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EVALUATION OF QUANTITATIVE ELEMENTAL COMPOSITIONS AND ANTIOXIDANT POTENTIALS OF *SPONDIAS MOMBIN* EXTRACTS (LINN), A PRECURSOR AGAINST INFECTIOUS DISEASES

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ABSTRACT

The purpose of this research work is to investigate the elemental composition and antioxidant potentials of *Spondias mombin* (leaf, root and stem bark) at concentration of 10, 20 and 5mg/ml. using different conventional laboratory methods, also to determine the antioxidant potentials of *Spondias mombin*. The element present are Sodium (Na), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Iron (Fe), Lead (Pb), Copper(Cu), Manganese (Mn), Potasium (K) and Phosphorus (P).it was observed that all elements were present at appreciable quantity. It was observed that the highest and lowest quantity were found in Zinc (20.34) and Cu (1.22) for the leaf extract, Potasium (24.98) and (2.14) for stem bark, Calcium (29.35) and Pb (2.91) for the root extract. Pb has minimal quantity in all parts of *Spondias mombin* extract. P value< 0.0001, Significantly different standard deviations ($P < 0.05$).The antioxidant composition are FRAP, DPPH, $Fe^{2+,}$ABTS, H_2O_2 and superoxide. The percentage composition of FRAP, DPPH, $Fe^{2+,}$ABTS, SO and H_2O_2, superoxide phenol and flavonoid were elucidated, it was observed that ABTS has the highest composition in the stem bark and superoxide (SO) has the lowest content in the aqueous root extract of *Spondias mombin*.it can be deduced that *Spondias mombin* is a very important medicinal plant which should not go into extinction, its uses, efficacy and importance should not be over emphasis for the fact that

it is rich in both elemental and antioxidants potential, therefore, its cultivation and day to day usage of *Spondias mombin* should be therefore be encouraged.

KEYWORD: Quantitative Elemental Compositions, Antioxidant Potentials, *Spondias mombin.*

1.0. INTRODUCTION

Spondias mombin L. (picture 1) (Family: Anacardiaceae), synonym Spondias lutea, commonly known as hog plum, yellow mombin or ubos. Locally called 'Iyeye' in Yoruba, is a deciduous erect tree which grows to 15 - 20 meters tall with a trunk 60-75 cm wide. The leaves are green, slender and tapest at the end, 15-30 cm long, most of which are shed prior to the production of numerous small, white fragrant flowers. The plant bears small (2 – 4 cm long, 1.5 – 2 cm wide), green, plum-like shaped fruits which turn yellow upon ripening (Gregory, 2000).

The leaves are used in the treatment of bacterial infections, the prevention and progression of viral infections, treatment of candida infections, and expelling parasites such as intestinal worms. It is also known to reduce anxiety, stop convulsions, calm and sedate, relieve pain, and suppress cough. And also aid digestion and stimulate the uterus (Ademola *et al*., 2005; Amadi *et al*., 2007). The stem bark is also used to reduce inflammation, relief pain, reduce spasms, kill fungi, kill bacteria, heal rashes, heal wound and stop bleeding. It is also used as a contraceptive (Uchendu *et al*., 2008).

The Spondias mombin extract contains potassium, sodium, calcium, phosphorus and magnesium (Njoku, 2007). The leaves are also rich in ascorbic acid and contain a good

amount of niacin. It also contains riboflavin and thiamin (Njoku, 2007). It was reported by Njoku, 2007 that beta-lactamase inhibitors isolated from *Spondias mombin* may contribute to the antimicrobial activity of the leaves.

Hinneburg, 2006 deduced that some vitamins (ascorbic acid and α-tocopherol), many herbs and spices (rosemary, thyme, oregano, sage, basil, pepper, clove, cinnamon, and nutmeg), and plant extracts (tea and grape seed) like *Spondias mombin* contains natural antioxidant components as well natural phenolic antioxidants, such as synthetics, can effectively scavenge free radicals, absorb light in the ultraviolet (UV) region (100 to 400 nm), and chelate transition metals, thus stopping progressive autoxidative damage and production of off-odors and off-tastes, this report was corroborated by Politeo *et al*., *c*2010.

Nawar.1996 observed that antioxidants are compounds that delay autoxidation by inhibiting formation of free radicals and by interrupting propagation of the free radical by one of several mechanisms. The mechanisms are as follows: (1) scavenging species that initiate peroxidation, (2) chelating metal ions such that they are unable to generate reactive species or de- compose lipid peroxides, (3) quenching O_{2-} preventing formation of peroxides, (4) breaking the autoxidative chain reaction, and/or (5) reducing localized O_2 concentrations (Wanatabe, 2010).

An antioxidant protects the key cell components by neutralizing the damaging effects of free radicals (Figure 1), which are natural by-products of cell metabolism (Miller *et al*., 2000). Anwar *et al*., 2009 reported that oxidative stress (OS) induced by reactive oxygen species (ROS) can be described as a dynamic imbalance between the amounts of free radicals generated in the body and levels of antioxidants to quench and/or scavenge them and protect the body against their deleterious effects (Shirwaikar *et al*., 2006).

Halliwell and Gutteridge, 2000 reported the excessive amounts of ROS may be harmful because they can initiated bimolecular oxidations which lead to cell injury and death, and create oxidative stress which results to numerous diseases and disorders such as aging, cancer therosclerosis, cirrhosis and cataracts. There has been a growing considerable interest to identify new sources of safe and inexpensive antioxidant and antimicrobial potential of natural origin (Anwar *et al*., 2009).Free radicals are formed when oxygen is metabolized or formed in the body and are chemical species which possess an unpaired electron in the outer

(valance) shell of the molecule. This is the reason why the free radicals are highly reactive and can react with proteins, lipids, carbohydrates and DNA.

Nawar, 1996 deduced that antioxidant effectiveness is related to activation energy, rate constants, oxidation–reduction (Redox) potential, ease with which the antioxidant is lost or destroyed (volatility and heat susceptibility), and antioxidant solubility**.** In addition, inhibitor and chain propagation reactions are both exothermic. As the A:H and R:H bond dissociation energies increase, the activation increases and the antioxidant efficiency decreases. Conversely, as these bond energies decrease, the antioxidant efficiency increases.

Aftab & Vieira. 2009 provided useful information about the most effective antioxidants that can interrupt the free radical chain reaction. Usually containing aromatic or phenolic rings, these antioxidants donate H to the free radicals formed during oxidation becoming a radical. These free radical intermediates are stabilized by the resonance delocalization of the electron within the aromatic ring and formation of quinone structures.

