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PRELIMINARY PHYTOCHEMICAL SCREENING OF ROOT OF *CARISSA CARANDUS* Linn.

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ABSTRACT

The plant kingdom is a treasure house of potential drugs and there has been an increasing awareness about their importance of medicinal plants. Phytochemicals, chemical compounds that occur naturally in plants are responsible for color and organoleptic properties. *Carissa carandas Linn* is a large branched evergreen shrub with short stem and strong thorns in pairs belonging to the family *Apocynacea*e. *Carissa carandas Linn* is an evergreen diffuse and spiny shrub occurring throughout the country. *Carissa carandas Linn* root contain chemical constituents of alkaloids, glycosides, flavonoids, terpenes, steroids and polysaccharides. Phytochemical screening of the plants with medicinal value is of great importance and has significance in pharmaceutical companies and research institutes as well for the production of the drugs for healing different ailments. The present investigation dealt with the screening of phyto constituents and physico-chemical analysis of the roots of *Carissa carandus Linn*. The results showed the presence of phytochemical constituents in the ethanolic extracts of roots of *Carissa carandus Linn* and it provides an evidence for their use in the folklore medicine for the treatment of various diseases. More such work is to be done to evaluate the exact mechanism of action of the extracts. The results obtained in this study indicate that phytochemical compounds are the bioactive constituents and this tropical plant of roots of *Carissa carandus Linn* are proving to be a valuable reservoir of bioactive compounds of potential health benefits.

KEYWORDS: Phytochemical screening, *Carissa carandus*, Ayurveda.

INTRODUCTION

Botanical information

Carissa carandus Linn belongs to the family *Apocynaceae* found to be widely distributed throughout India. The shrub is commonly known as karonda 'Christ's thorn'.[1]

Taxonomy of the plant: *Carissa carandus Linn*

Kingdom : Plantae

Class : Angiosperms

Sub-class : Dicots

Super order : Asterids

Order : Gentianales

Family : *Apocynaceae*

Genus : *Carissa*

Species : *Carandas*

Table 1: Vernacular names.[2]

Language	Name
Hindi	Koranda
Tamil	Kalakai
Malayalam	Karaka
Telugu	Pedakalavi
Marathi	Karvand
Sanskrit	Karamla, karamardaka
Bengali	Karamacha
Gujarati	Karamada
Kannada	Karayige
English	Cranberry Bengal currant
Urdu	Karamarda
Oriya	Kerendokuli

Plant description

Karonda is an evergreen deciduous small to big shrub usually 2-4 m tall. The stem is rich in white latex and the branches contain sharp spines. Flowers are small, measuring 3-5 cm in diameter with white colors. The fruit is a berry which is formed in clusters of 3-10 fruits.[3]

Table 02: Ethno medical information.[4]

Part used	Pharmacological activity
Fruit	Treat liver dysfunction and anemia, antipyretic, astringent, antiscorbuticS.
Root	Improve digestion, antidiabetic, stomachic, vermifuge, remedy for itches and insect repellent, anthelmintic, cardiotonic
Leaves	Used against fever, diarrhoea, ear ache, anticancer
Stem bark	Used to obstinate skin diseases
Seeds	Cardiotonic activity

Phytochemical Studies

Stem bark contain alkaloids.

Leaves contain triterpenes, tannins, ursolic acid and carissic acid. Fresh leaves isolated four pentacyclic triterpenoids including a new constituent, carissin (3beta-hydroxy-27-E-feruloyloxyurs-12-en-28-oic acid) and two previously unreported compounds. Volatile oil of fresh flowers yields myrcene, limonene, camphene, carene, dipentene, farnesol, nerolidol, dihydrojasmone, α-terpeneol, citronellal, β-ionone, nerylacetate, linalol and geranyl acetate.[5]

Fruits yield a mixture of volatile principles - 2-phenyl ethanol, linalool, ß- caryophyllene, iso amyl alcohol, benzyl acetate, lupeol, oxalic, tartaric, citric, malic, malonic and glycolic acids, glycine, alaline, phenyl alaline, cerine, glucose, galactose and a novel triterpenic alcohol (carissol -an epimer of α-amyrin).[6]

MATERIALS AND METHODS

Collection and Authentication

The root of *Carissa carandas Linn* was collected from the village **Kanai, Villuppuram District and Tamilnadu**. The collected root of *Carissa carandas Linn* was identified and authenticated by the Prof. **Dr. N. S. Jeganadhan, PROFESSOR AND HOD,** Department of Pharmacognosy, School of Pharmacy, Surya Group of Institutions, Vikiravandi.

Preparation of Extract

Roots were thoroughly washed under fresh tap water, shade dried and powdered by using a mechanical grinder. The preparation of Ethanolic Extract of roots of *Carissa carandas Linn was* done by using soxhlet apparatus in the Department of Pharmacognosy, Surya School of Pharmacy and Vikiravandi. About 200g of root powder was taken into the soxhlet apparatus and extracted using ethanol (95%). The extraction process was carried out for 18 - 20 hours till the appearance of colourless solvent in the side tube.

