African Journal of Pharmacy and Pharmacology Vol. 6(11), pp. 783-788, 22 March, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP11.435
ISSN 1996-0816 ©2012 Academic Journals

Full Length Research Paper

Evaluation of antibacterial activity of *Morinda citrifolia*, *Vitex trifolia* and *Chromolaena odorata*

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Accepted 15 February, 2012

Methanol, ethanol and ethyl acetate extracts of *Morinda citrifolia* L. (fruit, leaf and stem), *Vitex trifolia* and *Chromolaena odorata* (leaf) were prepared to evaluate the anti-bacterial activity. The antibacterial activities of extracts were tested against 12 bacterial isolates by *in vitro* disc diffusion method. Methanol extracts possessed higher level of antibacterial activity than ethanol and ethyl acetate extracts. When the plants were compared, *C. odorata* had better antibacterial activity than *V. trifolia* and *M. citrifolia* with the minimal inhibitory concentration (MIC) of about 25 mg/ml.

Key words: Antibacterial activity, disc diffusion method, *Chromolaena odorata*, *Morinda citrifolia* L., *Vitex trifolia*.

INTRODUCTION

Herbal and natural products have been used in folk medicine for centuries throughout the world because of biologically active compounds present therein to confer protection against microbial and insect attack (Kuruvilla, 2002). These compounds have been used in the form of a whole plants or plant extracts either as food or medicines in human (Wallace, 2004) for their, anti-bacterial, anti-cancer, analgesics, anti-diarrheal, antifungal or for some other therapeutic activities (Lucy and DaSilva, 1999). In the developed countries 25 percent of the medical drugs are based on plants (Principe, 2005), their active substances include alkaloids, flavonoids, *L*-asperuloside, caproic acid, caprylic acid (Wang et al., 2002), phenolic compounds/essential oils (Chowdhury, 2002) and, tannins (Lewis and Ausubel, 2006) etc.

Morinda citrifolia L. (Rubiaceae), *Vitex trifolia* (Verbenaceae) (Forest and Kim Starr) and *Chromolaena odorata* (*L.f.*) King and Robinson (synonym: *Eupatorium odoratum* L.) (Asteraceae) has a long history of use in traditional medicine for the treatment of infectious diseases (Chomnawang et al., 2005). *M. citrifolia* (Noni) is an important medicinal plant for many centuries throughout the south pacific and has been used in folk remedies by Polynesians over 2000 years (Whistler, 1985) and extracts of fruit, leaf and stem exhibit significant antibacterial activity (Selvam et al., 2009). It is a small shrub, their potential therapeutic properties are still unknown (Mc Clatchey, 2002) and is reported to have anti-bacterial, antiviral, antifungal, antihelminthic, analgesic, hypotensive, anti-inflammatory and immune enhancing effects (Liu et al., 2001; Duke et al., 2002). There is great demand for its fruit juice in treatment for different kinds of illness such as arthritis, diabetes, muscle aches, menstrual difficulties, heart diseases, cancers, gastric ulcers, blood vessel problems and drug addiction.

C. odorata (*L.f.*) King and Robinson (synonym: *Eupatorium odoratum* L.) (Asteraceae) is a perennial scandent or semi-woody shrub. In traditional medicine, a decoction of the leaf is used as a cough remedy and as an ingredient with lemon grass and guava leaves for the treatment of malaria (Doss et al., 2011). *C. odorata* leaf was found to possess antibacterial (Lavanya and Brahmaprakash, 2011), anti-inflammatory activity (Owoyele et al., 2005; Ayyanar and Ignacimuthu, 2009; Pauillac et al., 2009) and the fresh leaf is ground into a

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paste and applied topically on affected places to heal wounds (Kilani, 2006). In folk medicine, the aqueous leaf extracts of the plant is used as antiseptic wound dressing.

V. trifolia L. (Verbenaceae) is a tropical shrub widespread in Pacific-Asian countries and appears as one of the popular herbal medicine in Pacific region. *V. trifolia* L. is a stout, aromatic shrub or a small tree found wide in several parts of India, which is traditionally used by the tribes and native medicinal practitioners for the treatment of various ailments. The leaves are employed in maceration or decoction, internally or externally for various disorders like fever, inflammation, increase in body weight and found to possess wound healing, anti-bacterial, anti HIV, anticancer and antipyretic activities (Hossain et al., 2001; Li et al., 2005; Manjunath et al., 2007). Antimicrobial activity of the *V. trifolia* extract could be attributed due to the presence of phenolic compounds. The present study was carried out with the aim to evaluate the antibacterial activity of *M. citrifolia* L. (fruit, leaf and stem), *Vitex trifolia* and *Chromolaena odorata* (leaf) extracts.

