STUDIES OF PHYTOCHEMICALS AND *IN VITRO* RADICAL SCAVENGING POTENTIALS OF SIX DIFFERENT WILD FRUITS CONSUMED IN SOUTH-EASTERN NIGERIA

Abstract:

Objective: The study was carried out in order to document the phytochemical contents and radical scavenging capacities of different wild fruits consumed in south eastern Nigeria. **Methods:** Phytochemical screening and *in-vitro* antioxidant studies were carried out using standard method. **Result:** The results of quantitative phytochemical screening indicated the presence of flavonoids, tannins, phenols, terpenoids, alkaloids, saponins, steroids, oxalate and cardiac glycosides in varying concentrations. The in *vitro* antioxidant study showed that African elemi and African velvet tamarind with EC_{50} 1.68 ± 0.03 and 1.81 ± 0.01 µg/ml respectively scavenged DPPH radical more than other extracts compared to ascorbic acid EC_{50} 1.25 ± 0.02 while wild mango (EC_{50} 0.98 ± 0.01 µg/ml), pepper fruits (EC_{50} 1.75 ± 0.02 µg/ml) and date fruit (EC_{50} 1.88 ± 0.01 µg/ml) scavenged hydroxyl radical more than other extracts used in this study. All the extract showed a concentration dependency in reducing ferric iron to ferrous ion, also date fruit (0.11 ± 0.00 µg/ml) had more of the total antioxidant compounds in this study compared to ascorbic acid (0.22 ± 0.01µg/ml). **Conclusion:** The high quantity of phytochemicals and radical scavenging capacity of the wild fruits shows their importance in nutrition; therefore wild fruits should be included in our daily diet to improve the quality and nutritional values of food.

Keyword: *Phytochemical compositions, Antioxidant studies, wild fruits, radical scavenging and south eastern Nigeria.*

1.0 Introduction:

Plants are rich in antioxidants and much attention has been paid to the development of ethno medicines as they contain phenols, flavonoids, alkaloids, tannins, vitamins, terpenoids, and many other phytochemicals responsible for various pharmacological functions [1]. **Hog plum (*Spondiasmombin)*:** *Spondiasmombin*(Hog plum) (Plate 1.1) is a tropical fruit tree that grows to about 20 meters tall. It has diverse local names in Nigeria; in Igbo it is known as ‘Utu, Ngugulu or Ijikara’, Yoruba , ‘Iyeye or Ebo’ and in Hausa, ‘Isada’. The

plant has been found to be useful medicinally among many countries of the world where it commonly grows [2,3]. **African Velvet Tamarind (*Dialiumguineense*):** *Dialiumguineense*, known as African velvet tamarind (AVT), licky licky or black velvet tamarind (Plate 1.2) is a tropical forest fruit tree. AVT is known by different names in Nigeria. It is known as 'Tsamiyarkurmi' in Hausa, 'Icheku' in Igbo, 'Awin' in Yoruba, and 'Amugen' in Edo. Every part of this wild fruit has its own uses and health benefits. **Wild Mango (*Irvingia gabonensis*):** *Irvingiag abonensis*, also known as wild mango/bush mango or dika nut (Plate 1.3), is a native African fruit tree that produces edible fruits and seeds [4,5,6]. This fruit is locally called differently in Nigeria, in Igbo it is known as 'Ugiri', Yoruba- Oro and Hausa- Goron or Birin.In Cameroon, *I. gabonensis* kernels are widely marketed [7] **Date Fruit (*Phoenix dactylifera*):** Dates (*Phoenix dactylifera L.*) (Plate 1.4) is one of the oldest known fruit crops, cultivated for at least 5,000 years in North Africa and the Middle East [8]. It is locally called different names in Nigeria, in Igbo – Nkwuozara, Yoruba – Esoomeka and Hausa – Dabino. They are used in herbal medicine e.g it is eaten by women during pregnancy which helps to strengthen the uterine muscles.

