

Phytochemical and antimicrobial study of *Carissa carandas* L

Safeekun Nisha¹, Awadh Raj Singh²

¹ Research Scholar (Botany), S.G.S. Govt. P.G. College, Sidhi, Madhya Pradesh, India

² Principal, S.G.S. Govt. P.G. College, Sidhi, Madhya Pradesh, India

Abstract

During present investigation *Carissa carandas* has phytochemically analysed. The plant metabolites like and found in forests of Sidhi district of (M.P.). The fresh leaf and fruit of *Carissa carandas* were extracted using non polar solvent i.e. chloroform, followed by ethanol, dichloromethane, acetone, toluene, ethyl acetate. The extract was then tested for his or her antimicrobial activities by agar well diffusion technique. The methylene chloride and resolvent extract of leave showed higher results against the *Styphylococcus aureus sp.* and enterobacteria respiratory disease than the opposite solvent. The fruit extract of *C. carandas* in methylene chloride were showed the utmost bactericide activity against the E. coli among the opposite take a look at small organisms. A fruit extract in ester showed the higher result against the all strains. it's terminal that *C. carandas* fruit extract have antimicrobial activity, thus we will use it as seasoning medication.

Keywords: *Carissa carandas*, phytochemicals, antimicrobial

Introduction

Karonda (*C. carandas*) is a hardy, evergreen, spiny and indigenous shrub. Widely grown in India. Its fruits have sour and astringent taste, bearing the richest source of iron containing & good content of vitamin C. *Carissa carandas* is Very useful to cure anaemia, its fruits have anti-scorbutic properties also. (Bhaskar & Balakrishnan, 2009) ^[1]. This species is a rank-growing, straggly, woody, shrub climbing, usually growing on height of 10 or 15 ft (3-5 m) from the surface, sometimes ascending to the tops of tall trees; and rich in white, gummy latex. The branches are numerous and spreading, forming dense masses; are set with sharp thorns (Shariff *et al.*, 2001) ^[2].

C. carandas L. fruit is sour, acrid, astringent, appetizer, antipyretic, useful in disease of brain anorexia, indigestion, colic, hepatomegaly, splenomegaly, piles, cardiac diseases, oedema, amenorrhoea, fever and act as nervine in nervous disorder and having antiscorbutic properties. The leaves have antipyretic activity (Taylor *et al.*, 1995) ^[3]. The three pigment are present in fruit of *C. carandas* i.e. pelargonidin-3-o-glucoside. Cyanidin-3-o-rhamnoside and cyaniding-3-o-glucoside. The highest percentages of pelargonidin-3-oglucoside were presented in ripening stage. The content of cyaniding-3-oglucoside and cyanidin-3-o-rhamnoside were quite constant during early stage of development. But percentage reduced in over ripping fruit. (Shariff *et al.*, 2001)^[2]. An ethanolic root extract of *C. carandas* may produce its anticonvulsant effects via non-specific mechanisms (Karunakar Hegde *et al.*, 2009) ^[4].

Material and Methods

Plant material

The plant of *C. carandas* leaves and fruits were collected from various forest Sidhi district (M.P.) in the month of Oct. and Nov. The Plant was identified and the respective voucher specimens have been deposited in the Department of Botany, S.G.S. Govt. P.G. College, Sidhi (M.P.).

Preparation of extract of plant

The leaves and fruit of *C. carandas* were collected and washed with Lukewarm Water in bucket and dried. Then crushed, made its powder form with the help grinder. Powder were dissolved in different solvent and kept in shaker for 72 hrs at 24-28°C. The Extract was filtered out twice using cheese cloth, an extract were collected in Petridish and kept in incubator for drying the solvent. A Dried extract collected in falcon tubes and kept in freeze for further investigation.

Microorganism

The bacterial species used in this study were *Esherichia coli*, *S. aureus*, *S. epidermis*, *K. pneumonia*, *Enterococcus faecalis*. Above bacterial strain were cultured overnight at 37°C in nutrient broth media and used as inoculums using cell suspension with density of 105 CFU/ml. The bacterial species were cultured in nutrient broth for 24 hours and fresh inoculums were taken for the test. An Antimicrobial as-say performed with microbial cultures which were freshly grown at 37°C appo-priately diluted.

Antimicrobial activity

The modified agar diffusion method (Murray *et al.*, 1995 & Olurinola, 1996) ^[5-6] was used to evaluate the antimicrobial activity. The solid media of Muller Hilton agar was prepared by dissolving 38 gm in 1 litter distilled water. About 25 ml of Muller Hilton agar poured into Petridish. The inoculum for bacteria was prepared by culturing a large number of bacteria in a tube containing 10 ml of nutrient broth and incubating over night at 37°C. The inoculum of bacteria was transferred into Petridish containing solid Muller Hilton agar. Each agar plate was inoculated by bacterial strain. Circular well was prepared by the help of sterile micro tips, 50ul of plant extract added in agar well by using micropipette. Then covered the plate by the parafilm wax and kept the plate in incubator at 37°C for 24 hrs.

