International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 11-01-2022, Accepted: 26-01-2022, Published: 11-02-2022
Volume 7, Issue 2, 2022, Page No. 284-291

Studies on the influence of phytochemical and antioxident activity of Kalakai (*Carissa carandas* (L.) an effective medicinal plant

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Abstract
Medical plants are those that have active compounds that have been proven to treat sickness and relieve pain. Traditional medicines and medicinal plants are used as restorative agents in human activities, primarily in underdeveloped nations, for the preservation of health and the treatment of various maladies. Today's pharmacognacy, on the other hand, has at least 25% of medications originated from plants, as well as many synthetic counterparts based on prototype chemical compounds obtained from plants. With the rising costs of prescription pharmaceuticals in the preserving of customized health and wellbeing, as well as drug bio prospecting, interest in medicinal plants as a potential health aid has grown. *Carissa carandas* is a good medicinal plant with phytochemical content and antioxidant potential that can help treat and benefit various ailments in humans. The Carissa is a qualitative phytochemical, although quantitative phytochemicals performed well i n these tests as well.

Keywords: *Carissa carandas*, phytochemical, antioxidant etc

Introduction
Medicinal plants were employed to prevent numerous severe ailments in ancient India. Herbal medications can be found in abundance in the plant kingdom. Even in recent years, there has been a growing recognition of medicinal plants' usefulness. Herbal medicines are generally easy to find, safe, less priced, effective, and have few adverse effects. Medicinal plants, according to the World Health Organization, would be the best source of a range of pharmaceuticals. (Yada v and Agrawal.2011) [21].
The herb is used to purge, heal rheumatism and snake bites, and clean worm infested animal wounds in traditional medicine (Fatima, et al, 2013). Medicinal plants with active chemical components with high antioxidant properties have an important role in the prevention of a range of degenerative illnesses (Lukmanul *et al*., 2008) [12] and may have social benefits. In vitro, a large number of phytochemicals from various chemical classes have been shown to have inhibitory effects on various microorganisms. Botanical medicines, also known as phytomedicines, are medicinal plants whose seeds, berries, leaves, bark, roots, or flowers are used by a large number r of people. Knowledge of the chemical elements of plants is desirable since it will be useful in the Production of complicated chemical compounds (Mojab *et al*., 2003) [13]. As a result, Carissa carandas has a long history of use. Bitter, stomachic, anti-diarrheal, and antianthelmintic qualities are attributed to the root. Curries, tarts, and puddings, all use the ripe fruits. They are converted into jelly when they are only slightly under ripe. Pickles are produced from green, sour fruits in India. They've been used as a substitute fo r apples in tarts by removing the peel and seeds and seasoning them with sugar and cloves.
The unripe fruit is used as an astringent in medicine. The carissa fruit juice is fresh and delicious. Carissa congesta roots contain a number of volatile compounds, including 2-acetyl phenol. Triterpenoid components were detected in the leaves, as well as tannins, and a novel isomer of ur osolic acid, carissin acid, was discovered. (Siddiqui and colleagues, 2003). 2, phenyl ethanol, Lina loll, caryophylline, isoamyl alcohol, benzyl acetate, and carissol, a new triterpene alcohol, have all been discovered in the fruits of this plant. The plant's polar glycosides were mildly hydrolyze d by enzymes, yielding. A huge number of individuals are exposed to infectious pathogens in places with poor sanitation and a lack of knowledge about proper sanitary practices. Furthermore, many poor nations have tropical climates that encourage the survival and proliferation of diseases, causing organism s and vectors, creating a severe public health danger. Because plants contain medicinal components, they have been employed as a cure for human ailments since antiquity.

Materials and Methods
The purpose of this study was to determine the phytochemical and antioxidant properties of Carissa carandas (L.).

1. Quantitative phytochemical content analysis

2. Antioxidant activity of Carissa carandas was assessed utilizing a variety of techniques.
3. Quantitative phytochemical study of Carissa carandas fruit extract using methanol.

