A Review on Karaunda (Carissa Carandas Linn.)

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Abstract
Carissa Carandas Linn. (Karaunda) is a widely used medicinal plant by tribals throughout India and popular in various indigenous system of medicine like Ayurveda, Unani and Homoeopathy. All parts of the plant are used in traditional medicine. Traditionally the plant has been used in the treatment of scabies, intestinal worms, pruritus, biliousness and also used as antiscorbutic, anthelmintic. The notable biological activities reported are analgesic, anti inflammatory, anti pyretic, cardiotonic and histamine releasing. A higher gross heat value of this species indicates its higher potential to be used as good fuel source .The plant is also an alternative source of oil, hydrocarbon and phytochemicals. This review presents a detailed survey of the literature on phytochemistry, traditional and biologically evaluated medicinal uses of C. carandas.

Key words: Carissa carandas, Phytochemical constituents, Traditional uses, Pharmacological properties

Introduction
Carissa carandas is a species of flowering shrub in the dogbane family, Apocynaceae. (Youngken HW et al 1950) It produces berry-sized fruits that are commonly used as a condiment or additive to Indian pickles and spices. It is a very hardy, drought-tolerant plant that thrives well in a wide range of soils. Common names include Karonda (Devanagari: करोंडा), Vakkay in Telugu, Kalakai in Tamil.

Botanical Name: Carissa Carandas Linn,
Natural Order: Apocynaceae
Classical Names: Karamarda, Karmardaka, Sushena,
Vernacular Names: Eng: Karaunda, Jasmine flower Carissa
Hindi: Karaunta, Karanti, Karaunda, Karijige
Beng: Karamach
Guj: Karamda, Karamadan
Karonda (Carissa carandas) 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 colour. The fruit is a berry, which is formed in clusters of 3-10 fruits (Fig. 1). The fruit is globose to broad ovoid in shape and contains many seeds. Young fruits are pinkish white and become red to dark purple when ripe. Ripe fruit color vary from white, green and pinkish red depending on the genotype. Flowering starts in the month of January-February and fruits mature in May-June. Fruits are generally harvested at immature stage for vegetable purpose, fully ripen fruits are consumed. (Youngken HW et al 1950).

Leaves: The leaves are oblong and conical, 4-6 inch long and 2-3 inch wide, green on the top and brown below. If the leaves or stems are injured, the white milky sap is seen, which is characteristic of this group of plants. 
Flowers: White or yellowish flowers are found in groups.
Fruit: The are avoid with 5-1 hard angles curving upwards, glabrous with five to seven wings, woody and fibrous.
Bark: The bark is smooth gray. The bark is thick, soft and of red color from inside. (Evans WC et al 1996)

Karonda fruit is a rich source of iron and contains a fair amount of Vitamin C. It is antiscorbutic and very useful for cure of anaemia. Mature fruit contains high amount of pectin and, therefore, besides being used for making pickle, it can be exploited for making jelly, jam, squash, syrup and chutney. Ripe fruits exude a white latex when severed from the branch. The roots of the plant are heavily branched and make it suitable for stabilising eroding slopes. (Samuelsson G et al 1992). In traditional medicine the fruit is used to improve female libido and to remove worms from the intestinal tract. The fruits can also be used on old wounds which have become infected, as the juice will clean them and it has been found that the plant has anti-microbial and antifungal properties. The fruit have an analgesic action as well as an anti-inflammatory one.

The juice can be applied to the skin to relieve any skin problems. Histamine is emitted from the bruised roots. Traditionally Karonda has been used to treat anorexia and insanity. The stem is used to strengthen the tendons and the leaves contain the same triterpene acids as Lantana camara or Yellow Sage. It also has anti-pyretic activities, so can be helpful in reducing fever. (Bisset NG et al 1994). Modern medical research has shown that eating the fruit can lower cholesterol levels and the new lignans that have been found in the fruit are being investigated. Lignans are found in flax seeds, pumpkin seeds, cranberries and whole grains such as millet. They are used to treat atherosclerosis and are chemopreventives, as well as having the ability to lower cholesterol levels. (Youngken HW et al 1950)

