

## **Comparative studies of phytochemical screening of *Carissa carandus* Linn.**

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### **ABSTRACT**

Present study deals with the qualitative analysis of Ethanolic, Methanolic and Aqueous extract of various parts of *Carrisa carandus* Linn. *Carissa Carandas* Linn. The leaves and roots of plant were extracted by cold percolation method using organic solvents such as ethanol, methanol and distilled water. Karonda is a widely used medicinal plant by tribal's throughout India and popular in various indigenous system of medicine like Ayurveda, Unani and Homoeopathy. The Karonda tree has many uses as it is used in traditional medicine, and modern medical research has found that it has many beneficial properties. Its leaves feed the tussar silkworm; the wood is used for making household utensils, such as large cooking spoons, and the root can be pounded to a paste to make insect repellent. The plant is also an alternative source of oil, hydrocarbon and phytochemicals.

**Keywords:** *Carrisa carandus* Linn, Medicinal Use, Extracts and Phytochemistry

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### **INTRODUCTION**

The plant kingdom is a treasure house of potential drugs and there has been an increasing awareness about their importance of medicinal plants. They are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites. Different plants have been used as a source of inspiration in the development of novel drug. Phytochemicals, chemical compounds that occur naturally in plants are responsible for color and organoleptic properties, such as the deep purple of blueberries and smell of garlic. *Carissa carandas* Linn. Is a large dichotomously branched evergreen shrub with short stem and strong thorns in pairs, belonging to the family Apocynaceae. *Carissa carandas* Linn is an evergreen diffuse and spiny shrub occurring throughout the country. The plant is very valuable for the Indian System of medicine particularly Ayurveda. It is used for alleviating vata and pitta disorders. Its fruits and seed latex are used for treating rheumatoid arthritis, anorexia, indigestion, colic, hepatomegaly, splenomegaly, piles, cardiac diseases, oedema, amenorrhoea, fever and nerving disorder. [1].

The roots are useful in stomach disorder, intestinal worms, Scabies, diabetic, ulcer and pruitis [2]. Hegde & Joshi 2009 has been studied the Hepatoprotective effect of *Carrisa carendus* Linn root extract against CCl<sub>4</sub> and para acetamol [3]. Hegde et.al. 2010 has reported Anticonvulsant Activity of *Carissa carandas* Linn. Root Extract on Mice [4]. Physio-chemical test were carried out adopting, standard procedure [5, 6]. Alcoholic extract of the root exhibits cardiogenic effect [7]. The plant is also Useful to bring down blood pressure [8]. Various fatty acids such as palmitic (66.42%), stearic (9.36%), Oleic (2.04%) and linoleic (0.99%) acids were found in seed of *Carissa carandas* [9]. A leaf decoction of Karonda is used against fever, diarrhoea, and earache. The roots serve as a stomachic, vermifuge, remedy for itches and insect repellent. Sahoo et.al also determined secondary metabolites produced during different seasons in some arid medicinal plants [9].

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## MATERIALS AND METHODS

### Plant material

The leaves and roots of *Carrisa carendus* Linn. Were collected from Bhadvan Village, Ajara Tahsil of Kolhapur District, and Maharashtra State, India during Oct 2012. It was authenticated by Prof. R.S.Sawant, Department of Botany, Dr.Ghali College, Gadhinglaj, Kolhapur district, Maharashtra.

### Preparation of extract

#### Ethanollic Extract:

The collected roots of *Carrisa carandus* Linn were washed; the bark was peeled off and then dried under shade. The coarse powder of the roots (76.712g) was soaked in one Liter of 50% ethyl alcohol and extracted in the cold for 3 days with occasional shaking. The solvent from the total extract was filtered & concentrated on water bath for 8 hrs. The remaining was used for the analysis of Phytochemical test.

#### Aqueous Extract:

The collected roots of *Carrisa carandus* Linn were washed; the bark was peeled off and then dried under shade. The coarse powder of the roots (52.709g) was soaked in 500 ml of Distilled water and extracted in the cold for 3 days with occasional shaking. The solvent from the total extract was filtered & concentrated on water bath for 8 hrs. The remaining was used for the analysis of Phytochemical test.

#### Methanolic Extract:

The collected Leaves of *Carrisa carandus* Linn were washed and then dried under shade. The coarse powder of the leaves (128.045g) was soaked in 600 ml Methyl alcohol and extracted in the cold for 3 days with occasional shaking. The solvent from the total extract was filtered & then dried under shade. Powder was used for the analysis of Phytochemical test.