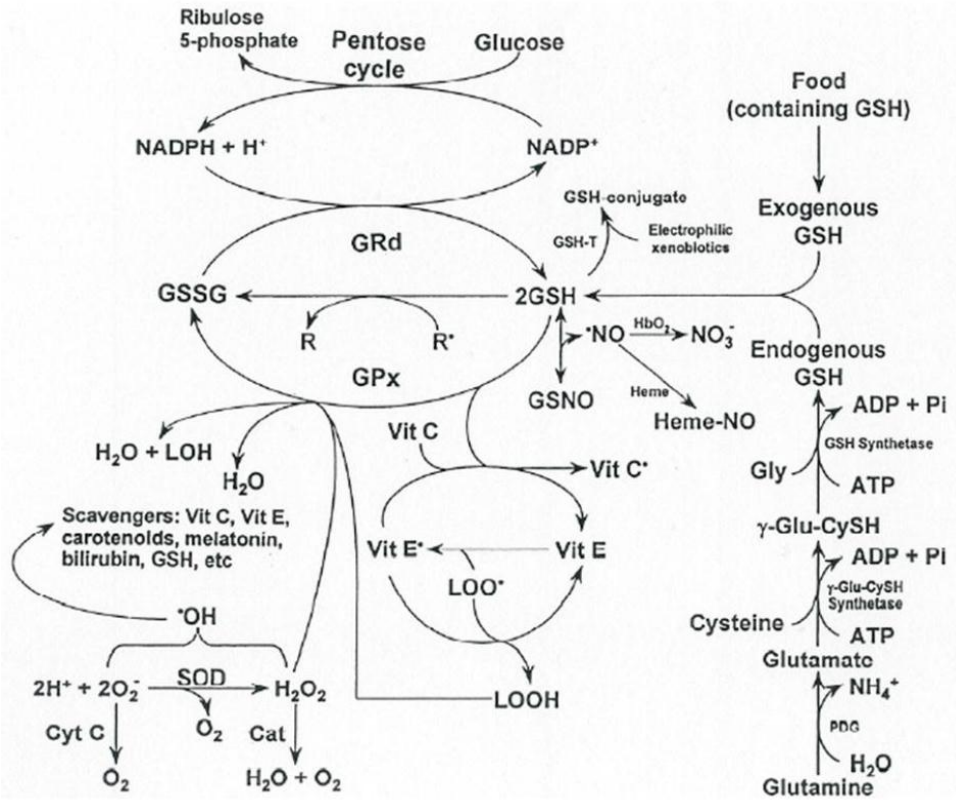


Figure 1: Free radicals and other reactants are enzymatically removed from cells by a series of antioxidative enzymes. Source: (Reiter *et al.* (2003).

Prior *et al.*, 1998; Cao *et al.*,1995 reported the simultaneous event that occur in the cell. These free radicals attack the nearest stable molecules, stealing its electron. When the attacked molecule loses its electron, it becomes a free radical itself beginning a chain reaction, finally resulting in the description of a living cell. Reiter *et al*. 2003 observed that free radicals may be either oxygen derived (ROS, reactive oxygen species) or nitrogen derived (RNS, reactive nitrogen species). The oxygen derived molecules are O-2(superoxide), HO. (hydroxyl), HO_2 (hydroperoxyl), ROO. (peroxyl), RO.(alkoxyl) as free radicals and H_2O_2 oxygen as non-radical. Nitrogen derived oxidant species are mainly NO. – (nitric oxide), ONOO. – (peroxy nitrate), NO_2 (nitrogen dioxide) and N_2O_3 (dinitrogen trioxide) as describe in Figure 2 below.

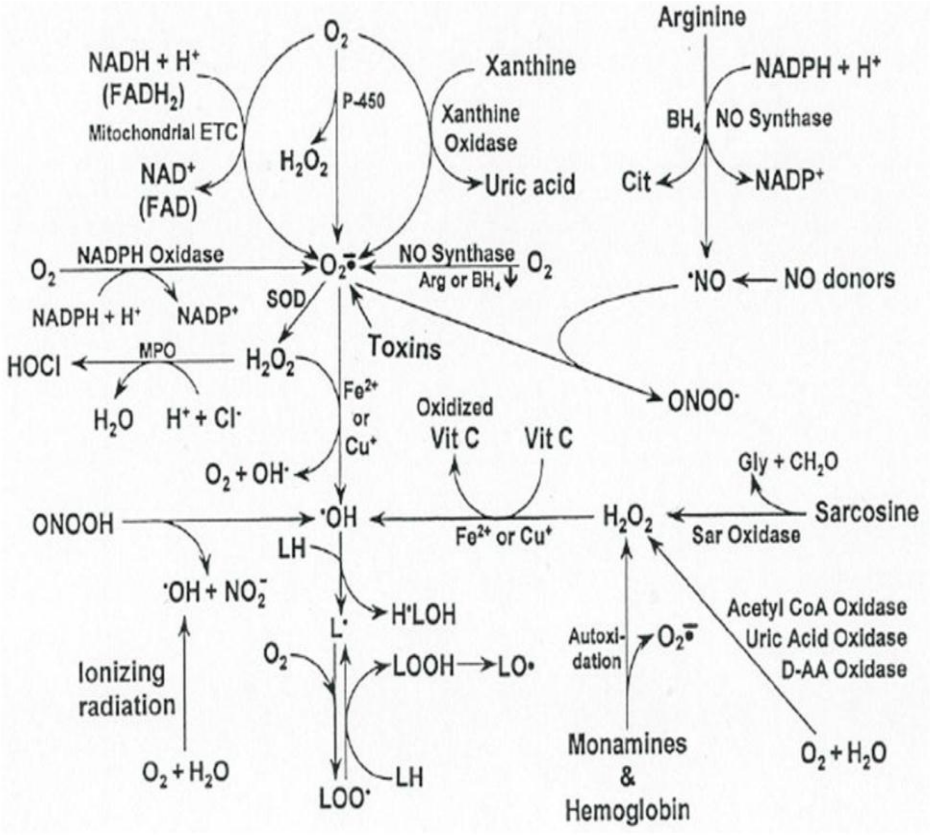


Figure 2: Oxygen and nitrogen-based free radicals and associated reactants that are generated in cells by various processes. Source: (Reiter *et al.*, (2003).

As describe in figure 1, the simultaneous event that occur in normal cell, there is appropriate oxidant: antioxidant balance. However, this balance may be shifted, when production of oxygen species is increased and when levels of antioxidants are diminished. This stage is called oxidative stress as reported by Vinson *et al.*, 1998; Cuvelier *et al.*, 1992. It was reported by Cuvelier *et al.*, 1992 that oxidative stress results in the damage of biopolymers

which include nucleic acids, proteins, poly-unsaturated fatty acids and carbohydrates (Naz *et al*,.2011).

2.0. MATERIALS AND METHODS

2.1. Elemental Analysis of *Spondias mombin.* (*L*inn), Extracts (Root, Leaf, and Stem bark)

The major elements comprising Calcium, Sodium, Potassium and trace elements (Fe and Zn) were determined according to the standard method and modified.(Osuntokun, 2016). The ground samples were sieved with a 2 mm rubber sieve and 2 g of each of the plant samples was subjected to dry ashing in porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO_3 /H_2O_2 (1:1) and heated gently on hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through a Whatman filter paper No 1 and the volume was made to mark with deionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer (AAS). Concentration of each element was calculated on percentage of dry matter (Selvakumar, 2011).

