Antifungal and antibacterial activity of *Carissa carandas* Linn.

CHANCHAL KUMAR MISHRA, A.K. PATTNAIK, ASHA RANI, D. SASMAL AND RAJESH KUMAR NEMA

Accepted : June, 2009

SUMMARY

The *in vitro* antibacterial and antifungal activity of methanolic and petroleum ether extracts of unripe roots and fruits of *Carissa carandas* has been evaluated using disc diffusion method against *Staphylococcus aureus, Bacilus pumulis* and *Vibrio chlorae* bacterial strain, while turbidity method against *C. albicans, A. oryzae* and *T. azoli* fungus strains. Comparatively, extracts showed significant antifungal activity with specific standard (fluconazole) and moderate antibacterial activity with specific standard (ciprofloxacin).

Key words : *Carissa carandas*, Antibacterial, Antifungal

Carissa carandas Linn. (Family : Apocynaceae) is a climbing shrub, usually growing to 10 or 15 feet (3-5 m) high. In Jharkhand and other states, it is commonly known as Karunda or Jasmin flower Carissa. Karunda may bloom and fruit off throughout the year. *Carissa* a genus of about 32 species is distributed mostly in the warmer parts. Of the 8 Indian species, 3 are of economic importance. It grows from sea level to 6000 feet and requirement is fully exposure to sun. For use, unripe fruits are collected from mid May to mid July. Ripening season is August and September (Vaidyaratnam and Arya, 1994).

MATERIALS AND METHODS

Bacteria's used for determination of antibacterial activity:

The bacterial strains used for this study are mentioned in Table 1. The details of fungal strains used for determination are given in Table 3. Strains were from various parts of India and Abroad. They were maintained at slant or stab cultures in nutrient agar media at 4°C temperature in refrigerator (Rhayour *et al.*, 2003).

Standard antibacterial agent used for comparison of antibacterial activity

Pure Ciprofloxacin (Dr. Reddy's Lab.) were used as the standard antibacterial agent 100 mg dissolved in 20 ml distilled water and filtered, each of two stock solution (2 mg/ml and 40 mg/ml) were prepared by proper dilution with distilled water.

Preparation of inoculum:

The preserve bacteria were cultured and sub-cultured as pure colonies as follows:

– One loopful (2 mm) of each bacterial suspension was inoculated in 5 ml of nutrient broth and all test tubes were incubated at 37°C for 24 hours.

– The overnight grown nutrient broth culture of each test organism was used for streaking over nutrient agar plates and subjected to incubation at 37°C for 24 hours and the same process was repeated until pure isolated colonies were obtained.

– From these isolated, colonies fresh sterile nutrient broth and media were re-incubated at 37 °C for 24 hours. These nutrient broth cultures served as inoculum for determination of anti-bacterial activities of the extract (Bagamboula *et al.*, 2003).

Determination of minimum inhibition concentration (MIC):

Disc diffusion techniques:

– Stock solution of root and fruit extract of *Carissa carandas* of 1 mg/ml and 10 mg/ml were prepared with sterile Dimethyl Sulphoxide (DMSO) and measured volume of stock solutions were dispensed in the conical flask to prepare concentration of 250, 500, 750, 1000 and 1500 ìg/ml of extracts.

– The sterile Petri plates were poured with molten agar medium and allowed to be solidified.

– The suspension of test organism or culture were flooded on the solidified nutrient agar medium and kept for 30 minutes in same position for proper inoculation.

– Location for each test extract concentration was marked at the back of Agar containing petriplate.

Correspondence to:
RAJESH KUMAR NEMA, Rishiraj College of Pharmacy, Village Revati, Sanwer Road, INDORE (M.P.) INDIA
Authors' affiliations:
CHANCHAL KUMAR MISHRA, A.K. PATTNAIK AND D. SASMAL, Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, RANCHI (JHARKHAND) INDIA
ASHA RANI, Govt. College of Pharmacy, Bariatu, RANCHI (JHARKHAND) INDIA

– The whatmann filter paper was dipped in appropriate stock extract concentration and placed on the respective place as the marked area on the back of the petriplate.

– The plates were incubated at 37°C for 24 hours.

– The results were analyzed on the basis of zone of inhibition mentioned in Table 2 (Friedman *et al.*, 2002; Joksiæ *et al.*, 2003; Dorman and Deans, 2004).

