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Effect of maturity indices of *Carissa carandus* L. fruit on its antibacterial activity

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ABSTRACT

Since a complex network of anabolic and catabolic reactions occurs within the fruits, which lead to the production of wide range of bioactive compounds. The present study aimed to identify the effect of maturity indices of Carissa carandus fruits on its antibacterial potential. Eight medically important bacterial strains were selected to identify the antibacterial potential by measuring their zone of inhibitions using agar well diffusion method. Among the various extracts used diethyl ether extract of the ripened fruit exhibited a maximum inhibition zone of 8 mm against Salmonella paratyphi. Methanol extract exhibited relatively high inhibition percentage while water extract showed the lowest inhibition percentage against the selected bacterial strains. Staphylococcus epidermidis and Salmonella paratyphi were most susceptible strains, while Bacillus cereus and Salmonella typhi were the most resistant bacterial strains. High inhibition was obtained using extracts of pre-mature, mature and pre-ripened stages. Thus the study proves significant difference between their antibacterial activities of C. carandus fruit with varying maturity.

Keywords: antibacterial activity, *Carissa carandus* L., fruit, maturity indices, underutilized.

INTRODUCTION

The fruits of *Carissa carandus* L. (Apocynaceae) are underutilized and used as a minor crop in the major parts of the country [1]. The fruits are berries ovoid-oblong or ellipsoidal in shape, green when young and turns purple-black when ripens. The fruits have been chiefly consumed by the tribal and local inhabitants of the forest areas; they use the unripe fruits for the preparation of pickles and chutney, while ripe fruits are utilized in preparation of curries, puddings and jellies [2]. The fruits have also been ascribed to possess cardiotonic, anticonvulsant, anthelmintic, antiviral and nematicidal activity [3-5].

The maturity indices of fruits have been defined as the stage of development at which the fruit has completed its natural growth and is ready for harvest. Maturity index of a particular

commodity is determined based on its importance at that particular stage. Since fruits undergo through a series of developmental transitions, involving coordinated changes in a number of catabolic and anabolic reactions, that results in ripening [5]. During this entire process, large range of bioactive compounds are either synthesized or degraded to form different bioactive compounds. Since, determination of specific maturity indices varies between commodities [6], it is used as a tool to define and measure the quality of the produce. Thus the present study has been carried out to identify the effect of maturity indices on the antibacterial potential of *C. carandus* fruit.

MATERIALS AND METHODS

Fruits of Karanda (*Carissa carandus* L.) were collected at their successive stages of growth and ripening from the Botanical Garden of Sardar Patel University, Vallabh Vidyanagar, Gujarat, India. The fruits were freshly collected, rinsed with distilled water and pulp along with the peel was dried at room temperature. The dried fruit samples were further grounded to powder and stored in air tight containers until further use.

Extraction was carried out using the method of Houghton and Raman [7] using different non polar to polar solvents like diethyl ether, ethyl acetate, acetone, methanol and water. The extracts obtained were later filtered using Whatman No. 4 filter paper, concentrated at room temperature and stored at 4°C until further use. The dried extracts were used for measuring their antibacterial activity after dissolving them in 5% Dimethyl sulfoxide (DMSO) to make a standardized solution of 10 mg/ml.

Microbial type pure cultures used in the study were obtained from Microbial type culture collection, Chandigarh, India (MTCC). Eight bacterial strains of which four were gram positive namely *Bacillus cereus* (MTCC-430), *Bacillus subtilis* (MTCC-121), *Micrococcus luteus* (MTCC-106), *Staphylococcus epidermidis* (MTCC-435) and four gram negative namely *Escherichia coli* (MTCC-443), *Klebsiella pneumoniae* (MTCC-109), *Salmonella paratyphi* (MTCC-735), *Salmonella typhi* (MTCC-734) were screened in the present study. Bacterial strains were maintained on a nutrient agar slant at 4°C and before using for the studies they were activated by culturing them on nutrient agar at 37°C for 24 hours.

The agar well diffusion method described by Perez *et al.* [8] was selected to ascertain the inhibitory spectrum against selected bacterial strains. The density of bacterial cells was measured using 0.5 Mc Farland turbidity standards [9]. Ciprofloxacin and Doxycycline (20 µg/ml) were used as positive controls, while (100 and 50 %) DMSO was used as negative controls. The cultured plates along with extract were incubated at 37°C for 24 hours and the antibacterial activity was assessed based on the diameter of the clear zone surrounding the well (excluding the well diameter) in millimeter. The tests were conducted in triplicates.

