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IN VITRO ANTIBACTERIAL ACTIVITY OF THE CRUDE METHANOL EXTRACT OF FRUIT OF *CARISSA CARANDAS*

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ABSTRACT

Infectious infections are the leading cause of morbidity and mortality in underdeveloped nations, making them a severe public health issue. The antibacterial activity of the crude methanol extract of *Carissa carandas* fruits was evaluated at three different concentrations by the agar well diffusion method. The methanol extract of the fruits exhibited antibacterial activity leaves against four pathogenic bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Staphylococcus aureus*. The methanol extract was most active against pathogenic bacteria. The inhibitory effect of the extract was compared with standard antibiotics ampicillin.

Keyword: Antibacterial Activity, *Carissa carandas*

INTRODUCTION

Since the mid-1970s, the emergence of a number of new pathogens and reemergence of older diseases has highlighted the fact that, contrary to expectations, epidemics of infectious disease remain a problem of public health concern [1]. Infectious diseases remain the largest global cause of death. Infectious

infections are the leading cause of morbidity and mortality in underdeveloped nations, making them a severe public health issue. Despite the availability of antibiotics, the situation is worse due to drug resistance. Antimicrobial resistance is a serious problem in hospitals these days due to the widespread

usage or misuse of these medications [2]. Drug resistance against human pathogenic microorganisms has been widely reported in recent years. Many scientists have recently focused their attention on biologically active compounds extracted from plants due to side effects and antimicrobial resistance. Because many infectious diseases have been recognized to be treated with herbal remedies throughout history, natural products can be chosen for biological screening based on ethno-medical use of the plants. Plant materials continue to play an important role in many underdeveloped countries as therapeutic medicines today.

Antimicrobial substances found in plants can stop bacteria from growing in a variety of ways. The primary active components in plants that are responsible for antibacterial action have been identified and isolated in several research [3].

Medicinal plants are effective in the treatment of infectious diseases and infections of various types of external wounds (chronic, deep suppurative, open, lacerated, incised, and ulcerated) and have been used for these purposes in humans and different species of animals. The use of medicinal plants has the added benefit of reducing many of the side effects often associated with synthetic antimicrobials [4].

Carissa carandas is an evergreen thorny shrub belongs to Apocynaceae family, which is commonly known as karonda. It has small berry-shaped fruits, used as additive in many pickles or as a spice in northern India. It is drought resistant plant that can be grown in a wide range of different types of soils. Approximately more than 25 species of genus *Carissa* are known, out of which five species are native to India. It is commonly used to make hedge for orchards. It has been found that the fruit is the richest source of iron, vitamin C and pectin. Even it is used as an ingredient in most of the edible preparations such as jam, jelly, squash and syrup [5]. Therefore, the present work aims to study the In vitro antibacterial activity of the crude methanol extract of fruit of *Carissa carandas*

MATERIAL AND METHODS

Plant material

Carissa carandas (Apocynaceae) plant and fruits were collected from the Satpuda region, Dhadagaon village in Nandurbar District, Maharashtra in the month of July 2022 (Figure 1). The plants and fruits of healthy and disease-free plants were used to test the medicinal properties of the plant.

Methanolic Extraction of Powdered Plant Material

The fruits of *Carissa carandas* (Apocynaceae) was collected and dried in the shade and then pulverized in a grinder. Material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction. The powdered was utilized for extraction by prescribed in standard reference using methanol as solvent for extraction of powdered plant [6].

Preliminary Phytochemical screening

Qualitative phytochemical analysis

Phytochemical tests performed on methanol extracts to determine the presence of different phytochemicals following established standard protocol. The plant extracts were subjected for the test of alkaloid, carbohydrates, fixed oils and fats, glycosides (Cardiac, Anthraquinone, Saponin), phenolic compounds and tannins, proteins and amino acids, flavonoids, lignin, terpenoids, and diterpenes. Qualitative phytochemical examinations were carried out for all extracts of fruit as per the standard methods [6, 7].