Varieties
Formerly there were believed to be 2 distinct varieties: C. carandas var. amara–with oval, dark-purple, red-fleshed fruits, of acid flavor; and var. dulcis–round, maroon, with pink flesh and sweet-subacid flavor. However, David Sturrock, a Florida horticulturist who took a special interest in the karanda, observed these and other variations throughout seedling populations. (Youngken HW et al 1950)

Food Value
Analyses made in India and the Philippines show the following values for the ripe karanda: calories, 338 to 342/lb (745-753/kg); moisture, 83.17-83.24%; protein, 0.39-0.66%; fat, 2.57-4.63%; carbohydrate, 0.51-0.94%; sugar, 7.35-11.58%; fiber, 0.62-1.81%; ash, 0.66-0.78 %. Ascorbic acid content has been reported as 9 to 11 mg per 100 g. (Winton A.L et al 2001)

For best growth, fertilize carissas three to four times a year with a general-purpose fertilizer containing all micronutrients. Once established, plants need very little irrigation, and in fact, do poorly in wet soils or sites that are frequently flooded during heavy rainfall. (Blaschek W et al 1998)

Fertilizer Application
In the early years of the planting, plants require plenty of nitrogen for its growth. The fertilizer should be applied at bimonthly intervals after dividing the fertilizer in six parts. The fertilizer requirement of karonda is given in table 1. Phosphorus and potassium fertilizers should be applied after soil testing. In most cases, it is advisable to supplement the fertilizer applications with micronutrient sprayings. Make three applications each year using Micron-Z.
Pests and Diseases
Nursery plants are probably prone to the same pests that attack young carissas. Fungus diseases recorded on the karanda in Florida are algal leaf spot and green scurf caused by *Cephaleuros virescens*; twig dieback from *Diplodia natalensis*; and stem canker induced by *Dithiorella* sp.

Insect Pests and Diseases
As in other crops, here too the pests and diseases reduce the yield. (Göbel H *et al* 1994)

**A. Pests**
There is no serious pest problem in karonda. In the early stages, leaf eating caterpillar feed voraciously on the leaves and cause mortality of the plants. These caterpillars can be controlled by spraying Thiodan or Endosulfan @ 2 ml/litre of water or Nuvacron @ 1 ml/litre of water.

**B. Diseases**
Among the diseases, anthracnose and bacterial canker are of common occurrence.

**Anthracnose**
This is a fungal disease caused by *Colletotrichum inamdarii*. This disease is widespread in Uttar Pradesh and can be seen in the form of black grey spots on the leaves. Spray of copper sulphate @ 0.1 % can control this disease.

**Bacterial canker**
This is a bacterial disease and caused by *Xanthomonas carissa*. Symptoms first appear as spots on the lower surface of the leaf. These are small, round and water soaked which turn dark brown. A yellowish green halo may surround the spots which causes necrosis. The disease can be checked by removing diseased leaves followed by a spray of Phytomycin (200 ppm).

Physical Constants
Foreign Matter – Not more than 2%; Total ash – Not more than 12%; Acid insoluble ash – Not more than 3%; Alcohol soluble extractive – Not less than 4%; Water soluble extractive – Not less than 8%. (Vdevmurari *et al* 2009)

Phytochemical Constituents
Carissone, carindone, carinol, odoroside H, digitoxigenin, glucose and D-digitalose (roots); alkaloids (root, stem bark); carissol (an epimer of α-amyrin), lupeol, tartaric, citric, malic and glycolic acids, glycine, alkaline, phenyl alkaline, cerine, glucose and galactose (fruit); fatty acids, viz., palmitic, stearic, oleic, arachidic, linoleic acids (seeds); triterpene alcohol, ursolic acid (leaves) myrcene, limonene, camphene, carene, dipentene, farnesol, nerolidol, dihydrojasmine, α-terpeneol, citronellal, β-ionone, nerylacetate, linalool and geranyl acetate (volatile oils of fresh flowers). (Bruneton *et al* 1995). Various fatty acids such as palmitic (66.42%), stearic (9.36%), arachidic (21.19%), oleic (2.04%) and linoleic (0.99%) acids were found to occur in the seeds of Carissa. A terpinic alcohol Carissol (I), which is an epimer of α-amyrin was isolated from its fresh fruit. Glucose and galactose as well as amino acids such as serine, glutamine, alanine, valine, phenylalanine and glycine in the fruit were also reported by the same group of researchers (Zafar *et al* 1985, Hasnain *et al* 1990).

