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EVALUATION OF THE PHYTOCHEMICAL COMPOSITION OF MANGO (*MANGIFERA INDICA LINN*) STEM BARK AND LEAVES

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ABSTRACT

The phytochemical contents and medicinal values of *Mangifera Indica* stem barks and leaves were investigated. The results showed that the plant parts contain the presence of bioactive compounds comprising alkaloids (0.84 – 9.66 mg 100 g $^{-1}$), flavonoids (6.86 – 11.24 mg 100 g $^{-1}$), saponins (3.22 – 8.48 mg 100 g $^{-1}$), tannins (0.09 – 0.75 mg 100 g $^{-1}$) and phenols (0.09 – 0.75 mg. 100 g $^{-1}$). The plant also contains water soluble vitamins comprising ascorbic acid (7.92 – 29.92 mg. 100 g $^{-1}$), riboflavin (0.08 – 0.09 mg. 100 g $^{-1}$), niacin (0.55 – 0.75 mg 100 g $^{-1}$) and thiamine (0.02 – 0.45 mg. 100 g $^{-1}$). Both plant parts are good sources of minerals such as Ca, Mg, K, Zn, Cu and Cd. These results showed that extract from mango (*Mangifera indica)* stem barks and leaves may have antioxidant, anti-inflammatory and immunomodulatory functions.

Key words: *Mangifera indica*, Bioactive compounds, Antioxidant, Anti-inflammatory, Immunomodulatory, Pharmaceutical agents.

INTRODUCTION

There has been a tremendous pressure on medicinal plants for their extensive utilization as sources of raw materials for the pharmaceutical industries. Demands for medicinal plants are rapidly increasing not only in developing countries but also in the developed ones. Medicinal plants have various effects on living systems. Some are sedatives, analgesic, antipyretics, cardio-protective, anti-inflammatory, antioxidants, antispasmodics, and immunomodulatory functions.

The research for the novel compounds effective against plasmodium strains resistant to widely used synthetic drugs, coronary arteriosclerosis, cardiovascular,

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neurological, endocrine, respiratory, immune and self-immune, ischeamia, gastric disorder, tumor progression and carcinogensis has led to increased interest in new and existing information about the remedies of these diseases from natural sources [1-5]. Plants form the major part of treatments used by traditional healers in many societies.

The mango tree (*Mangifera indica* Linn Anacardiaceae) is naturalized in West Africa. The tree originated from India to West Africa[6, 7]. The bark and leaves have astringent prosperities and are used in Nigeria as lotion to relieve toothache, sore gums, sore throat or as an infusion in malaria, diarrhea and dysentery treatment [4,7]. All the organs of the plants are rich in tannins and flavonoids[5,7]. In the leaves of the West African species, four anthocyanidins (3 – monosides of delphinidin, paeonidin, and cyanidin) leucoanthocyanins, catechic and gallic tannins, mangiferin (flavoric heteroside), kaempferol and quercitin (both free and glycosides) were reported to be phytoconstituents of West African species[7]. The mango tree is rich in phytochemicals, which are vital in health promotion, disease prevention and drug production. Phytochemicals act as antioxidant, stimulate the human system, induce protective enzymes in the liver or block damage to genetic materials[8, 9]. Phytochemicals exhibit a wide range of biological functions due to their antioxidant properties. Several types of polyphenols (phenolic acid, hydroly-sable tannins and flavonoids) show anti-carcinogenic and anti-mutagenic effects[8-11]. Polyphenols interfere in many steps of malignant tumors, inactivating carcinogens, inhibiting the expression of mutagens and the activity of enzymes involved in the activation of procarcinogens. Polyphenols activate enzymatic systems, which are responsible for the detoxification of xenobiotics [8-10]. Polyphenolic flavonoids inhibit the initiation, promotion and progression of tumors[8]. Recently, plant flavonoids have attracted the attention of researchers as potentially important dietary cancer chemo-protective and preventive agents[12]. Naturally occurring flavonoids are potentially antiallergic, anticarcinogenic, anti-viral and antioxidant[13]. Phytochemicals regulates, protects and control prostrate and testicular cancer and semen quality in men[14]. Isoflavones, which are effective phytoestrogens modulate estrogen levels in humans. They are of clinical value in low astrogens states like menopause, or imbalanced and toxic estrogen – sensitive conditions such as breast, uterine and prostrate tumor growth[15, 16]. Isoflavones prevent breast cancer, cystic ovaries and endometriosis among women[14].