2.2. Determination of Antioxidant Constituents of *Spondias mombin*.(*L*inn),extracts (Root, Leaf, and Stem bark)

2.1. Reagent and Chemicals

All the chemicals and solvents were of analytical grade and obtained from Merck and Hi-Media, Mumbai, India.

2.2. Preparation of *Spondias mombin.* (*L*inn)Extracts

The ***Spondias mombin* (*L*inn) extracts (Root, Leaf, and Stem bark)** were collected in July from Adekunle Ajasin University, Akungba Akoko, and properly authenticated. A voucher of specimen (AAUA 133) was stored in the laboratory for further reference. The **extracts** were grounded to coarse powder. The extract was filtered and dried in rotavapour.

2.2.1. Determination of total phenolic content of *Spondias mombin.* (*L*inn)Extracts

The extract was determined based on the Folin -Ciocalteau's method, modified by Singleton *et al*. (1999) and Ayoola *et al*. (2008). The total phenolic content was determined as garlic acid equivalents (GAE). To a mixture of 0.1 ml of *Spondias mombin* extract and 0.9 ml of water was added 0.2 ml of folin ciocalteu' s phenol reagent and the resulting mixture was

vortexed. After 5 minutes of standing, 1 ml of 7 % (w/w) Na_2CO_3 solution was added and the solution was then diluted to 2.5 ml before incubation for 90 minutes at room temperature. The absorbance against a negative control containing 1 ml of methanol in place of the sample was then taken at 750 nm. The standard used was the garlic acid at 0.1 mg/ml in order to determine Garlic Acid Equivalent (GAE) of the sample, after preparing a calibration curve. Distilled water were used as blank.

2.2.2 Determination of total flavonoid content of *Spondias mombin.* (*L*inn)Extracts

The total flavonoid content of the extract was estimated according to methods of Miiiauskas *et al*. (2004) and Ayoola *et al*. (2008). The total flavonoid content of the extract was calculated as quercetin equivalents. Standard quercetin with varying concentration 0.1, 0.2, 0.3, 0.4, 0.5 mg/mi was used as standard in comparison to the sample extract. This was carried out based on the aluminum chloride colorimetric assay method described by Neergheen *et al*. (2014),(Neergheen *et al*. (2009).

To the solution of the standard quercetin with varying concentrations as above or varying concentration of 100 p1 and 50 p1 (made up to 100 p1 with distilled water) of sample in a test tube was added 4 ml of distilled water, 0.3 ml of 5 % sodium nitrite was then added after which it was left to stand for 5 minutes. After standing for 5 minutes, 0.1 ml of 10 % aluminum chloride was added and shaken which was immediately followed by the addition of 0.1 ml of 1 M sodium hydroxide solution and the volume made up to 10 ml with distilled water. The resulting assay mixture was then vortexed and the absorbance was measured at 510 nm against a negative control containing 1 ml of methanol in place of the sample. Calibration curve was prepared and absorbance readings were taken at 510 nm and compared to that of the standard quercetin in order to obtain quercetin equivalent (QE) of sample, distilled water was used as blank (Bao, 2005).

2.2.3 Estimation of ferric reducing antioxidant power (FRAP) of *Spondias mombin.*(*L*inn) Extracts

The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method with absorbance measured with a spectrophotometer (Benzie and Strain, 1999). The principle of this method is based on the reduction of a colorless ferric-tripyridyltriazine complex to its blue ferrous coloured form owing to the action of electron donor in the presence of antioxidants.

A 300 mmol/L acetate buffer of pH 3.6,10 mmolJL, 2, 4, 6-tri-(2-pyridyi)-1, 3, 5- triazine and 20 mmolJL $Fec1_3.6H_2O$ was mixed together in the ratio of 10:1:1, respectively, to give the working FRAP reagent. A 50 pl aliquot of the extract at 1 mg/ml and 50 i of standard solutions of ascorbic acid (20, 40, 60, 80, 100 pjg/mi) was added to 1 ml of FRAP reagent. Absorbance measurement was taken at 593 nm exactly 10 minutes after mixing against reagent blank containing 50ml of distilled water and 1 ml of FRAP reagent. The reducing power was expressed as equivalent concentration (EC) which is defined as the concentration of antioxidant that gave a ferric reducing ability equivalent to that of the ascorbic acid standard (Pulido *et al*., 2000).

2.2.4 Determination of diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity of *Spondias mombin*.**(*L*inn)** Extracts

Free radical scavenging capacity of ***Spondias mombin*.(*L*inn)** extracts against 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated by a modified method of Szabo *et al*. (2007). The radical scavenging ability of the extract was determined using the stable radical DPPH (2, 2- diphenyl-2-picrylhydrazyl hydrate) as described by Miliauskas *et al*. (2004). The reaction of DPPH with an antioxidant compound which can donate hydrogen, leads to its reduction. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm. To 1 ml of different concentrations (0.5, 0.25, 0.125, 0.0625, 0.03 125 mg/ml) of the extract or standard (vitamin C) in a test tube was added 1 ml of 0.3 mM DPPH in methanol. The mixture was incubated in the dark for 30 minutes after which the absorbance was read at 517 nm against a DPPH control containing only 1 ml methanol in place of the extract.

The percent of inhibition was calculated as follow:

$$I\ \% = \left[\frac{(A\text{control} - A\text{sample})}{A\text{control}]}\right] \quad X\ 100$$

Where A control is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Sample concentration providing 50,20,10,5 % inhibition ($1C_{50,20,10,5}$) was calculated from the graph plotting inhibition percentage against extract Concentration.

2.2.5 Determination of Fe^{2+} chelation of *Spondias mombin*.**(*L*inn)** Extracts

The potential of the ***Spondias mombin*.(*L*inn)** extracts to chelate Fe^{2+} was determined using a modified method of Puntel *et al*., (2005). Briefly, 150 mM $FeSO_4$ was added to a reaction

mixture containing 168 ml of 0.1M Tris-HCl pH 7.4, 218 ml saline of *Spondias mombin* extracts and the volume is made up 1 ml with distilled water. The reaction mixture was incubated for 5 min, before the addition of 13 ml of 1, 10-phenantroline and the absorbance was read at 510nm.

2.2.6 The 2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid (ABTS) scavenging ability of *Spondias mombin.* **(*L*inn)** Extracts.