MATERIALS AND METHODS

Collection of plants

The *Morinda citrifolia* (fruit, leaf and stem), *Vitex trifolia* and *Chromolaena odorata* (leaf) were collected from Central Agricultural Research Institute, Andaman and Nicobar Islands, India.

Preparation crude extracts

The fresh fruit, leaf and stem of *M. citrifolia*, leaf samples of *V. trifolia* and *C. odorata* were washed with distilled water immediately after collection. The collected fruit, leaf and stem were chopped into pieces, dried in shade and powdered, then mixed in 1:5 ratios with solvents namely ethanol, methanol and ethyl acetate separately. The contents were kept or incubated as such in room temperature for 48 h with constant stirring at regular intervals. After 48 h, the contents were filtered through Whatmann No.1 filter paper followed by Wong et al. (1994) with slight modification. Then filtrates were vacuum dried using rotary evaporator and concentrates were stored at 4°C. The residues were redissolved with the appropriate solvents from which they were prepared and used for the antibacterial assay.

Test organisms

Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus* sp., *Klebsiella pneumoniae*, *Shigella flexneri*, *Proteus mirabilis*, *Pseudomonas diminuta*, *Pseudomonas fluorescens* and *Enterobacter cloacae* were collected from Division of Animal Science, Central Agricultural Research Institute, Andaman and Nicobar Islands and compared with the reference strains such as *S. aureus* ATCC 6538 and *E. coli* ATCC 25922 were used for the study.

In vitro antibacterial assay

The disc diffusion method was employed for determining antibacterial activity of *M. citrifolia* (fruit, leaf and stem), *V. trifolia* and *C. odorata* (leaf) extracts as described by Bauer et al. (1966). The 4 to 6 h old cultures were used for the bioassay. The sterile cotton swab were dipped in the cultures and swabbed over the surface of Mueller Hinton agar. The cultures were allowed to dry for 5 min and the sterile filter paper discs (5 mm) with known concentration of plant extracts (1000 µg/disc) were impregnated over the surface of Mueller Hinton agar medium using sterile forceps. Chloramphenicol (30 µg/ml) was used as positive control and solvent discs were used as negative control instead of extracts (Parekh and Chanda, 2007). Then, plates incubated at 37 °C for 24 h and the diameter of “zone of inhibition” was measured and recorded.

Determination of minimal inhibitory concentration (MIC)

Six bacterial isolates namely *E. coli*, *Shigella flexneri*, *Proteus mirabilis*, *P. diminuta*, *Enterobacter cloacae* and *S. aureus* ATCC 6538 were selected for MIC studies because of their potential inhibitory effect against the plant extracts when compared to other bacterial isolates. The MIC of plant extracts of *M. citrifolia* (fruit, leaf and stem), *V. trifolia* and *C. odorata* was determined as described by Greenwood (1989). Serial dilution of different concentrations 100, 50, 25 and 12.5 mg/ml were used to determine the MIC. The MIC was recorded as the least concentrations of the extract that completely inhibited the growth of the test organisms. About 0.1 ml of the contents of the tubes was further sub-cultured on nutrient agar by pour late method and the contents were incubated for 24 h to determine bactericidal or bacteriostatic activity. Bactericidal effect was determined when no growth occurred on the sub-culture medium after the MIC determination.

RESULTS

The *in vitro* antibacterial activity of ethanol, methanol and ethyl acetate extracts of *M. citrifolia* (fruit, leaf and stem) are presented in Table 1, and *V. trifolia* and *C. odorata* (leaf) are shown in Table 2. The results obtained from the disc diffusion assay showed that the plant extracts posses antibacterial activity against the tested microorganism. The extracts showed good inhibitory activity on almost all the microbes tested. Among the three solvents tested, methanol extracts showed maximum inhibitory potential against all the tested organisms than ethanol and ethyl acetate extracts. Chloramphenicol is used as a standard antibacterial agent inhibited the growth of all the organisms and the zones of inhibition produced were far greater than those produced by the different extract concentration. When comparing among the plants, *C. odorata* extracts showed maximum antibacterial activity than *M. citrifolia* and *V. trifolia*.

