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ORIGINAL ARTICLE **OPEN ACCESS**

Antibacterial Potential and Phytoconstituents of *Carissa carandas* Flower, Leaf and Root Extracts

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ABSTRACT

Carissa carandas is an ethnopharmacologically important plant and shows various medicinal properties. The objective of this study is to evaluate the phytochemical constituents and antimicrobial activity of methanolic extracts of Carissa carandas flower, leaf and root against pathogenic strains such as Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, Candida albicans and Aspergillus niger. Further, the antimicrobial activity was determined by using the agar well diffusion method. The zone of inhibition of extracts was compared with that of reference drug i.e. streptomycin and ketoconazole. The results show that antimicrobial activity was observed against Staphylococcus aureus, Escherichia coli and Bacillus cereus. The phytochemical analyses of the plants were carried out and confirmed the presence of alkaloids, phenol, tannin, saponin, glycoside, terpenoids, flavonoids, carbohydrates, amino acids, and protein. The antimicrobial activity of the Carissa carandas was due to the presence of various secondary metabolites. Hence, the study concluded the presence of antimicrobial agents present in Carissa carandas and may be used to develop of new pharmaceutical applications.
Keywords: *Antibacterial, Antifungal, Carissa carandas, Micro broth dilution assay.*

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INTRODUCTION

Medicinal plants have historically been used as an alternate source for the treatment of bacterial illnesses. Globally, the bacterial infection is the leading cause of death. According to statistical forecasts, there were 1.27 million (95 percent UI 0.911-1.71) deaths attributed to bacterial AMR in 2019, among the 4.95 million (3.62-6.57 million) fatalities linked to bacterial AMR overall (1). Plants-derived bioactive compounds prove to be an alternative or complementary treatment of disease. As more than 80% of the world's population heavily depends on traditional medicines for health care. To resolve human ailments due to pathogenic microbes, search for natural products and bioactive compounds are the major sources (2). However, less than 1% of plants are characterized for their secondary metabolites, phytochemicals constituents, and pharmacologically active ingredient. In this regard, traditional medicinal plants (TMP), are the most valuable source of new bioactive chemical entities due to their ecological biodiversity and diverse chemical endowment within each species. As per experimental investigations, medicinal plants include important components such as tannins, essential oils, coumarins, flavonoids, phenolics, alkaloids, terpenoids, lectin, polypeptides, and polyacetylenes (3). The presence of bioactive chemicals in medicinal plants varies depending on plant species, soil type, and microbial interaction. Infectious illnesses caused by bacterial and viral pathogens continue to be a major source of concern for global health. Antibiotics have considerably reduced the occurrence of bacterial infections, but we are increasingly witnessing antimicrobial resistance (AMR) to these antibiotics, and the development of novel antibacterial is trailing behind the advent of resistance. During 1950 and 1980, growth in the development of new antibiotics resulted in the introduction of about 200 new medications to the market. However, in the twenty-first century, just 12 new antibiotics have been approved (4).Some plants may exhibit a broad spectrum of antimicrobial effects, which possibly control the impairments associated with multidrug-resistant microbes. *C. carandas* is a flowering shrub species of the Apocynaceae (dogbane) family and distributed in tropical and subtropical regions. The plant exhibits therapeutic potential due to the presence of phytochemical components. *C. carandas* leaf, bark, fruit, and root have been used in traditional medicine to treat a variety of human

ailments including hepatomegaly, indigestion, amenorrhea, oedema, colic, piles, antipyretic, fever, liver dysfunction, stomach pain, skin infections, intestinal worms, antimicrobial, and antifungal. *C. carandas* leaf exhibits anticancer, antibacterial, antioxidant, and non-mutagenic properties (5).Since traditional use of this medicinal plant and very less information is available on different organs of this plants. Aim to investigate phytochemical screening and antimicrobial activity of the methanolic extract of *Carissa carandas* flower, leaf, and root part by well diffusion assay.

MATERIAL AND METHODS

Collection of Plant Samples

The fresh parts of *C. carandas* were collected from Forest nursery situated in Jhansi, U.P (India). Authentication of the plant was done by Central Ayurveda Research Institute, Central Council for Research in Ayurvedic Sciences Ministry of Ayush, Govt. of India Gwalior Road, Jhansi (U.P). And reference voucher no. 28758 was assigned for preserving herbarium.

Preparation of Plant Extracts

Different parts of *C.carandas* were shed dried until getting properly dried. And further ground into a fine powder by mortar and pestle. Subsequently, extracts were prepared in presence of methanol solvent using Soxhlet apparatus and filtered using Whatman no. 1 filter paper, further solvent was evaporated in a rotary vacuum evaporator at 55°C. The extracts were stored at -4°C and used for *in vitro* screening of antimicrobial activity.

