

***In vitro* study of antibacterial activity of *Carissa carandas* leaf extracts**

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ABSTRACT

*In the present study we focus on the need for the alternative sources of the antibiotics as the pathogenic microbes are gaining resistance against standard antibiotics. Cold Aqueous, Methanol, Ethanol, Ethyl Acetate extracts of leaves of *Carissa carandas* in a final concentration of 500 mg/ml were evaluated for their antibacterial properties against some selected pathogenic microorganisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* using agar well diffusion method. Methanolic, Ethyl Acetate and Ethanolic extracts of *Carissa carandas* leaves show an average inhibitory zone diameter of 23.5, 22.0 and 21.5 mm respectively which indicate that the Methanolic extract has shown the best result having Zone of Inhibition greater than that of the standard antibiotic Tetracycline (17.0 mm).*

Key words: Antibacterial properties, *Carissa carandas*, Pathogens, Solvent extraction, Agar Well Diffusion.

INTRODUCTION

An antimicrobial is a substance that kills or inhibits the growth of micro-organisms such as bacteria, fungi, protozoans, etc. On the basis of mode of action, antimicrobials are classified into two broad categories as Microbicidal that kill microbes without leaving any option for their survival and Microbistatic that cease all the metabolic activities of microbes that are important for their survival so they are called as growth inhibitors of microbes [1].

The widespread use of commercially available antimicrobials led to the consequence of emergence of antimicrobial resistant pathogens that ultimately led to the threat to global public health [2]. All commercially available antibiotics with prolonged use may have negative effect on human health because they kill gut flora, so human beings need to take probiotics to replace the killed gut flora. Therefore there is a constant and urgent need to develop new antimicrobial drugs for the treatment of infectious disease from medicinal plant [3].

According to WHO (1993), 80% of the world's population is dependent on the traditional medicines and a major part of the traditional therapies involve the use of plant extracts or their bioactive components [4]. Plant based natural constituents can be derived from any part of the plant like bark, leaves, roots, fruits, seed, fruit rind, etc [5]. Plants have been studied in detail for their antimicrobial properties and some secondary metabolites have been found to be responsible for the same.

Carissa carandas is a species of flowering shrub in the dogbane family, Apocynaceae. It produces berry-sized fruits. *Carissa carandas* traditionally used as stomachic, antidiarrheal and anthelmintic; stem is used to strengthen tendons; fruits are used in skin infections and leaves are remedy for fevers, earache and syphilitic pain. Alcoholic extract of root material decrease the blood pressure and aqueous extract of root have been reported various pharmacological activities like histamine releasing, anthelmintic, sapsmolytic and cardiogenic. Fruits have also been studied for its analgesic, anti inflammatory and lipase activity [6].

MATERIALS AND METHODS

Sample Collection

Fresh and healthy leaves of *Carissa carandas* collected RDSO, Alambagh area after proper identification. The leaves were washed with tap water followed by distilled water and then dried. Dried sample was ground into fine powder by the help of a grinder.

Bacterial strains and culture preparations

Three pathogenic strains namely *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*, available at Amity University, Lucknow were subcultured and used throughout the study.

Preparation of Plant Extract

Antimicrobial metabolites from the dried leaves were extracted in various solvents such as Cold Aqueous, Methanol (80%), Ethanol (70%), and Ethyl Acetate (80%). 2 gm of dried powdered sample was soaked in 20 ml of the respective solvents (1:10) and kept in dark for 3-4 days so that secondary metabolites diffuse out into the solvents. It was then filtered in weighed petri plate and dried in hot air oven at 50°C, so that solvents get evaporated. The dried metabolite extract was dissolved in double volume of DMSO (Dimethyl Sulfoxide) thus giving the final concentration of extract to 500 mg/ml.

Antibacterial Susceptibility Assay

Antibacterial susceptibility assay was carried out by well diffusion method [7] wherein sterile Nutrient agar plates were prepared and spreaded with 50 µl of the available bacterial cultures against which antibacterial activity was tested. Thereafter 3 wells of 8 mm diameter were dug with the help of sterile borer. Two plates were prepared for each microbial strain.

In the plate 1; the 1st, 2nd and 3rd well was filled with 60 µl of standard antibiotic Tetracycline, Methanolic and Ethanolic extract respectively.

In the plate 2; the 1st, 2nd and 3rd well was filled with 60 µl of standard antibiotic Tetracycline, Cold Aqueous and Ethyl acetate extract respectively.

Plates were incubated at 37°C for 24 hours. The antibacterial activity of each extract was expressed in terms of mean of diameter of Zone Of Inhibition (in mm) produced by each extract at the end of incubation period.

RESULTS

Antibiogram analysis:

In order to check the antimicrobial activity of extracted plant samples, agar well diffusion method was used. With the help of this test we can determine whatever the extract being tested has antibacterial property or not.

Table 1 below shows the results of Zone of Inhibitions (ZOI) observed for the antimicrobial properties of *Carissa carandas* leaf extracts and the standard antibiotic tetracycline used throughout the study.

Table 1: Antibacterial Susceptibility Assay of *Carissa carandas* Leaves Extracts.