The 2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) (ABTS) scavenging ability of the *Spondias mombin* extract was determined according to the method described by Re *et al*. (1999). The ABTS was generated by reacting an (7 mM) ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM/l, final conc) in the dark for 16 hours and adjusting the absorbance at 734 nm to 0.7. Then 0.2ml of the appropriate dilution of the extracts was then added to 2.0 ml of ABTS solution and the absorbance was read at 732 nm after 15 minutes. The standard trolox equivalent antioxidant capacity was subsequently calculated (264.32g). The percent inhibition was calculated from the following equation:

$$\text{Percentage of inhibition} = \left(\frac{AC - AS}{AC} \right) \times 100$$

2.2.7 Determination of superoxide anion scavenging activity of *Spondias mombin.***(*L*inn)** Extracts.

The superoxide anion radicals were produced in 2 ml of phosphate buffer (100 mM, pH 7.4) with 78 µM β- nicotinamide adenine dinucleotide (NADH), 50µM nitro blue tetrazolium chloride (NBT) and test samples at different concentrations. The reaction mixture was kept for incubation at room temperature (30^0C) for 5 minutes. Then 5-methylphenazinium methosulphate (PMS) (10 µM) was added to the reacting mixture to initiate the reaction and was incubated for 5 minutes at room temperature. The colour reaction between superoxide anion radical and NBT was read at 560 nm. Gallic acid was used as a positive control agent for comparative analysis. The reaction mixture without test sample was used as control and without PMS was used as blank (Selvakumar *et al*., 2001).

2.2.8. Hydroxyl Radical Scavenging Activity Assay **of** *Spondias mombin.***(*L*inn)** Extracts.

The scavenging activity for hydroxyl radicals was measured with Fenton reaction (Yu,2002, Ganu. 2010).Reaction mixture contained 60 µl of 1.0 mM $FeCl_3$, 90 µl of 1 mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 µl of 0.17 M H_2O_2, and 1.5 ml

of *Spondias mombin* extract at various concentrations. After incubation at room temperature for 5 min, the absorbance of reaction mixture was noted at 560 nm. The hydroxyl radicals scavenging activity was calculated according to the following equation and compared with ascorbic acid as standard:

$$\text{Inhibition} = \frac{AB - AE}{AB} \times 100$$

Where AB was the absorbance of blank (without extract) and AE was the absorbance of tested samples.

2.2.9. Statistical Analysis

Data were expressed as Mean ± standard error of mean, n= 5. The presence of significant differences among means of groups was determined by One-way Analysis of Variance (ANOVA), using GraphPad Prism (GraphPad Software, San Diego, CA, USA). Differences were considered to be significant if $p < 0.05$.

3.0: RESULTS

Figure 3.1-3.8 reveals the percentage of phenol, flavonoid, DPPH (1-1, diphenyl-2-picrydrazyl), FRAP (Ferric Reducing Antioxidant Property), H_2O_2 (Hydrogen peroxide), Fe^{2+} (Iron), OH (Hydroxide), ABTS - 2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) and SO (Sulphur monoxide) in the leaf, Stem Bark and root extracts of *Spondias mombin*. Ferric Reducing Antioxidant Property (FRAP) was highest in the leaf and stem bark while ABTS (2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) was highest in the stem bark and lowest in the root extracts. The Diphenyl-2-picryl-hydrazyl Radical Scavenging Activity (DPPH) and Superoxide anions **(**SO) were present at significant quantity at p<0.01> and all values were significant at p<0.05).

Table 3.1 reveals the quantitative elemental compositions of leaf, stem bark and root extracts of *Spondias mombin* (mg/100g).the element present are Sodium (Na), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Iron (Fe), Lead (Pb), Copper(Cu), Manganese (Mn), Potasium (K) and Phosphorus (P).it was observed that all elements were present at appreciable quantity.The highest and lowest quantity were found in Zinc (20.34) and Cu (1.22) for the leaf extract, Potasium (24.98) and (2.14) for stem bark , Calcium (29.35) and Pb (2.91) for the root extract. Pb has minimal quantity in all parts of *Spondias mombin* extract. P value< 0.0001, Significantly different standard deviations ($P < 0.05$).

Table 3.1: Quantitative Elemental Compositions of Leaf, Stem Bark and Root Extracts of *Spondias mombin* (mg/100g).

Plant sample used	Na	K	Ca	Mg	Zn	Fe	Pb	Cu	Mn	P
Leaf	14.60	15.21	19.76	20.11	20.34	21.11	1.25	1.22	6.24	18.92
Bark Stem	19.00	24.98	23.12	20.34	16.89	22.12	2.56	2.14	5.72	17.78
Root	20.91	23.11	29.35	24.71	17.37	20.32	2.71	3.02	4.96	27.23

P value< 0.0001, P value summary**** Significantly different standard deviations (P < 0.05).

Figure 3.1 Total polyphenol content expressed as gallic acid equivalents (μg GAE)/mg plant extract in *Spondias mombin.* **Figure 3.1** shows the percentage composition of phenol content of crude extract of *Spondias mombin*. This reveals the presents of phenol in *Spondias mombin.* The ethanolic leaf and stem bark extracts of *Spondias mombin* has the highest content of phenol and aqueous root extract of *S. mombin* has the lowest content of phenol at concentration 5, 10, 20 and 50 mg/ml respectively in *S.mombin* extract, using gallic acid as the standard.

