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ANTIOXIDANT AND PHYTOCHEMICAL CONCENTRATION OF THE METHANOL EXTRACT THE LEAVE AND STEM OF THE TWO COMMON VARIETIES OF *MANGIFERA INDICA*.

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ABSTRACT: *Phytochemical and antioxidant concentration was determined two common varieties of the stem, and leaves of Mangifera indica that was collected in the school environment in Ekpoma, Edo State, Nigeria. The plant sample of each variety where labeled based on their Varieties common name (Opioro mango and Sweet mango) and method of extraction. After extraction, they were further concentrated and subjected to antioxidant and phytochemical analysis, which include phenol, flavonoid, saponins, alkaloid and DPPH assay. The result obtain from this study revealed that methanol is an effective method of preparation of plants sample varieties studied and also, plant varieties significantly showed inhibition of the dpph radicals. In comparism among all extract, methanol extract of opioro (MOL) mango leaves was highest in phenol content (7.55±0.077mg/g), methanol extract of sweet mango stem (MSS) revealed high total flavonoid content (309.45±16.99), while methanol extract of opioro mango stem (MOS) showed significant concentration of saponin (40.847±1.276) and these findings support the use of M. indica extracts for pharmaceutical purposes.*

KEYWORDS: *Mangifera Indica*, phytochemical, antioxidant properties

INTRODUCTION

Global production of Mango has doubled in the last thirty years, mango production is centered in Indian, China, Thailand, Pakistan and Mexico, but there are currently more than 90 countries that grow Mango commercially, countries in Americas and Africa produce small percentage of Worlds mango crop each yea, 13% and 10% respectively. Asia, where the Mango is native, is the largest Mango-producing region, producing 77% of global supply annually. Within the U.S. a limited number of Mango are grown in Florida, California, Puerto Rico and Hawaii. (Mossler and Jonathan 2013).

The leaves are simple without stipules. When the leaves are young they are Orange – Pink rapidly changing to a dark glossy red, then dark green as they mature. The leaves are variable I shape like oval-lanceolate, lanceolate, oblong, linear-oblong, ovate, oborate-lanceolate or roundish – oblong. Mango tree stem bark is usually dark grey-brown to black, rather smooth, superficially cracked or inconspicuously fissured, peeling off in irregular, rather thick pieces.
Most of the early Indian varieties were monoembryonic, which produced seedlings that not reproduce true to type. Planting and selection of these seedlings has changes these varieties with many becoming known as a "common" variety. Common variety are generally of inferior fruit quality to named, introduced and usually found growing wild or on roadsides and abandon home sites. (Illoh and Olorede 1991).

Herbal medicine is more accessible to most of the population. About 60 to 85% of the populations of every country of the developing world rely on herbal or indigenous forms of medicine (Onyeka *et al.*, 2012). The reasons for the high patronage of herbal medicine are the high cost of very effective antibiotics and the problem of antibiotic resistance which is very common in developing countries (Hack, 2006).

Kanwal *et al.*, 2010 reported that isolated flavonoids from the leaves of mangos showed potent antifungal activity against *Alternaria alernata* (Fr) Keissler, *Aspegillus fumigates*, *Aspergillus niger* Van Tieghan and Penicillium cetril. Mango leaf, bark and root and its polyphenols possess some aritiallcer activities along with its antioxidant properties (Priya et al., 2009). Mango leaves (young leaves), still rose or orange colour, can be boiled to render them edible. Although the cooked leaves hold their shape and are attractive, their reserved flavour in an acquired taste. Some varieties are more suitable for eating in this manner. (Martin et al., 1998)Mango stem bark extract (Vimang) has been traditionally used in many countries for the treatment of menorrhagia, diarrhea, syphilis, diabetes, scabies, cutaneous infections and anemia (Scartezzini and Speroni, 2000). Stem extract which has main ingredient *mangifera* is used as a nutritional supplement
Oxidation reaction ensures that molecular oxygen is completely reduced to water. These products of partial reduction of oxygen are highly reactive and create havoc in the living system. Hence, they are called reactive oxygen species (ROS). Members of this group (ROS) are superoxide Anion Radical, Hydroperoxy Radical, Hydroxyl Radical, Hydrogen Peroxide, lipid peroxide Radical, Singlet Oxygen, Nitric Oxide and Peroxyl Nitrate. These free radicals are naturally produced by the body. Antioxidant are also defined as a substance which are capable of inhibiting a specific oxidising enzymes or a substance that reacts with oxidizing agents prior to causing damage to other molecules or a substance that sequester metal ions or even a substance capable of repairing system such as iron transporting protein (Brewer, 2011). As such, production of free radicals and other reactive oxygen species in the human body by numerous physiological and biochemical processes is reported (Halliwell ,1994). This work was aimed to determine the phytochemical concentration of the methanol extract of the leave and stem of two common varieties of *Mangifera indica* commercially known in Nigeria (opioro Mango and sweet mango).

METHODOLOGY

Materials

Apparatus: Digital weighing balance, test tubes, beakers, conical flask, rotary evaporator, electric hot plate, stop watch, funnel, cotton wool, water bath, mortar and pestle, scissors, petri dish, hand gloves, sample bottles and laboratory record book, spectrophotometer, beaker, measuring cylinder, volumetric flask.

Chemicals: Reagent: 1,1-Diphenyl 1-2-Picrylhydrazyl (DPPH), 2, 2 azinobis-3-elhylbenzolhiazoline-6-sulfanic acid, 24 THriazine – 4 4 – d sulfanic acid, sodium carbonate, Potassium Taricyanide Catechin Butylated hydroxytoluene (BHT), ascorbic acid, Catechin, tannic acid, quercetin and $FeCl_3$, vanillin, folin-ciocalteusis phenol reagent and sodium carbonate.

