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Research Article

PHARMACOGNOSTICAL STANDARIZATION AND PHYTOCHEMICAL IDENTIFICATION OF FRUIT AND ROOT OF *CARISSA CARANDAS* LINN.

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ABSTRACT

Objective: *Carissa carandas* Linn is a member of Apocynaceae family and has climbing shrub, usually growing to 10 or 15 feet (3-5 m) high. In Jharkhand, Bihar, Rajasthan and other near states commonly known as 'Karunda'or 'Jasmin flower Carissa' has been proven multipurpose tree. The objective of the research was to study standardization parameters such as Pharmacognostical studies which include macroscopical and microscopic evaluation. Fluroescence analysis, Physicochemical studies were carried out for detection of adulterants and confirm the identity of plant. Total Ash values, extractive values and loss on drying were also determined and recorded.

Methods: Extraction was done by maceration process by using pet. ether (60-80),Chloroform, ethyl acetate, ethanol and water as solvents. Evaluation was done by using different parameters like Fluroescence Analysis, ash value, extractive value, loss on drying and foreign matter.

Results: The *Carissa carandas* unripe fruit and root extract shows the presence of phytoconstituents such as alkaloids, flavonoids, glycosides, reducing sugar, steroids, terpenoids, tannins and saponin and different values of standardization parameters.

Conclusion: Above studied Pharmacognostical, Fluroescence Analysis and Phytochemical parameters are used to check its quality, purity and for its identification that can be use for the purpose of safety monitoring and overall quality assurance of herbal medicines. The present investigation are encouraging and can be used as an effective reference data for the standardization of *Carissa carandas* Linn fruit and roots.

Keywords: *Carissa carandas*, Microscopy, Fluroescence, Physiochemical, Quality.

INTRODUCTION

Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main sources of new pharmaceuticals and healthcare products. The history of plants being used for medicinal purpose is probably as old as the history of mankind. The use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicine. One of the survey conducted by the WHO reports that more than 80% of the world's population still depends upon the traditional medicines for various diseases.

Consumption of various types of fruit provides excellent health benefits because they are a good source of phytochemicals that are good for preventing diseases. The objective of the present study was to generate information about the potential health-enhancing properties of fruits and roots of *Carissa carandas* Linn.

Carissa carandas Linn (F; Apocynaceae) a genus of about 32 species distributed mostly in the warmer parts. Of the 8 Indian species, 3 are of economic importance. The Plant is native and common throughout much of India, Sri lanka, Java, Malaysia, Myanmar and Pakistan. (Karunakar Hegde et. al., 2009) [1]. Its Leaves are simple, opposite, oblong-oval or oblong-lanceolate, subacute at the base, obtuse at the apex, glabrous and thin. The flowers are regular and bisexual. (Jayaweera, DMA et. al., 1991) [2]. The bark light gray, scaly; branch lets usually alternate, with thin stout sharp horizontal glabrous spines 2.5-3.8 cm long at their base. (Kiritikar. K. R., 1980) [3]. The Fruit cluster of 3 to 10 is oblong, broad-ovoid or round, has fairly thin but tough skin, purplish-red turning dark-purple or nearly black and shiny when ripe [4]. It grows from sea level to 6000 feet and requirement is fully exposure to sun. Karunda may bloom and fruit off throughout the year. For use, unripe fruits are collected from mid May to mid July. Ripening season is August to September. Knowledge of chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances [5,6].

Photograph of *Carissa carandas* Linn.

Taxonomy of *Carissa carandas* Linn.

About *Carissa carandas*	
Kingdom- Plant	
Class-	Angispermae
Order-	Gentianales
Family-	Apocynaceae
Genus-	*Carissa*
Species-	*carandas*
Scientific Name-	*Carissa carandas*

Herbarium sheet No. - H-397/CM/D-2007

MATERIAL AND METHODS

Plant fruits was collected in May to July 2011 from Garhawa; Daltanganj and also procured from daily Market, Ranchi (Jharkhand) & Kotputli, Ajmeri Gate Market, Jaipur (Rajasthan). Plant roots were brought from Daltanganj, Department of Horticulture, Palandu, Ranchi (Jharkhand) in June to August 2011. The plant herbarium (Herbarium sheet No.- H-397/CM/D-2007) has been identified by Dr. S. Jha, Professor, Department of Pharmaceutical Sciences, BIT Mesra, Ranchi. Also identified from Department of Botany, University of Rajasthan, Jaipur (Herbarium sheet No.- RUBL 211312). A Set of voucher Specimen made from aerial part of the plant. Plants materials were air dried (Fruit and Roots) and powdered. Crude powder microscopy of fruits and roots was done with the help of high resolution Camera.