Pharmacological Activites
The roots are anthelmimitic, stomachic, antiscorbutic and are useful in stomach disorders, intestinal worms, scabies, diabetic ulcer and pruritus. The unripe fruit is bitter, sour, astringent, thermogenic, constipating, appetiser, antipyretic and useful in hyperdiropsia, anorexia, diarrhea, disease of brain, nematicidal and intermittent fevers. The ripe fruit is sweet, cooling, appetiser, antiscorbutic and is useful in bilious complaints, anorexia, burning sensation, scabies, pruritus and other skin diseases. Decoction of leaves is given at the commencement of remittent fever. Various plant parts are reported to be used in dropsy, anasarca, madness, rheumatism, hemeplegia, epilepsy, convulsions, postnatal complaints, sores and bite of rabid jackal or dog. (Swami *et al* 2010)

Toxicology
Plant extract caused vomiting, rhinorrhoea, tachypnea, exhaustion and death in conscious cats.

Organoleptic identification of *Carissa carandas* Linn
Organoleptic identification are described in table 2. (Bruneton *et al* 1995)

Standardisation of Crude Drugs (Alok Sharma *et al* 2007)

**Determination of Foreign Matter**
100 g of the drug sample examined, was weighed and spread out in a thin layer. The foreign matter was detected by inspection with the naked eye. It was separated and weighed and the percentage was calculated. Drug which was
undertaken for further study was free from moulds, insects, animal faecal matter and other contamination such as soil, stones and extraneous material

**Determination of Moisture Content**

The azeotropic distillation apparatus consisted of a glass flask connected by a tube to a cylindrical tube fitted with a graduated receiving tube and a reflux condenser. The receiving tube was graduated in 0.1ml division so that the error of reading does not exceed 0.05ml. The preferred source of heat was an electric heater with a thermostat control. Accurately weighed drug expected to give about 2-3ml of water and a few piece of porous porcelain was placed in heating flask. When as the boiling started initially the distillation rate was adjusted to the 2 drops per second was adjusted until most of the water had distilled over and then it was readjusted to a rate of 4 drops per second. As soon as the water was completely distilled, the inside of the condenser tube was rinsed with toluene. The receiving tube was allowed to cool at room temperature and the water droplets adhering to the walls of the receiving tube was dislodged by tapping the tube. The layer of water and toluene layers were separated and the volume of water was measured. The percentage water content (%v/w) was calculated using the formula:

\[
100\left(\frac{N1-N}{w}\right)
\]

Where \( w \) = the weight in g of the material being examined.
\( N \) = the number of ml of water obtained in the first distillation.
\( N1 \) = the total number of ml of water obtained in both distillations.

**Determination of Ash**

**Determination of Total Ash**

About 2.0g of powdered drug was incinerated in a tared silica dish at a temperature not exceeding 450°C until free carbon was left, then it was cooled and the final weight was taken. The percentage of ash with reference to the air-dried drug was calculated (PASF 1987).

**Determination of Acid Insoluble Ash**

The ash obtained by above method was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and the insoluble matter was collected on an ash-less filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air dried drug was calculated (PASF, 1987).

**Determination of Extractable Matter**

**Method I. Hot Extraction**

About 4.0 g of accurately weighed coarsely powdered air-dried drug was placed in a thumble and refluxed with various organic solvents over soxhlet extraction. The successive extraction was carried out in the order of solvent Pet. ether, alcohol and water. After recovery of solvents under vacuum and drying in desiccator, the percentage extractable matter was calculated.