Test Microorganisms and Growth Media

Escherichia coli, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Staphylococcus aureus* bacterial strain were chosen for their clinical and pharmacological importance in Clinitech Solution Excellence in Clinical Research Lab, Hyderabad. On nutrient agar,

the bacterial cultures were cultured for 24 hours at 37°C. The bacteria were cultivated at 37°C in Mueller-Hinton agar (MHA) plates (the bacteria were cultured in nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the yeasts and moulds were grown at 28°C in Sabouraud dextrose agar and PDA medium, respectively. The stock cultures were kept at a temperature of 4°C.

Preparation of the inoculum

A loopful of test microorganism was collected and sub-cultured into a second test tube containing the nutrient agar broth to make the bacterial inoculum. The tub was then incubated for 24 hours at 37°C, and the resulting broth with microorganisms was standardized to have a consistent population density [8].

In vitro antibacterial activities [9-11]

Determination of zone of inhibition method

Agar Well Diffusion Method (Zone of Inhibition)

The agar disc diffusion method was used to test the antibacterial activity Methanolic extracts of *Carissa carandas* (Apocynaceae) fruits against four pathogenic bacteria (two Gram-positive and negative). The agar well diffusion method was used to assess the antibacterial activity of the crude extracts.

The media was allowed to cool at 45 to 50°C immediately after autoclaving. To achieve a uniform depth of almost 4 mm, the freshly prepared and cooled media was poured into flat-bottomed Petri dishes (90 mm in diameter) and put on a level and horizontal surface. The agar material was allowed to cool and solidify at room temperature before being used to check sterility, and the plates were incubated at 35°C for 18–20 hours before being utilised. Then, using a sterile spreader, 0.1 mL of the tested inoculum was equally placed on the surface of the solidified agar. On the agar plate, four equidistant wells of 8 mm diameter and 3 mm depth were created. Antibacterial activity of all extracts was tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes*.

Methanolic extracts of *Carissa carandas*, as well as standard drug, were ready in double distilled water using nutrient agar tubes in five dilutions (5, 25, 50, 100, and 250 µg/ml). The wells were filled with about 100 µL methanol extracts. The antibacterial activity of ampicillin was tested in control trials under identical conditions. At 37 °C, the bacterial agar plates were incubated aerobically for 24 hours. The sizes of inhibitory zones (including the diameter of the disc) on the agar surface around the discs

were measured to determine the microorganism species' sensitivities to the plant extracts, and values less than 8 mm were declared inactive against microorganisms

RESULTS AND DISCUSSION

In the present study the methanolic extract of *Carissa carandas* (Apocynaceae) was evaluated for In vitro antibacterial activities which revealed the following results.

Taxonomy [12]

Kingdom	: Plantae
Clade	: Tracheophytes
Clade	: Angiosperms
Order	: Gentianales
Family	: Apocynaceae
Genus	: <i>Carissa</i>
Species	: <i>C. carandas</i>

Common Name [12]

Hindi	: Karonda
Malayalam	: Karakka
Telugu	: Peddakalavi
Marathi	: karvand
Tamil	: Kalakkai
Sanskrit	: Karamard

Methanolic extract of *Carissa carandas*

Dried fruits were selected for further study, phytochemical evaluation and subjected for extraction by methanol as a solvent (Table 1).

Preliminary Phytochemical screening

Qualitative phytochemical analysis

Qualitative phytochemical analyses were carried out on methanol extracts. The results reveal that methanolic extract contains saponins, alkaloids, flavonoids, tannins, carbohydrates, proteins and amino acids (Table 2). It gives a preliminary insight into various compounds present in a plant, based on which further study towards the biological activities of the compounds can be tracked.

In vitro antibacterial activities

The bactericidal activity of methanolic extract of *Carissa carandas* (Apocynaceae) increased linearly when the concentration of extracts ($\mu\text{g}/\text{ml}$) was increased. The results

showed that the methanolic extract at $250 \mu\text{g}/\text{ml}$ has a good growth inhibition zone, with bacterial activity of 29 ± 0.8 , 28 ± 0.2 , 29 ± 0.6 , and 29 ± 0.8 against *E. coli*, *P. aeruginosa*, *S. pyogenes*, and *S. aureus*, respectively as compared to other concentration when compared with standard drug ampicillin. The methanolic extracts of *Carissa carandas* (Apocynaceae) at $250 \mu\text{g}/\text{ml}$ were shown to be more efficient against all of the microorganisms tested when compared to standard drug (Figure 2 and Table 3).