Powder Microscopy

Shaded dried root of the plant were powdered with the help of an electric grinder till a fine powder was obtained. Powdered materials of roots were cleared with sodium hydroxide and stained with phloroglucinol; concentrated hydrochloric acid (1:1) and mounted in glycerine medium.

Fluroescence Analysis

The root powder was individually mixed with different solvents like dilute hydrochloric acid, dilute sulphuric acid, 50% nitric acid, 5% potassium hydroxide, 40% sodium hydroxide and acetone. The were observed under the short UV light (254 nm) and long UV light (365 nm) to detect the emission of fluorescence [7,8]. The 25 gm of root powder was exhaustively extracted in a soxhlet apparatus with different solvents like petroleum ether, chloroform, ethyl acetate and ethanol.

Dertemination of Physico chemical parameters

The various physicochemical parameters like ash values and extractive values were determined. They were performed according to the official methods prescribed in standard books [9,10].

Extraction

The dried *Carissa carandas* roots (150gm) and unripe fruits of (250 gm) powder were subjected into hot successive extraction in a soxhelet apparatus with Pet. ether (60-80), Chloroform, Ethyl acetate and Ethanol solvents. The average time period for extraction was 48 hours. The solvents were evaporated with the help of rotary vapor and calculate the yield values of each extracts.