Phytochemical screening

The phytochemical study was done to identify chemical constituents present in methanol extract of *C. carandas* (6)**.**

Antibacterial and Antifungal Activity

The antimicrobial activity of extracts was evaluated against following pathogenic microorganisms *E.coli* (MCC-2246), *Klebsiella pneumonia* (MCC-2570), *Staphylococcus aureus*(MCC-2408), *Pseudomonas aeruginosa* (MCC-2080), *Bacillus cereus* (2217) *Candida albicans*(3102), and *Aspergillus niger.*

The prepared plant extract was dissolved in dimethyl sulfoxide (DMSO), antimicrobial activity of the extracts was tested using the agar well-diffusion method according to the guidelines set by the clinical and laboratory standards institute (7). Standardized test bacterial suspensions were prepared in Nutrient broth (HiMedia Laboratories Pvt.Ltd, Mumbai, India, M002) (equivalent to 0.5 McFarland standard) and fungal suspension in Potatao Dextrose Broth (Himedia Laboratories Pvt.Ltd, Mumbai, India,GM403) were inoculated uniformly on the entire surface of freshly prepared Nutrient agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India, MV001) and Potato Dextrose Agar (M096F) using sterile cotton swabs. Wells were made using a sterile cock borer (6 mm). Wells were filled with 20µL of each concentration (100 mg/mL) of the crude extracts, streptomycin and Ketaconazole20 µL used as positive controls,while 10% DMSO was used as a negative control. The inoculated plates were left at room temperature for 30 min for the extract to diffuse and later, the plates were incubated at 37°C for 24 hours. After the incubation period, the diameter of zones of inhibition were measured in millimetres (mm) using a ruler, and results were interpreted according to guidelines. The experiments were performed in triplicates.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the Flower, leaf and root of *C. carandas* was determined by broth microdilution method.The two-fold serial dilution of extract was prepared by using Stock concentration of 50mg/ml ranging from 25, 12.5, 6.25, 3.125, 1.563, 0.78, 0.391, 0.195, 0.975, 0.49 mg/ml. Add 50 µl of nutrient broth from column 2-10, and 100µl was added in column12. Add 50 µl of two-fold serially diluted extract from column 2-10. The standardised microorganism suspension adjusted to 5 x105 CFU ml−1 was then added 10µl to all wells (except column no.11). After incubation for 24 h at 37 °C, resazurin (0.015 %) was added to all wells (25 µl per well), and further incubated for 2–4 h for the observation of colour change. On completion of the incubation, columns with no colour change (blue resazurin colour remained unchanged) were scored as above the MIC value (8).

RESULTS

Phytochemical Screening

The phytochemical analyses of the different parts of the plant i.e. flower, leaves and roots were carried out and confirmed the presence of various secondary metabolites such as alkaloids, phenol, tannin, saponin, glycoside, terpenoids, flavonoids, carbohydrates, amino acids, and protein (Table 1).

Table 1: Preliminary phytochemical analysis of *Carissa carandas* Flower, Leaf and Root Extracts

S. No.	Phytochemical Tests	*Carissa carandas*		
		Flower	Leaves	Roots
1	Alkaloid Test			
	Mayner's Test	-	+	+
	Wagner's Test	+	+	+
2	Phenol	+	+	+
3	Tannin	+	+	+
	Saponin	+	+	+
4	Glycoside			
	Libermann's Test	+	+	+
	Salkowski's Test	+	+	+
5	Steroid	-	+	-
6	Terpenoids	-	+	+
7	Flavanoids	+	-	-
8	Carbohydrate			
	Molish's Test	+	+	+
9	Amino acids			
	Million's Test	+	+	+
	Biuret Test	+	-	+
	Proteins	+	-	+

Antimicrobial activity

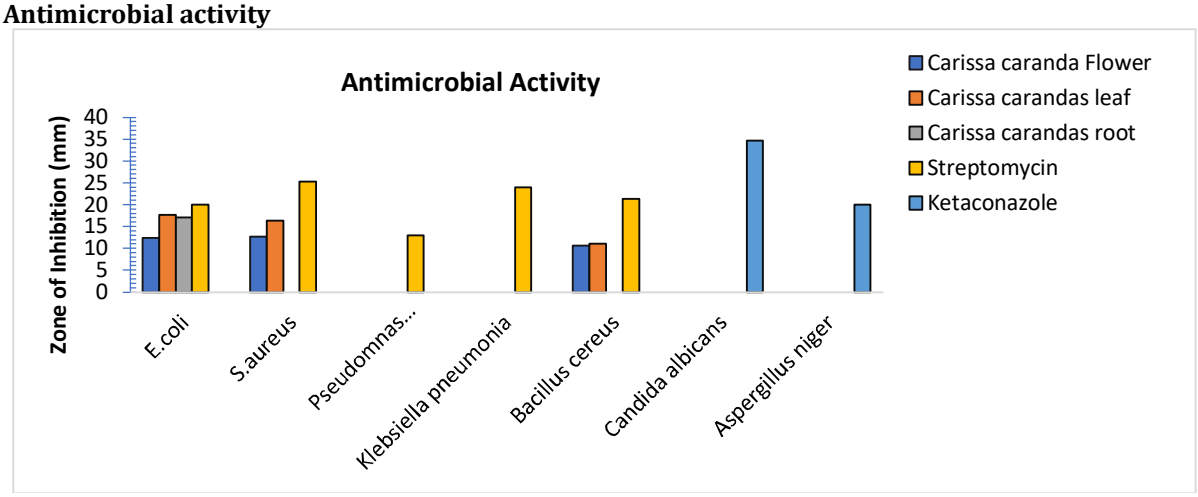

Figure 1: Antibacterial and antifungal activity of *Carissa carandas* flower, leaves and root extracts.