The MIC of methanol, ethanol and ethyl acetate extract of *Morinda citrifolia*, *Vitex trifolia* and *Chromolaena odorata* for the six bacterial isolates tested is presented in Table 3. *Escherichia coli* and *Staphylococcus aureus* ATCC 6538 were found to be most sensitive to the extracts since their growth was inhibited at a relatively lower concentration (12.5 mg/ml) than the other organism. *Shigella flexneri*, *Proteus mirabilis*, *Pseudomonas diminuta*

Table 1. Antibacterial activity of *Morinda citrifolia* fruit, leaf and stem extracts against bacterial pathogens (in mm).

Organism	*Morinda citrifolia* (mm)									Chloramphenicol
	Fruit extract			Leaf extract			Stem extract			
	E	M	EA	E	M	EA	E	M	EA	
Escherichia coli	6	7	6	7	8	6	R	7	R	27
Pseudomonas aeruginosa	7	7	R	R	6	R	R	6	R	23
Staphylococcus aureus	8	7	R	8	9	7	6	6	6	29
Klebsiella pneumonia	R	8	6	6	7	6	R	R	R	25
Streptococcus sp.	8	6	R	R	6	6	6	6	6	25
Shigella flexneri	9	7	7	8	8	7	6	8	R	32
Proteus mirabilis	6	8	R	6	6	6	R	6	6	27
Pseudomonas diminuta	7	8	R	10	11	9	10	6	7	24
Pseudomonas fluorescens	6	6	R	6	8	7	R	R	R	16
Enterobacter cloacae	6	8	6	6	7	R	6	6	6	30
S. aureus ATCC 6538	6	7	6	13	15	10	6	6	6	32
E. coli ATCC 25922	R	7	R	R	7	7	6	6	6	31

E – Ethanol; M – methanol; EA – ethyl acetate; R – bacterial resistance.

Table 2. Antibacterial activity of *Vitex trifoliata* and *Eupatorium odoratum* leaf extracts against bacterial pathogens (in mm).

Organism	*V. trifolia* Leaf			*C. odorata* Leaf			Chloramphenicol
	E	M	EA	E	M	EA	
Escherichia coli	6	7	6	6	8	6	27
Pseudomonas aeruginosa	R	6	8	6	7	6	23
Staphylococcus aureus	R	7	6	11	12	8	29
Klebsiella pneumonia	6	6	6	6	9	R	25
Streptococcus sp.	R	6	6	6	7	R	25
Shigella flexneri	7	8	6	7	10	7	32
Proteus mirabilis	6	6	8	6	6	6	27
Pseudomonas diminuta	7	7	R	8	11	7	24
Pseudomonas fluorescens	R	7	R	R	7	R	16
Enterobacter cloacae	6	7	7	6	8	7	30
S. aureus ATCC 6538	8	9	6	12	15	9	32
E. coli ATCC 25922	7	8	7	7	9	6	31

E – Ethanol; M – methanol; EA – ethyl acetate; R – bacterial resistance.

and *Enterobacter cloacae* were inhibited at concentrations of the extracts above 25 mg/ml. There was no growth of tested bacteria following sub-culture of the contents of the tubes above the MIC.

DISCUSSION

The increased frequency of resistance to commonly used antibiotics led to search for newer, effective, cheap and easily affordable drugs in the management of infectious diseases. In this study, our results showed that methanol extracts of *M. citrifolia* (fruit, leaf and stem), *V. trifolia* and *C. odorata* gave favourable results against all the 12 bacterial isolates tested than ethanol and ethyl acetate extracts. The methanol extracts inhibited most of the bacterial isolates significantly compared to ethanol and ethyl acetate extract (Isami et al., 2007; Satish Kumar et al., 2008). Many studies revealed that the methanol extracts of plants inhibit the growth of bacteria more than aqueous extracts of plants and found to possess more inhibitory effect than other extracts (Sukanya et al., 2009). This trend to show that the active ingredients of plant parts are better extracted with methanol than other solvents. Since methanol has high polarity, it could dissolve both the polar and non polar compounds in it. Generally, plant extracts are usually more active against gram positive bacteria than gram negative bacteria (Basri and Fan, 2005). This may be due to the permeability barrier provided by the cellwall or to the membrane

Table 3. Minimal Inhibitory Concentration (MIC) of *Morinda citrifolia* (Fruit, leaf and stem), *Eupatorium odoratum* (leaf) and *Vitex trifolia* (leaf) extracts against some bacteria.

