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Pharmacognostic and Phytochemical Investigation of the seed of Manilkaraobovata

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Abstract: *A dried powdered seed of Manilkaraobovata was analysed for chemical constituents, mineral elements and vitamin compositions. Some analytical profile for the powder like total ash, acid insoluble ash, sulphated ash, water and alcohol extractive values were equally determined. The analytical standardization of the powder showed the total ash, acid insoluble ash and sulphated ash values to be 5.5, 1.0 and 30.5% respectively. The alcohol and water extractive values were 10.0 and 13.8% while the water content was 11.0%.Vitamins E and C were 3.70 and 18.78 mg/100g of powder while β-carotene was 16.24 IU. The antioxidant mineral element compositions (mg/100g) were zinc (34.11), copper (4.71), manganese (7.89) and selenium (1.20). The chemical classes of constituents present in the powder include alkaloids, flavonoids, phenolics, steroids, saponins, pseudotannins, tannins, triterpenes and volatile oils. The high levels of the antioxidant mineral elements and vitamin including flavonoids suggest that the powder may be a good antioxidant.*

Keywords: *Manilkaraobovata, vitamins, mineral elements, phytocompounds, antioxidants*.

I. Introduction

Standardization of plant products which eventually enter the commercial market is of considerable importance. This involves determination of quality parameters for them. This analysis becomes very necessary in view of the indiscriminate manner in which the unskilled herbalist, whose primary purpose is gain, adulterates herbal products.

Manilkaraobovatais widely distributed in tropical Africa (Angola, Benin, Democratic Republic of Congo, Ghana, Uganda, United Republic of Tanzania, and Zambia). It is found in lowland, riverine and ground water forest. The tree grows up to 14m or more. It has a pale grey/ dark brown bark. The leaves are simple, alternate and cluster at ends of branches. The petiole measures 0.5-1.8 cm. The lamina, which is obovate-obong measures 3-10 cm by 1.6-5.5 cm, with the lower surface being hairy whenyoung. Flowers clustered in leaf axils, with pedicels 1 cm long. Calyx is 5 mm long. The flower is white with fascicles in axils of older or fallen leaves. The yellow fruit, which is obovoid to subglobose, measures up to 2-5 cm long. The seed is 1 cm long. The wood is hard and is used for timber, building and carving. The fruits are edible. The fruit and stem bark are used as spice for cooking. The plant is used in traditional medicine for treatment of cardiovascular disorders (Hemsl 1963; Haragu Chi, et al 2003).

This work determined the mineral element and vitamins related antioxidants in powdered seed of Manilkaraobovata. It went further to determine some analytical standards like total ash, acid insoluble ash values for the powder. The chemical classes of constituents present in the powder were determined.

II. Materials and Methods

Plant: The seeds of Manilkaraobovatawere purchased from Onueke market, Abakiliki, Ebonyi State. The seeds were authenticated by Mr. A. Ozioko of the Bioresources Development and Conservative Programme, Nsukka, Enugu State. The photography of the seed was taken and developed. The voucher specimen of the seeds is deposited at the Herbarium of the Department of Pharmacognosy, University of Nigeria, Nsukka. The seeds were pulverized and stored in a cool dried air-tight container.

Reagentsandinstrument: The reagents were sourced commercially and were used as supplied. Ethanol, sodium thiosulfate, 2,6-dichlorophenol indophenol, hydrochloric acid, sulfuric acid were products of Sigma Aldrich, Germany. Other standard laboratory reagents were used. PyUnican Spectrometer, England was used to measure absorbances.

Phytochemicaltest: The chemical classes of constituents present in the powder were detected following standard procedures (Harborne, 1998; Sofowara, 1982; Cook, 1961). The classes tested for include phenolics, triterpenes, sterols, alkaloids, tannins, pseudotannins, saponins, flavonoids, glycosides and volatile oils.

Determination of some pharmacognostic parameters for the powder: Analytical standards determined include total ash, acid insoluble ash, water soluble ash, sulphated ash, alcohol and water extractive values. The moisture content of the seed was also determined by loss on drying method (Brain and Turner, 1975). The standardization parameters were determined following the methods outlined in British pharmacopoeia (1988).

