

**Pharmacognostic Standardization and Anti-microbial studies
of dried *Carissa carandas* fruits****N. Anupama¹, G. Madhumitha^{1*}****¹Chemistry of Heterocycles and Natural Product Research Laboratory, Organic
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Abstract: The Pharmacognostic evaluation of the plant material helps to keep a proper documentation about the quality of many important medicinal plants. Our work was focused on the pharmacognostic evaluation, nutritional analysis and to find out the microbial inhibition of the crude extract. The bioactive crude extracts were prepared by the successive extraction methods by using petroleum ether and methanol.

Key Words: Pharmacognostic evaluation, Nutritional analysis, Petroleum ether, Methanol, Bioactive crude extract.

Introduction

The natural drugs obtained from plants play an important role in health care systems especially in the developing countries. The pharmacognostic standardization and physicochemical analysis were very essential enough to decide the authenticity of the plant material which will contribute to its safety and efficacy¹. The search for novel and suitable antibiotic agents from natural origin like plants has been of great interest in the last few decades and the discovery of antibiotics have substantially decreased the rate of public health hazards resulting from the bacterial infections². The various natural products obtained from plant origin were either pure compounds or standardized plant extracts which provide unlimited opportunities for novel and drug treatments because of their unmatched range of chemical diversity. In various cases the world population mainly depends on plants and its extracts due to its safety, their fewer side effects, easy availability and low cost. The natural products from plant origin are extractable and found useful in a number of pharmaceutical preparations³.

The plant of study *Carissa carandas* L. belonging to Apocynaceae family is an evergreen shrub with light grey or yellowish brown bark which is rough and scaly. It is commonly known as karaunda, is a widely used medicinal plant. The fruits, leaves, barks and roots of *C. carandas* have been used for ethnomedicine in the treatment of human diseases, such as, diarrhea, stomachic, anorexia, intermittent fever, mouth ulcer, sore throat, syphilitic pain, burning sensation, scabies, and epilepsy. The fruits are berries, ovoid-oblong or ellipsoidal in shape in size⁴. The chemical constituents present in the species include steroids, terpenes, tannins, flavonoid, benzenoides, phenylpropanoid, lignans, sesquiterpenes and coumarins⁵.

Therefore, the fruit of *Carissa carandas* could be used as a source of functional food material. The physicochemical analysis of dried fruits of *Carissa carandas* including total ash content, water insoluble ash content, acid insoluble ash content and anti microbial activity were carried out⁶.

Experimental

Collection and authentication of Plant material

The *Carissa carandas* fruits were collected from in and around of Vellore District, Tamil Nadu, India during the month of October, 2012. The sample was authenticated by Dr. M. Palanisamy and a voucher specimen was deposited at the Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2013-14/Tech.1119). The fresh fruits of *Carissa carandas* were air dried under shade and pulverized to a coarse powder. This powder was used to carry out the extraction process to obtain the petroleum ether and methanol extract.

Microscopic analysis

The microscopy of the plant was studied according to the method of Brain and Turner^{7,8}.

Physico chemical analysis

The physicochemical parameters of *Carissacarandas* fruit powder such as total ash, acid insoluble ash, and water soluble ash were determined⁹ and solvent extractive index value was determined to find out the amount of solvent soluble compounds in the sample.

Preliminary phytochemical screening

The methanol extract of dried fruit powder was screened for the presence or absence of the major class of compounds by standard method¹⁰.

Preparation of the extract

The powdered sample material(500 g) was defatted with petroleum ether (1000 mL) and then subjected for distillation to get a thick dark green mass called petroleum ether extract (PTE). Then the residue was extracted with methanol (1000 mL) at room temperature for effective extraction. The extract was filtered through a Whatman No 1 filter paper, and the process was repeated. Then concentrate the methanol extract was subjected in a rotary evaporator to yield dark brown mass (30 g) called methanol extract (MTE). This methanol extract was subjected to *in-vitro* antimicrobial activity¹¹.

Anti bacterial studies

Bacterial strains used for the study

The crude methanol extract was studied against two gram negative and two gram positive bacteria for the anti microbial assay. All pathogens used in this study were in ATCC cultures which includes Gram positive organisms *Staphylococcus aureus*(ATCC 33591), *Bacillus cereus*(ATCC 14579) and Gram negative organisms *Salmonellaparatyphi* A (ATCC 9150) and *Proteus vulgaris*(ATCC 6380).

Antibacterial activity

The antibacterial activity was determined by well diffusion method and the bacterial cultures were grown on nutrient agar medium. The culture (24h) of test organisms was swabbed on Muller Hinton agar (MHA). Wells were cut using sterile well borer on the agar surface and fermentation broth (200µl) was added to each well aseptically. The petri dishes were incubated for 24hours at 30°C. The inhibition zones formed were visually detected and measured (mm) after 24 hours¹².