1. Plant species selection

Carissa carandas plant materials (fruits) were taken from Kadamangudi village in Ngapatti nam and Nagapattinum Districts in Tamil Nadu. The plant pieces were then shade dried and roughly pulverized separately before being kept in tightly sealed vials for laboratory examination. Plant Materials Authentication, Rabinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamil Nadu, and the Botanical Survey of India (BSI), Southern Circle, Coimbatore, India, have certified the species. The item was tagged, numbered, and noted with the collecting date as well as the location.

2. Extraction of Fruit materials

The fresh fruit was cleaned with running water and dried in shade. The Sox let equipment was used to extract the coarse particles (25g) in 250ml of methanol solvent. The extra acted data was saved and used for future investigation. For these extracts, DMSO (dimethyl sulfoxide) is used as a dissolved solvent.

3. Qualitative phytochemical examination

Preliminary phytochemical analysis of the extract was performed using Evans' standard procedures (1996).

Alkaloids

Were detected by dissolving extracts in dilute hydrochloric acid and filtering the m separately. The presence of alkaloids was determined using the filtrates.

Mayer's test.

The filtrates were subjected to Mayer's reagent treatment. The presence of alkaloids is indicated by the production of a yellow-cream precipitate. Wagner's test: Filtrates were subjected to Wagner's reagent treatment. The presence of alkaloids is indicated by the production of a brown or reddish brown precipitate.

4. Flavonoid detection

1. Lead acetate test.

A few drops of lead acetate solution were added to the extracts. The discorded of lead acetate is formed. The presence of flavonoids is indicated by a yellow-colored precipitate.

2. H_2SO_4 Test

The extracts were subjected to few drops of H_2So_4.The presence of flavonoids indicate Congo red color.

3. H_2SO4 test

4. Steroid detection.

To five milliliters of extract, two milliliters of acetic anhydride were added, each with two milliliters of H2SO4.In certain samples, the color shifted from violet to blue or green, suggest ing the presence of steroids.

5. Terpenoid detection

I Salkowski's test: five mg of leaf, flower, and seed extract was combined with two ml of chloroform, then concentrated H2SO4 (3 ml) was added slowly to produce a layer. The presence of terpenoids is indicated by the emergence of a reddish brown color in the inner face.

6. Anthroquinone detection

1. Born Trager's Test.

In a water bath, around 5 mg of the extract was cooked with 10% HCl for a few minutes. To the filtrate, an equal amount of CHCl3 was added. The liquid was heated after a few drops of 10% NH3 were added. The presence of anthroquinones is indicated by the production of a pink cooler.

2. Phenol detection

3. Ferric chloride test.

Few drops of ferric chloride solution were added to 10mg extracts. It was discovered that ferric chloride was formed. The presence of phenol is indicated by a blue black color.

4. **Lead acetate test.**

A few drops of lead acetate solution were added to 10mg of extracts. It was discovered that lead acetate is formed.

5. Saponin detection.

0.5 mg of the extract was mixed with five ml of distilled water and shaken. The presence of saponins is indicated by the formation of foaming (which looks as a creamy mist of little bubbles).

6. Tannin detection.
A tiny amount of extract was combined with water and boiled in a water bath to detect tannins. The mixture was filtered, and the filtrate was treated with ferric chloride.
The result was a dark green color. It suggests that tannins are present.

7. Carbohydrate detection.
0.5 mg of extracts were diluted in five ml of distilled water and filtered separately. The presence of carbohydrates was determined using the filtrate.

5. Detection of proteins and amino acids
In a biuret test, 0.5 mg of extract was mixed with an equivalent volume of 40 percent Na OH solution and two drops of 1 percent copper sulphate solution. The presence of protein is indicate by the formation of violet-colored dots. The development of pink or purple hue indicates the presence of proteins, peptides, or amino acid ds.

6. Detection of oils and resins.
Filter paper was treated with a test solution. On the filter paper, it takes on a clear look.
It suggests that oils and resins are present.