S.NO.	EXTRACTS	ZONE OF INHIBITION (ZOI) AGAINST (in mm)					
		<i>Escherichia coli</i>		<i>Pseudomonas aeruginosa</i>		<i>Staphylococcus aureus</i>	
		By Extract	By Tetracycline	By Extract	By Tetracycline	By Extract	By Tetracycline
1	Ethanol	21.0	16.5	17.0	14.0	21.5	17.0
2	Methanol	19.5	16.5	15.0	14.0	23.5	17.0
3	Ethyl Acetate	22.0	15.0	16.0	13.5	20.0	14.0
4	Cold Aqueous	20.5	15.0	18.5	13.5	16.0	14.0

Note: Well diameter = 8 mm.

Figure 1-2 below show the photographs of the antibiogram of *Carissa carandas* leaves extracts in various solvents performed against available pathogens.

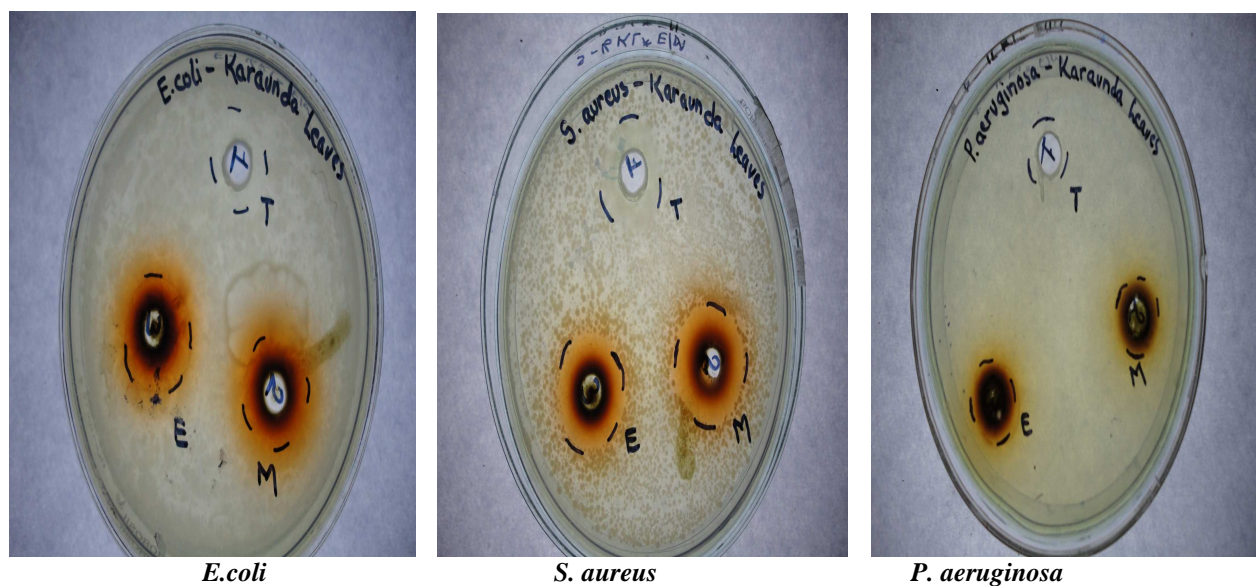


Figure 1: Ethanolic (E) and Methanolic (M) Extracts of *Carissa carandas* leaves.