**Method II. Cold Maceration**

About 4.0 g of coarsely powdered air dried material, was accurately weighed in a glass stoppered conical flask and macerated for 6 hours with 100ml of the solvent specified for the plant material concerned, shaking frequently and then allowed to stand for 18 hours. It was then filtered rapidly taking care not to lose any solvent in the process. The extracted matter was dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and then weighed. The percentage extractable matter was calculated.

**Determination of Volatile Oils**

About 20-40g of coarsely powdered air dried material, was accurately weighed and placed in a round bottomed flask. Volatile oils were determined by steam distillation adopting standard procedure. The distillate was collected in a graduate tube, using xylene. The aqueous phase was allowed to re-circulate into the distillation flask for all determinations and the rate of distillation was read from the marks engraved on the apparatus. The increase in the volume of xylene was noted and the difference in initial and final value of xylene was used to calculate the volatile oil content of the drug.

**Determination of Swelling Index**

About 4.0 g of fine powdered accurately weighed material was taken in a 25ml glass stoppered measuring cylinder. The internal diameter of the cylinder was about 16mm, the length of the graduate portion about 125mm, marked in 0.2ml division from 0 to 25 ml in an upward direction. 25 ml of water was added and the mixture was thoroughly shaken for every 10 minutes for an hour. Kept for 3 hours at room temperature and the volume in ml occupied by the plant material, including any sticky mucilage was measured. The mean value of the individual determination, related to 1.0g of plant material was calculated (WHO, 1980).
**Determination of Foaming Index**

About 1.0 g of a coarse powder of the drug was placed into a 500ml conical flask containing 100ml of boiling water. Moderate boiling was maintained for 30 minutes. Then it was cooled and filtered into a 100 ml volumetric flask and the volume was made up to the mark with distilled water. The decoction was then poured into 10 stoppered test-tubes (height 16cm, diameter 16 mm) in successive portions of 1ml, 2ml, 3ml etc. up to 10ml, and adjusted the volume of the liquid in each tube with water to 10ml. The tubes were stoppered and shaken them in a length wise motion for 15 seconds, two shakes per second. After 15 minutes, the height of the foam was measured. The results are assessed as follows.

1. If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100 &
2. If a height of foam 1 cm was measured in any tube, the volume of the plant material decoction in this tube (a) was used to determine the index. If this tube is the first or second tube in a series, an intermediate dilution was prepared in a similar manner to obtain a more precise result.
3. If the height of the foam was more than 1 cm in every tube, the foaming index was over 1000. In this case the determination was repeated using a new series of dilution of the decoction in order to obtain a precise result.

The foaming index was calculated using the following formula:

\[ \text{Foaming Index} = \frac{1000}{a} \]

Where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm was observed.

**Determination of Pesticide Residues**

Organochloride pesticides were extracted from powdered drug of *Carissa carandas* adopting the procedures mentioned in WHO guidelines (WHO, 1998). The organochloride pesticides standard and the sample extract was applied on to GLC (Nuccon 5767) under the following conditions. Column 6”x1/8” (ID) glass column filled with 80-100 mesh gas chrom. coated with mixture of 1.5% OV- 17 and 1.95 OV 210, temperature 190°C, Detector: Ni63 ECD, 250°C, Injector: temp. 250°C. Carrier gas IOLAR grade Nitrogen, flow rate 60 ml/min. Using the above mentioned analytical condition the level of the detection (LOD) and level of the quantitation (LOQ) of the organochlorine pesticide were in the range of 0.1-0.5µg/L and 1-3µg/L, respectively. Recoveries in the fortified samples were found to be 85-95% (Khare et al 2003)

**Preliminary Screening of Phytochemicals**

The preliminary phytochemical studies were performed for testing the different chemical groups present in the drug. 10% (w/v) solution of the extract was taken for the studies unless otherwise mentioned in the respective individual test. The chemical tests were performed.

General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them (Bruneton J et al 1 983)

**Alkaloids**

**Dragendorff’s test:**

A few mg of alcoholic or aq. extract of the drug was dissolved in 5 ml of distilled water, to which 2 N hydrochloric acid was added until an acid reaction occurred, then 1 ml of Dragendorff’s reagent was added. An orange or orange-red precipitate was considered positive test for alkaloids.