RESULTS AND DISCUSSION

The fruit of *C. carandus* was used to measure the effect of different extracts of the fruit on the growth of some selected bacterial strains. Among the various extracts used diethyl ether extract of the ripened fruit exhibited a maximum inhibition zone of 8 mm against *Salmonella paratyphi* (Table 1). However, the growth of *Salmonella paratyphi* and *Staphylococcus epidermidis* were also inhibited by 6 mm zone using diethyl extract of pre-ripened fruit. Similarly, growth of *Escherichia coli* was inhibited by a zone of 6 mm when diethyl ether extract of young and pre-mature fruits were used. In contrast, ethyl acetate fraction of all the growth stages exhibited least

or no activity against the selected bacterial strains, where as studies proved that *Bacillus subtilis* and *Salmonella paratyphi* were the most resistant bacterial strains (Table 1). Acetone extract of the young fruit exhibited moderate inhibitory zone against *Salmonella paratyphi* with 6 mm inhibitory zone, while *Bacillus cereus*, *Micrococcus luteus*, *Escherichia coli* and *Salmonella typhi* were found to be highly resistant and exhibited no inhibitory zone against the acetone fractions.

Table 1. Antibacterial activity of *Carissa carandus* fruit at their sequential stages of growth

Extracts used	Stages of fruit growth	Zone of Inhibition (mm)							
		Gram $^{+ve}$ Bacteria				Gram $^{-ve}$ Bacteria			
		BC	BS	ML	SE	EC	KP	SP	ST
Diethyl ether	Young	1	-	-	4	**6**	1	-	-
	Pre-mature	2	-	-	1	**6**	2	-	-
	Mature	-	-	-	3	**5**	2	-	-
	Pre-ripened	-	4	-	**6**	2	2	**6**	2
	Ripened	-	4	-	-	-	2	**8**	-
Ethyl acetate	Young	-	-	3	**4**	1	1	-	**4**
	Pre-mature	-	-	2	**3**	-	2	-	-
	Mature	-	-	-	-	-	**2**	-	-
	Pre-ripened	**2**	-	-	-	1	1	-	**2**
	Ripened	**1**	-	-	-	-	-	-	**1**
Acetone	Young	1	1	-	4	-	1	**6**	-
	Pre-mature	-	2	-	2	-	2	**4**	-
	Mature	-	**2**	-	1	-	-	**2**	-
	Pre-ripened	-	2	-	-	-	2	**4**	-
	Ripened	-	1	-	**4**	-	1	**4**	-
Methanol	Young	-	**4**	1	-	1	-	-	2
	Pre-mature	4	4	4	4	2	4	**6**	-
	Mature	-	4	4	**6**	4	-	**6**	2
	Pre-ripened	-	**4**	-	**4**	1	2	-	-
	Ripened	-	**5**	4	2	-	**5**	-	-
Water	Young	-	-	-	**1**	-	-	-	-
	Pre-mature	-	-	-	**1**	-	-	-	-
	Mature	-	-	-	-	-	-	-	-
	Pre-ripened	-	-	-	-	-	-	-	-
	Ripened	-	-	-	-	-	-	-	-

BC – *Bacillus cereus*, **BS –** *Bacillus subtilis*, **EC –** *Escherichia coli*, **KP –** *Klebsiella pneumoniae*, **ML –** *Micrococcus luteus*, **SE –** *Staphylococcus epidermidis*, **SP –** *Salmonella paratyphi*, **ST –** *Salmonella typhi*

Among the various extract used, methanol extract exhibited relatively high inhibition percentage against the selected bacterial strains. However, water extract exhibited no activity against all the selected bacterial stains used for the present study. High inhibition was obtained using extracts of pre-mature, mature and pre-ripened stages. Besides Tian *et al.* [10] have observed differences in the solvents used for extraction and revealed that the difference in the polarity of solvents affect the bioactivities and with the increasing polarity the antibacterial activity generally decreases. *Staphylococcus epidermidis* and *Salmonella paratyphi* were most susceptible strains, while *Bacillus cereus* and *Salmonella typhi* were the most resistant bacterial strains. The resistance observed in some bacteria may be due to several factors like genetic makeup of the species, sporulating conditions, water content, coatings and mineral content [11]. The results are in accordance to Negi and Jayaprakasha [12], who observed no clear trend on the different types of bacteria on the antibacterial activity. However, Taylor *et al.* [13] reasoned that the active compounds may be present in the extract but in insufficient quantities. Lack of activity according to Farnsworth [14] can thus only be proven by using large doses. Alternatively, Jager *et al*. [15] found that the active principle may be present in high quantities, but there could also be other constituents exerting antagonistic effect or negating the positive effects of the bioactive agents.

Fruits of *C. carandus* at all successive stages of growth and ripening had more or less some inhibitory activity against selected bacterial strains. Hence, fruits with varying maturity had varying activity and extracts of pre-mature, mature and pre-ripened fruits were found promising for its inhibitory effect on the bacterial strains tested. Thus the study proves that the difference in maturity indices plays important role in synthesis of different bioactive compounds with antibacterial potential.

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