et al.,* 2005; Satish, *et al.,* 2010), *Millingtonia hortensis* (Jetty, *et al.,* 2000), *Pedalium murex* (*Chitravadivu et al., 2009;) Ocimum gratissimum* (Matasyoh, *et al.,* 2008; Ramanoelina, *et al.,* 1987; Junaid, *et al.,* 2006; Adebayo-Tayo, *et al.,* 2008) on human disease management but reports are not available on the exploitation of these plants in plant disease management.

The finding of the present investigation is an important step towards crop protection strategies for bacterial disease management. Methanol extract showed highly significant activity when compared with bacterimycin against plant pathogenic bacteria. This tends to express that the active ingredients is an effective antibiotic and plant parts may be better extracted for the active principle with methanol than other organic solvents. The results of the present investigation is 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.

The probability of plant secondary products being involved in plant-pest interactions, the strategy of randomly isolating, identifying, and bioassaying these compounds may also be an effective method of pesticide discovery (Satish *et al.,* 1999; Bisignano *et al.,* 2000). The results reveal that *Acorus calamus, Carum copticum*, *Emblica officinalis, Eupatorium odaratum, Hyptis suaveolens, Millingtonia hortensis, Pedalium murex* and *Ocimum gratissimum* extract against phytopathogenic bacteria for the first time. Biologically active compounds from plants will often have activity against organisms with which the producing plant does not have to cope.

Table 3. Zone of Inhibitory activity (in millimeter) of aqueous and solvent extracts of nine plant spp and synthetic antibiotic against some plant pathogenic pathovars of *Xanthomonas* and *Erwinia carotovora* at 50 µl concentrations((150mg ml^{-1}).

Test plants			**Zone of inhibition in mm**			
	Plant part used	**Solvent extract**	**Plant pathogenic bacteria**			
			X. a.* pv. *m	***X. c.* pv. *v.***	***X. o.* pv. *o***	***E. c***
Acacia nilotica	**Leaf**	Petroleum ether	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
		Chloroform	12.33±0.33	11.00±1.00	11.67±0.67	12.33±0.67
		Methanol	14.33±0.33	18.67±0.67	15.00±1.17	17.33±0.33
		Ethanol	12.67±0.67	15.00±0.00	12.33±0.67	12.67±0.89
Acorus calamus	**Rhizome**	Petroleum ether	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
		Chloroform	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
		Methanol	09.67±0.67	11.00±3.66	09.67±0.67	11.00±3.66
		Ethanol	10.67±0.67	11.00±3.66	09.67±0.67	11.00±3.66

Table 3. (Continue)

Test plants	Plant part used	Solvent extract	Zone of inhibition in mm — Plant pathogenic bacteria: *X. a.* pv. *m*	*X. c.* pv. *v.*	*X. o.* pv. *o*	*E. c*
Carum capticum	**Seed**	Petroleum ether	14.33±1.45	14.67±0.33	14.33±1.45	14.67±0.33
		Chloroform	12.67±1.21	11.67±0.33	13.00±0.00	12.67±1.21
		Methanol	12.67±0.33	15.00±0.57	21.33±0.89	12.00±0.57
		Ethanol	14.00±0.00	18.33±0.33	16.67±1.29	10.00±1.55
Emblica officinalis	**Leaf**	Petroleum ether	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
		Chloroform	12.33±0.33	11.00±1.00	11.67±0.67	12.33±0.67
		Methanol	14.33±0.33	18.67±0.67	15.00±1.17	17.33±0.33
		Ethanol	12.67±0.67	15.00±0.00	12.33±0.67	12.67±0.89
Eupatorium odaratum	**Leaf**	Petroleum ether	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
		Chloroform	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
		Methanol	19.67±0.67	20.67±2.97	20.33±0.89	17.67±0.33
		Ethanol	20.33±0,89	18.67±0.67	21.33±0.89	16.00±1.00
Hyptis suaveolens	**Leaf**	Petroleum ether	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
		Chloroform	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
		Methanol	17.67±0.33	19.33±1.33	18.66±0.33	16.00±1.00
		Ethanol	15.00±1.71	13.00±0.57	16.33±0.57	12.67±0.33
Millingtonia hortensis	**Leaf**	Petroleum ether	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
		Chloroform	12.67±0.33	14.67±0.33	12.67±0.33	11.67±0.33
		Methanol	15.00±0.57	17.00±0.00	18.66±0.33	11.33±0.33
		Ethanol	14.00±0.57	17.33±0.33	12.00±0.57	11.00±1.17
Ocimum gratissimum	**Leaf**	Petroleum ether	10.67±0.33	11.33±0.67	11.00±0.57	10.67±0.89
		Chloroform	12.00±0.00	11.00±0.57	10.67±0.33	10.00±0.57
		Methanol	09.67±0.33	09.67±0.33	10.00±0.57	09.67±0.33
		Ethanol	11.67±0.89	10.00±0.74	10.33±0.33	11.67±0.67
Pedalium murex	**Fruits**	Petroleum ether	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
		Chloroform	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
		Methanol	00.00±0.00	14.33±0.67	10.00±0.00	00.00±0.00
		Ethanol	09.67±0.33	16.00±1.00	16.33±0.67	08.33±0.00
Pedalium murex.	**Leaf**	Petroleum ether	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
		Chloroform	07.00±3.66	10.33±3.67	11.67±0.33	08.67±0.33
		Methanol	12.67±0.33	13.00±0.00	10.00±0.00	11.33±0.33
		Ethanol	09.00±0.00	09.00±0.00	08.00±0.57	10.33±0.33
Bacterimycin			21.66±0.40	14.66±0.40	13.00±0.00	14.67±0.33

X. a. pv. m.: Xanthomonas axonopodis pv. malvacearum.
X. o. pv. o: Xanthomonas oryzae pv oryzae
X. c. pv. v.: Xanthomonas campestris pv. vesicatoria.
E.c: *Erwinia carotovora* (MTCC 1428)

Table 4. Phytochemical analysis of methanol extract of nine plant species.

Medicinal Plants	Parts used	Cardiac glycoside	Steroids	Saponins	Phenols	Gum & mucilage	Flavonoids	Alkaloid
Acacia nilotica	Leaf	--	++	--	++	--	++	++
Acorus calamus	Rhizome	--	++	--	++	++	++	--
Carum capticum	Seeds	++	++	--	++	--	++	--
Emblica officinalis	Leaf	++	++	++	++	--	++	++
Eupatorium odaratum	Leaf	++	++	--	++	--	++	++
Hyptis suaveolens	Leaf	--	++	--	--	--	++	++
Millingtonia hortensis	Leaf	--	--	++	++	--	--	++
Ocimum gratissimum	Leaf	++	--	--	++	++	++	++
Pedalium murex	Fruits	--	--	++	--	++	++	++
Pedalium murex	Leaf	--	++	++	++	++	++	--

++: Present

--: Absent

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