Mango extracts from leaves, fruit, seed kernel, fruit pulp, roots, bark and stem bark have been used extensively for medicinal purposes in many countries[5]. The ethnomedical use of mango stem bark aqueous extract in Cuba has been documented widely[5]. It has been extensively used in cancer, diabetes, asthma, infertility, lupus, prostatisis, prostatic hyperplasia, gastric disorders, arthralgies, mouth sores and tooth pain[5].

The phytochemical profile of mango stem barks has been reported[5]. Mangiferin is the major component in mango stem bark. Other flavonoids and flavonol constituents include quercetin, catachin and epicatechin[5] (Fig. 1). The phytochemical screening of the raw material showed that polyphenols, terpenoids, sugars and saponins were present in mango stem bark. Many phenolic constituents, benzoic acids and its propylester (Fig. 2), three free sugars (galactose, glucose and arabinose) and three polyalcohols (sorbitol, myoinositol and xylitol) were identified and tested from the mango fruits and stem bark related constituents of mango stem barks. Volatile components of mango stem bark were extracted, and the major constituents identified were β-relemens, aromandrene, α-guaiene, β-endesmol, β-sitrosterol and β-campesterp (Fig. 3). The amount of sesquiterpenoid hydrocarbons was higher than the oxygenated compounds. The composition of the fatty acid fraction was also determined as their trimethylsilyl esters derivatives and the major constituents were palmitic, oleic and linoleic acids with a minor proportion of myristic and estearic acids [5]. Many polyunsaturated and dicarboxylic acids of biological importance such as eicosatriencoic, succinic and malonic were found to be present in mango stem bark in trace levels [5].

Mangifera indica stem bark has antioxidant, anti-inflammatory and immunoduletory properties and have been developed in Cuba as nutritional supplement or functional food in several formulations (antioxidant) or anti-inflamatory, analgesic and immunomodulator (tablet, capsule, syrup and cream) and have been extensively used to prevent disease progress or improve the patient's quality of life in diseases like HIV/AIDS, cancer, asthma, gastric and dermatological disorders[5].

The present investigation was undertaken to quantify the percentage constituent of crude phytochemicals, vitamins and minerals present in the leaves and stem bark of *Mangifera indica* and consequently assess the potential raw materials for drug formulation.

EXPERIMENTAL

Materials and method

The experiment was carried out in the Department of Chemistry. Michael Okpara University of Agriculture, Umudike, Nigeria. Fresh leaves and stem barks of *Mangifera indica* Voucher No. *MF201* was collected from Ekwelu Village, Ariam Usaka Ikwuano Local Government, Abia State, Nigeria. The plant was botanically identified by Dr. A. Nmeregini of the Taxonomy unit of the Department of Forestry of this University. The stem bark and leaves were dried at the laboratory bench of 28^{o}C for two weeks. The stem

bark and leaves were each weighed to 500 g and separately ground into uniform powder using a Thomas Wiley Machine (Model – 5 USA). The powdered samples were then dried and stored in airtight bottles for chemical analysis. The yield was 386.2 g. The minerals: calcium, sodium, potassium, magnesium and trace elements (zinc, cadmium and copper) all of which were determined according to the method of Shahidi et al.[17] Phosphorus content of the digest was determined colorimetrically according to the method described by Nahapetain and Bassivi[18]. Alkaloids and phenols were determined according to the methods of Harborne [19] while tannin was determined using the method of Van-Burden and Robinson[20]. Saponins' content was determined using the method of Obadoni and Ochuko. Flavonoids were determined according to the method of Boham and Kocipai[22]. The B-complex vitamins (thiamine, riboflavin and niacin) were determined according to the methods of SKALAR Analyzers [23] while ascorbic acid (vitamin C) was determined using the method of Baraket et al.[24].