Plant Collection

The leaf, stem, bark and root of two varieties (Alphonso, Benue Mango, Opiolo Mango and Julie Mango) were collected from Ekpoma, Esan West Local Government Area, Edo State, Nigeria. These areas consist of villages which are generally classified as rural and poor. The plants were identified by the vernacular names and later validated by the Department of Botany, Ambrose Alli University

Extract Preparation

Plants materials were dried and grinded into fine powder. 100g of powdered sample was weighed and soaked separately in 250 ml of methanol and 250ml of water respectively in a clean sterilized and flat bottomed glass container. The container with this content was covered and maintained for 48hr (2days) and were accompanied with occasional stirring and agitation. The complete mixture was then subjected to coarse filtration on a piece of clean, white sterilized cotton materials and whatman's filter paper. The resulting filtrate was evaporated on water bath and allowed maintain 60^{o}C to dryness. The resulting concentrates were regarded as the crude methanol and water extracts. (Delower *et al.,* 2013)

Methods

Determination of Total Flavonoid Content

Total flavonoid content of the extracts was quantified following the method reported by Meda *et al.,* (2005) 0.5ml of extract was mixed with 1.5ml of methanol, 0.1ml of aluminum chloride (1%) 0.1ml of 1m Potassium acetate and 2.8ml of distilled water. The reaction mixture was allowed to stand at room temperature for 30mins, before the absorbance was read at 514nm against a reagent blank. Total flavonoid was expressed as Quercetin equivalent (QE)

Determination of Total Phenolics Content

Using modified folin-ciocalteu method (Wolf *et al.,* 2003) total phenol content in the extract was determined. An aliquots of the extract was mixed with 5ml folin – ciocalteus reagent of sodium carbonate. The tubes were votexed for 15sec and was allowed to stand for 30mins at 40^{0}C for colour development. Absorbance was then measured at 765nm using the Hewlett Parckard Uv-Vs spectrophotometer. Total phenolics content is expressed as mg/g Tannic acid equivalent.

Determination of Total Saponin Content

Total saponin content of extract was quantified according to the method described by Hial *et al.,* (1976). 0.25ml of the extract was mixed with 0.2ml of vanillin reagent (8% vanillin in ethanol) and 2.5ml of 72% aqueous H_2SO_4. The reaction mixtures were heated in a water bath at 60^{0}C for 10 minutes. The tubes were cooled in ice for 4 minutes and allowed to acclimatize to room temperature, subsequently, the absorbance was measured at 544nm, and total saponin content of sample was expressed as standard saponin equivalent in mg/g of sample dry weight.

Determination of Total Alkaloid Content

Total alkaloid level of the extract was determined following the method reported by Singh *et al.,* (2004) 1ml of the extract was mixed with 1ml of $FeCl_3$ solution (0.025M $FeCl_3$ in 0.5M HCl) and 1ml of 0.05M of 1, 10-phenanthrolme in ethanol. The reaction mixture was incubated in hot water bath of 70^{0}C for 30minutes. The absorbance of red coloured complex formed was measured at 510nm against reagent blank. Alkaloid content of sample was separated as quinine equivalent in mg/g of sample dry weight.

Determination of Total Tannin Content

Total tannins content of the extract was quantified using the method of Swan (1979) 1ml of sample extract was pipette into 50ml volume flask 20ml distilled water, 2.5ml of folin derrus reagent and 10ml of 17% Na_2CO_3 were added and mixed properly. The mixture was made up to mark with distilled water, mixed well and allowed to stand for 20minutes when a blueish – green colouration developed. The absorbance was read after colour development of a wavelength of 760mm. total tannin content was expressed as tannin acid-equivalent.

Determination of Antioxidant Activity

DPPH Radical Scavenging Assay

The effect of the extract on DPPH radical was estimated using the method of Livana-pathiranan and Shahidi. (2005). A solution of 24mg DPPH in methanol and 0.010g in 10ml of methanol was prepared and 1.85ml of DPPH solution was mixed 0.15ml of extract in methanol containing 0.4 - 2.0ml (five different concentrations in triplicate) of the extract. Control contained DPPH and ethanol. The reaction mixture left in the dark at room temperature for 30min. the absorbance of the mixture was measured spectrophotometrically at 515nm. Ascorbic acid and BHT were used as reference.

Statistical Analysis

Data were analyzed using Statistical package (Graphpad prism 8.0). Result were expressed in their mean and standard error of mean and analysis of variance was used to compare means at $p< 0.05$ level of significance.

RESULTS

Table 1 show the phytochemical and antioxidant properties of methanol extract of the stem and leaves of two varieties of *Mangifera Indica*. Mango varieties include; Sweet Mango and Opioro Mango. The result reveals the phytochemical concentration obtained from **MOL**- methanol extract of opioro mango leaves, **MOR**- methanol extract of Opioro mango root, **MOS**- methanol extract of opioro mango stem, , **MSL**- methanol extract of sweet mango leaves, **MSR**- methanol extract of sweet mango root and **MSS**- methanol extract of sweet mango stem,. The result for the total phenol content, total flavonoid, total tannin, total saponins and total alkaloid content of plant extract are represented in figure 1 to 5 respectively. While Figure 7-10 showed result of the DPPH radical scavenging ability of different extract concentration (0.2 -1.0 mg/mol)

Table 1. Phytochemical and Antioxidant Properties of Methanol Extract Of the Stem and Leaves of Two Varieties of *Mangifera Indica*. Mango Varieties Include; Sweet Mango and Opioro Mango

Plant Extract	Phenolic (TAEmg/g)	Flavonoids (QEmg/g)	Saponins (SEmg/g)	Alkaloid (QEmg/g)	Tannin (TAEmg/g)
M.O.L	7.55±0.077	368.62±49.82	59.08±15.93	181.22±0.375	19.54±0.063
M.O.S	2.96±0.154	430.91±28.85	40.847±1.276	166.41±11.820	10.343±1.832
M.O.R	7.52±0.019	361.21±29.169	48.103±1.805	172.90±3.920	18.573±1.112
M.S.S	4.81±0.021	309.45±16.99	88.55±1.46	158.34±7.46	14.59±0.143
M.S.R	5.34±0.024	257.41±2.96	75.24±0.370	151.96±1.227	15.69±0.700
M.S.L	4.34±0.060	540.63±15.47	87.78±2.4420	126.46±2.454	16.52±0.624

Result where expressed in their Mean and Standard error of mean and analysis of variance was used to compare means at $p< 0.05$ level of significance