**Hager’s test:**

To 1 ml of alcoholic extract of the drug in a test tube, a few drops of Hager’s reagent was added. Formation of yellow precipitate confirmed the presence of alkaloids.

**Wagner’s test:**

1 ml of alcoholic extract of the drug was acidified with 1.5% v/v of hydrochloric acid and a few drops of Wagner’s reagent was added. A yellow or brown precipitate considered the presence of alkaloids.

**Mayer’s test:**

A few drops of Mayer’s reagent was added to 1 ml of acidic aqueous extract of the drug. White or pale yellow precipitate was considered positive for the presence of alkaloids.

**Carbohydrates:**

**Benedict’s test:**

To 0.5 ml of aqueous extract of the drug 5 ml of Benedict’s solution was added and boiled for 5 mins. Formation of a brick red coloured precipitate indicated the presence of carbohydrates.
**Fehling’s test:**
To 2 ml of aqueous extract of the drug 1 ml of a mixture of equal parts of Fehling’s solution ‘A’ and Fehling’s solution ‘B’ was added and contents of the test tube were boiled for few mins. A red or brick red precipitate indicates the presence of carbohydrates.

**Molisch’s test:**
In a test tube containing 2 ml of aqueous extract of the drug 2 drops of a freshly prepared 20% alcoholic solution of β-naphthol was added and mixed and then 2 ml of conc. sulphuric acid was poured so as to from a layer below the mixture. Carbohydrates, if present, produce a red-violet ring, which disappears on the addition of an excess of alkali solution.

**Flavonoids**

**Shinoda’s test:** In a test tube containing 0.5 ml of alcoholic extract of the drug, 5-10 drops of dil. hydrochloric acid followed by a small piece of magnesium was added. In the presence of flavonoids a pink, reddish pink or brown colour indicated the presence of flavonoids.

**Triterpenoids**

Liebermann-Burchard’s test: 2 ml of acetic anhydride solution was added to 1 ml of petroleum ether extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid. A violet coloured ring indicated the presence of triterpenoids.

**Proteins**

**Biuret’s test:**
1 ml of hot aq extract of the drug was added and then 5-8 drops of 10% w/v sodium hydroxide solution was added followed by 1 to 2 drops of 3% w/v copper sulphate solution. A red or violet colour indicated the presence of protein.

**Millon’s test:**
Aqueous extract of the drug in 1 ml of distilled water was dissolved and 5-6 drops of Millon’s reagent was added. A white precipitate which turns red on heating indicates the presence of protein.

**Resins**
The extract was dissolved in acetone and the solution was poured into distilled water. Turbidity indicated the presence of resins.

**Saponins**
5 ml of an aqueous extract of the drug was taken in a test tube then a drop of sodium bicarbonate solution was added, the mixture was shaken vigorously and left for 3 mins. Honeycomb like froth showed the presence of saponins.

**Quantitative Analysis of Phytochemicals**

Estimation of Tannin By Spectrophotometric (William DH et al 2004)

**Regents used**

[A] Sodium carbonate saturated solution: 35g anhydrous sodium carbonate was dissolved in 100ml distilled water at 70-80°C and cooled overnight. Supersaturated solution was seeded with crystals of Na₂CO₃ H₂O and after crystallisation filtered through glass wool.

[B] Tannic acid standard solution: Different concentration of tannic acid solution was prepared by dissolving the appropriate amount of pure tannic acid in known volume of water to obtain the standard curve for estimating the unknown concentration of the samples.

[C] Folin ceocalteus Reagent

**Preparation of Standard Curve**
0.1ml aliquots standard tannic acid solutions of different known concentration were put into 100ml volumetric flask and a volume of 10ml sodium carbonate solution was added, the volume was made up with water. The final mixture was mixed well and absorbance was taken at 760nm after 30min. A graph was plotted between the absorption and the concentration.

