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PHYSICOCHEMICAL AND PHYTOCHEMICAL EVALUATION OF *Moringa oleifera* POD AND SEED

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder without a known care hence the need for discovery of therapies for its treatment. The present study was under taken to investigate the physical properties and secondary metabolites in *Moringa oleifera* pod and seed with a view to identify the bioactive compounds with antidiabetic potentials in them. The extraction of *Moringa oleifera* pod and seed separately was carried out via maceration using 70% methanol. Fractionation of each macerate was done by liquid- liquid partitioning using solvents of varied polarity. Physicochemical properties and phytochemical screening were evaluated using standard laboratories procedures. The total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive value, water-soluble extractive value and moisture content were 6.5%, 3%,1%,3.2%4.2% and 5% for the pod and 3.0%,1%,0.5%,3.4%,4.2% and 5.3% respectively. The qualitative phytochemical screening revealed the presence of alkaloids, cardiac glycosides, flavonoids, glycosides, steroids, tannins and triterpenes in the 70% methanol extract and some of its fractions. Alkaloids, carbohydrates, cardiac glycosides, flavonoids, glycosides, saponins, steroids, tannins and triterpenes were present in the 70% methanol extract of the seed and some of its fractions. Anthraquinones (free and combined) and phlobatinnins were absent in the various extracts of the pod and seed. The result of the study shows that *Moringa oleifera* pod and seeds contain some of the phytochemicals with reported anti-diabetic properties. The presence of these bioactive compounds give credence to its use for management of diabetes mellitus in the rural areas in Nigeria. *Moringa oleifera* fruits can be explored for development of nutraceuticals with pure phytochemicals for management of diabetes mellitus.

Keywords: Diabetes mellitus, nutraceuticals, Phytochemicals.

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INTRODUCTION

Man has relied so much on medicinal plants for health and food needs. The traditional use of medicinal plants both physically and spiritually for curing, preventing illnesses and for promoting wellbeing among human beings has a long historical record. Diabetes mellitus is a metabolic disorder which has no known cure (Lucy *et al.,* 2002). The increase in prevalence rate of diabetes mellitus in developing countries follow the trend of urbanization and lifestyle changes including high consumption of "Western-diet" (Wild *et al.,* 2004). This alarming increase is a great cause for concern. There are different modes of managing the disease; diabetes mellitus. Conventional medicines, dietary therapy and herbal preparations are used for managing this disease. A lot of side effects have been associated with the orthodox medicine, search for alternative medicine with no/less adverse effects among medicinal plant is on-going worldwide.

Moringa oleifera is the most widely cultivated species of the genus Moringa which is the only genus in the family Moringaceae. Its common names include Moringa, Drumstick tree, Horseradish tree, Ben oil tree. In Nigeria, it is known in Hausa language as zogalla, Odudu oyibo, and in Yoruba language, it is known as ewe igbale (Duke, 1982). *Moringa oleifera* has numerous medicinal uses which have long been recognised in Ayurvedic medicine and others system of alternative medicine. This study investigated phytochemical and physicochemical evaluation of *Moringa oleifera* as a preliminary study to identify secondary metabolites in various extract of the pod and seed with emphasises on those with antidiabetic potential.

MATERIALS AND METHODS

Plant material

The fresh fruits (pods with the seeds inside) of *Moringa oleifera* were collected from Karraworo District of Adavi Local Government Area of Kogi State in the month of January 2016. The plant was identified and authenticated with an authentic voucher of *Moringa oleifera* (Voucher number 197) deposited in the herbarium of Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

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Plate 1: *Moringa oleifera* fresh pods, dried pods, and powdered pods.

Plate 2. *Moringa oleifera* dried seeds, and powdered seeds.

Reagents and Chemicals

All chemicals used in this study were of analytical grade, products of Guangdong Guanghua Sci-Tech Co. LTD, China and British Drug House chemicals limited, Poole, England.

Extraction Procedure

Four hundred grammes of powdered sample of moringa pods and seeds were separately extracted by maceration using seventy percent methanol for 4 days. The extracts was concentrated at low-temperature(50-60 ^{0}C).The concentrates were then solubilized in distilled water(100g in 1 litre) and exhaustively partitioned using solvents of

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varied polarity (n-hexane, chloroform, ethyl acetate, and n-butanol).The residual fraction(mother-liquor) and other fractions obtained were concentrated. The percentage yield (w/w) of each extract was calculated in terms of the weight of initial air-dried powdery plant material**.**

Physicochemical Analysis

Powder samples of *Moringa oleifera* pod and seed were analysed for physicochemical parameters such as total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive value, water-soluble extractive value and moisture conten t(% loss on drying) using standard laboratory procedures (Yarnalkar, 1991; Khandelwal, 2004; Kar, 2005).