The extracts of the studied plants exhibited varying degrees of inhibition activity against the tested microbes and the results were expressed in terms of the diameter of the growth-inhibition zone (clear zones). According to the study, methanolic extract of *C. carandas* flower, leaf and root shows antibacterial activity. The *C. carandas* flower at concentration 100mg/ml shows significant zone of inhibition against *E.coli* (12.33±2.52) , *S. aureus* (12.6±1.52) and *B. cereus* (10.66±0.57) whereas no activity was found against *P.aerogenosa* and *K. pneumonia.* The activity of methanolic extract of *C. carandas* leaf shows Inhibition against *E.coli* (17.67±2.89) *S. aureus* (16.3±1.52) and *B. cereus* (16.3±1.52) and no zone of inhibition was observed against *P.aerogenosa*, *K. pneumonia*, *A.niger, C. albicans* respectively and is shown in Figure 1 and Table 2. Highest activity was obtained by leaf extract against *E.coli*. Furthermore root of *C. carandas* exhibited activity towards *E.coil* (17±1) and no zone of inhibition was observed against rest of culture. Positive control shows higher zone of inhibition as compared to extract, whereas no activity was reported by negative control DMSO shown in Figure 2 respectively.

Table 2: Diameter of inhibition zone (mm) of *Carissa carandas* flower, leaves and root extracts against different Bacterial and Fungal strain.

Plant parts	Gram positive and Gram negative Bacteria					Fungi	
	E.coli	***S. aureus***	***P.aerogenosa***	***K.pneumonia***	***B. cereus***	***C.albicans***	***A. niger***
Flower	12.33±2.52	12.6±1.52	0	0	10.66±0.57	0	0
Leaves	17.67±2.89	16.3±1.52	0	0	11±1	0	0
Root	17±1	0	0	0	0	0	0
Antibiotic	20±0	25±0.5	23.3±1.5	22.6±4.04	21.3±1.15	34.6±0.5	20±0

Figure 2: Antibacterial activity *Carissa carandas* leaf extract against *E. coli* and *S. aureus*

Minimum Inhibitory Concentration

The minimum inhibitory concentration of methanol extract of *Carissa carandas* flower, leaf and root is shown in Table 3. MIC *of Carissa carandas* flower had the lowest MIC value of 1.56mg/ml as compared to other extract.

Table 3: Minimum Inhibitory Concentration (mg/ml) of of *Carissa carandas* flower, leaves and root extracts against test Bacteria and Fungi

Plant parts	*Bacteria*					Fungi	
	E.coli	***S. aureus***	***P.aerogenosa***	***K.pneumonia***	***B. cereus***	***C.albicans***	***A. niger***
Flower	6.25	25	0	0	1.56	0	0
Leaves	3.125	12.5	0	0	6.25	0	0
Root	3.125	0	0	0	0	0	0
Antibiotic	2µg/ml	4µg/ml	0	0	0.0625µg/ml	4µg/ml	16µg/ml

DISCUSSION

This research focused on the antimicrobial activity of different parts of *C. carandas*. Flower, leaf and root show antimicrobial activity against selected pathogenic microorganisms. The phytochemical screening of the plants reported important phytochemicals which could be responsible for antimicrobial activity. *C. carandas* flower and leaf shows zone of inhibition against *E. coli, S. aureus* and *B. cereus* and root inhibit the growth of *E.coli*, However no activity was found against *P.aerogenosa, K. pneumonia.* These results support previous results in which methanol and methanol-water extract of *C. carandas* leaf inhibited the growth of *S. aureus, S. mutans*, and Mycobacterium smegmatis in a dose-dependent manner (9). Whereas some work are reported on antimicrobial activity of silver nanoparticles (AgNPs) of *C. carandas* leaf using an aqueous extract of methicillin-resistant *S.aureus* significant activity observed against *S.aureus, S.sonnei*, *Shigella boydii* and *Salmonella typhimurium* (10). In another study 60% ethanolic extract *C.carandas* leaves exhibit activity against *S.aureus* (11).Although 50% ethanol extract of *C.carandas* fruit exhibit activity against *S. aureus, E.coli, K.pneumoniae, P. aeruginosa* and *B. subtilis* (12).

CONCLUSION

In conclusion, the investigation of our study proved whole plant parts of *Carissa carandas* possess antimicrobial activity against pathogenic microbes. The phytochemicals compound present in plants such as flavonoids, tannins, cardiac glycosides, saponins, steroids, reducing sugars, and anthraquinones responsible for various pharmaceutical applications, and indicate their relevance in fighting against

infectious diseases. Hence It can be concluded from the study that it can be explored for its potential effect in the field of pharmaceutical applications.

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CONFLICT OF INTEREST
Authors do not have any conflict of interest.

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