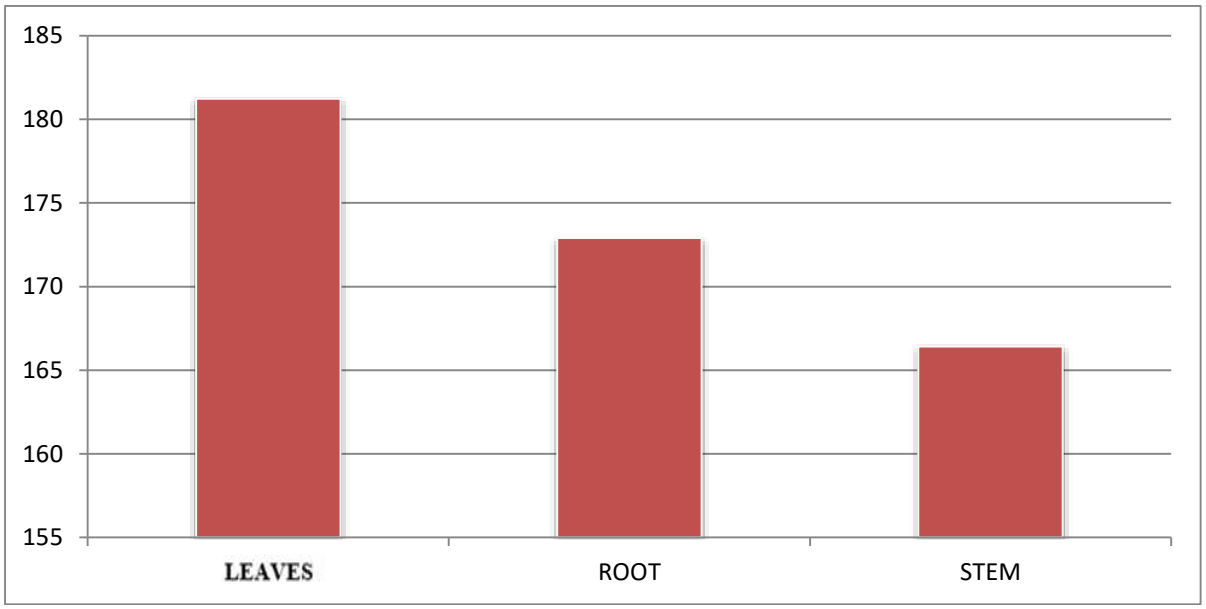

Figure 1: Total Alkaloid content for methanol extract of root, stem and leaves of Opioro Mango with concentration of Quinine milligram per gram. Mean ± standard error of mean of triplicate determination.

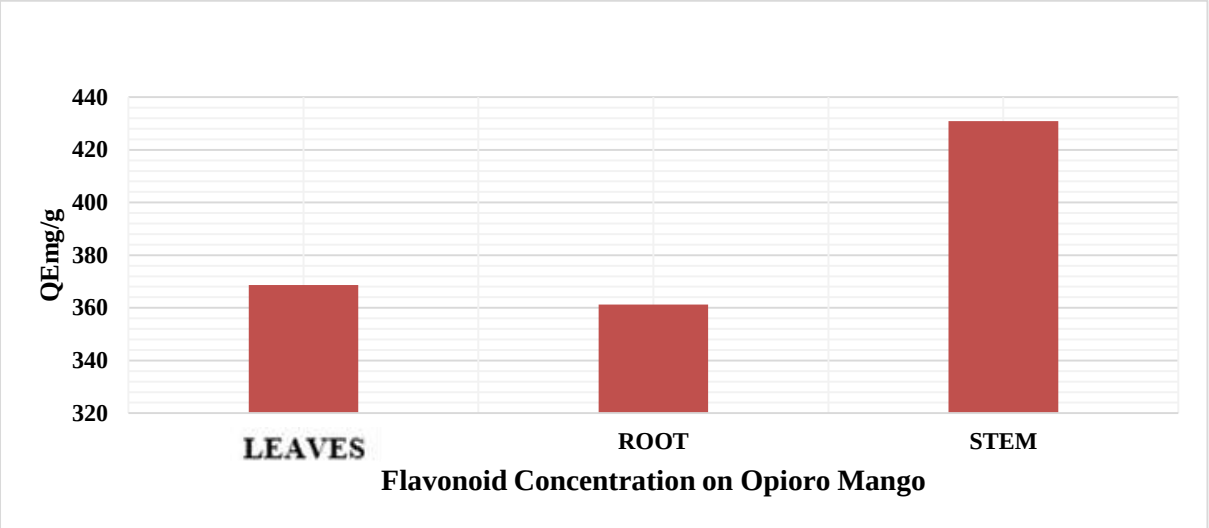

Figure 2: Total Flavonoid content for methanol extract of root, stem and leaves of Opioro Mango with concentration of Quercetine milligram per gram. Mean ± standard error of mean of triplicate determination.