Phytochemical Screening

Qualitative procedures were adopted for phytochemical screening of *Moringa oleifera* pod and seed (Klyne, 1970; Dominguez, 1973; Harbone, 1973; Brain & Turner, 1975; Trease & Evans, 1983; Sofowora, 1993; Evans, 2002).

RESULTS

The percentage value of physicochemical parameters analyzed are presented using bar charts in figure 1 and 2.The values are mean of three determinations. The percentage yields of crude extract, n-hexane, chloroform, ethyl acetate, n-butanol and aqueous fractions are 12.56, 0.47, 1.12, 0.67, 2.66, 4.99 for the seed and 12.91, 0.85, 0.68, 0.78, 1.64, 8.45 for the pod. The phytochemical screening of *Moringa oleifera* fruit (pod and seed) revealed the presence of alkaloids, carbohydrates (reducing sugars) cardiac glycosides, glycosides, flavonoids, steroids, tannins, and triterpenes as shown in table 1 and 2. Anthraquinones and phlobatannins were absent.

Figure 1. Physicochemical properties of Moringa oleifera pod.

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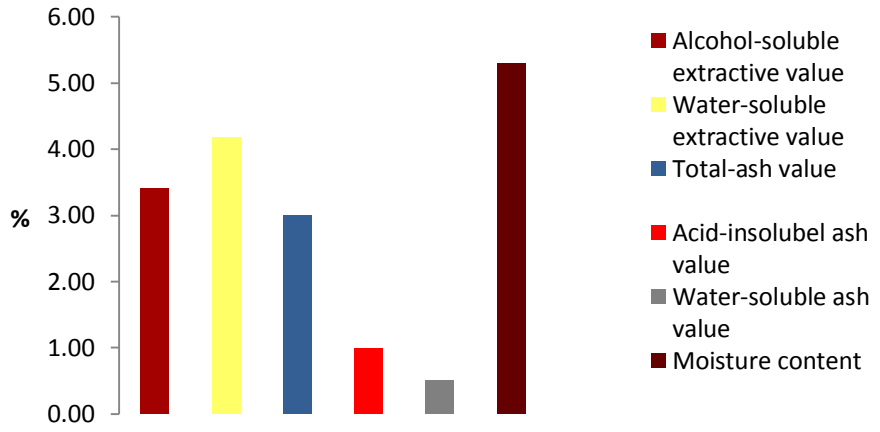

Fig 2. Physicochemical properties of *Moringa oleifera* seed.

Table 1. Phytochemical Screening of various Extracts of *Moringa oleifera* pod.

Phytochemical Constituent	Crude Extract	n-hexane Fraction	Chloroform Fraction	Ethyl acetate Fraction	n-butanol Fraction	Aqueous Fraction
Alkaloids	+	-	-	+	-	+
Anthraquinones (Free)	-	-	-	-	-	-
Anthraquinones (combined)	-	-	-	-	-	-
Cardiac glycosides	+	+	-	+	+	+
Carbohydrates (Reducing Sugar)	+	-	-	-	+	+
Flavonoids	+	-	-	+	+	+
Glycosides	+	-	+	+	-	-
Phlobatannins	-	-	-	-	-	-
Saponins	-	-	-	-	-	+
Steroids	+	+	+	+	+	+
Tannins	+	-	-	+	+	+
Triterpenes	-	+	-	-	-	+

+ indicates presence, - indicates absence.

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Table 2. Phytochemical Screening of various Extracts of *Moringa oleifera* seed.