Results and Discussion

Microscopic characteristics

The transverse section of dried fruit of *Carissa carandas* was done. The microscopic method is useful to establish the botanical identification of the source material. The fruits are circular in nature, pericarp consists of epicarpic cells and the outermost region consists of single layer of tangentially elongated cells with thick cuticle; hypodermis consisting of tangentially elongated parenchymatous cells and mesocarp is filled with irregular to elongated parenchymatous cells with numerous branching laticiferous tubes. Innermost layer of thin

walled tangentially elongated cells were seen in endocarp. Transverse section and powder microscopy of dried fruit¹³ is showed in Figure 1 and 2 (A, B, C, D)



Figure 1: Transverse section of *Carissa carandas* fruit

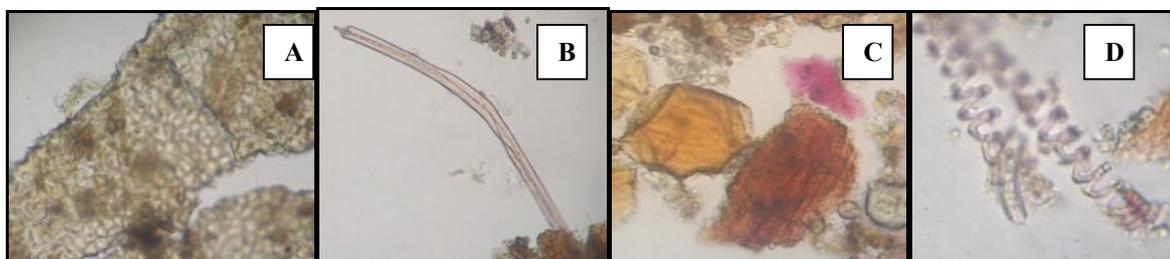


Figure 2: Powder microscopy of *Carissa carandas* fruit powder, A- Epicarpic cells, B- Fibre, C- Vessel, D- Vascular elements

Physico chemical analysis

The physical constant evaluation of the drug is an important parameter. The total ash was approximately 2 to 4 times more than acid insoluble and water soluble ash respectively. The *Carissa carandas* dried fruit powder showed the presence of total ash of 5.05 % w/w, acid insoluble ash of 9.17 % w/w, and water soluble ash of 0.74 % w/w (Table 1). The extractive index values of petroleum ether, ethyl acetate, chloroform and methanol are 10, 4.25, 1.95 and 1.54 % respectively as shown in Table 2 and Figure 3.

Table 1: Physicochemical analysis of dried fruits of *Carissa carandas*

Physicochemical Parameters	Value (% w/w)
Total ash	5.05
Acid insoluble ash	9.17
Water soluble ash	0.74

Table 2: Extractive index values of different extracts by various solvents

S. No	Extractive Value	Percentage of extract(%)
1	Petroleum ether	10
2	Ethyl acetate	4.25
3	Chloroform	1.94
4	Methanol	1.54

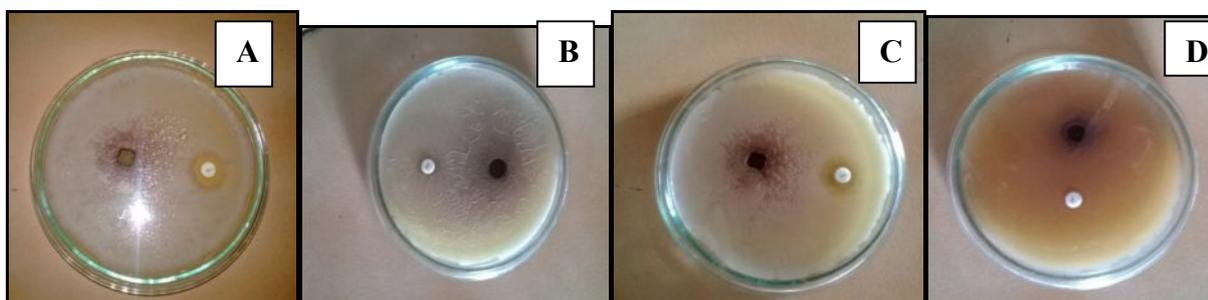
Table 3: Physicochemical analysis of dried fruits of *Carissa carandas*

Physicochemical Parameters	Value (% w/w)
Total ash	5.05
Acid insoluble ash	9.17
Water soluble ash	0.74

**Figure 3: Successive extraction of the sample with various solvent systems**

Anti bacterial studies

The methanol extract exhibited moderate antibacterial activity against *Bacillus cereus* (16mm), *Salmonellaparatyphi* A (15mm) followed by *Proteus vulgaris*(15.7mm) and *Staphylococcus aureus*(14.6mm).The standard antibiotic streptomycin disc (25 μ g) was used as positive control¹⁴. The results were showed in **Table 3& Figure 4 (A,B,C,D)**.

**Figure 4 (A, B, C, D): Anti bacterial studies of crude extract by well diffusion method****Table 3: Anti-bacterial activity of dried fruits of *Carissa carandas* methanol extract against standard bacterial pathogens**

Bacterial pathogens	Methanol extract (75 μ g/ml) Zone of inhibition (mm)
Gram positive bacteria:	
<i>Staphylococcus aureus</i> (ATCC 33591)	14.6 \pm 0.0
<i>Bacillus cereus</i> (ATCC 14579)	16 \pm 0.85
Gram negative bacteria:	
<i>Salmonella paratyphi</i> A (ATCC 9150)	15 \pm 0.38
<i>Proteus vulgaris</i> (ATCC 6380)	15.7 \pm 1.54

Conclusion

The present study testifies to supplement the information regarding its standardization and identification and in carrying out further research and its use in traditional system of medicine. The

pharmacognostic parameters of fruits were carried out and this will enable to identify, evaluate the purity of the crude drug. In conclusion, the parameters reported here can be taken as distinctive enough to decide the authenticity of this drug. The methanol extract of dried fruits of *Carissa carandas* was subjected to anti bacterial studies and it showed moderate activity to the organisms.

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