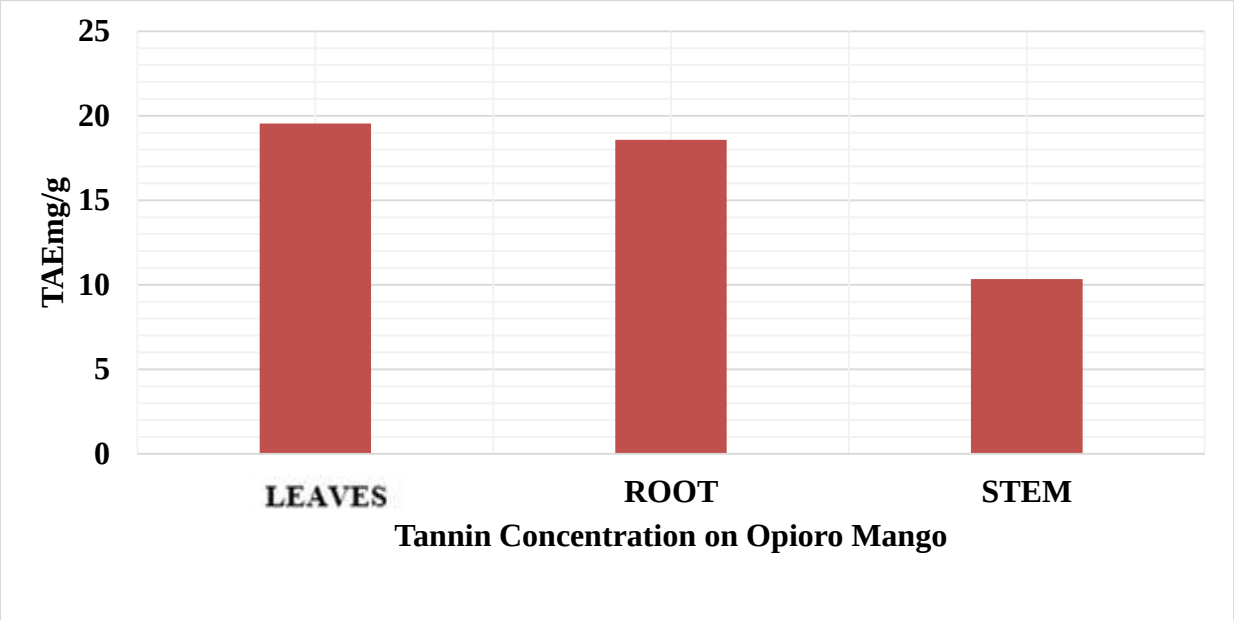

Figure 3: Total Tannin content for methanol extract of root, stem and leaves of Opioro Mango with concentration of Tannic acid milligram per gram. Mean ± standard error of mean of triplicate determination.

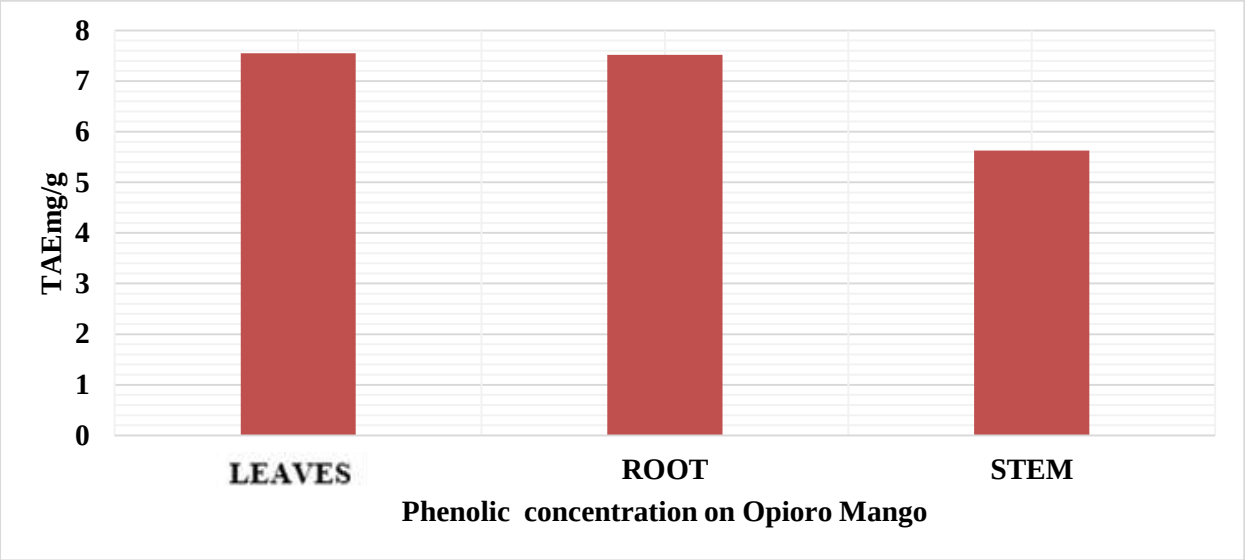

Figure 4: Total phenol content for methanol extract of root, stem and leaves of Opioro Mango with concentration of Tannic acid milligram per gram. Mean ± standard error of mean of triplicate determination.

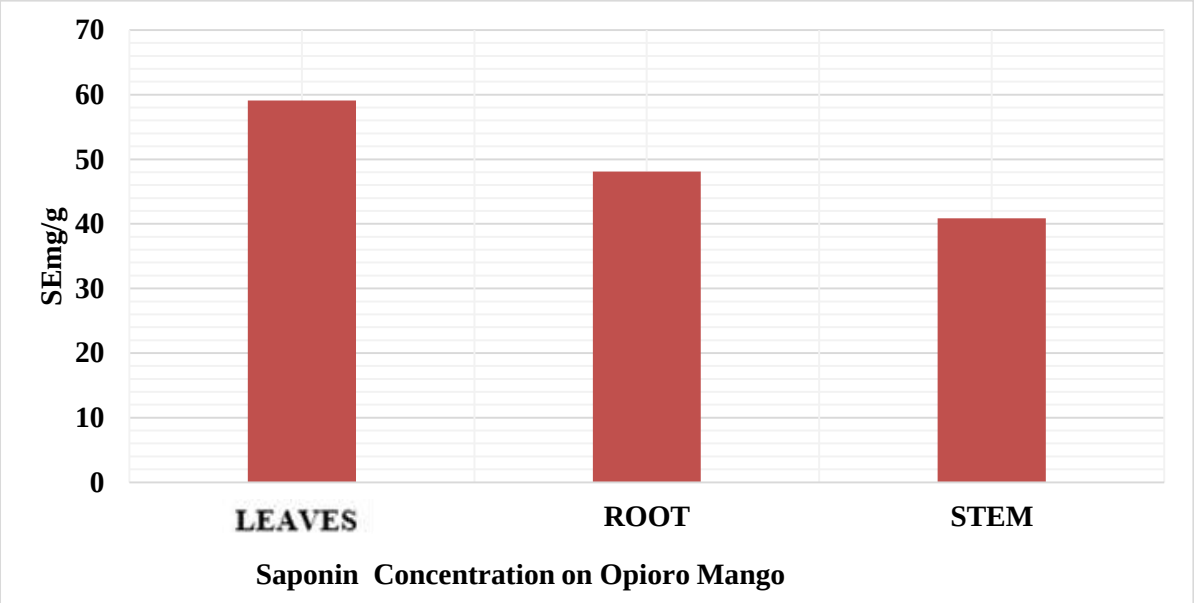

Figure 5: Total Saponin content for methanol extract of root, stem and leave of Opioro Mango with concentration of Standard Saponin milligram per gram. Mean ± standard error of mean of triplicate determination.