Disc diffusion method for determination of antibacterial potency of the test compound and its comparison with standard antibacterial agent ciprofloxacin:

– Sterile disc of Whatman filter paper no.1 of 6 mm diameter was prepared.

– Nutrient agar medium was prepared, sterilized and poured on to sterile Petri dishes and then kept in the incubator at 37°C for 24 hrs.

– One set of two dilutions, each of extract and ciprofloxacin was prepared and stored in a properly capped volumetric flask.

– All plates were flooded with corresponding culture of the test organism under Laminar Airflow and left for 30 minutes.

– The excess inoculum was discarded using a sterile Pasteur pipette.

– Sterile discs were soaked in these dilutions and placed on the corresponding quadrants of the flooded nutrient agar plates marked at the back with same concentration. This was done both for the test compounds as well as for ciprofloxacin.

– The plates were kept overnight in the incubator at 37°C. After incubation, the diameter of zone of inhibition around each disc was measured and the results were tabulated for both compounds and ciprofloxacin (Dorman and Deans, 2000; Bagamboula *et al.*, 2003).

Fungal strain used for determination of antifungal activity:

Required strains were procured from Institute of Microbial Technology, Chandigardh (Table 3). Fluconazole (FDC Ltd.) was used as standard drug for the comparison of antifungal activity. Stock solutions of each 25 ml were prepared in sterile DMSO (Dimethyl sulfoxide) at a concentration of 1mg/ml.

Standard anti-fungal agent used for comparison of antifungal activity:

Fuconazole was used as standard for the comparison of antifungal activity. Stock solution of each 30 ml were prepared in sterile DMSO (Dimethyl Sulfoxide) at a concentration of 1mg/ml (Gulluce *et al.*, 2003; Carillo-Munoz *et al*., 2005).

Media:

– Liquid media and – Nutrient broth

Activation of culture:

– The ampoule was marked near the middle of cotton wool with a sharp file.

– Surface around the mark was disinfected with alcohol.

– Thick cotton wool was wrapped around the ampoule and broken at the marked area.

– The cotton plug was removed carefully and about 0.3 to 0.4 ml of specified medium was added to make suspension of the culture.

– The suspension was transferred to 3 test tubes, each containing 5 ml of recommended medium (liquid).

– The test tubes were incubated at appropriate temperature and conditions recommended for the culture.

Preparation of inoculum:

The suspensions of the fungi obtained after revival were used for streaking over the Nutrient malt yeast extract agar medium in Petridishes and subjected to incubation at 37°C for 12 hrs. From the cultures obtained from streaking in Petridishes culturing and sub-culturing until the pure growth culture was obtained. From these pure growth cultures, fresh sterile nutrient broth media were reinoculated and all these test tubes were incubated at 37°C for 12 hrs. These nutrient broth cultures served as inoculum for the determination of antifungal activity of the compounds (Iida *et al.*,1999; Kim *et al.*,1999; Karaman *et al.*, 2001).

Determination of minimum inhibition concentration (MIC):

The different extracts of *Carissa carandas* plant were first made the stock solution of 40 mg/ml and placed with sterile Dimethyl Sulphoxide (DMSO) and measured quantities of stock solutions were dispensed in the test tubes containing molten sterile agar media to prepare concentration of 250 µg/ml, 500 µg/ml, 750 µg/ml, 1000 µg/ml and 1500 µg/ml of extract. The molten agar media contained in test tubes with increasing concentration of the plant extracts were made into slants and were marked accordingly. One sterile test tube with no extract but with equal volume of solvent DMSO, served as the vehicle control. All the marked test tubes were filled with five volumes of increasing concentration and 5 volumes of

malt dextrose liquid media with the extract concentration ranging from 250, 500, 750, 1000 and 1500 µg/ml and was done in triplicate to know the extract concentration of inhibition. All the test tubes with extract as well as vehicle control were incubated at 30°C for 24 hrs. The turbidity was measured at 567 nm using Systronic UV Spectrophotometer. The reading was tabulated according to table prepared using the absorption data from Spectrophotometer. The minimum inhibition concentration (MIC) of the extract was indicated by the list concentration of the drug at which no organism was recorded in Table 4 (Goff *et al.*, 1995; Sanglard and Odds, 2002).