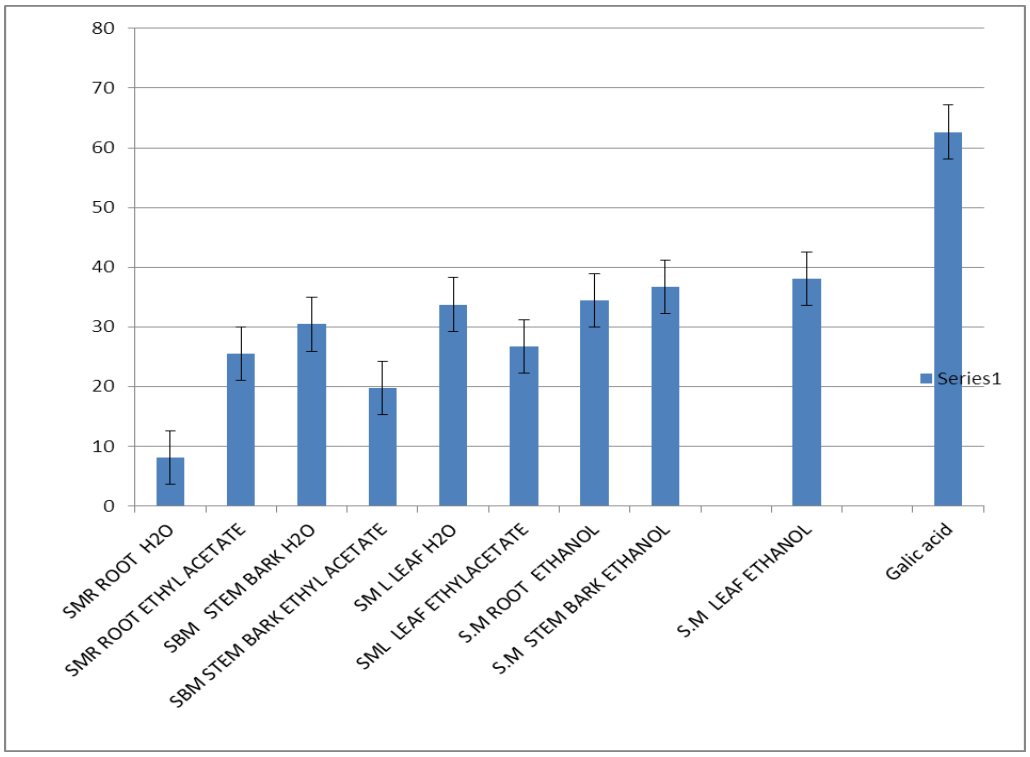
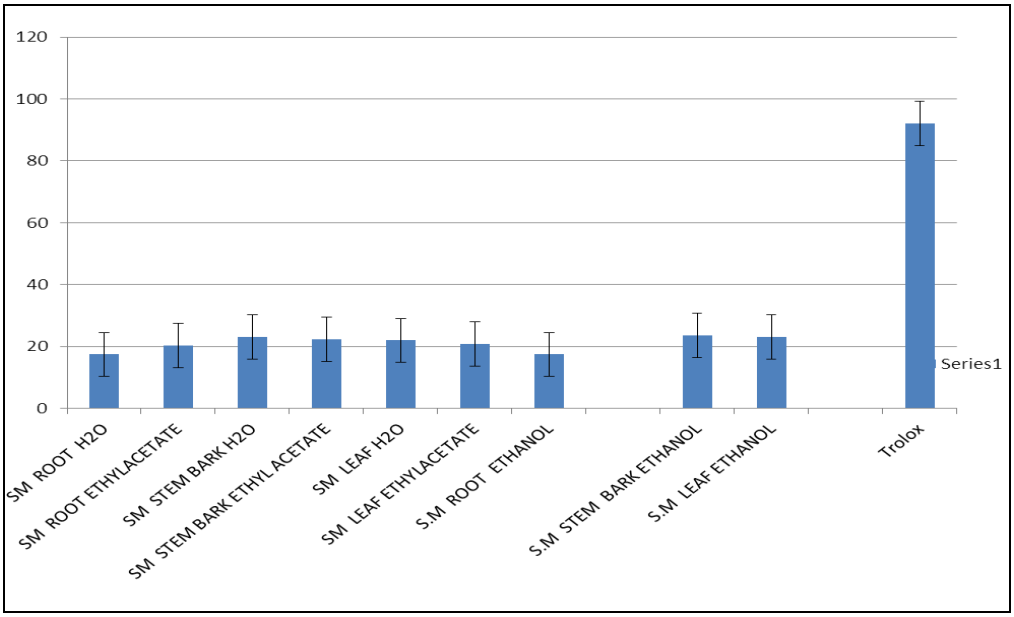


Figure 3.1: Percentage Composition of Phenol Content of Crude Extracts of *Spondias mombin.(Linn) Extracts.*

Figure 3.2 shows the percentage composition of ABTS content of crude extract of *Spondias mombin*. Using trolox as the preferable standard, the aqueous stem bark and ethyl acetate has

the highest content of ABTS at concentration 5, 10, 20 and 50 mg/ml respectively in *S.mombin* extract.



Figures 3.2: The Percentage Composition of ABTS 2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) Content of Crude Extracts of *Spondias mombin*. (Linn) Extracts.

Figure 3.3. Total flavonoid content expressed as quercetin equivalents (µg QE)/mg plant extract in *Spondias mombin* Figure 3.3 shows the percentage composition of flavonoids content of crude extract of *Spondias mombin*. Using rutin as the standard, it was observed that ethanolic leaf extract has the highest and ethyl acetate has the lowest content of flavonoids at concentration 5, 10, 20 and 50 mg/ml respectively in *S. mombin* extract.

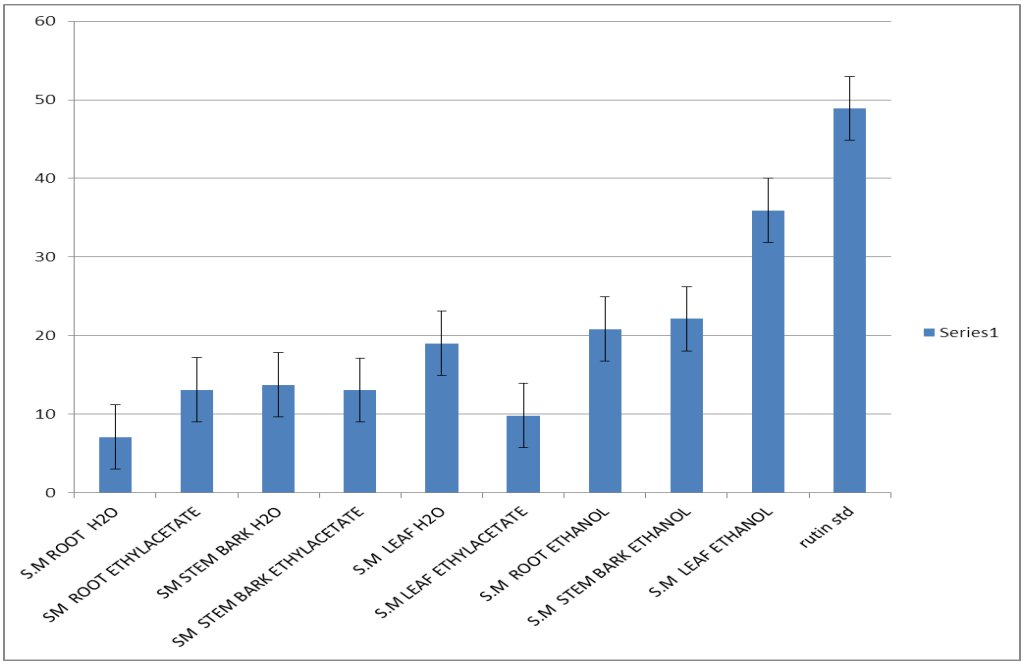


Figure 3.3; Percentage Composition of Flavonoid Content of Crude Extracts of *Spondias mombin*. (Linn) Extracts.

Figure 3.4 reveals the percentage composition of Fe2+ content of crude extract of *Spondias mombin.* Using EDTA at concentration 5, 10, 20 and 50 mg/ml respectively as the standard, ethyl acetate leaf extract has the highest content of Fe^{2+} and ethanolic root extract has the lowest content of Fe2+ in *S. mombin* extract.