Antibacterial activity was evaluated by measuring the diameter of the inhibition zones and the mean of the diameters of the inhibition zones was calculated.

Phytochemical Screening: Freshly prepared water, chloroform, ethyl acetate extract of leave and fruit of *C. carandas* were subjected to preliminary photochemical screening for detection of the following major constituents:

Alkaloids: Presence of alkaloids was reported by subjecting 0.5g aqueous extract in 5ml 1% HCl, boiled, filtered and Mayer's reagent was added. Yellow precipitation was shown presence of alkaloids.

Reducing sugar: 5gm. Extract 1ml of distilled water and 5-8 drops Fehling's solution was heated and observed for brick red precipitate.

Tannins : Extract was mixed with distilled water and heated on water bath. Then filtered and ferric chloride was added to the filtrate. A dark green colour indicates presence of tannins.

Flavonoids: Plant extract added in distilled water and diluted with NaOH and HCl. A yellow solution that turns colourless indicates the presence of flavonoids.

Phlobatanins: Plant extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCL solution. Red precipitate shows the presence of Phlobatanins.

Terpenoids: Plant extract added with 2ml of chloroform and conc. H₂SO₄ gently added to form a layer. A reddish brown coloration of the interface was formed, which showed presence of terpenoids.

Cardiac Glycoside: Plant extract treated with 2ml of glacial acid containing a drop of FeCl₃ solution. A brown colour ring indicates the presence of Cardiac Glycoside.

Result and discussion

In the phytochemical study we tend to used the leaves and a fruit extracts in numerous solvents and located the various metabolic compounds shown in table 1. In Phytochemical test we found that extract of leave and fruit of *C. carandas*, different chemical constituent alkaloid, glycosides, saponin, terpenoids, flavanoids, tennins and steroids are present, when we react with water, chloroform, ethyl acetate. Phytochemical investigations distributed on the plant, to elucidate the multifarious role of this medicative plant.

Table 1: Phytochemical screening of leave and fruits extract of *C. carandas*.

Phytochemical	Plant Part					
	Leaves			Fruits		
	Water Reaction	Chloroform Reaction	Ethyl acetate Reaction	Water Reaction	Chloroform Reaction	Ethyl acetate Reaction
Alkaloid	+	+	+	+	-	+
Glycosides	+	-	-	+	+	+
Reducing sugar	-	+	+	-	-	-
Tannins	+	+	-	-	-	-
Saponin	+	-	+	+	-	+
Flavanoids	-	+	+	-	-	+
Terpenoid	+	+	+	+	+	+
Cordial glycosides	+	-	-	+	+	-
Steroids	+	-	+	+	+	+
Photo tannins	-	-	-	-	-	-
Anthraquinone	+	-	-	-	-	-

+ = Positive ; - = Negative

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ethyl acetate.

The antimicrobial activity of *C. carandas* against the different bacterial strains is shown as below in table 2.

Table 2: Screening of antimicrobial activity of leave and fruit extract of *Carissa carandas* with different solvents

Part of plants	Strain	Zone of Inhibition (Diameter)					
		Acetone	Ethanol	Chloroform	Ethyl acetate	Dichloro-methane	Toluene
Leaves	<i>E. coli</i>	9mm	5mm	-	-	-	-
	<i>S. aureus</i>	-	6mm	-	-	11mm	-
	<i>K. pneumonia</i>	-	9mm	-	-	10mm	12mm
	<i>S. epidermis</i>	6mm	-	-	-	-	-
	<i>E. facealis</i>	8mm	-	10mm	8mm	8mm	-
Fruits	<i>E. coli</i>	12mm	-	6mm	8mm	20mm	6mm
	<i>S. aureus</i>	-	6mm	-	14mm	-	-
	<i>K. pneumonia</i>	-	-	8mm	10mm	4mm	6mm
	<i>S. epidermis</i>	-	-	-	12mm	-	-
	<i>E. facealis</i>	6mm	-	-	10mm	-	-