African Elemi (*Canariumschweinfurthii*): African elemi, known as bush candle, Incense tree, Purple canary tree, or Gum resin tree in English (*Canariumschweinfurthii*) (Plate 1.5) is a big tree with a long and straight bowl of more than 50m. In Nigeria, the tree is known as 'Ube mgba', 'ubeosa', 'ubeokpoko', 'ubeigwe' (Igbo), 'Atili' or 'Atile' (Hausa), or 'Origbo' (Yoruba)[9]. **Pepper fruit (*Dennettiatripetala*):** *Dennettiatripetala*(Plate 1.6) also have different names in Nigeria which are 'mmimi' in Igbo, 'ataigbere' in Yoruba while 'ako' in Hausa. This fruit is use in traditional medicine as a remedy for cough, fever, toothache, diarrhea, diabetes, and nausea in pregnant women. This study documented the phytochemical contents of some wild fruits in Nigeria and also compared the radical scavenging potentials of the fruits using various methods.

2 Materials and methods

2.1 Chemicals and drugs

All chemicals used in this study were of analytical grade and products of Sigma Aldrich, Germany

2.1.1 Sample Collection

Hog plum (*Sapondiasmombin*), African velvet tamarind (*Dialiumguineense*), Wild mango (*Irvingiagabonensis),* Date (*Phoenix dactylifera)*, African elemi (*Canariumschweinfurthii*) and Pepper fruit(*Dennettiatripetala*) were obtained from Eke Awka market in Awka and identified by a taxonomist Dr. Ukpaka, C.J of Biological Science Department, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria.

2.2 Sample Preparation

Some of the samples (African velvet tamarind and wild mango) were peeled while the other samples (hog plum, date fruit, African elemi) were de-seeded and sun dried for four days, they were ground using manual grinder. The powder was extracted with 80 % ethanol and concentrated with rotary evaporator and stored at 40 ^{0}C until being used.

2.2.1 Quantitative Phytochemical Analysis

Quantitative phytochemical analysis were carried out on the ethanol extract of the selected wild fruits using standard phytochemical tests as described by [10,11]. The following phytochemicals were assayed: cardiac glycosides, flavonoids, phenol, tanins, alkaloid, terpenoids, saponins, oxalate an steroids.

2.3 In *vitro* antioxidant assay

2.3.1 DPPH radical - scavenging assay

The method reported by [12] was used to assay for the capacity of the extracts to scavenge DPPH radical. Briefly, a 2.0 ml solution of the extract at different concentrations (62.5 – 500 µg/ml) diluted two-fold in methanol was mixed with 1.0 ml of 0.3 mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 25 min. Blank solutions were prepared with each test sample solution (2.0 ml) and 1.0 ml of methanol while the negative control was 1.0 ml of 0.3 mM DPPH solution plus 2.0 ml of methanol. Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with a UV visible spectrophotometer. DPPH Radical inhibition was calculated using the equation:

$$\% \text{ inhibition} = \frac{A_0 - A_S}{A_0} \times 100$$

Where A_0 is the absorbance of the control, and As is the absorbance of the tested sample. The EC_{50} represented the concentration of the extract that inhibited 50% of radical.

2.3.2 Hydroxyl radical ($OH^{\cdot}$) Scavenging Assay

The method of [13] was used to assay for hydroxyl radical, different concentrations (62.5 – 500 µg/ml) of the extracts were added to a reaction mixture containing, the following, 2-deoxyribose (2.5 µM), potassium phosphate buffer (pH 7.4, 20 mM), $FeCl_3$ (100 µM), EDTA (104 µM), H_2O_2 (1 mM), and L-ascorbic acid (100 µM). The mixtures were incubated for 1 h at 37 ^{o}C, followed by addition of 1.0 ml of 1% (w/v) TBA in 0.05 M NaOH and 1.0 ml of 2.8 % (w/v) TCA. The resulting mixture was heated for 15 min at 100^{o}C. After cooling on ice, absorbance was measured at 532 nm. Inhibition of 2-deoxyribose degradation expressed in percentage was calculated as per the equation:

$$\% \text{ inhibition} = \frac{A_O - A_S}{A_O} \times 100$$

A_O = Absorbance of the control, As = Absorbance of the test sample.

2.3.3 Ferric reducing power assay

Ferric ions reducing power was measured according to the method described by [14]. Ethanol extract of the different wild fruits at different concentrations of 62.5 – 500 µg/ml were mixed with 2.5 ml of 20 mM phosphate buffer and 2.5 ml 1% potassium ferricyanide, and then the mixture was incubated at 50 ^{0}C for 30 minutes. Afterwards, 2.5 ml of 10%, w/v trichloroacetic acid and 0.5 ml 0.1% w/v ferric chloride were added to the mixture, which was kept aside for 10 minutes. Finally the absorbance was measured at 700 nm.