Figure 1: Fruits of *Carissa carandas* (Apocynaceae) Plant

Table 1: Yield of methanol extracts obtained from *Carissa carandas*

Sr. No.	Extract	Color	Yield (%)w/w
01	Methanol	Dark brown	9.53

Table 2: Preliminary phytochemical analysis of various extracts of *Carissa carandas*

Sr. No.	Parameter	Methanol Extract
1	Carbohydrate	+
2	Protein	+
3	Amino acid	+
4	Steroids	-
5	Cardiac glycosides	-
6	Anthraquinone glycosides	-
7	Saponin glycosides	+
8	Cyanogenetic glycosides	-
9	Coumarin glycosides	-
10	Alkaloids	+

11	Flavonoids	+
12	Tannins	+

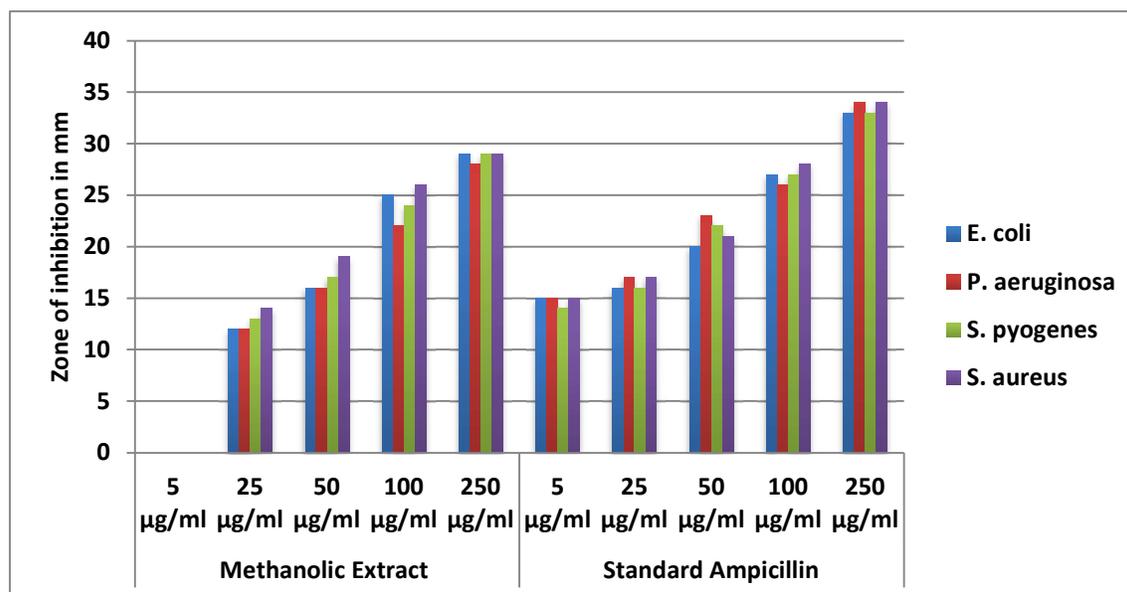
+ Positive

- Negative

Table 3: Antibacterial activity of Methanolic extract by Agar well diffusion Method

Sample with code	Antibacterial activity				
	Zone of inhibition in mm				
	Concentration in $\mu\text{g/ml}$	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. pyogenes</i>	<i>S. aureus</i>
Methanol Extract (PM)	5 $\mu\text{g/ml}$	NA	NA	NA	NA
	25 $\mu\text{g/ml}$	12 \pm 0.7	12 \pm 1.2	13 \pm 1.3	14 \pm 1.2
	50 $\mu\text{g/ml}$	16 \pm 0.9	16 \pm 0.9	17 \pm 1.1	19 \pm 0.8
	100 $\mu\text{g/ml}$	25 \pm 0.2	22 \pm 0.9	24 \pm 0.9	26 \pm 0.7
	250 $\mu\text{g/ml}$	29 \pm 0.8	28 \pm 0.2	29 \pm 0.6	29 \pm 0.8
Standard Ampicillin	5 $\mu\text{g/ml}$	15 \pm 0.8	15 \pm 0.9	14 \pm 1.3	15 \pm 0.9
	25 $\mu\text{g/ml}$	16 \pm 0.4	17 \pm 0.3	16 \pm 0.9	17 \pm 1.3
	50 $\mu\text{g/ml}$	20 \pm 0.6	23 \pm 1.4	22 \pm 0.7	21 \pm 0.7
	100 $\mu\text{g/ml}$	27 \pm 1.5	26 \pm 1.5	27 \pm 0.6	28 \pm 0.9
	250 $\mu\text{g/ml}$	33 \pm 0.8	34 \pm 0.5	33 \pm 0.9	34 \pm 1.3