7. Antioxidant activity
(i) The Ferric, Reducing/Antioxidant Power (FRAP) test developed by Pulido *et al*. in 2000. The reaction mixture's absorbance was measured at 593 nm. The concentrations are given in mm. Fe (II)/g extract. The ABTS + test was used by Re *et al*. (1999) to assess antioxidant activity. The extracts' radical scavenging activity was measured spectrophotometrically. (2, 20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] 2-ethylbenzothiazoline-6-sulfonic acid (2, 20-azino- bis (3-ethylbenzothiazoline-6, sulfonic acid). The absorbance was measured at 734 nm in the ABTS+ cation decolorization experiment (Re *et al*., 1999). The concentration of Trolox with comparable antioxidant activity represented a s l mol/g is the unit of total antioxidant activity. Metal chelating activity was established by Dinis *et al*. (1994) [6]. At 562 nm, the solution's absorbance was measured spectrophotometrically. The results were represented in milligrammes of ethylene diamine tetraacetic acid (EDTA) equivalents per gram of extract.

1. Phosphomolybdenum test by Prieto *et al*. (1999) [15].
The antioxidant activity of extracts was previously's established technique of green phos phomolybdenum complex production (1999). The mixture's absorbance was measured at 695 nm.
The data are presented as averages in grams of ascorbic acid.
AAE is the number of equivalents per 100 g of extract.On DPPH, Blios (1958) [4] showed free radical al scavenging action. The action of hydroxyl radical scavenging was established by Klein *et al*. (1991). Spectroscopically, the intensity of the color generated was measured at 412 nm against a reagent blank. The sample extracts' hydroxyl radical scavenging activity was calculated as a perc ntage of their antioxidant activity.

1. B-Carotene/linoleic acid antioxidant activity (Taga *et al*., 1984) [20].
According to the previously published approach, the antioxidant activity of the antioxidant t (leaf extracts, or tocopherol (a T), 100 lL) solution was assessed in terms of carotene-b/linoleic acid (Taga *et al*., 1984) [20]. at a wavelength of 470 nm The bark's antioxidant properties is also noteworthy. Klein *et al*. established the action of hydroxyl radical scavenging (1991).The intensity of the color created was measured spectroscopically at 412 nm against a reagent blank. The hydroxyl radical scavenging activity of the sample extracts was measured as a percentage of their antio oxidant activity.

2. Antioxidant action of B-carotene and linoleic acid (Taga *et al*., 1984) [20].
The antioxidant activity of the antioxidant (leaf extracts, or tocopherol (a T), 100 L) solution was measured in terms of b, carotene/linoleic acid using a previously described method (Taga *et al*., 1984) [20]. at a 470 nm wavel length Antioxidant properties of the bark

Phenol Total Estimation
Five milliliters of the extract were pipetted into a 50 milliliter flask, followed by ten milliliters of distilled water. In addition, two milliliters of NH4OH solution and five milliliters of strong amyl alcohol were added. The samples were manufactured to the required specification and then left to react for 30 minutes to allow for color development. This was measured at a wavelength of 505 nm.

Estimation of carbohydrate by Krishnaveni *et al*., 1984 [9].
100 mg of material was hydrolyzed in a boiling tube with 5 ml of 2.5 N HCl for 3 hours i n a boiling water bath. It was kept at room temperature.

Results and Discussion

The goal of this study was to determine the phytochemical and antioxidant capabilities of Carissa carandas (L.), a commonly used medicinal plant in India.

Qualitative phytochemical analysis

The presence and absence of alkaloids, glycosides, flavonoids, steroids, terpenoids, anthroquinone, phenols, saponins, tannins, carbohydrates, protein, amino acids, oil, and resin in Carissa a carandas fruits were investigated. (Table.1)

Table 1: Qualitative Phytochemical Analysis of *Carissa carandas* fruit extracted with methanol solvent

S. No	Phytochemical test		Inference
1.	**Alkaloids**	Mayer ‘s test	+
		Wagner ‘s test	+
2.	Glycosides		+
3.	Flavonoids	Lead acetate test	++
		H2SO4 test	
4.	Steroids: Liebermann-Burchard test		-
5.	Terpenoids: Salkowski test		+
6.	Arthroquinone:Borntrager’ s test		-
7.	Phenols	Ferric chloride test	-
		Lead acetate test	
8.	Saponin		-
9.	Tannin		-
10.	Carbohydrates		+
11.	Proteins and Amino acids	Biuret test	+
		Ninhydrin test	+
12	Oils and Resins	-	

(+-positice, - negative)

Carissa Carandas is high in antioxidant potentials.