Statistical analysis

All measurements were replicated three times and standard deviation determined. The students t-test at $p < 0.05$ was applied to assess the difference between the means [25].

RESULTS AND DISCUSSION

The phytochemical content of *Mangifera indica* stem bark and leaves is shown in Table 1. The alkaloids content was very high on the bark (9.66 mg. 100 g^{-1}) and the leaf contained 0.84 mg. 100 g^{-1} of alkaloids. Pure isolated plant alkaloids are used as a basic medicinal agent for their analgesic, antispasmodic and bactericidal effects[25]. Most of the plant barks used in the cure of diseases have been reported to contain traces of alkaloids, for instance, *Azadirachta indica* used in the cure of malaria contain alkaloids[4]. Quinine, isolated from *Cinchona* bark is the oldest known effective anti-malarial agent[27]. The presence of alkaloids in *Mangifera indica* stem barks and leaves supported the use of this plant parts in the treatment of malaria and fever in Nigerian folk medicine[4]. The flavonoids content was very high in the leaves (11.24 mg. 100 g^{-1}) while the bark contained (6.86 mg 100 g^{-1}) of flavonoids. This quantification supported the findings that flavonoids and flavonol like mangiferin, catachin, epicatachin and quercetin have been isolated from mango stem bark [5] (Fig. 1).

The biological functions of flavonoids include protection against allergies, inflammation, free radical scavenging, platelets aggregation, microbes, ulcers, hepatoxins, viruses and tumors. Mangiferin, catechin and epicatechin are the major phyto-constituents

of *Mangifera indica* [5,7]. These flavonoids are responsible for the antioxidant effects of the mango stem bark and leaves. The mango stem barks contain polyphenols, which have the ability not only to protect the human organism from the attack of oxidative chemical species (OCS) but also are able to reach the target organs and tissues. The antioxidant activity of all those polyphonols is governed by the number and location of these aromatic hydroxyl groups[28,29]. The antioxidant activity of mangiferin may be due to its ability to scavange OCS associated to the initiation of lipid preoxidation. The mango stem bark have 0.75 mg 100 g^{-1} of phenols while the mango leaves contained 0.09 mg 100 g^{-1} of phenols. The benzoic acid available in the plant undergo hydrolysis and esterification respectively to form gallic, methyl and propyl esters (Fig. 2). Phenols protect plants from oxidative damage and perform the same functions for humans[15]. The outstanding phytonutrients feature of phenols is their ability to block specific enzymes that causes inflammations. They also modify the prostaglandin pathways, thereby protecting platelet from clumping. Mango stem barks contained 8.48 mg 100 g^{-1} of saponins and the leaves contained 3.22 mg 100 g^{-1} of saponins. Saponins natural tendency to ward off microbes makes them good candidates for treating fungal and yeast infections. These compounds served as natural antibiotics, helping the body to fight infections and microbial invasion [26].

These compounds also appear to greatly enhance the effectiveness of certain vaccines. Plant saponins help humans to fight fungal infections, combat microbes and viruses, boost the effectiveness of certain vaccine and knock out some kinds of tumor cells particularly lung and blood cancers [26]. They also lower blood cholesterol thereby reducing heart disease. The most outstanding and exciting prospects for saponins are how they inhibit or kill cancer cells. They may also be able to do it without killing normal cells in the process, as is the mode of some cancer fighting drugs [26]. Cancer cells have more cholesterol type compounds on their membranes than normal cells. Saponins therefore bind cholesterol and thus, interfere with cell proliferation.