The extract collected was dried by evaporating the solvents on a water bath and percentage yield of alcoholic extract was recorded with respect to the total quantity of powder used for the extraction.

Phytochemical Analysis[7]

Preliminary Phytochemical Analysis

The Ethanolic extracts of the powdered root of *Carissa carandas Linn* was subjected to various qualitative tests for identification of plant constituents present in this species.

Test for Alkaloids

Hager's Test

To 1ml of the extract add 3ml of Hager's reagent (saturated solution of picric Acid). Formation of yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test

To 1ml of the extract add 2ml of Wagner's reagent (Iodine in potassium Iodide). Formation of reddish brown precipitate indicates the presence of alkaloids.

Test for Saponins

Foam test

Take small quantity of extract and add 20ml of distilled water and shake in a graduated cylinder for 15mins lengthwise. A 1cm layer of foam indicates the presence of Saponin.

Test for Cardiac Glycosides

Legal's Test

Dissolve the extract in pyridine and add alkaline sodium nitroprusside solution. The formation of blood red colour shows the presence of glycosides.

Keller-kiliani Test

1g of powdered drug is extracted with 10ml of 70% alcohol for 2mins, filtered. To the filtrate add 10ml of water and 0.5ml of strong solution of lead acetate and filtered. The filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cool residue in 3mlof glacial acetic acid and 2 drops of ferric chloride solution.

Transfer this solution to the surface of 2ml of concentrated sulphuric acid. A Reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

Baljet Test

To 1ml of the test extract add 1ml of sodium picrate solution. Formation of yellow to orange colour shows the presence of glycosides.

Test for Carbohydrates and Sugars

Molisch's Test

To 2 ml of the extract add 1ml of α-naphthol solution add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the liquid indicates the presence of carbohydrates.

Fehling's Test

To 1ml of the extract add equal quantities of Fehling's solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

Benedict's Test

To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2mins and cool. Formation of red precipitate shows presence of sugars.

Test for Tannins

Ferric chloride Test

To 1ml of extract add ferric chloride solution, formation of dark blue or greenish black product shows the presence of tannins.

Test for Flavonoids

Shinoda's Test To the extract solution add few fragments of magnesium ribbon and add concentrated hydrochloric acid drop wise gives cherry red or pink scarlet or occasionally green to blue colour appears after few minutes shows the presence of flavonoids.

Alkaline reagent Test

To the extract add few drops of sodium hydroxide solution, formation of an intense yellow colour which turns to colourless on addition of few drops of dilute acid indicates the presence of flavonoids.

Test for Steroids

Libermann- Burchard Test

1 gm of test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and few drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.

Salkowski Test

Dissolve the extract in chloroform and add equal volume of concentrated sulphuric acid. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

Test for Proteins and Amino Acids

Ninhydrin Test

Add 2drops of freshly prepared 0.2% ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. Development of blue colour reveals the presence of proteins, peptides or amino acid.

Xanthoprotein Test

To 1ml of the extract add 1ml of concentrated nitric acid. A white precipitate is formed, it was boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. Formation of orange colour indicates the presence of aromatic amino acids.

Test for Diterpenes

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.

Test for Phenol

Ferric Chloride test

Test extract were treated with 4 drops of Alcoholic FeCl3 solution. Formation of bluish black colour indicate the presence of Phenol.

Physico-Chemical Analysis[8]

Ash value represents the inorganic salts naturally occurring in the drug and adhering to it. Total ash is the residue remaining after incineration. The acid insoluble ash is the part of total

ash which is insoluble in dilute hydrochloric acid. Mixing of sulphuric acid with powdered crude drug before ashing and this sulphated ash is normally less fusible than ordinary ash. Extractive value which is an indicative of approximate measures of chemical constituents and nature of the constituents was performed using ethanol as solvent. The moisture content was determined in to air-dry sample by loss on drying method. The Total ash, water and alcohol insoluble ash was also determined. Alcohol and water soluble extractive values, foreign organic matter was determined.

Total ASH

Place about 2-4g of the ground air-dried material accurately weighed in a previously ignited and tarred crucible (usually of platinum or silica).

Spread the material in an even layer and ignite it by gradually increasing the heat to 450°C until it is white indicating the absence of carbon. Cool in desiccators and weigh. Ash value can be calculated by using formula: -

$$\text{Ash value} = \frac{\text{Initial Weight} - \text{Final Weight} \times 100}{\text{Initial Weight}}$$

ACID Insoluble ASH

The total ash obtained was boiled with 25 ml of dilute hydrochloric acid for 5 minutes. The insoluble matter was collected on tarred grouch crucible, washed with hot acidulated water, ignited, cooled and weighed. The percentage acid insoluble ash was calculated with reference to the air dried drug.