Phytochemical Constituent	Crude Extract	n-hexane Fraction	Chloroform Fraction	Ethyl acetate Fraction	n-butanol Fraction	Aqueous Fraction
Alkaloids	+	-	+	+	+	+
Anthraquinones (Free)	-	-	-	-	-	-
Anthraquinones (combined)	-	-	-	-	-	-
Cardiac glycosides	+	+	+	+	+	+
Carbohydrates (Reducing Sugar)	+	-	+	+	+	+
Flavonoids	+	-	-	+	+	+
Glycosides	+	+	+	+	+	+
Phlobatannins	-	-	-	-	-	-
saponins	+	-	-	+	+	+
Steroids	-	+	+	+	-	-
Tannins	+	-	-	+	+	+
Terpenoids	+	+	+	+	+	+

+ indicates presence, - indicates absence

DISCUSSION

The ash content of plant-inclined drug is generally taken as the residue after incineration. The total ash content of the pod and seed is 6.5 and 3.0% respectively, indicating the pod is richer in inorganic matter than the seed. The toal ash represents the inorganic salts naturally occurring in the drug and adhering to it. Its determination gives a basis for judging the identity and adulteration of inorganic matter in a vegetable drug. The amount of the inorganic matter that is insoluble in acid is less than half of the total ash in both pod and seed. Acid insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the water soluble portion of the total ash (Kokate et al., 2007; Evans, 2002). Obtained results, shows that the amount of water soluble ash is less than that of acid insoluble ash The ash content gives an idea about the quality of the plant-inclined drugs been assessed. The moisture

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content (percentage loss on drying) of the powdered pod and seed of *M.oleifera* were found to be 5 and 5.3% respectively (%w/w) which shows that they contain little amount of moisture and other volatile matter, the drying process used can be described as effective. Moisture content determination is important for the plant drugs because insufficient drying may lead to possible enzymatic deterioration of active principles (Kokate et al., 2007).

Extractive values are useful for the evaluation of plant-inclined drugs, they give informations about the nature of its constituents. The water extractive value of both the pod and seed of *M.oleifera* were found to be higher than that of the alcohol extractive value which indicates the presence of more water soluble components such as sugar, acids and inorganic compounds. The alcohol soluble extractive value of pod and seed was found to be 3.4 and 3.2% respectively indicating the presence of polar constituents like alkaloids, glycosides, flavonoids, phenols, and steroids. The extractive values recorded for polar extracts further indicated that *M.oleifera* pod and seed are richer in polar constituents. Correct identification and quality assurance of the starting materials is an essential pre-requisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy.

Phytochemicals are natural bioactive compounds often referred to as secondary metabolites present in plants. These bioactive compounds have been search targets for extraction and development of new drugs. Diabetes is a major challenge to World health. The number of people living with diabetes has nearly quadrupled since 1980 to 422million adults, with most living in developing countries. Diabetes prevalence has been rising more rapidly in middle- and low-income countries including Asia and Africa where most patients will be found by 2030(WHO, 2016). This rapidly increasing prevalence is a significant cause of concern. The disease is estimated to be 9% among adults aged more than 18years, hence its economic impact is huge (WHO, 2016).

Screening of the seed of *M.oleifera* revealed glycosides, cardiac glycosides, triterpenes and alkaloids (except in the n-hexane fraction). Polar fractions contain tannins, saponins and carbohydrates in the form of reducing sugars. Steriods are present in all extracts of *M. oleifera* pod. Flavonoids, tannins, cardiac glycosides in the polar fractions. Steroids and terpenes occur free, but most are found as glycosides in plants. Steroidal and triterpenoid glycosides collectively referred to as saponins are known to possess potent hypoglycaemic and hyperglycaemic activity (Rao & Gurfinkel, 2000; Mbaze *et al.,* 2007; Ragasa, Tsai, & Shen, 2009; Narender *et al*., 2009). Triterpene saponins are frequently the object of phytochemical and pharmacological investigations. The curative potential of triterpenes is very high yet still poorly recognized. Numerous *in vitro* and *in vivo*

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studies have revealed their multidirectional properties: anti-cancer (Laszczyk, 2009), antioxidant (Ramachandran & Prasad, 2008), anti-inflammatory (Yasukawa *et al.,* 1996), and hypolipidaemic (Perez & Vargas, 2002), among others.

Flavonoids represnt another group of bioactive compounds with antidiabetic potentials. Reports of such activities have been documented by some researchers across the world (Walter-Law *et a*l., 2002; Vessal, Hemmati, & Vasei, 2003;Jayaprakasam, Vareed, Olson, & Nair, 2005; Tsuda, Ueno, Yoshikawa Kojo, &Osawa, 2006; Gaikwad, Mohan, & Rani, 2014). Moringa oleifera is widely known as a miracle tree basically because ever portion of the tree is considered medically useful. This study highlights phytochemicals in various extract of the seed and pod of *Moringa oleifera*. The polar extracts of the seed and pod should be explored as potential sources of secondary metabolites for the treatment of diabetes mellitus.

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