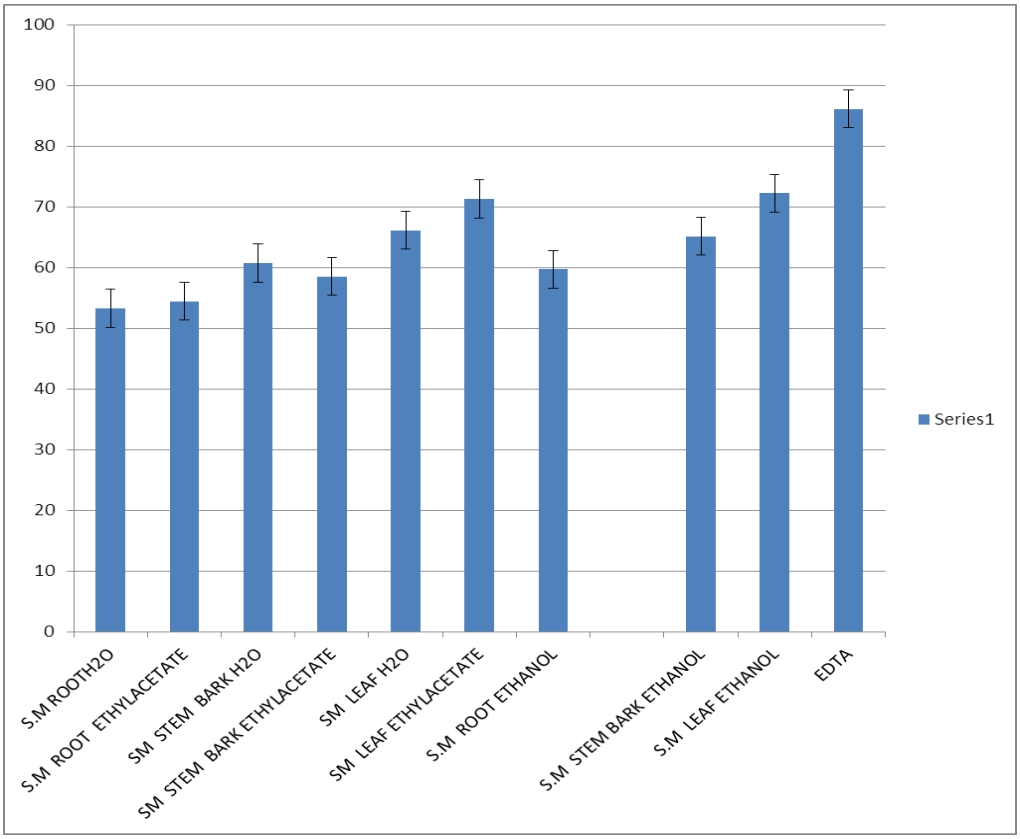


Figure 3.4: Percentage Composition of Fe $^{2+}$ (Iron) Content of Crude Extracts of *Spondias mombin*.(Linn)Extracts.

Figure 3.5 reveals the percentage composition of FRAP content of crude extract *of Spondias mombin*. Using Vit C as the preferred standard, ethanolic leaf extract has the highest content of FRAP and aqueous root extract has the lowest content of FRAP at concentration 5, 10, 20 and 50 mg/ml respectively in *S. mombin* extract.

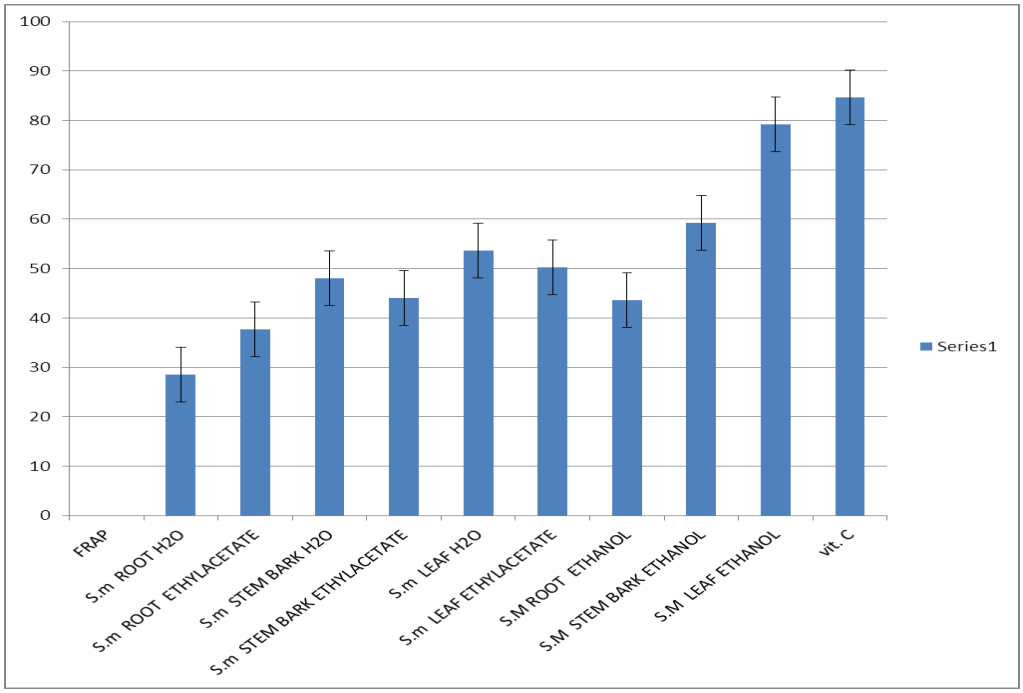


Figure 3.5: Percentage Composition of FRAP (Ferric Reducing Antioxidant Property). Content of Crude Extracts of *Spondias mombin. (Linn) Extracts.*

Figure 3.6 shows the percentage composition of DPPH content of crude extract of *Spondias mombin.* Using trolox as the preferred standard, it was observed that ethanolic stem bark has the highest and aqueous root extract has the lowest content of DPPH at concentration 5, 10, 20 and 50 mg/ml respectively in *S. mombin* extract.