RESULT

Powder Microscopy

The following Microscopical characteristics observed: -

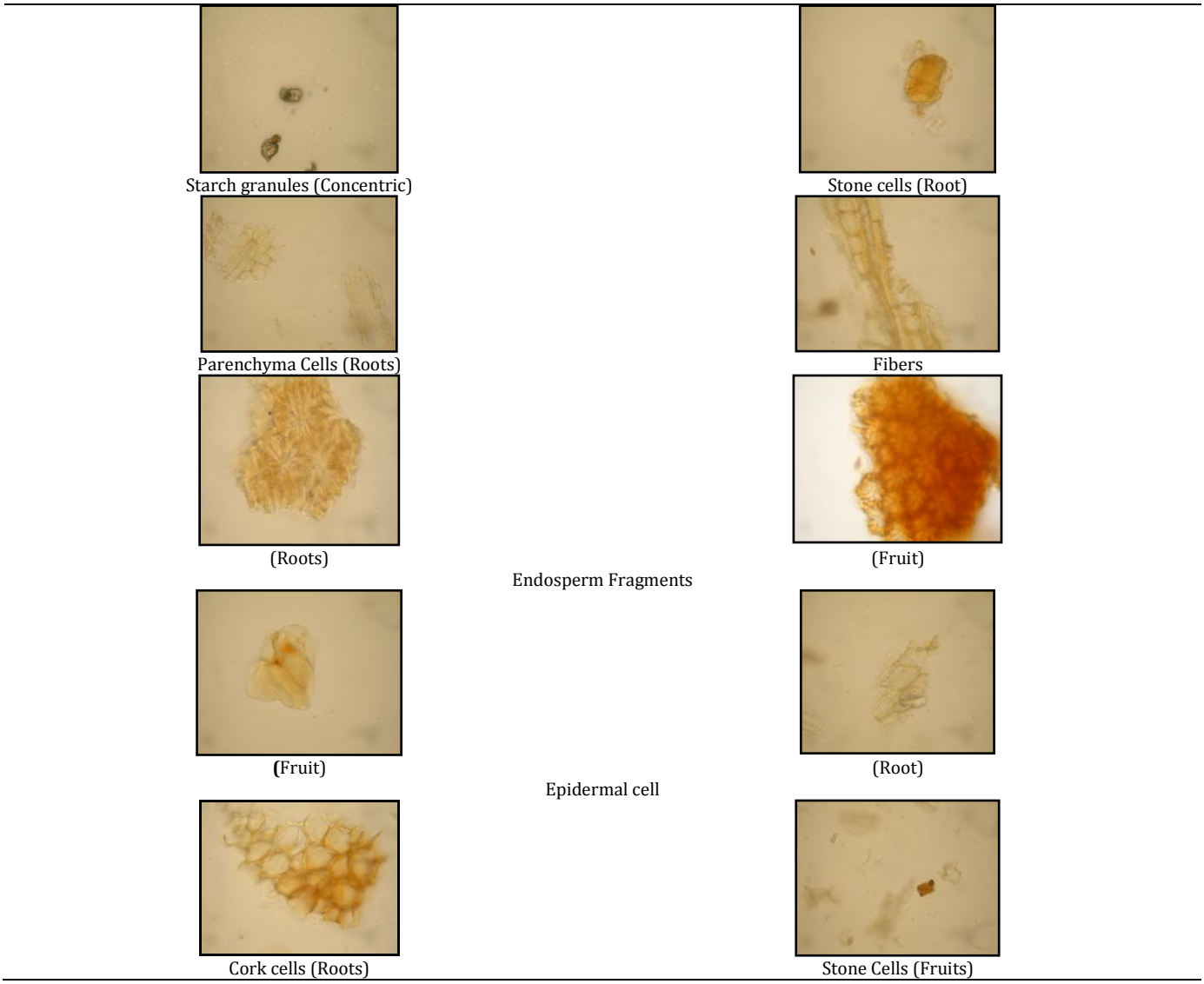

Fig. 1: Microscopical characteristics of fruits and roots of *Carissa carandas* Linn.

Phytochemical Studies

Endosperm- The tissue produced inside the seeds of most flowering plants around the time of fertilization. It surrounds the embryo and provides nutrition in the form of starch, though it can also contain oils and protein. This can make endosperm a source of nutrition in human diet.

Starch Granules- It is found in plant cells as the storage energy molecule. The shapes vary from near perfect spheres to flattened ovoids, elongated disks, polygons and many others. The granule structure is not just a loose agglomeration of glucose polymers. It is systematically structured with the starch molecules oriented in specific spherocrystalline patterns. Plants synthesize glucose which is difficult to store as it is soluble in water. So, glucose is converted into starch which is insoluble in water and can easily be stored in plant cells. Starch is a polysaccharide so many glucose molecules are required to prepare one starch molecule so ultimately many glucose molecules are stored in one starch molecule.

Stone cells- These are more or less iso-diametric, elongated or branched forms. Pitting or stratification may be also observed. They are found in groups, single or complete layers.

Epidermal Cells- It is a simple permanent tissue forming the outermost layer of plant structure and normally only one cell thick. It occurs as surface layer of leaves, flowers, fruits, seeds and younger parts of roots and stems. Its function is to conserve the moisture supply to the inner tissues and protection to extent against mechanical infection.

Parenchyma cells- This is another type of permanent tissues of almost all organs of higher plants. Parenchyma cells are thin-walled polyhedral in shape and with large central vacuole in many cases. The functions of parenchyma cells are storage of food and water, food manufacture, excretion, secretion and also assimilation. Parenchyma cell are alive at maturity.

Fibers- Usually developed in bundles or in layers. Usually thick walled and have a narrow lumen and pointed ends. Walls of the fibers are usually lignified, but may also contain cellulose. Depending upon the seat of their occurrence they are said to be pericyclic fibers, xylem fibers or phloem fibers.

Cork Cells- it is a simple permanent tissues made up of compactly arranged approximately prismatic cells found in radial rows. No intercellular spaces are present between them. It forms the bark of stems or roots. It protective in function and guards against mechanical injury to inner bark or cambium and also prevents loss of water. Protoplasm in the cork cell dies and hence matured cork cells are dead.

The different pharmacognostical characteristics and preliminary phytochemical test that gives the description and confirmation of *Carissa carandas* Linn plants and its parts [11,14].

Table 1: Fluroescence Analysis of fruit extract

Extract	Long UV Light (365 nm)	Short UV light (254 nm)	Day light
Petroleum ether	Light Black	Greenish Black	Light Brown
Ethyl acetate	Black	Brownish Black	Light Brown
Chloroform	Greenish Brown	Light Black	Light Brown
Ethanol	Light Green	Olive Green	Reddish Brown

Table 2: Fluroescence Analysis of root extract

Extract	Long UV Light (365 nm)	Short UV light (254 nm)	Day light
Petroleum ether	Light Black	Greenish Black	Dark Brown
Ethyl acetate	Black	Brownish	Dark Brown
Chloroform	Greenish Brown	Light Brown	Dark Brown
Ethanol	Parrot Green	Olive Green	Reddish Brown

Table 3: Physicochemical Values

Parameters	Values
Total Ash	20 % w/w
Acid insoluble Ash	18 % w/w
Water Soluble Ash	16 % w/w
Alcohol Soluble Extractive	1.2 % w/w
Water Soluble Extractive	2.0 % w/w
Loss on Drying	14%

Table 4: Phytoconstituents present in unripe fruit extracts of *Carissa carandas* Linn.