Organism	Concentration of extracts (mg/ml)	*Morinda citrifolia*			*Vitex trifolia*	*Chromolaena odorata*
		Fruit	Leaf	Stem		
Escherichia coli	100	-	-	-	-	-
	50	-	-	-	-	-
	25	-	-	-	-	-
	12.5	+	+	+	+	+
Shigella flexneri	100	-	-	-	-	-
	50	-	-	-	-	-
	25	+	+	+	+	+
	12.5	+	+	+	+	+
Proteus mirabilis	100	-	-	-	-	-
	50	-	-	-	-	-
	25	+	+	+	+	+
	12.5	+	+	+	+	+
Pseudomonas diminuta	100	-	-	-	-	-
	50	-	-	-	-	-
	25	+	+	+	+	+
	12.5	+	+	+	+	+
Enterobacter cloacae	100	-	-	-	-	-
	50	-	-	-	-	-
	25	+	+	+	+	+
	12.5	+	+	+	+	+
S. aureus ATCC 6538	100	-	-	-	-	-
	50	-	-	-	-	-
	25	-	-	-	-	-
	12.5	+	+	+	+	+

'+' Growth appears, '-' No growth appears.

accumulator mechanisms.

M. citrifolia has been classified as a medicinal herb due to its therapeutic properties. Various parts of the plant, including roots, stems, leaves and fruit have been consumed solely on the basis of the assumption that it possesses healing properties. In the present study, *M. citrifolia* (fruit, leaf and stem) antibacterial property has been screened and the results showed effect on the growth of most of the tested bacteria and MIC values auspiciously determines that plant parts of *M. citrifolia* can be used in the treatment of infectious disease (Wei et al., 2008; Selvam et al., 2009; Usha et al., 2010). In the present study, *C. odorata* leaf extracts inhibited most of the bacteria tested when compared to other plant extracts. Among the ethanol, methanol and ethyl acetate, methanol extracts recorded significant antibacterial activity and MIC values against *S. aureus* ATCC 6538 and *E. coli* were more significant. Gram positive organisms were more susceptible than gram a negative organism which correlates with the earlier reports (Vital and Rivera, 2009). This might be due to the difference in sensitivity between gram positive and gram negative could be ascribed to the morphological differences between these microorganisms (El Astal et al., 2005). This suggests that *C*. *odorata* antimicrobial mode of action might be due to the presence of flavonoids and tannins that binds to bacterial cell wall and inhibits its biosynthesis (Anyasor et al., 2011).

V. trifolia leaf extracts showed significant antibacterial activity against gram positive and gram negative organisms. Methanolic extracts inhibited most the tested microorganisms than ethanol and ethyl acetate extracts (Leit et al., 2001; Meena et al., 2010; Abd Aziz et al., 2011). Acceptance of medicines from plant origin as an alternative form of healthcare is increasing because they are serving as promising sources of novel antibiotic

prototypes (Kodura et al., 2006). Some of the phytochemical compounds e.g. glycosides, saponin, tannin, flavonoids, trepenoid, alkaloid have variously been reported to have anti-microbial activity (Okeke et al., 2001; Rahman et al., 2010).

The complications in the antimicrobial activity of propolis could be due to differences in its chemical components. It has also been reported that the samples collected from different geographic origin with different climates and vegetations show different antibacterial activities (Kashi et al., 2011).

The present study shows that the methanol, ethanol and ethyl acetate extracts have inhibitory activity against most of the bacterial organisms. The inhibition of the growth of these organisms *in vitro* by the extracts may be due to the presence of some active constituents in the extracts. These active principles may have acted alone or in combination to inhibit the growth of the bacterial organisms. The medicinal uses of these plants to heal diseases including infectious one has been extensively applied by people. The problem of microbial resistance is growing and the outlook for use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

Conclusion

The methanol, ethanol and ethyl acetate extracts showed comparable antibacterial activity towards bacterial isolates, supporting the traditional use of these plants and suggesting a need to isolate and evaluate active constituents. Researches on the pharmacological properties of the plant extracts have several limits, due to unknown composition of all the components of the plant source investigated. The potential uses of solvent extracts of *M. citrifolia* (fruit, leaf and stem), *V. trifolia* and *C. odorata* is for the treatment of infectious disease and for the development of novel chemotherapeutic agents.

ACKNOWLEDGEMENT

We thank to Dr. Jai Sunder, Senior Scientist, Central Agricultural Research Institute and K. Riyas, Lab Technician, Malabar Medical Institute and Hospital, Cochin for support on collecting the samples and constant support to complete this study.

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