Water Soluble Extractive Value

Place about 15gm of coarsely powdered air-dried material, accurately weighed in a glass stoppered conical flask. Add 300ml of water and weigh to obtain the total weight including the flask. Shake well and allow standing for 1 hour. Attach a reflux condenser to the flask and boil gently for 6 hours, cool and weigh and filter rapidly through a dry filter. Dry the extracted powder in oven till the weight is constant. Calculate the content of extractable matter in mg per gm of air dried material.

$$\text{Extractive value} = \frac{\text{Initial weight-Final weight} \times 100}{\text{Initial weight}}$$

Alcohol Soluble Extractive Value

Place about 15gm of coarsely powdered air-dried material, accurately weighed in a glass stoppered conical flask. Add 300ml of water and weigh to obtain the total weight including the flask. Shake well and allow standing for 1 hour.

Attach a reflux condenser to the flask and boil gently for 6 hours; cool, weigh and filter rapidly through a dry filter. Dry the extracted powder in oven till the weight is constant. Calculate the content of extractable matter in mg per gm of air-dried material.

Loss on Drying

The powdered root of *Carissa carandas Linn* was dried in the oven at 100- 105^0C to constant weight.

RESULTS

Phytochemical Studies

Table 3: Phytochemical constituents present in ethanolic extract of the root of *Carissa carandas Linn*.

S.NO	Phytoconstituents	Ethanolic Extract of root
1.	Steroid	+
2.	**Tannin**	
3.	b.Ferric chloride test	+
4.	Saponin: Foam test	+
5.	**Alkaloids**	
6.	a.Wagner's reagent	+
7.	b.Hager's reagent	+
8.	Proteins: Xanthoprotein test	-
9.	Amino acids: Ninhydrin test	-
10.	**Carbohydrate**	
11.	a.Molisch's test	+
12.	b.Benedict test	+
13.	c.Fehling's test	-
14.	**Flavonoid**	
15.	a. Alkaline reagent test	+
16.	b.Magnesium turning test	+
17.	Diterpenes: Copper acetate test	+
18.	Phenols: Ferric chloride test	+
19.	CardiacGlycosides	+

Physico-Chemical Analysis

Table 4: Results of physicochemical parameters in roots of *Carissa carandas Linn*.

Parameters	Results
Total Ash	20%w/w
Acid Insoluble Ash	18%w/w
Alcohol Soluble Extractive	1.2%w/w
Water Soluble Extractive	2.0%w/w
Loss On Drying	1.4%

DISCUSSION

The root of *Carissa carandas Linn* belonging to the family A*pocynaceae* is a widely growing plant throughout India. The plant also has many valuable medicinal properties. Hence this study deals with the phytochemical screening of the selected plant. A detailed study was done on this plant. The Ethanolic extract of *Carissa carandas Linn* root was subjected to preliminary identification of phytoconstituents which showed the presence of carbohydrates, flavonoids, terpenes, cardiac glycosides, alkaloids, tannins, phenolic compounds and steroids. The physico chemical evaluation like total Ash, acid insoluble Ash, alcohol soluble extractive, water soluble extractive, loss on drying are determined. The information obtained from ash values and extractive values are also useful during the collection and also during the extraction process. These values are helpful to identify the sample of genuine drug**.** Therefore screening intimates presence of many bioactive chemical constituents which act as antioxidant, antidiabetic and antimalarial agent. It is suggested that further work should be carried out to isolate, purify and possibly characterize the active constituent s responsible for the activity of the plant. Scientific validation is necessary before put into practice.

CONCLUSION

Preliminary phytochemical screening of ethanolic root extract of *Carissa carandus Linn* which showed the presence of carbohydrates, flavonoids, terpenes, cardiac glycosides, alkaloids, tannins, phenolic compounds and steroids. Further this study directs future research in separating the bioactive compound responsible for this activity.

REFERENCES

1. Wealth of India- Raw material 82, fig 32, FI Br ind, 630; volume-3; ca-ci series; Published by Dr. k. s Krishnan marg, New Delhi, 1992.
2. Ayurvedic Pharmacopoeia of India; Part 1, 2001; 3: 86.

3. Chanchalkumarmishra, Shrivastava B, International Journal of Pharmacy and Pharmaceutical Sciences, 2013; 5.
4. ReshuVirmani, TarunVirmani, Reasearch in Pharmacy and Health Science, 2017.
5. Rajaram sawant, Ashoksutar, Rasayan Journal, 2012; 5.
6. Anupama N; Madhumitha G, Journal of Chemical and Pharmaceutical Research, 2015; 7(10): 841-847.
7. Rajaram S, Sawant and Ashvin G. Godghate, Asian Journal of Plant Science and Research, 2013; 3(1): 21-25.
8. Upreti. K, International Journal of Herbal Medicine, 2013.