On a fresh weight basis, the antioxidant capabilities of Carissa carandas fruits were measured in milligrammes per gramme. FRAP if plasma is 734.4., ABTS 8569.7, MCPs is 25.4, DPPH is 131 and linoleic acid is 110 respectively. (Table 2).

Table 2: Effect of antioxidant property of *Carissa carandas* fruit by various method

S.no	Antioxidant properties (%)	Fruit (mg/g)
1.	FRAP	734.4± 19.40
2.	ABTS	8569.7±994.50
3.	Metal chelating property(MCP)	0.9 ±0.10
4.	Phosphomolybdenum	25.4± 0.80
5.	Hydroxyl radical scavenging activity	189.5±10.40
6.	DPPH	131.0±4.80
7.	b-carotene/linoleic acid	110±4.70

±Standard deviation

Quantitative analysis

Carissa carandas fruit extract phytochemical analysis in methanol solvent.

Alkaloids (02.150.06), saponins (5.80.174), flavonoids (6.41.3), phenols (11.540.7), terpenoids (7.80.234), glycosides (6.80.204), and carbohydrates (05.890.17) mg/g are found in Carissa carandas fruits.

Table 3: Quantitative phytochemical analysis of *Carissa carandas* fruits extracted with Methanol solvent

S. No	Phytochemicals test	Methanol extract (fruit)
1	Alkaloids	02.15±0.06
2.	Saponins	5.8±0.174
3.	Flavonoids	10.08±0.30
4.	Phenols	07.49±0.22

5.	Terpenoids	7.8±0.234
6.	Glycosides	6.8±0.204
7.	Carbohydrates	05.89±0.17

± Standard deviation

Individuals and communities benefit greatly from the use of medicinal plants (Pascaline *et al*., 2011). Carissa carandas is a flowering shrub of the Apocynaceae (dogbane) family. Because the fruit is high in iron, it is occasionally used to cure anaemia. Because it includes a significant quantity of vitamin C, it is an antiscorbutic. Carissa carandas (Apocynaceae) is an evergreen shrub b or small crooked tree with dichotomous branches armed with simple or forked, 2,4 cm long, pa ired axillary thorns that can grow up to 3 meters tall. The bark peels in square flakes and is yellowish brown in color. Petiole is short, light green, leathery, globous, and globous. Herbal medicines are now commonly utilized as cures for a variety of diseases.

Carissa carandas products are widely available on the market and are commonly used by natural health practitioners to treat rheumatism. Carissa congesta is a cardio tonic, hepatoprotective, free radical scavenger, xanthine oxidase inhibitor, histamine releasing agent, antirhumatic, stomachic, antidiarrheal, vermifuge, anthelmintic, astringent, antiscorbutic, antibacterial, antiviral, and anticonvulsant plant used in traditional medicine. It also has the potential to be employed as a powerful energy source. The results of this study show that C. carandus extracts have antibacterial activity against the two pathogens examined, S. aureus, a gram, positive bacteria, and E. col i, a gram-negative bacteria.

When compared to S. aureus, the plant extracts had lesser antibacterial efficacy against E. coli. The structural differences between S. aureus and E. coli may explain why S. aureus is more sensitive to antibacterial drugs (Nikaido Vaara, 1985). Gram, negative bacteria have an exterior phospholipid membrane with structural polysaccharide components that prevent antimicrobial chemicals from penetrating their cell walls, but Gram, positive bacteria have merely an outer peptido glycan that isn't a good permeability barrier.

Fig 1: KALAKAI (*Carissa carandas* L.) plant

However, another study on the antibacterial activity of methanol root extracts from Carica papaya found that gram, negative bacteria were more susceptible to antibacterial agents than gr am, positive bacteria, and suggested that the discrepancy be attributed to the nature of the medium used, which affected the agent's diffuse ability (Doughari *et al*., 2007). C. spinarum L. root me thanolic extract's zone of inhibition (13.331.53 mm and

11.660.47 mm against S. aureus and E. coli, respectively) is a solid indication of the maximum effectiveness against these bacteria.

The presence of phytochemicals examined was related to the different degrees of antibacterial activity identified in the current investigation.