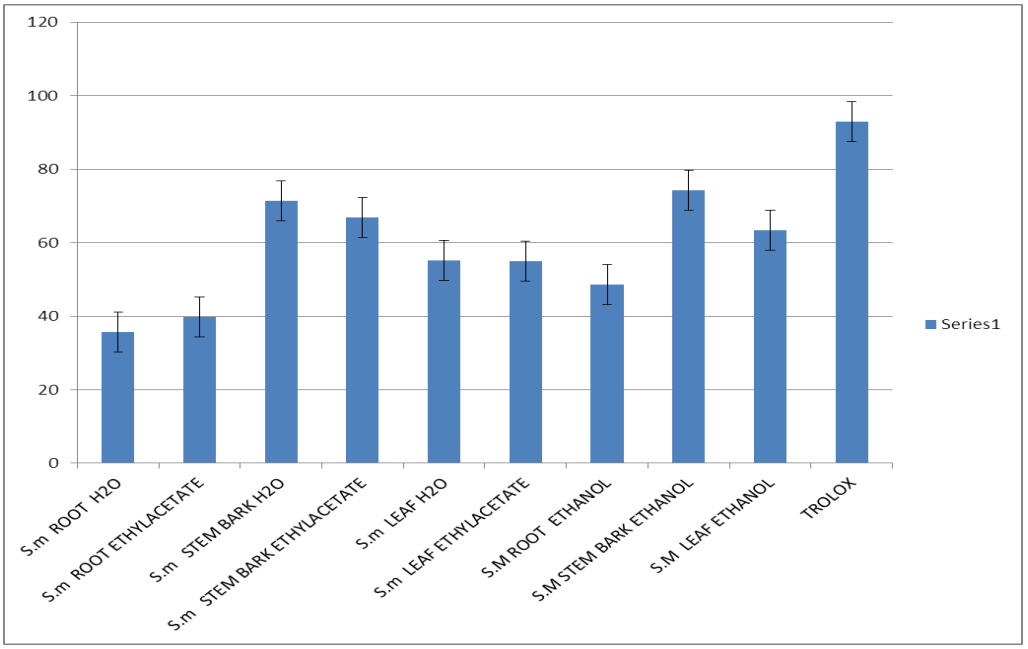


Figure 3.6: Percentage Composition of DPPH (1-1, diphenyl-2-picrydrazyl) **Content of Crude Extracts of *Spondias mombin*. (Linn) Extracts.**

Figure 3.7 shows the percentage composition of H2O2 content of crude extract of *Spondias mombin*. Using Vit C as the standard, it was observed that ethanolic leaf has the highest and ethyl acetate stem bark has the lowest content of H_2O_2 at concentration 5, 10, 20 and 50 mg/ml respectively in *S. mombin* extract**.**

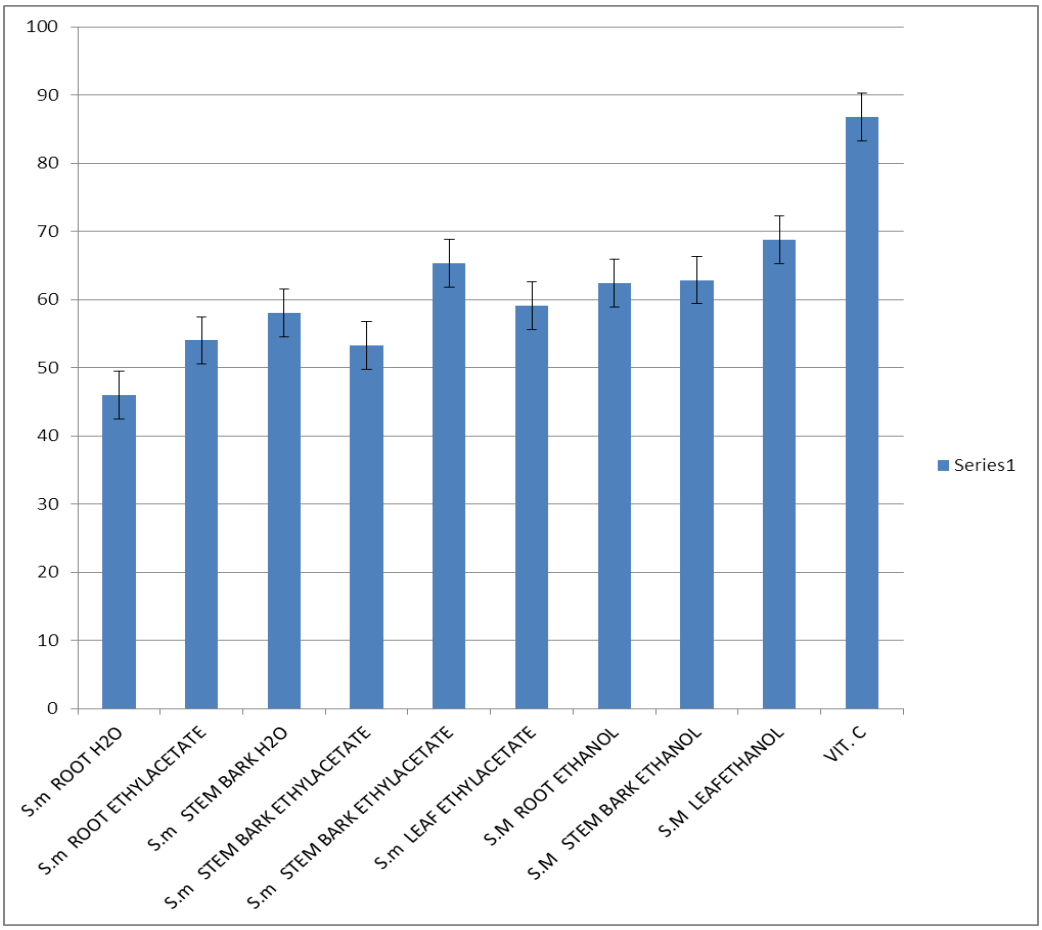


Figure 3.7: Percentage Composition of H_20_2 (Hydrogen peroxide) **Content of Crude Extracts of *Spondias mombin*. (Linn) Extracts.**

Figure 3.8 shows the percentage composition of SO content of crude extract of *Spondias mombin.* Using Vit C as the standard, it was observed that ethanolic leaf has the highest and aqueous root extract has the lowest content of SO at concentration 5, 10, 20 and 50 mg/ml respectively in *S. mombin* extract.