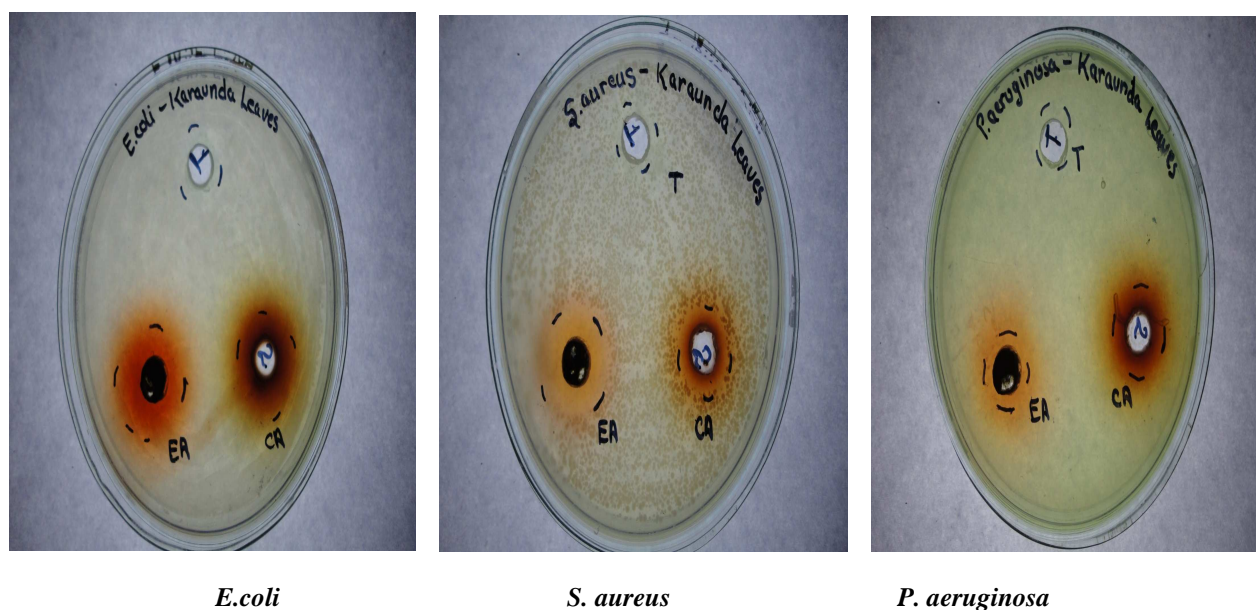
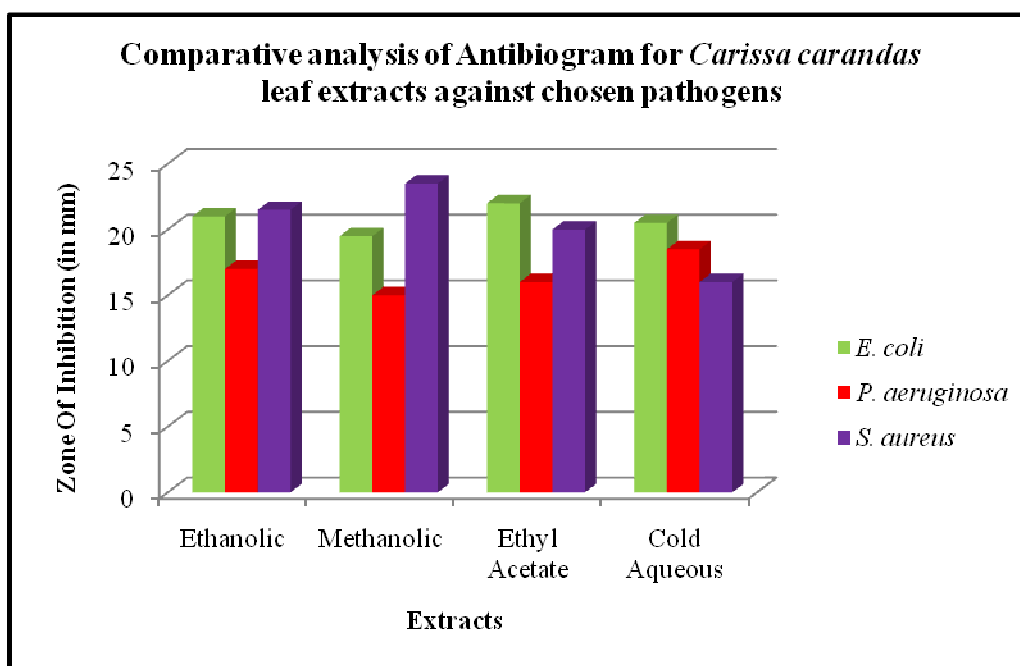


Figure 2: Ethyl Acetate (EA) and Cold Aqueous (CA) Extracts of *Carissa carandas* leaves.

DISCUSSION

Researchers have extensively studied the biological properties of *Carissa carandas* and their results showed that this plant is ethno-medically valuable. Some antibiotics have become almost obsolete because of the problem of drug resistance [8]. *Carissa carandas* leaf extracts were found to be active against used pathogens and this could be the drugs without side effects for the future.

Agar well diffusion method was used here in order to determine the antimicrobial properties of the plant extracts against the pathogens.

Figure 3: Comparative analysis of *Carissa carandas* leaf extracts against chosen pathogens.

Methanolic extract showed the maximum Zone of Inhibition (23.5 mm) against *Staphylococcus aureus*, followed by zone of Ethyl Acetate extract of 22.0 mm against *Escherichia coli*. Ethanolic extracts also showed the zone of 21.5 mm and 21.0 mm respectively, against *Staphylococcus aureus* and *Escherichia coli* respectively. The *Pseudomonas aeruginosa* showed less sensitivity against *Carissa carandas* leaf extracts with maximum Zone of Inhibition of only 18.5 mm in case of Cold Aqueous extracts.

The antibacterial activity of leaves of *Carissa carandas* may be indicative of presence of metabolic toxins or broad spectrum antimicrobial compounds that act against gram +ve as well as gram –ve bacteria.

Methanolic extracts exhibited higher degree of antibacterial activity as compared to that of other extracts tested against bacteria that cause gut infection, stomachache, and diarrhoea.

CONCLUSION

Based on the above research work it can be concluded that the leaves of *Carissa carandas* can be a very good source for herbal drugs and specially the solvents like Methanol, Ethanol, Ethyl Acetate and Cold aqueous can be explored further for the extraction of antimicrobial compounds by more sophisticated procedures for extraction in order to increase the yield.

The future prospects of present research work includes isolation and purification of the therapeutic antimicrobial agents from the active extract and there further pharmacological evaluation by several method such as – NMR, MS, GC-MS, TLC, HPLC.

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