Table 1 : Details of bacterial strain used for determination of antibacterial activity

Name of strains	Sources	No. of strain
Staphylococcus aureus	Inst. of Microbial Technology, Chandigarh	MTCC 757
Bacilus pumulis	Inst. of Microbial Technology, Chandigarh	MICM 2327
Vibrio chlorae	All India Inst. of Hygiene and Public Health, Kolkata	824

RESULTS AND DISCUSSION

The results indicate that all the test extracts showed good inhibitory activity against all these bacterial strains but not better than fungal strains:

– Methanolic extract of root showed antibacterial activity against *Staphylococcus aureus, Bacillus pumulis* and *Vibrio chlorae* at 1500 µg/ml, 1500 µg/ml and 1000 µg/ml, respectively (Table 2), while antifungal activity against *T.azori, A. oryzae* and *C. albicans* at 1000 µg/ml, 1000 µg/ml and 750 µg/ml, respectively (Table 4).

– Pet. ether extract of roots showed antibacterial activity against *Staphylococcus aureus, Bacillus pumulis* and *Vibrio chlorae* at 1500 µg/ml, 1500 µg/ml and 1000 µg/ml, respectively (Table 2), while antifungal activity against *T. azori, A. oryzae* and *C. albicans* at 1000 µg/ml, 750 µg/ml and 750 µg/ml, respectively.

– Methanolic extract of fruits showed antibacterial activity against *Staphylococcus aureus, Bacillus pumulis* and *Vibrio chlorae* at 1500 µg/ml for all bacterial strains (Table 2) while antifungal activity against *T. azori, A. oryzae* and *C. albicans* at 1000 µg/ml for all the fungal strains (Table 4).

Table 2 : Determination of minimum inhibitory concentration (MIC) of the various extracts of *Carissa carandas* plant

Sr. No.	Test compounds	Name of the bacteria	Growth media containing different concentration of the extract in µg/ml				
			250 µg/ml	500 µg/ml	750 µg/ml	1000 µg/ml	1500 µg/ml
1.	Methanolic extract of roots	*Staphylococcus aureus*	+	+	+	±	--
		B. pumulis	+	+	+	±	--
		Vibrio chlorae	+	±	±	--	--
2.	Pet. Ether extract of roots	*Staphylococcus aureus*	+	+	+	±	--
		B. pumulis	+	+	+	±	--
		Vibrio chlorae	+	+	±	--	--
3.	Methanolic extract of rruits	*Staphylococcus aureus*	+	+	+	±	--
		B. pumulis	+	+	+	±	--
		Vibrio chlorae	+	+	+	±	--
4.	Pet. ether extract of fruits	*Staphylococcus aureus*	+	+	+	±	--
		B. pumulis	+	+	+	±	--
		Vibrio chlorae	+	+	±	±	--

+ — Growth, ± — Partial inhibition -- — Complete inhibition,

Table 3 : Details of fungal strain used for determination of antifungal activity

Name	***Candida albicans***	*Aspergillus* oryzae	***T. azori***
MTCC code	MTCC 227	MTCC 282	MTCC 113
Growth medium	Malt Yeast Agar	Malt yeast peptone	Malt Yeast peptone
Growth condition	Aerobic	Aerobic	Aerobic
Incubation temp.	20-25 °C	30 °C	30 °C
Incubation time	48 hrs.	24 hrs.	24 hrs.
Subculture	30 days	30 days	30 days
Sources	Inst. of Microbial Tech., Chandigarh	Inst. of Microbial Tech., Chandigarh	Inst. of Microbial Tech., Chandigarh

Table 4 : Determination of minimum inhibitory concentration (MIC) of the various extract of *Carissa carandas* plant

Sr. No.	Test compounds	Name of the fungus	Growth in Malt dextrose liquid media containing different concentrations of the extract and isolated compounds µg/ml				
			250 µg/ml	500 µg/ml	750 µg/ml	1000 µg/ml	1500 µg/ml
1.	Methanolic extract of roots	*T. azori*	+	+	±	--	--
		Aspergillus oryzae	+	+	±	--	--
		Candida albicans	+	±	--	--	--
2.	Pet. ether extract of roots	*T. azori*	+	+	±	--	--
		Aspergillus oryzae	+	±	--	--	--
		Candida albicans	+	±	--	--	--
3.	Methanolic extract of fruits	*T. azori*	+	+	±	--	--
		Aspergillus oryzae	+	+	±	--	--
		Candida albicans	+	+	±	--	--
4.	Pet. ether extract of fruits	*T. azori*	+	+	±	--	--
		Aspergillus oryzae	+	+	±	--	--
		Candida albicans	+	±	--	--	--
5.	Fluconazole	*T. azori*	+	±	--	--	--
		Aspergillus oryzae	+	±	--	--	--
		Candida albicans	+	±	--	--	--

+ — Growth, ± — Partial inhibition, -- — Complete Inhibition

– Pet. ether extract of fruits showed partial inhibition of antibacterial activity at 1000 µg/ml against all bacterial strains and complete inhibition of at 1500 µg/ml for all the bacterial strains (Table 2), while antifungal activity against *T. azori*, *A. oryzae* and *C. albicans* at 1000 µg/ml, 1000 µg/ml and 750 µg/ml, respectively (Table 4).