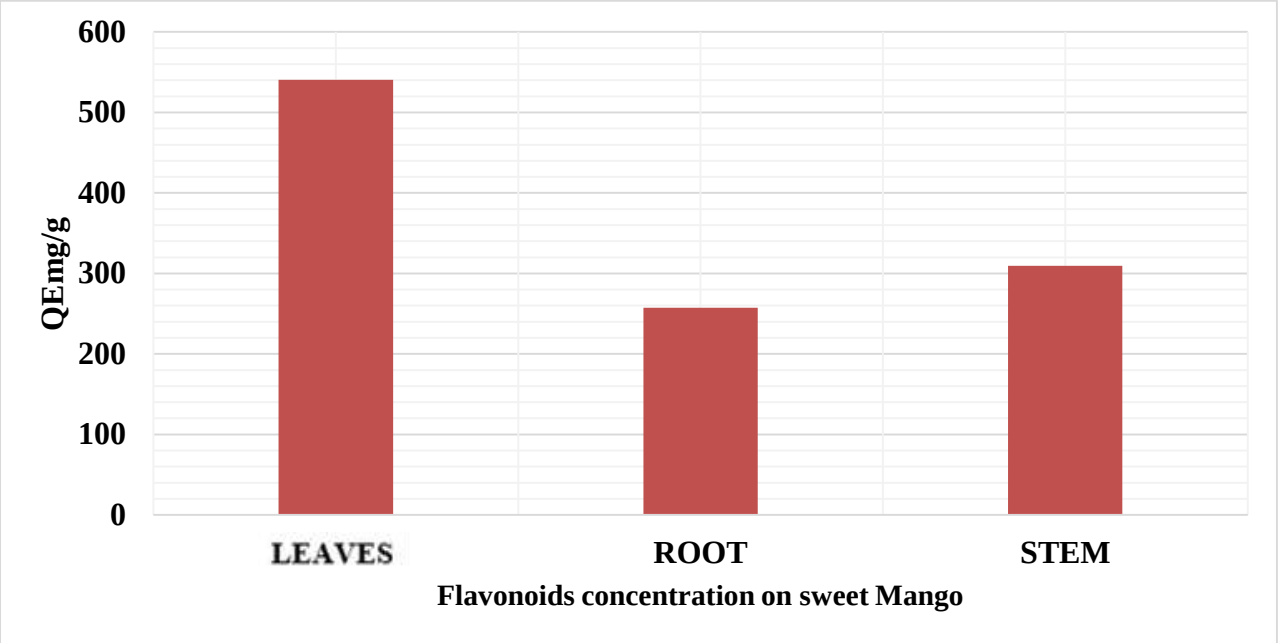

Figure 6: Total flavonoid content for methanol extract of root, stem and leave of Sweet Mango with concentration of quercetin milligram per gram. Mean ± standard error of mean of triplicate determination

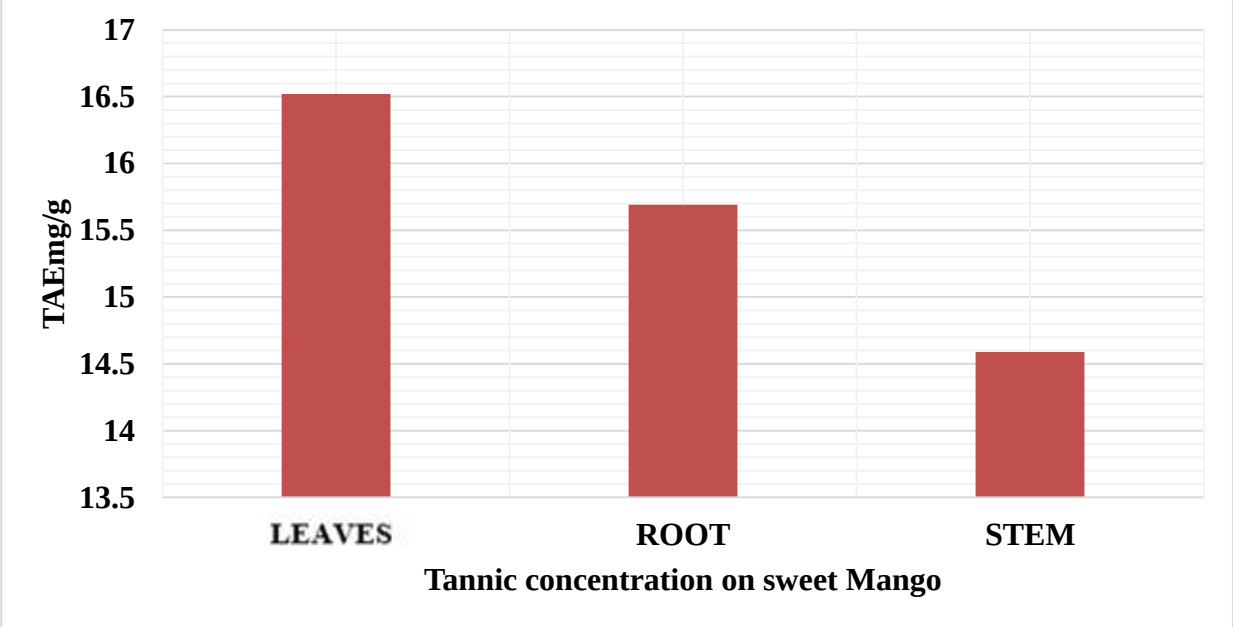

Figure 7: Total Tannin content for methanol extract of root, stem and leaves of Sweet Mango with concentration of tannic acid milligram per gram. Mean ± standard error of mean of triplicate determination.