Phytoconstituents	Petroleum ether Extract (60-80) (PECC)	Chloroform Extract (CECC)	Ethyl Acetate Extract (EACC)	Ethanolic Extract (EECC)
Alkaloids	--	--	+	+
Flavonoids	--	--	+	+
Glycosides	--	--	+	+
Carbohydrates	--	--	--	+
Sterols	+	+	+	+
Terpenoids	+	+	+	+
Tannins	+	+	+	+
Saponin	--	--	--	+

- Absent, + Present

Table 5: Phytoconstituents present in root extract of *Carissa carandas* Linn.

Phytoconstituents	Petroleum ether Extract (60-80) (PECC)	Chloroform Extract (CECC)	Ethyl Acetate Extract (EACC)	Ethanolic Extract (EECC)
Alkaloids	--	--	+	+
Flavonoids	--	--	+	+
Glycosides	--	--	+	+
Carbohydrates	--	--	--	+
Sterols	+	+	+	+
Terpenoids	+	+	+	+
Tannins	+	+	+	+
Saponin	--	--	--	+

- Absent, + Present

Fluroescence Analysis

The Fluroescence Analysis performed for the different extract is tabulated. (Table-1) For the unripe fruit extract of petroleum ether extract, chloroform and ethyl acetate extract produced Colours ranging with in black and green, whereas ethanolic extract of fruits exhibited different colours like light green, olive green and reddish brown colours. Similarly, The Fluroescence Analysis performed for the different solvents extract of roots is similar as like unripe fruits which have been tabulated. (Table- 2) Whereas ethanolic extract of roots exhibited little different colours like parrot green, olive green and reddish brown colour [15].

Physico chemical parameters

The physic chemical parameters like ash values and extractive values are tabulated. (Table- 3) Total ash, loss on drying and water soluble extractive was found to be 20 % w/w, 14 % w/w and 2.0 % w/w which can be assumed as a standard for the drug [16,17].

Phytochemical Screening

Phytochemical Screening was performed for identification or presence of alkaloids, flavonoids, glycosides, carbohydrates, sterol, terpenoids, tannins and saponin etc. (Table- 4 & 5)

DISCUSSION

Microscopical evaluation is indispensable in the initial identification of herbs as well as in detection of adulterants and identifying the plant by characteristic tissue features. In powdered microscopy abundant fibers and parenchyma were seen which has been identifying tool for the root of the plant.

The phytochemical Screening reveals the presence of phytoconstituents like; alkaloids, flavonoids, glycosides, reducing sugar, steroids, terpenoids and tannins which are present in ethanolic extracts of unripe fruits and roots. Alkaloids, flavanoid, glycoside, terpenoid and tannins are present in ethyl acetate extract of unripe fruits and roots, while sterols, terpenoids and tannins are present in both Pet. ether (60-80) and similar for chloroform extract of fruits and roots, which is best proved about the different traditional use and different of pharmacological activities.

Fluorescence analysis of drug extract helps to identify the drug with specific fluorescent colours and also to find out the fluorescent impurities. The study of fluorescence analysis can be used as a diagnostic tool for testing adulteration. The fruits and root powder has exhibited different colour shades in the long and UV light which can be utilized in detecting impurities. In the case of the root extracts the petroleum ether, chloroform and ethyl acetate extract produced similar kind of fluorescent colour but the ethanolic extract produced entirely different fluorescence which can be an effective tool while setting the standards. The significance of performing the ash values is to find out the amount of inorganic impurities, resistant materials like sand, soil and stone in crude drugs. The ash values obtained after incinerating the powdered of fruits and roots is of significance as this usually consists mainly the carbonates, phosphates, silicates and silica.

Standardization is the prime need of time. These help in the establishment of quality and identity profile that can be used for the purpose of safety monitoring and overall quality assurance of herbal medicines. Hence it is very essential to establish a pharmacognostical standardization. The results obtained in the present investigation are encouraging and can be used as an effective reference data for the standardization of *Carissa carandas* Linn fruit and roots.

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