– Standard Fluconazole showed antifungal activity against *T. azori*, *A. oryzae* and *C. albicans* at 750 ìg/ml for all fungal strains (Table 4).

REFERENCES

Bagamboula, C.F., Uyttendaele, M. and Debevere, J. (2003). Antimicrobial effect of spices and herbs on *Shigella sonnei* and *Shigella flexneri*. *J. Food Prot.*, **66**: 668-673.

Carillo- Munoz, A.J., Quindosa, G., Ruesga, M. (2005). Antifungal activity of posaconazole compared with fluconazole and amphotericin B aganist yeast from oropharyngeal candidasis and other infections. *J. Antimicrob. Chemother,* **55** : 317-19.

Dorman, H.J.D. and Deans, S.G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol.*, **88** : 308-316.

Dorman, H.J.D. and Deans, S.G. (2004). Chemical composition, antimicrobial and in vitro antioxidant properties of *Monarda citriodora* var. *citriodora*, *Myristica fragrans*, *Origanum vulgare Hirtum*, *Pelargonium* sp. and *Thymus zygis* oils. *J. Essent. Oil Res.*, **16**: 145-150.

Friedman, M., Henika, P.R. and Mandrell, R.E. (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes* and *Salmonella enterica*. *J. Food Prot.*, **65**: 1545-1560.

Goff, D.A., Koletar, S.L., Buesching, W.J., Barnishan, J. and Fass, R.J. (1995). Isolation of Fluconazole-resistent *Candida albicans* from human immunodeficiency virus-negative patients never treated with azoles. *Clin. Infect. Dis.*, **20**: 77-83.

Gulluce, M., Sokmen, M., Daferera, D., Agar, G., Ozkan, H., Kartal, N., Polissiou, M., Sokmen, A. and Sahin, F. (2003). The *in vitro* antibacterial, antifungal and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. *J. Agric. Food Chem.*, **51**: 3958-3965.

Iida, Y., Oh, K. B., Saito, M., Matsuoka, H., Kurata, H., Natsume, M. and Abe, H. (1999). Detection of antifungal activity in Anemarrhena asphodeloides by sensitive BCT method and isolation of its active compound. *J. Agric. Food Chem.*, **47**: 584-587.

Joksic, G., Stankovic, M. and Novak, A. (2003). Antibacterial medicinal plants *Equiseti herba* and *Ononidis radix* modulate micronucleus formation in human lymphocytes *in vitro*. *J. Environ Pathol. Toxicol. Oncol.*, **22**: 41–48.

Karaman, S., Digrak, M., Ravid, U. and Ilcim, A. (2001). Antibacterial and Antifungal Activity of the Essential Oils of Thymus reVolutus Celak. *Turkey. J. Ethnopharmacol.* **76**: 183-186.

Kim, B.S., Moon, S.S. and Hwang, B.K. (1999). Isolation, antifungal activity and structure elucidation of the glutarimide antibiotic, streptimidone, produced by *Micromonospora coerulea*. *J. agric. Food Chem.*, **47**: 3372-3380.

Rhayour, K., Bouchilkhi, T., Tantaoui-Elaraki, A., Sendide, K. and Remmal, A. (2003). The mechanism of bactericidal action of oregano and clove essential oils and of their phenolic major components on *Escherichia coli* and *Bacillus subtilis*. Essent. *Oil Res.*, **15**: 356-362.

Sanglard, D. and Odds, F.C. (2002). Resistence of *Candida* species to antifungal agents: molecular mechanism and clinical consequences. *Lancet Infect Dis.*, **2**: 73-85

Vaidyaratnam, P.S.Vareir. and Arya Vaidya Sala (1994). Indian medicinal plants, Volume-I, pp. 389-396, Orient Langman Publication.

****
*****