Tannin content was more in mango stem bark having 1.0 mg 100 g^{-1} of tannins and the leaves contained 0.45 mg 100 g^{-1} of tannins. The second largest chemical constituent in mango stem bark, which may contribute to its antioxidant activity, was the terpenoids family[5]. β-elemene, one of the major components in the terpenoids fraction, has exhibited anti-tumor activity on several types of leukemia by inducing the apoptosis of tumor cells[5]. β-Elemence exhibited a marked antiproliferative effect on glioma cells and could therefore induce apoptosis. Other volatile phytoconstituents comprises linesol, β-endesmol, α-guaience and β-seline. They also have antioxidant activity. The volatile phytoconstituents may have anti-parasitary against *P. falcipatum* and may be responsible

for the use of mango stem barks and leaves as infusion in malaria treatment [5].

The mineral composition of *M. indica* stem bark and leaves is shown in Table 2. Calcium was more abundant in the leaves having 3.82 mg 100 g $^{-1}$ of calcium while the mango stem bark contained 1.41 mg 100 g^{-1} of calcium. Among the trace element, copper was more abundant with the leaves having 8.68 mg 100 g^{-1} and the bark contained 8.33 mg 100 $^{-1}$ of copper. Zinc and cadmium were at high level with the leaves containing 7.85 mg 100 $^{-1}$ of zinc while the stem bark contained 5.30 mg 100 g $^{-1}$ of zinc. The value of cadmium was more on the leaves containing 1.50 mg 100 g^{-1} and the mango stem barks contained 0.98 mg 100 g^{-1} of cadmium. The trace elements play a key role for the activation of enzymes with the inflammatory response and Zn has been relevant on service physiological systems. Mineral deficiencies such as Ca, Zn and Fe are the major health problems in developing countries particularly for infants [30]. Zn or Fe deficiency causes poor growth, impaired immune function and consequently delayed mental development [30].

The vitamin content showed that the mango leaves contained 29.92 mg 100 g^{-1} and mango stem barks contained 7.92 mg 100 g^{-1} ascorbic acid. The plant parts examined also contained the B-Vitamins such as niacin, riboflavin and thiamine (Table 3). Deficiency of any of the vitamins can cause widespread clinical symptoms. The clinical manifestation of survey 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 [31]. This function 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 function [30].

Table 1. Phytochemical composition of mango stem bark and leaves mg 100 g $^{-1}$

Phytochemicals	Mango stem bark	Mango leaves
Alkaloids	9.66 ± 0.20^{a}	0.84 ± 0.11^{b}
Phenols	0.75 ± 0.22^{b}	0.09 ± 0.20^{c}
Flavonoids	6.86 ± 0.20^{a}	11.24 ± 0.10^{a}
Saponins	8.48 ± 0.10^{a}	3.22 ± 0.10^{a}
Tannins	1.10 ± 0.20^{b}	0.45 ± 0.10^{b}

Data are means ± standard deviation of triplicate determinations on dry weight basis.
Values with superscript that are the same are not significantly different as $p < 0.05$

Mangifera indica exhibits antioxidant, anti-inflammatory and immuno-modulatory properties. These findings justify the traditional use of mango stem bark and leaves in traditional medical practice.

Table 2. Mineral composition of mango stem bark and leaves mg 100 g $^{-1}$

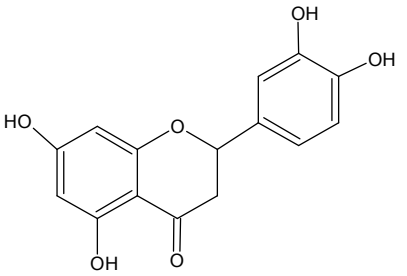
Minerals	Mango stem bark	Mango leaves
	Macro elements	
Calcium	1.41 ± 0.22^{b}	3.82 ± 0.10^{a}
Magnesium	0.46 ± 0.10^{c}	0.91 ± 0.20^{c}
Potassium	0.60 ± 0.11^{c}	0.83 ± 0.10^{c}
Phosphorus	0.55 ± 0.20^{c}	0.78 ± 0.10^{c}
Sodium	0.45 ± 10^{c}	0.38 ± 0.11^{c}
	Micro elements	
Copper	8.33 ± 0.20^{a}	8.68 ± 0.10^{a}
Zinc	5.30 ± 0.11^{a}	7.88 ± 0.10^{a}
Cadmium	0.98 ± 0.22^{c}	1.50 ± 0.20^{b}