Determination of some antioxidant vitamins: β-carotene, vitamins E and C were determined. Furter-Meyer method was used in the determination of Vitamins E (Jakulowicz, et al, 1997). One gram of the powdered seed was weighed into 100 ml flask fitted with reflux condenser containing 10 ml ethanol and 20 mlethanolic sulfuric acid. The mixture was refluxed for 45 minutes, cooled and 50 ml of water added. The mixture was separated with a separating funnel and the unsaponifiable matter extracted with diethyl ether. The diethyl ether extract was washed with water and dried with anhydrous sodium sulphate. It was evaporated to dryness and dissolved in ethanol. Different concentrations of the pure vitamin E were prepared in ethanol. One milliliter of nitric acid was added to known volumes of each of the solution and incubated for 3 minutes at 90℃, cooled and the absorbances measured at 470 nm against a blank of 5 ml ethanol and 1 ml of nitric acid treated in a similar manner.
Vitamin C was determined using dichlorophenol indophenols visual titration method (Beisy, et al 1946). β-carotene was determined using adsorptiometric method (Beisy, et al,1946).
Determination of some mineral elements: A 10 g of powdered seed was incubated with a mixture of nitric acid and perchloric acid for 12 hours – a method known as wet digestion (Harris, 1996). The incubated material was extracted with 30% HCl and diluted to 1000 ml with 30% HCl. Selenium was determined by inductively coupled plasma emission (Kenkel, 1991). Different standard solutions of zinc were prepared. To a known volume of the standard solution was added dithizone solution, sodium thiosulfate solution, mixed thoroughly and absorbance measured at 535 nm against a blank of mixture of sodium thiosufate and dithizone solution. Manganese and copper were determined colorimetrically using persulfate and neocuproine methods, respectively (Parkinson and Allen, 1975; Miller and MeFee, 1983; Jackson, 1967)

Phytochemical test: The chemical classes of the compound present in the extract were determined following standard procedure (Harborne,1998). The classes tested for include phenolics, triterpenes, sterols, alkaloids, tannins, saponins, flavonoids and volatile oils.

III. Results and Discussion

The seeds ofManilkaraobovataare shown as fig 1. The powder was faint yellow in colour with sweet smell. The smell lasted for 24 hours when touched. The pharmacogonstic standards are shown as table 1 while mineral elements and antioxidant vitamin compositions are shown as tables 2. The percentage yields of the successive extracts are given in table 3. The powder was found to contain triterpenes, steroids phenolics, pseudotannins, saponins, flavonoids, volatile oil, alkaloids, tannins, and glycosides.

Fig 1. Seeds of Manilkaraobovata

Table 1: Pharmacognostic Parameters of ManikaraobovataSeed

S/N	Pharmacognostic parameters	Percentage value (w/w) %
1.	Total Ash	5.5
2.	Sulphated ash	30.5
3.	Acid-insoluble ash	1.0
4.	Moisture content	11.0
5.	Water extractive yield	6.9
6.	Alcohol extractive yield	5.0

Table 2: The mineral element and vitamin composition of seed

S/N	Parameter determined	Composition (mg/100g)
1.	Vitamin E	3.70
2.	Vitamin C	18.78
3.	β-carotene	16.24 IU
4.	Zinc	34.11
5.	Copper	4.71
6.	Manganese	7.89
7.	Selenium	1.20

Percentage Yield

The percentage yield of the successive solvent fractions are tabulated in Table 3. The highest yield was observed for methanol (35%).