Values are means of three replicates. NA: No activity exhibited by extracts against microorganism

Figure 2: Antibacterial activities of extracts of *Carissa carandas*

CONCLUSION

The present study justified the claimed uses of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes. However, higher concentration of alkaloids, flavonoids, tannins, and phenolic components. The

findings of this study back up claims that leaves have been utilised in traditional medicine to cure a variety of infectious diseases caused by bacteria. Researchers believe that antimicrobial components of plant extracts (terpenoid, alkaloid, and phenolic compounds) interact with enzymes

and proteins of the microbial cell membrane, disrupting it and causing a flux of protons towards the cell exterior, causing cell death or inhibiting enzymes involved in amino acid biosynthesis.

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REFERENCES

- [1] Thuy Thu Vu, Hyungrok Kim, Vu Khac Tran, Quang Le Dang, Hoa Thi Nguyen, Hun Kim, In Seon Kim, Gyung Ja Choi, Jin-Cheol Kim. In vitro antibacterial activity of selected medicinal plants traditionally used in Vietnam against human pathogenic bacteria. *Complementary and Alternative Medicine* (2016) 16:32
- [2] Joshi B, Panda SK, Jouneghani RS, Liu M, Parajuli N, Leyssen P, Neyts J, Luyten W. Antibacterial, Antifungal, Antiviral, and Anthelmintic Activities of Medicinal Plants of Nepal Selected Based on Ethnobotanical Evidence, Evidence-Based Complementary and Alternative Medicine. 2020; 14.
- [3] Britto, John DA, Sebastain, Roshan S, Mary SR. Antibacterial activity of selected of lamiaceae against human pathogens. *Indian J of Nat Products and resources*. 2012; 3(3): 334-342.
- [4] Bahar Mammed, Ashebr Abraha, Teka Feyera, Adugna Nigusse, Solomon Assefa. In Vitro Antibacterial Activity of Selected Medicinal Plants in the Traditional Treatment of Skin and Wound Infections in Eastern Ethiopia. *BioMed Research International*, Volume 2018.
- [5] Sonia Singh, Meenakshi Bajpai and Pradeep Mishra, *Carissa carandas* L. – phyto-pharmacological review. *Journal of Pharmacy and Pharmacology*, (2020), 72: 1694–1714.
- [6] Khandelwal KR. *Practical Pharmacognosy. Techniques and Experiments*. 19th edition. Pune: Nirali Prakashan; 2005.
- [7] Kadabadi SS, Deore SL, Baviskar BA. *Experimental phytopharmacognosy: A Comprehensive Guide*. Pune: Nirali Prakashan; 2011.
- [8] Harborne JB. (Eds). *Phytochemical methods: A guide to Modern techniques of Plant analysis*. 3rd ed. Chapman and hall, London. 1998; 302.
- [9] Bhalodia NR, Shukla VJ. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: An ethnomedicinal plant, *Journal of*

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- Advanced Pharmaceutical Technology & Research. 2011; 2(2).
- [10] Rojas JJ, Ochoa VJ, Ocampo SA, Munoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in the treatment of nonnosocomial infections. BMC Complement Altern Med. 2006; 6: 2.
- [11] Gupta D, Dubey J, Kumar M. Phytochemical analysis and antimicrobial activity of some medicinal plants against selected common human pathogenic microorganisms, Asian Pacific Journal of Tropical Disease. 2016; 6(1): 15-20.
- [12] <http://www.flowersofindia.net/catalog/slides/Karanda.html>