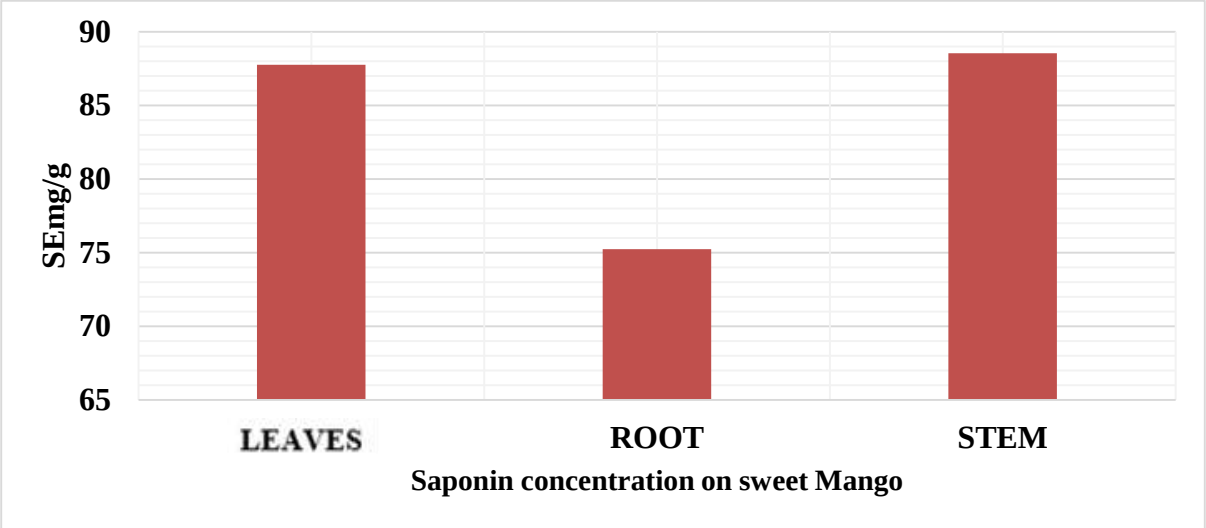

Figure 8: Total Saponin content for aqueous extract and methanol extract of root, stem and leaves of Sweet Mango with concentration of Saponin milligram per gram. Mean ± standard error of mean of triplicate determination.

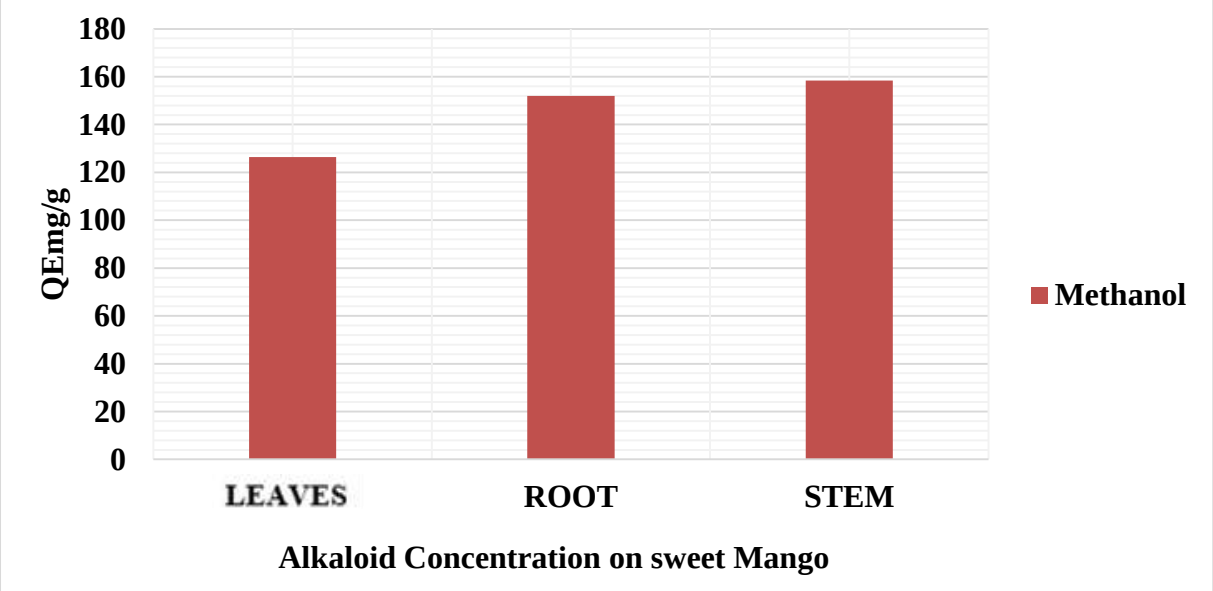

Figure 9: Total Alkaloid content for methanol extract of root, stem and leaves of Sweet Mango with concentration of quinine milligram per gram. Mean ± standard error of mean of triplicate determination