#### Aqueous + Methanol Extract:

The residue obtained from Methanolic extract was mixed with Distilled water (500 ml) and extracted in the cold for 3 days with occasional shaking. The solvent from the total extract was filtered & concentrated on water bath for 8 hrs. The remaining was used for the analysis of Phytochemical test.

### Chemicals and drugs

All the chemicals and solvents were of analytical grade from SD Fine Chemicals Pvt. Ltd, Bombay.

### Phytochemical Screening

#### Steroid:

1ml extract was dissolved in 10 ml of chloroform & equal volume of concentrated  $H_2SO_4$  acid was added from the side of test tube. The upper layer turns red and  $H_2SO_4$  layer showed yellow with green fluorescence. This indicates the presence of steroid.

#### Tannin:

- 2ml extract was added to 1% lead acetate a yellowish precipitate indicates the presence of tannins.
- 4ml extract was treated with 4 ml  $FeCl_3$  formation of green colour indicates that presence of condensed tannin

#### Saponin:

5 ml extract was mixed with 20 ml of distilled water then agitated in graduated cylinder for 15 min formation of foam indicates Saponin.

#### Anthocyanin:

2 ml of aqueous extract is added to 2 ml of 2N HCl &  $NH_3$ , the appearance of pink red turns blue violet indicates presence of Anthocyanin.

#### Coumarin:

3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates coumarins.

**Emodins:**

2 ml of  $\text{NH}_4\text{OH}$  and 3 ml of benzene was added to extract appearance of red colour indicates presence of emodins.

**Alkaloids:**

A quantity (3 ml) of concentrated extract was taken into a test tube and 1 ml HCl was added the mixture was heated gently for 20 min cooled and filter, the filtrate was used for following test.

- a) Wagner test: Filtrate was treated with Wagner's reagent; formation of brown reddish precipitate indicates presence of alkaloids.
- b) Hager's test: Filtrate was treated with Hager's reagent, presence of alkaloids confirmed by the yellow colored precipitate.

**Proteins:**

- a) Xanthoproteic test: Extract was treated with few drops of concentrated  $\text{HNO}_3$  formation of yellow indicates the presence of proteins.

**Amino acids:**

- a) Ninhydrin test: To the 2 ml extract 2 ml on ninhydrin reagent was added & boil for few minutes, formation of blue colour indicates the presence of amino acid.

**Carbohydrates:**

Extract were dissolved individually in 5 ml of distilled water and filtered. The filtrate were used for the following test

- a) Molisch's test: Filtrate were treated with 2 drops of alcoholic  $\alpha$ -naphthol solution in the test tube, formation of violet ring at the junction indicates the presence of carbohydrate.
- b) Benedict's test: Filtrate were treated with Benedict's reagent and heated gently, orange red ppt indicates presence of reducing sugars.
- c) Fehling test: 2 ml extract were hydrolyzed with dilute HCl & neutralized with alkali & heated with Fehling's solution A and B, formation of red ppt indicates the presence of reducing sugar.
- d) Iodine test: 2 ml of extract were treated with 5 drops of Iodine solution gives blue colour indicates that presence of carbohydrates.

**Flavonoid:**

- a) Alkaline reagent test: Extract was treated with 10 % NaOH solution, formation of intense yellow colour indicates presence of Flavonoid.
- b)  $\text{NH}_4\text{OH}$  test: 3 ml of extract were 10 %  $\text{NH}_4\text{OH}$  solution development of yellow fluorescence indicates positive test.
- c) Mg turning test: Extract were treated with Mg turning and add conc.HCl to this solution add 5ml of 95 % ethanol, formation of crimson red colour indicates Flavonoid.
- d) Zn test: 2 ml extract were treated with Zn dust and conc.HCl development of red colour indicates presence of Flavonoid.

**Diterpenes:**

- a) Copper acetate test: Extract were dissolved in water and treated with 10 drops of copper acetate solution, formation of emerald green colour indicates presence of diterpenes.

**Phytosterol:**

- a) Salkowski's test: Extract was treated with chloroform and filtered. The filtrate was treated with few drops of concentrated  $\text{H}_2\text{SO}_4$  and shakes, allow standing, appearance of golden red indicates the positive test.

**Phenol:**

- a) Ferric Chloride test:

Test extract were treated with 4 drops of Alcoholic  $\text{FeCl}_3$  solution. Formation of bluish black colour indicate the presence of Phenol

**Phlobatannins:**

Deposition of red ppt when aqueous extract of each plant sample is boiled with 1% aqueous HCl was taken as evidence for presence of Phlobatannins.

**Leucoanthocyanin:**

5 ml of isoamyl alcohol added to 5 ml of aqueous extract, upper layer appear red in colour indicates the presence of Leucoanthocyanin.