Table 3: Percentage yield of successive extract

S/N	Extract	Percentage yield %
1.	Methanol Extract	35
2.	Pet Ether	7.6
3.	F1 Fraction (Chloroform Extract)	3.7
4.	F2 Fraction (Acetone-HCl extract)	4.0

Table 4: Preliminary phytochemical profile of Manikaraobovata

S/N	Phytochemical Constituents	Petroleum ether	methanol	F_1	F_2
1.	Triterpenes	+	-	-	-
2.	Steroids	+	-	-	-
3.	Phenolics	-	+	+	+
4.	Pseudotannins	-	+		+
5.	Flavonids	+	+	+	+
6.	Volatile oil	+	-	-	-
7.	Alkaloids	-	+		+
8.	Tannins	-	+	+	+
9.	Glycosides	+	+	+	+
10	Saponins	-	+	+	+

Key: + means present while – means absent
F1 Fraction (Chloroform Extract; F2 Fraction Acetone-HCl extract

The sweet scent of the powder can be found valuable in flavouring of food, drugs or even use in perfumery. The pharmacognostic standards can used in identification and subsequent evaluation for quality and purity of the drug. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components. The high difference between the total ash and acid–insoluble ash indicates moderate level of the mineral elements and less adhesion of poisonous earthy matters. The moisture content (11.0%) which is relatively small showed that there is less chance for microbial degradation of the seed during storage, as the general requirement for moisture content in crude drug is not more than 14% w/w (African pharmacopoeia, 1986) and that the seed can be stored with little or no drying. They were found to contain many classes of phytocompounds which the methanol extract had the highest which include phenolics, saponins, flavonoids, alkaloids, tannins, pseudotannins, and glycosides, while the petroleum ether extract had triterpenes, steroids, volatile oils and glycosides**.** These secondary plant metabolites are known to possess various pharmacological effects. Some of these phytocompounds like phenolics and flavonoids have been implicated in redox reaction. The powder can be used as antioxidant. Okafor et al (2015) showed that the seed of M. obovate has both antioxidant and hepatoprotective activities. Saponins possess a carbohydrate moiety attached to a triterpenoid or a steroidal aglycone. Saponins form a group of compounds, which on consumption causes deleterious effects such as heamolysis and permeabilization of the intestine (Cheeke, 1996; Price et al., 1987). Saponins have also been shown to have hypocholesterolemic as well as anticarcinogenic effects (Koratkar and Rao, 1997). The cholesterol lowering effect in animals and

humans is reported to be through the formation of mixed micelles and bile acids into micellerbile acid molecules (Okenfull et al., 1984).

Vitamins C, E and beta-carotene are well known antioxidants and were found in moderate quantities in the powdered seed. Supplementing with those vitamins antioxidants for 2 weeks increased the glutathione concentration of the blood by 50% (Johson, et al, 1993). This glutathione is one of the body's most important antioxidant. M. obovatacontained appreciable amount of Ascorbic acid. Lack of ascorbic acid impairs the normal formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine. A striking pathological changes resulting from this defect is the weakening of the endothelial wall of the capillaries due to a reduction in the amount of intracellular substance. Consequently, the clinical manifestation of scurvy from mucous membrane of the mouth and gastrointestinal tract, anemia, pains in the joints and defect in skeletal calcification can be related to the association of ascorbic acid and normal connective tissue metabolism (Hunt et al., 1980). These functions of ascorbic acid also accounts for its requirement for normal wound healing. Ascorbic acid is essential to prevent diseases associated with connective tissue and to improve the immune functions (Zhao, 2007)

The antioxidant related mineral elements (table 2) were found in reasonable quantities. These metals have been confirmed to contribute to the building of the body's antioxidant. For example, Selenium is incorporated to proteins to form selenoproteins, which helps prevent cellular damage from free radicals. Manganese, zinc, and copper are building blocks for superoxide dismutase and selenium for glutathione peroxidase, which are natural antioxidants.

And as antioxidants, they strengthen the immune system as antioxidants (Talwar et al., 1989). Also, magnesium, zinc and selenium are also known to prevent cardiomyopathy, muscle degeneration, growth retardation, alopecia, dermatitis, immunologic dysfunction, gonadal atrophy, impaired spermatogenesis, congenital malformations and bleeding disorders (Chaturvedi et al., 2004).

IV. Conclusion

Seeds of Manilkaraobovatacan found wide applications in food and drug industries as flavour. It contains a reasonable phytocompounds, mineral elements and vitamins and can be used as an antioxidant.

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