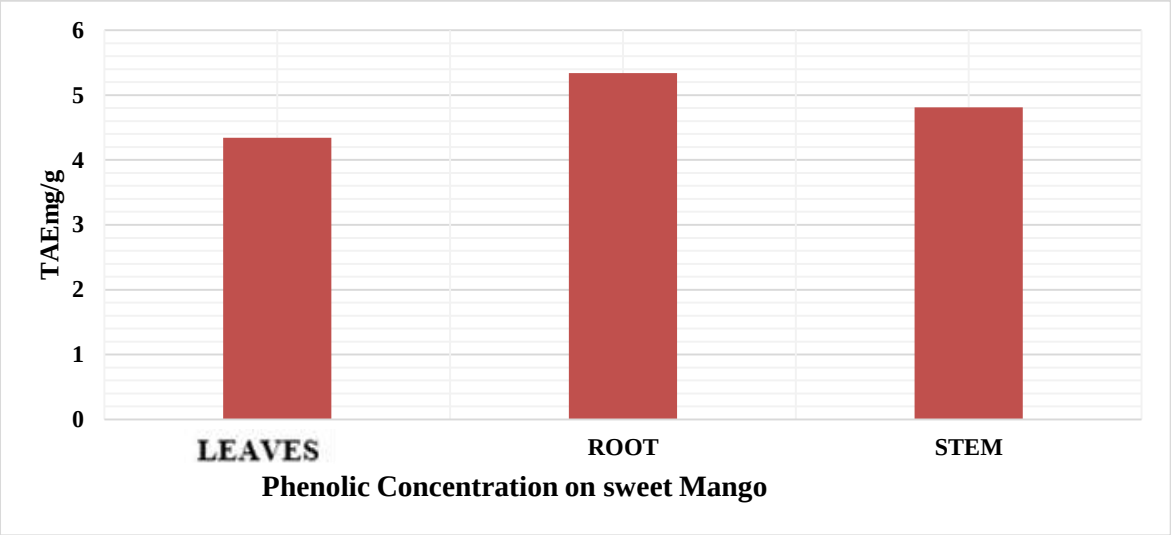

Figure 10: Total Phenol content for methanol extract of root, stem and leaves of Sweet Mango with concentration of tannic acid milligram per gram. Mean ± standard error of mean of triplicate determination.

ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging Assay

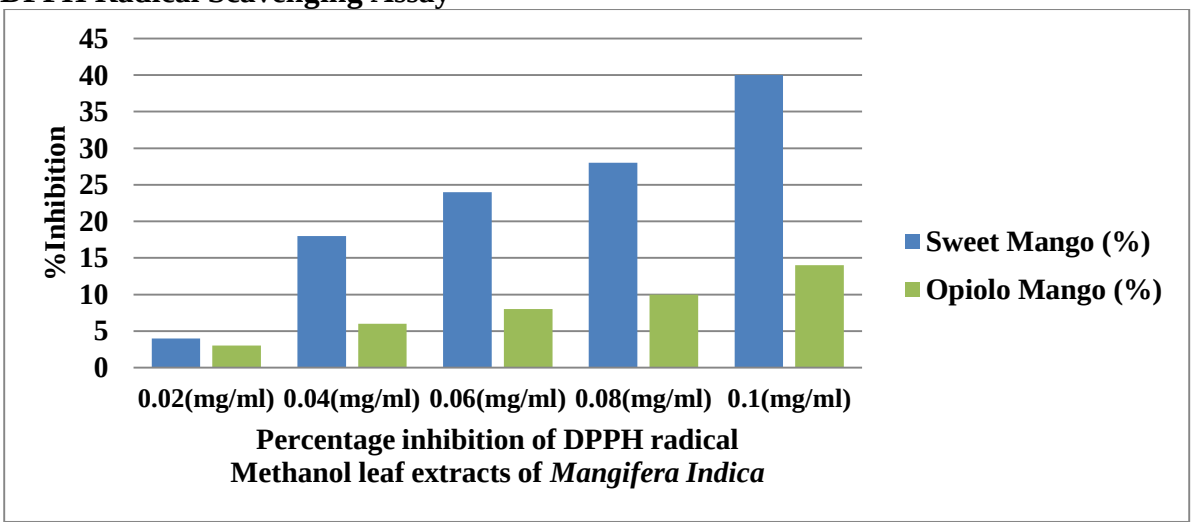

Figure 11: Percentage inhibition of Methanol leaves extract of *Mangifera Indica* varieties (sweet mango and opioro mango). Sweet mango showed the higher percentage inhibition. This shows change in concentration of the Methanol leave extract affect the level of inhibition of DPPH radical.

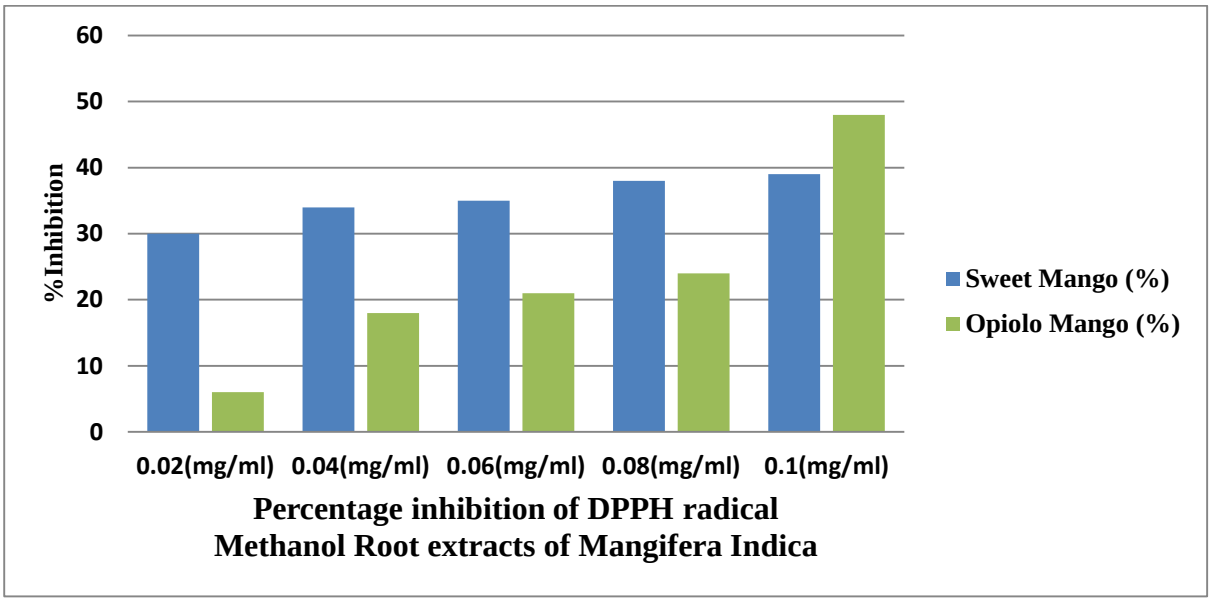

Figure 12: Percentage inhibition of Methanol root extract of *Mangifera Indica* varieties (sweet mango and opioro mango). Sweet mango showed higher scavenging activity in all concentration except root extract at 0.1mg/g. This shows change in concentration of the Methanol root extract affect the level of inhibition of DPPH radical