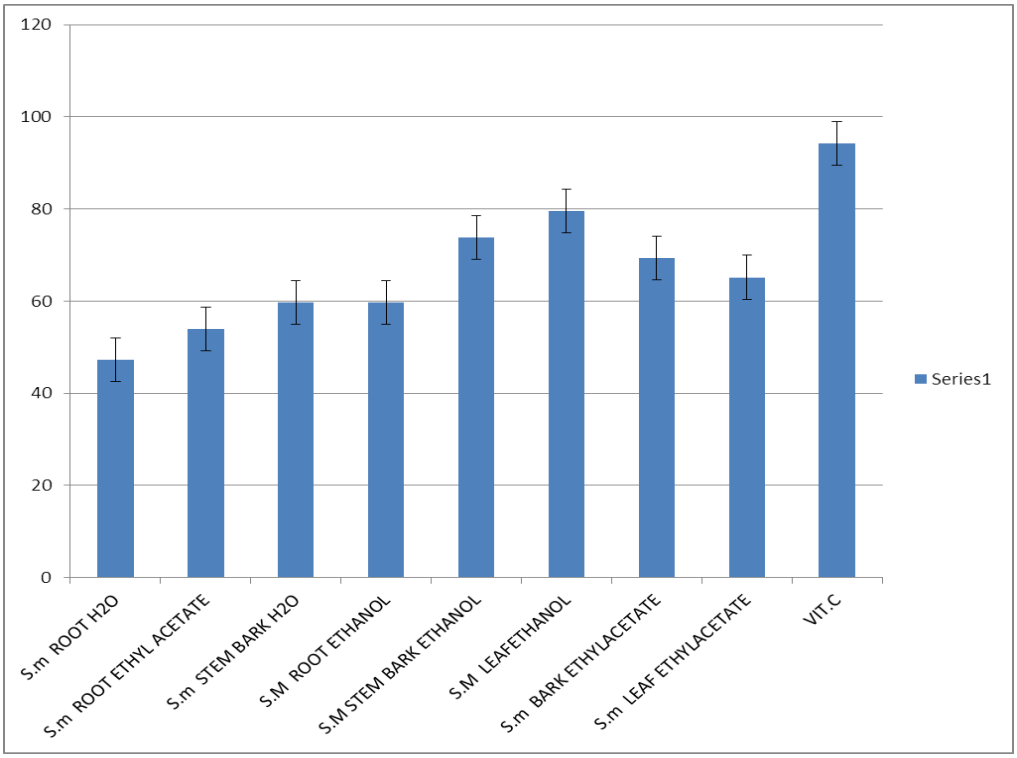


Figure 3.8: Percentage Composition of SO (Sulphur monoxide). Content of Crude Extracts of *Spondias mombin.(Linn) Extracts.*

Key to Figures

Phenol, DPPH (1-1, diphenyl-2-picrydrazyl)

FRAP (Ferric Reducing Antioxidant Property). H_2O_2 (Hydrogen peroxide)

Fe^{2+} (Iron), OH (Hydroxide). ABTS - 2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid)

SO (Sulphur monoxide).

SAMPLE A	S.M ROOT H_2O
SAMPLE B	S.M ROOT ETHANOL
SAMPLE C	S.M ROOT ETHYLACETATE
SAMPLE D	S.M STEM BARK H_2O
SAMPLE E	S.M STEM BARK ETHANOL
SAMPLE F	S.M STEM BARK ETHYLACETATE
SAMPLE H	S.M LEAF H_2O
SAMPLE I	S.M LEAF ETHANOL
SAMPLE O	S.M LEAF ETHYLACETATE

4.0 DISCUSSION

Oxidative stress has been implicated in several diseases including diabetes, rheumatoid arthritis, cardiovascular diseases, atherosclerosis, neurodegenerative diseases (Parkinson, Alzheimer, and Huntington), cancer, and aging (Hybertson *et al.*, 2011). Natural antioxidants such as phenolic acids and flavonoid compounds from plants may offer resistance against the

oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, and by other mechanisms (Liang *et al.,* 2011). Thus the aim present study is to show the antioxidant potentials of leaf, stem bark and root of *Spondias mombin*. (Linn) extracts, to be used in folk medicine in Nigeria (DPPH, ABTS, FRAP, Phenol, Flavonoid, H_2O_2) inclusive and also to determine the quantitative elemental composition of *Spondias mombin* extracts (leaf, stem and bark) were investigated in the present study. It was observed that the plant contains many antioxidant components. Antioxidant plays an important role in the human body system in different ways by reducing the concentration of reactive oxygen species (ROS) which was reported by Zhoe *et al*. (2008). Zhoe *et al*., 2008 also reported that DPPH measure the hydrogen atom and electron donor capacity of the *Spondias mombin*. (Linn) extracts to the stable oxygen forming free radical.

Iron is essential for the life because it is required for oxygen transport, respiration and activity of many enzymes is reported in table 3.1 where Iron (Fe3+) where found to be 22.12% in the Stem bark extract of *Spondias mombin* and 21 and 20.32% in the leaf and root respectively. Other element found in the extract of *Spondias mombin* are Na (20.91%) in the root , K (24.98%) in the stem bark, Ca (29.35%) in the root, Mg (24.71%) in root, 20.34 % in Zinc in leaf, Pb 2.71 in the root, Cu 3.02 % in root, Mn 6.24% in leaf and P^+ 27.23% in root extract of *Spondias mombin* extracts. Since all this elements were present in the *Spondias mombin* extracts at a very appreciable quantity, physiological activity were enhanced which makes the plant vital to both human, animal and even microorganism and also against infectious diseases.

Zhao *et al*. (2008) reported that chelating agents inhibits radical generations by stabilizing transition metals consequently reducing free radicals damage. In addition, some phenolic compounds exhibit antioxidant activity through the chelating of metal ions Fe^{2+} present in the *Spondias mombin* extracts. It must be noted that this chelating the transition metal catalyst is an important attribute of an antioxidant which is a group of compounds serving as a function of sequestration of transition metals that are well established in pro-oxidant (Nosiri *et al*., 2010).

Osuntokun *et al*., 2017 reported that some phenols which identified in *Spondias mombin* can polymerize into polyphenols that can bind minerals. Proanthocyanidins often occur as oligomers or polymers of monomeric flavonoids, polyhydroxy flavan-3-ols such as [+]-catechin and [−]-epicatechin. The polymeric procyanidins are better antioxidants than the

corresponding monomers, catechin, and epicatechin. Catechin and epicatechin were observed that can combine to form esters, such as catechin/epicatechin gallate, or bond with sugars and proteins to yield glycosides and polyphenolic proteins. Glycosylation of flavonoids at the 3 −OH group usually decreases the antioxidative activity due to the reduction of the number of phenolic groups (quercetin/rutin) (Osuntokun *et al*., 2017).

Formanek et al., 2001 reported that antioxidants especially phenolic, flavonoid, FRAP and DPPH are suggested to be a major and essential compounds that protect human cell from oxidative stress, damage and cell proliferation, flavonoid and phenols shows anti-carcinogenic potentials and mutagenic capabilities. Flavonoids are considered to be very beneficial compounds to the treatment of major infectious diseases due to the facts that they contain a natural potent antioxidants.

Flavonoids have been reported by (Priyadarsini *et al.*, 2003) to have antiviral, anti-allergic, anti-platelet, anti-inflammatory, antitumor and antioxidant potentials (Osuntokun *et al*., 2017). They are free radical scavengers. Super oxidant (Wojdyło *et al*., 2007) permits oxidative cell damage and have strong anticancer activity. It should be mention that flavonoids and their consumption has been associated with a variety of beneficial health effects including increase activity and erythrocyte superoxide dismutase, a decrease in lymphocyte DNA damage, a decrease in urinary 8-hydroxy-2-deoxygenase, and increase in plasma antioxidant capacity, this shows the efficacious use of *Spondias mombin* in our day to day activity which must be encouraged (Osuntokun, 2016).

5.0. CONCLUSION

This research work presented demonstrated that different parts of *Spondias mombin* are biologically active due to its elemental and a antioxidant composition. The current findings are a step forward to increase knowledge and usefulness and efficacy of *Spondias mombin* as a medicinal plant. The free radical scavenging potential and confirmation of the antioxidant activity add to the importance of *Spondias mombin* and details studies are warranted.

6.0 RECOMMENDATION

It is thereby recommended that the medicinal plant such as *Spondias mombin* and other types of medicinal plants should be studied and exploited for future use.

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