Leave extract of toluene shown the maximum activity against *K. Pneumonia* with zone of inhibition around 12mm. Leave extract of *C. carandas* in acetone, ethanol and dichloromethane was shown the average antimicrobial

activity against the different bacterial strain. Leaves extract of *C. carandas* in ethanol and in chloroform also shown the antimicrobial activity against the *S. aureus* and *E. facealis*. The result of this study showed that leave and fruit extracts

of *C. carandas* have varied antimicrobial activity against tested (*E. coli*, *S. epidermis*, *Enterococcus sp.*, *S. Aureus* and *K. pneumonia*). In comparative study in between leaf and fruit of *C. carandas*. We found that fruit extract of plant showed more and highest microbial activity against the bacteria used in study. Out of all the extract from *C. carandas* fruit, the dichloromethane extract was most active around 20mm zone of inhibition against the *E. coli*. Fruit extract of *C. carandas* in chloroform also show the maximum activity against the *K. pneumonia*. Ethyl acetate extract of fruit showed antibacterial activities against the all bacterial strains used in this study. This result shows that the plant might have important compounds that can be used for the treatment of bacterial infection.

One hundred and fifty volatile components of karonda were first identified. This fruit flavor showed some metabolites as follows: alcohols comprised the largest class of volatiles (29.8%), terpenoids (24.0%); esters (23.1%); fatty acids (11.9%); carbonyls (2.2%); furanoids (1.7%) and others (4.2%). Major constituents found in karonda flavor were isoamyl alcohol (6.65 ppm), isobutanol (6.06 ppm) and [beta]-caryophyllene (5.94 ppm). Some compounds present, e.g. furfural, could probably be degradation products of ascorbic acid and sugars. It has been reported that acid treatment and even distillation induce the oxidation of ascorbic acid (Sturock,1959) [7].

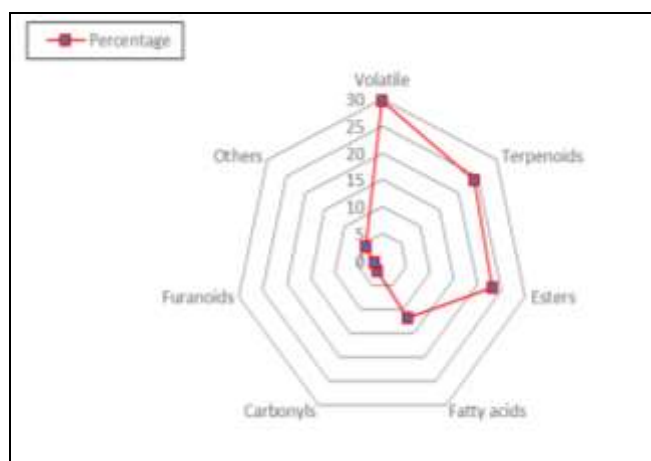


Fig 1: graphics analysis alcohols comprised of *C. carandas*

C. carandas Linn have potent cardioprotective mechanism. *C. carandas* involved cardiac remodeling, improvement in renal performance, antioxidant properties and improved serum nitric oxide synthesis. *C. carandas* methanolic crude extract has potent cardiac remodelling activity and possibly useful in treating and halting progression to heart failure (Rabbani *et al.*, 2010) [8].

Conclusion

A critical analysis of literatures on *C. carandas* have shown the interesting fact that crude latex and its various organic and dichloromethane extract posses an array of multidimensional pharmacological activities via, indigestion, colic, hepatomegaly, splenomegaly, piles, oedema, amenorrhoea, fever and nervine disorder and antiscorbutic properties, The leaves have antipyretic activity. The roots showed bitter, rheumatism, disease of brain, stomach disorder, intestinal worms, diabetic, ulcer and pruiti, cardiogenic properties. Other parts of plant roots, bark, leaves, fruit also showed biological activity such as

hepatoprotective, antianthelmintic, astringent, antiscorbutic wound healing.

In general, most of the acetone, chloroform and toluene extract of the plant also showed antibacterial activities against different bacteria. This indicates the potential of the plants to be used as antibacterial agents against wound causing pathogens. However, further studies should be conducted with different extraction solvents and toxicity and Phytochemical analysis should also be performed on these plants to use them as sources and templates for the synthesis of drugs to control wound and other disease-causing bacteria.

Antibacterial activity investigation has been found to offer important clues in the identification and development of traditionally used herbal plants into modern drugs. Toluene extract of *C. carandas* leave shown the maximum inhibition against *K. pneumonia*. Acetone, ethanol and dichloromethane were also shown the inhibition against the different bacteria used in this study. Chloroform and ethyl acetate extract of leave also shown antibacterial activity against *E. faecalis*.

This work is focused on the antimicrobial activity of extracts from leaves and fruits of *Carissa carandas* and has found to be significant against *E. coli*, *S. epidermis*, *Enterococcus sp.*, *S. aureus sp.*, *K. pneumonia*. Future investigation is required to isolate the active ingredients which may act as less costly medicines.

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