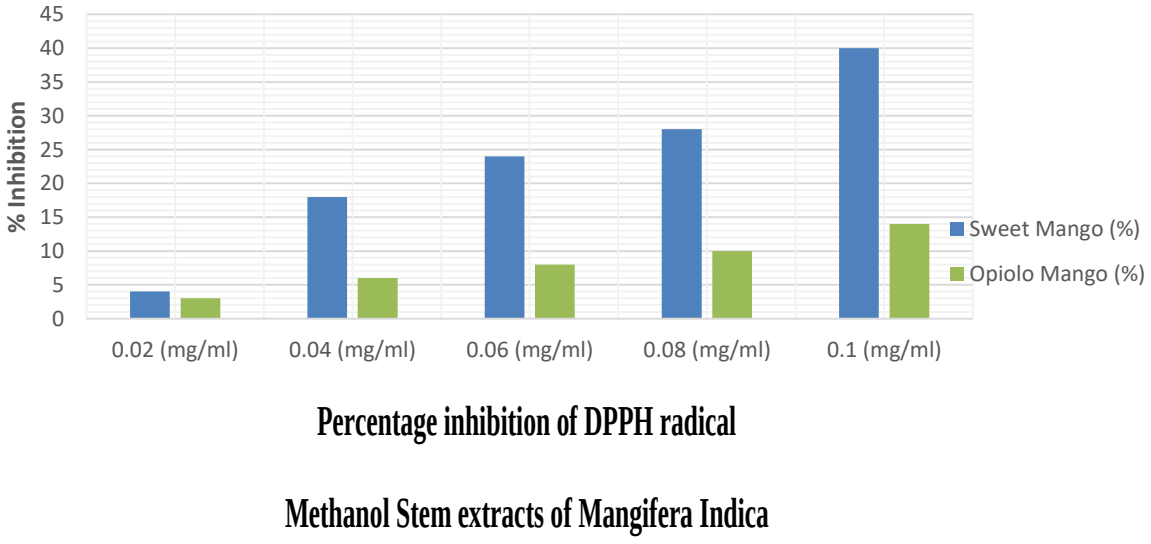

Figure 13: Percentage inhibition of Methanol stem extract of *Mangifera Indica* varieties (sweet mango and opioro mango). Sweat mango showed higher percentage inhibition. This shows change in concentration of the Methanol stem extract affect the level of inhibition of DPPH radical

DISCUSSION

The result obtained from this study revealed that methanol is a very effective method of preparation of mango extract of the varieties studied; sweet mango and opioro mango (local names). Phytochemical and antioxidant concentration was determined on the root, stem, and leaves of these two varieties of *Mangifera indica* that was collected in the school environment in Ekpoma, Edo State. The plant sample of each variety where labeled based on their plant

Varieties. After extraction, they were further concentrated and subjected to antioxidant and phytochemical analysis, which include phenol, flavonoid, saponins, tanni alkaloid and DPPH assay.

According to result in **table 1, t**he total phenolic concentration of opioro mango leave was higher, followed by opioro mango root. While Sweet mango leaves revealed the lowest (4.39±0.060) phenolic concentration compared to other extract studied. Total flavonoid determination carried out showed that there was a significant amount of the total flavonoid found in all extract studied ranging from 257.41±2.96 to 540.63±15.470, sweet mango leave extract being the highest flavonoid concentrated plant extract among all extract studied. This explains that mango leaves, stem and root are a very good source of phytochemicals. After ingestion of green tea, flavonoid content if absorbed rapidly as shown by their elevated level in plasma and urine. They enter the system circulation soon after ingestion and cause increase significantly in plasma antioxidant status (Benzie *et al*., 1999).

In this study, result also showed that sweet mango had higher saponin concentration significantly than that of the opioro mango. Opioro mango stem bark recorded the lowest saponin concentration (40.84±1.27) among the opioro mango extract. While opioro mangos leave 59.08±15.95 was the highest opioro mango extract. Among the sweet mango, the stem bark contained the highest opioro mango (87.85±1.46) compared to the leaves and root. Tannin concentration was determined and result showed that opioro mango leave had highest tannin concentration (19.54±0.063), followed by the opioro mango root (18.573±1.112). Total alkaloid determined carried out revealed significant concentration in all extracts. Opioro mango leave and hifh alkaloid concentration (181.22±0.375) followed by the opioro mango root (172.90±3.920). Tannins can cause regression of tumors that are already present in tissue, but if used excessively over time, they can cause tumors in healthy tissue. They have been also reported to have anti-viral antibacterial (Akiyama *et al.,* 2001) and antiparasitic effects (Kolodziej and Kiderlen 2005).

Free radical scavenging ability was also determined using DPPH ASSAY. The DPPH decolorizes in the presence of compounds that are capable of either transferring an electron or donating hydrogen. The change in dpph absorbance after addition of a least materials is used as an index of the antioxidant capacity of the materials. In the study, plant varieties significantly showed inhibition of the DPPH radicals. This dpph radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant extract. The figure (11-13) showed the antioxidant activities of the methanol extract of the root, leaf and stem bark of two varieties of *Mangifera indica* assessed. Using the DPPH radical scavenging in different concentration 0.02ml-0.1ml of methanol extract produced moderate to high DPPH scavenging ability in all the *Mangifera indica* plant extract of plant varieties studied.

Implication to Research and Practice

This study ascertained that *Mangifera indica* plant extract contains essential phytochemicals and antioxidants upon isolation and chemical characterization will promote optimal health and reduce the risk of chronic diseases.

CONCLUSION

This research revealed that these two mango varieties exhibited a reasonable concentration of phytochemicals with great potentials (phenols, flavonoids, saponin, tannin and alkaloid) and also exhibited properties as a good source of antioxidants. For the esteem phytochemical potentials observed, this study suggest its application for further research and usefulness in food and pharmaceutical industries.

Future Research

Further studies should be done on experimental animals and human studies that evaluate not only their safety and efficacy but also their absorption, metabolism, excretion and mechanism of action of *Mangifera* plant extracts. As our knowledge of these *Mangifera indical* potentials grow, we will learn how best to create new product through altering their concentration, combination and their bioactivity

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