Data are means ± standard deviation of triplicate determinations on dry weight basis. Values with superscript that are the same are not significantly different at $p < 0.08$

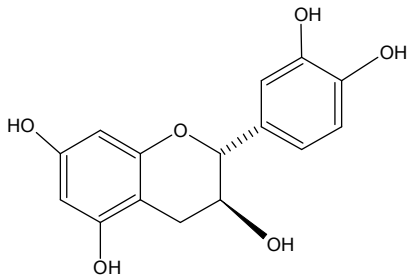
Table 3. Vitamin content of mango stem bark and leaves mg 100 g $^{-1}$

Vitamins	Mango Stem Bark	Mango Leaves
Ascorbic acid	7.92 ± 0.10^{b}	29.92 ± 0.11^{a}
Riboflavin	0.08 ± 0.20^{d}	0.09 ± 0.10^{d}
Niacin	0.55 ± 0.22^{c}	0.75 ± 0.20^{c}
Thiamine	0.02 ± 0.10^{d}	0.45 ± 0.11^{c}

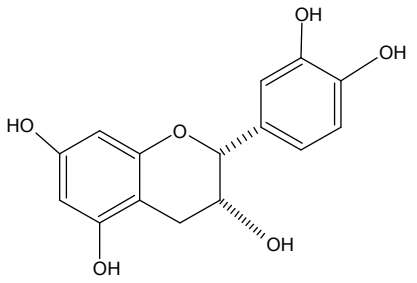
Data are means ± standard deviation of triplicate determinations on dry weight basis. Values with Superscript that are the same are not significantly different at $p < 0.05$



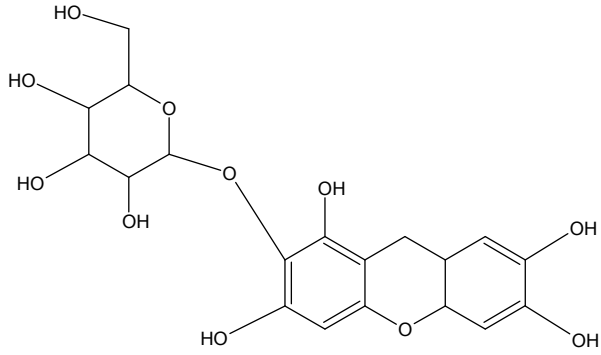
Quercetin



(+) Catechin

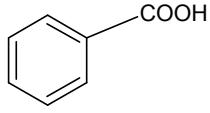


(-) Epicatechin

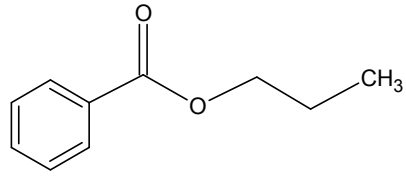


Mangiferin

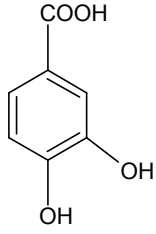
Fig. 1: Flavoniod constituents of mango stem barks