**Cardial Glycosides:**

a) Keller-Killani Test: Plant extract treated with 2 ml glacial acetic acid containing a drop of  $\text{FeCl}_3$ . A brown colour ring indicates the presence of positive test.

**Table: 1 Phytochemical Analysis of root and leaf extract of *Carissa carandas* Linn.**

Sr. No.	Secondary metabolite	E.R.E.	A.R.E.	M.L.E.	A.M.L.E.
1	Steroid	+	+	+	+
2	Tannin				
	a. Lead Acetate test	+	+	-	+
	b. $\text{FeCl}_3$	+	-	+	+
3	Saponin: Foam test	+	+	+	+
4	Anthocyanin	-	+	-	-
5	Coumarin	+	-	+	-
6	Emodins	-	+	-	-
	Alkaloids				
7	a. Wagner's reagent	+	+	+	+
	b. Hager's reagent	+	+	+	+
8	Proteins: Xanthoproteic test	-	+	-	-
9	Amino acids: Ninhydrin test	-	+	-	+
	Carbohydrate				
10	a. Molisch's test	+	+	-	+
	b. Benedict test	+	+	+	+
	c. Fehling test	-	-	-	-
	d. Iodine test	-	-	-	-
	Flavonoid				
11	a. Alkaline reagent test	+	+	+	+
	b. $\text{NH}_4\text{OH}$ test	+	+	+	+
	c. Mg turning test	+	+	-	+
	e. Zinc Dust test	-	-	-	-
12	Diterpenes: Copper acetate test	+	+	+	+
13	Phytosterol: Salkowski's test	+	+	+	+
14	Phenols: $\text{FeCl}_3$ test	+	+	+	+
15	Phlobatannins	-	+	+	-
16	Leucoanthocyanin	-	+	-	-
17	Antraquinone	-	-	-	-
18	Cardial Glycosides	-	+	+	+
19	Chalcones	-	+	-	-

E.R.E.-Ethanollic Root Extract, A.R.E.-Aqueous Root Extract

M.L.E.-Methanolic Leaf Extract, A.M.L.E.- Aqueous + Methanolic Leaf Extract

Note: ( + ) = Present, ( - ) = Absent

## RESULTS AND DISCUSSION

### Phytochemical screening

Table No.1. Shows the result of Phytochemical screening of *Carrisa carandus* Linn. Preliminary Phytochemical investigation of the ethanolic extract of the roots of the plant *Carrisa carandus* Linn led to the presence of Flavonoids, Saponin, Steroids, Coumarin, Alkaloids, Carbohydrate, Diterpenes, Phytosterol, Phenols and Tannins whereas Anthocyanin, Emodins, Proteins, Amino acids, Phlobatannins, Leucoanthocyanin, Anthraquinone, Cardiac glycosides and Chalcones were absent. Also the aqueous extract of root led to the presence of Flavonoids, Saponin, Steroids, Proteins, Amino acids, Alkaloids, Carbohydrate, Diterpenes, Phlobatannins, Leucoanthocyanin, Phytosterol, Cardiac glycosides, Chalcones, Emodins, Phenols and Tannins whereas Anthocyanin, Coumarin and Anthraquinone were absent. Also the Methanolic extract of leaves led to the presence of Flavonoids, Saponin, Steroids, Alkaloids, Carbohydrate, Diterpenes, Phlobatannins, Phytosterol, Cardiac glycosides, Coumarin, Phenols and Tannins whereas Anthocyanin, Proteins, Amino acids, Leucoanthocyanin, Emodins, Chalcones and

Anthraquinone were absent. Also the Methanolic and aqueous leaves extract contains Flavonoids, Saponin, Steroids, Alkaloids, Carbohydrate, Diterpenes, Phytosterol, Amino acids, Cardiac glycosides, Phenols and Tannins whereas Anthocyanin, Proteins, Leucoanthocyanin, Emodins, Chalcones and Phlobatannins, Coumarin, Anthraquinone were absent.

Present study deals with qualitative analysis of root extract of *Carrisa carandus* Linn, On the basis of these data researcher easily isolated particular metabolite from the root extract quantitatively.

Alok Sharma *et al* 2007 reported ten Phytochemical such as Carbohydrate, Flavonoid, Protein, Resin, Saponin, Starch, Steroids, Tannin and Triterpenoid from the root of *Carrisa carandus* Linn [10].

### CONCLUSION

It is concluded that in the present study, Aqueous root extract contain more number of phytochemicals than the remaining extract whereas in the ethanolic root extract shows lesser number of the phytochemicals.

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