The antibacterial effects of medicinal plants h have been connected to the existence of bioactive chemicals in medicinal plants. Tannins like tannic acid and propylgallate stop germs from growing in food (Sharma *et al*., 2011) [18]. Microbial enzymes, cell envelopes, transport proteins, and polysaccharides can all be inactivated by tannins (Akiyama *et al*., 2001) [2]. Antibacterial activity of flavonoids extracted from the leaves of Osmum sanctum has been demonstrated against S. aureus, Staphylococcus cohni, E. coli, Proteus, and Klebsiella pneumoniae (Ali, Dixit, 2012) [3]. Flavonoids' hydroxyl group is thought to work as a n antioxidant and chelator, enhancing antibacterial activity (Heim, 2002). Saponin, a natural detergent, may produce a stable foam in water (Abid *et al*., 2012) [1].

Fig 2: Kalakai (*Carissa carandas* L.) fruit

Plant extracts with strong antimicrobial activity have a low MIC value, whereas extracts with limited antimicrobial activity have a high MIC value. When tested against S. aureus, the current study found that C. spinarum L. leaf petroleum ether extract and C. spinarum L. root methanol extract have stronger activity than other extracts. Furthermore, when tested against E. coli, C. spinarum L. root ethanoic and methanol extracts showed significant activity. Low MIC values in E. coli and S. aureus bacteria suggest that bioactive chemicals in the roots of C. spinarum L. exhibit a broad spectrum of antibacterial action. At lower concentrations, ampicillin and ciprofloxacin, which were employed as positive controls, generated wider zones of inhibition against the pathogens t tested. Plant samples were subjected to phytochemical analysis, which confirmed the existence of elements with medicinal and physiological properties (Sofowora, 1993). Phytochemicals such as proteins, carbohydrate, phenols, tannins, flavonoids, saponins, glycoside s, steroids, terpenoids, and alkaloids were found in the plant extracts.

Several studies have examined the antioxidant effects of various portions of medicinal plants high in phenolic compounds. Vitamins, terpenoids, phenolic acids, lignin's, stilbenes, tannins, flavonoids, Quinone's, coumarins, alkaloids, amines, betalains, and other metabolites are among the many phytochemical compounds found in plants that have antioxidant action. Many of these antioxidant compounds have been demonstrated to have anti-inflammatory, anti- atherosclerotic, anticancer, antimutagenic, anticarcinogenic, antibacterial, and antiviral properties in studies.

The purpose of this study was to determine the phytochemical and antioxidant properties of Caris sa carandas (L.).

Biological activity of Carissa Carandas such as Qualitative phytochemical content analysis, Various techniques were used to determine the antioxidant activity of Carissa carandas, and Carissa carandas fruit extract was subjected to a quantitative phytochemical analysis using m ethanol. Alkaloids, glycosides, flavonoids, steroids, terpenoids, arthoquinone, phenols, sapiens, tannin, carbohydrates, proteins, amino acids, oil, and resins are among the phytochemical tests to be examined. Ferric Reduction of Plasma (FRAP), Azino-Bis (3-Ethyl enzothazoline 6-Sulphonic acid (ABTS), metal chelating property, phosphomolybdenum, hydroxyl radical scavenging, 2,2,Diphenyl 1,Picryll Hydrazyl (DPPH), and b,carotene were shown to have antioxidant capabilities. Qualitative phytochemicals such as alkaloids, saponins, flavonoids, phenols, terpenoi ds, glycosides, and carbohydrates were analyzed in Carissa carndas fruits.

Conclusion

The medication administration levels were determined using an aqueous extract of Carissa carndas fruits. These are recognized for their therapeutic characteristics and are commonly used in eastern nations. They are harmless, non-toxic, and have a great natural antioxidant potential. Based on the favorable results of the experiment, methanol extract of dried Carissa carandas fruits might be utilized as a dietary supplement to provide anti-inflammatory benefits, as we did here with dried fruit extract.

Acknowledgement

The authors are thankful to Professor and Head, Mr. S. Rajasekaran, Department of Botany, A.V.C. College (Autonomous), Mannampandal, for providing the necessary lab facilities, kind help, and encouragement for my research work.

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