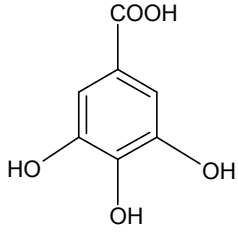
Benzoic acid



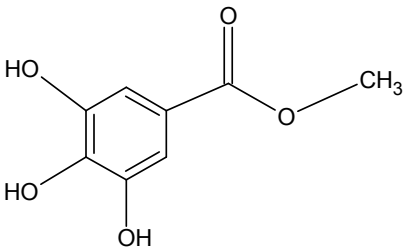
Benzoic acid propyl ester



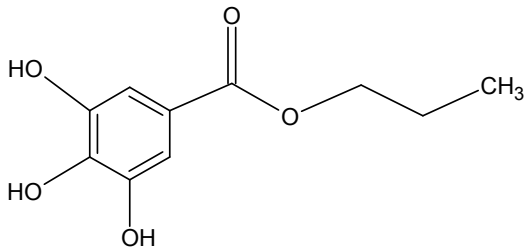
3, 4-Dihydroxybenzoic acid



Gallic acid

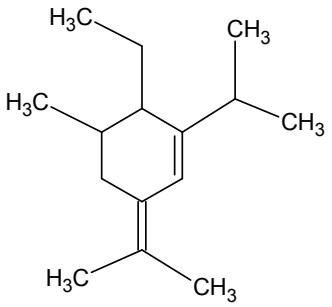


Gallic acid methyl ester

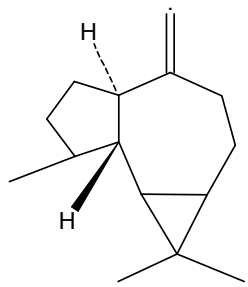


Gallic acid propyl ester

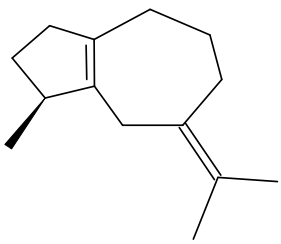
Fig. 2: Phenolic constituents, benzoic acid and benzyl ester derivatives from mango stem barks



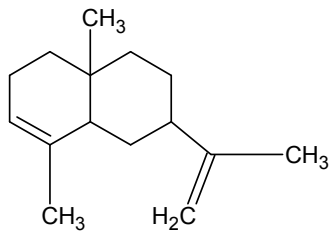
β - Elemen



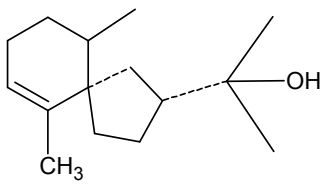
Aromandrene



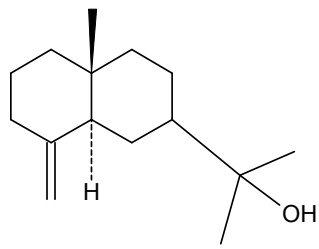
α - Guainine



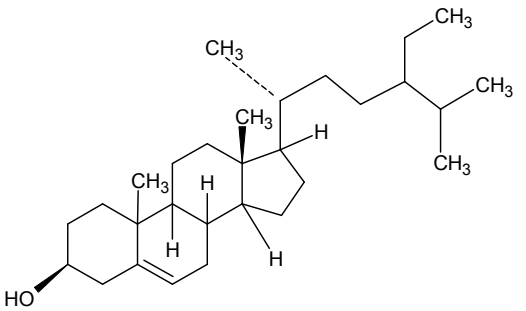
β - Selinene



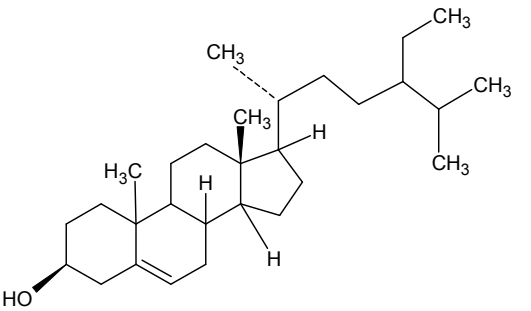
Hinesol



β - Eudesmol



β - Sitosterol



β - Campesterol

Fig. 3: The volatile constituents of mango stem bark

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