**Procedure for Plant Material**
2.0g powdered drug material was extracted with 100ml distilled water in water bath for 24 hours. It was filtered and the volume was made upto 100 ml volumetric flask. To 1ml aliquot, 5ml folin ceocalteus reagent and 10ml saturated sodium carbonate were added and the volume made upto 100ml in volumetric flask. Corresponding blank was also
prepared. The instrument was set to zero through blank and the corresponding absorbance of different samples were taken using 760nm by Spectrophotometer. Calculation was made according to the following formula.
Percentage of tannin = Absorbance x factor (slop) x dilution factor x 100/ powdered drug (mg)

Estimation of Sugar
Sugar estimation (Mont Gomery et al 1957) [Spectrophotometric method]
10% homogenous solution of the plant tissue was prepared in 80% Ethanol. It was centrifuged at 2000 rpm for 15 minutes. The supernatant was obtained and made up to known volume (generally up to 10 ml or depending on the expected concentration of sugar). 0.1 ml aliquot was taken, 0.1 ml of 80% phenol and 5 ml conc. H$_2$SO$_4$ was added. It was cooled and then the absorbance was noted at 490 nm. The standard curve was prepared with known concentration of sugar. Calculation was made according to the following formula.
Percentage of Sugar = Absorbance x Eq. factor x Conc.

Estimation of Starch
Starch estimation (Plummer et al 1971) [Spectrophotometric method]
Extraction of starch from plant tissue
10% homogenate of the plant tissue in 80% Ethanol was prepared. Then it was centrifuged at 3000 rpm for 15 minutes. The supernatant was discarded. The pellet was dissolved in 10 ml of distilled water and it was boiled for 40 mins in a water bath to dissolve starch. After cooling, the volume was made and centrifuged for 10 mins. The supernatant was taken for estimation.

Preparation of (I$_2$-KI) reagent
Starch was estimated with iodine- potassium iodide (I$_2$-KI) reagent. 60 mg of iodine was dissolved in 10ml of 6% KI solution. From this 1 ml was taken and added in distilled water along with 0.43 ml of HCl (0.05N) and the total volume was made up to 100 ml with distilled water.
The reaction mixture consisted of 1ml starch extract, 4ml distilled water and 1ml reagent. The absorbance of the reaction mixture was measured at 640nm. The starch content of the drug was calculated from calibration curve prepared by using a standard and it was calculated as follows:

\[
\text{Starch content} = \frac{K \times \text{Abs. At 640} + B \times \text{Total Volume}}{\text{Wt of the plant material}}
\]
The values of K and B were obtained by preparing the calibration curve for starch using the starch of AR grade (Merck reagent). The value of K was 592.02 and value of B was 10.769.

Extraction, isolation and elucidation of chemical constituents
Extraction:
Air dried and coarsely powdered material (80 kg) was extracted exhaustively with EtOH (95%) in a Soxhlet apparatus. The combined extracts were dried under reduced pressure to obtained a brown-coloured viscous residue (1040 g,13 %). It was acidic to litmus..

Isolation and elucidation of chemical constituents:
The crude alcohol extract (1040gram) was defatted with 8 l. of petroleum ether (40-60°) and then extracted with 9 l. ether in a similar manner. The ether was distilled to leave 300g of a residue. A 150-g . portion of the residue was dissolved in a mixture of 200ml methanol and 30ml water and again defatted three times with petroleum ether (40-60°) using 300ml each time. At the interphase of the petroleum ether-methanol separated 100mg of white crystalline substance (A). The white crystalline substance (A) was found to be insoluble in most of the organic solvents. It was crystallized from glacial acetic acid in short white needles, m.p 272-275 °C .The Liebermann-burchard test was positive.(Jafer et al 1985)

Conclusion
Nowadays, herbal drugs are widely used as curative agent for different ailments. Concentrated extract of C. congesta can be found in various herbal preparations, which are readily available in the market. C. congesta preparations have broad range of distribution in the market and employed by practioner of natural health for the treatment of rheumatism. In the traditional system of medicine, C. congesta plant is used as cardiotonic, hepatoprotective, free radical scavenger and xanthine oxidase inhibiting agent, histamine releasing agent, antiarthritic, stomachic; antidiarrhoeal, vermifuge and anthelmintic, astringent, antiscorbutic, antibacterial, antiviral and anticonvulsant. Moreover, it has good potential to be used as potent energy source.
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