2.3.4 Total antioxidant capacity

Total antioxidant capacity assay as reported by [15]. Ethanol extract of the wild fruits at different concentration ranging from 62.5 to 500 µg/ml were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95 ^{0}C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as positive reference standard

3.0 Statistical Analysis

All data were reported as mean ± standard deviation, where appropriate. One – way analysis of variance (ANOVA) was used to analyze the data results using Statistical Package for Social Science (SPSS) version 20 software. Group mean obtained after each analysis were compared using Ducan multiple comparison test and difference were considered significant at $p<0.05$.

4.0 RESULTS

4.1 Quantitative Phytochemical Analysis

Result of the quantitative phytochemical composition of the six selected wild fruits Fig 1.0 showed that, African velvet tamarind has the highest amount of flavonoids compared to other fruits. The alkaloid concentration was highest in hog plum, while the wild mango showed the highest concentrations saponin, oxalate and steroids compared to the other fruits. Date fruit and African velvet tamarind had the highest concentrations of glycosides. Tannin concentration was highest in African elemi followed by hog plum and least was observed in wild mango, while pepper showed the highest phenolic content.

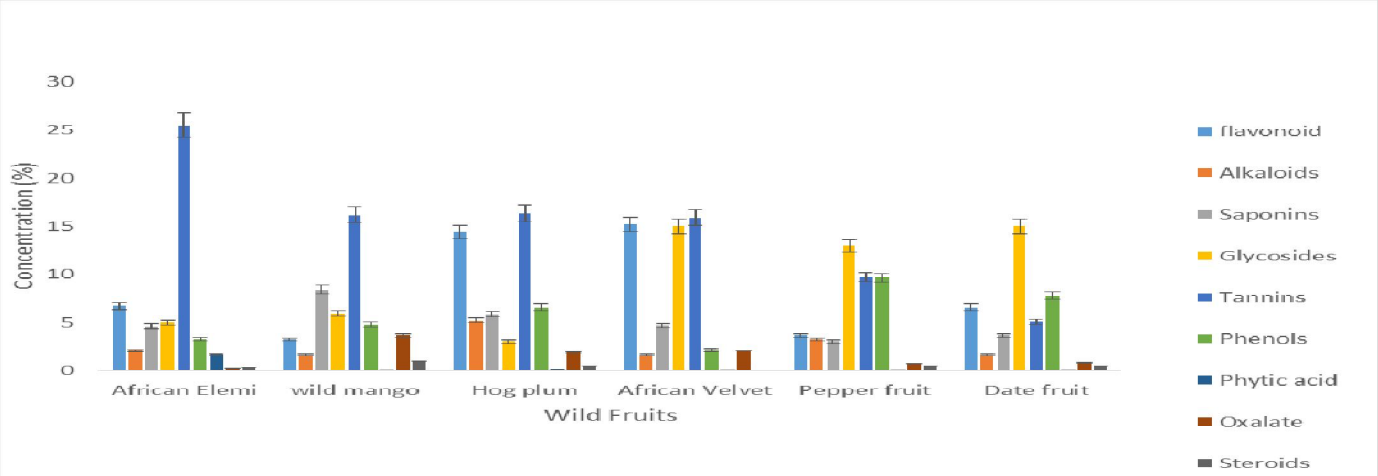


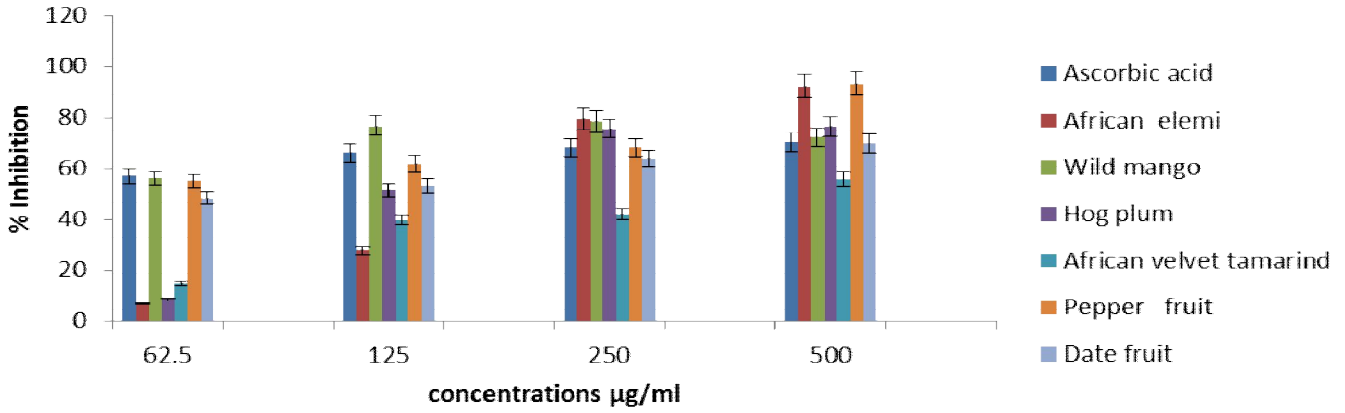
Fig 1.2: Phytochemical Composition of the selected Wild Fruits

4.2 In *Vitro* Antioxidant Assay

Results of the *in vitro* antioxidant assay of the six selected wild fruits and their difference in percentage inhibition concentrations are shown in Figure 1.1- 1.4.

4.2.1 DPPH (2-2 Diphenyl-1-Picrylhydrazyl) Radical Scavenging potentials of selected wild fruits

Figure 1.2 showed the ability of different selected wild fruits extracts to scavenge DPPH radical compared to ascorbic acid, the result shows that African elemi and African velvet tamarind with EC_{50} 1.68±0.03 and 1.81±0.01 µg/ml respectively scavenged DPPH radical more than other extracts compared to ascorbic acid EC_{50} 1.25 ± 0.02.

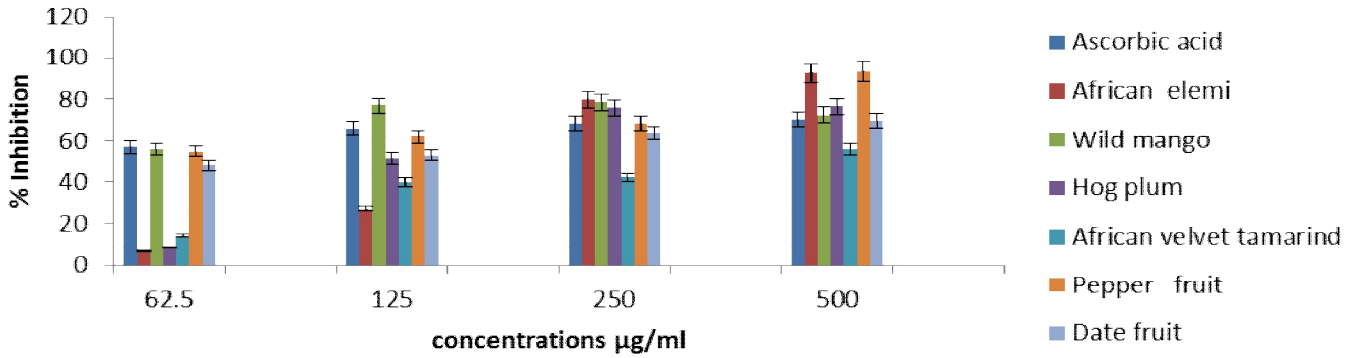


Values are mean ± standard deviation of triplicate determinations

Fig 1.2: DPPH (2-2 Diphenyl-1-Picrylhydrazyl) Radical Scavenging Assay

4.2.2 Hydroxyl (OH^-) Radical Scavenging potentials of Selected Wild Fruits

The hydroxyl radical (OH^-) scavenging capacities of the different selected wild fruits are presented in Figure 1.3. The result showed that wild mango, pepper fruit and date fruit with EC_{50} 0.98 ± 0.01, 1.75±0.02 and 1.88±0.01 µg/ml respectively scavenged (OH^-) more than other extracts compared to ascorbic acid.

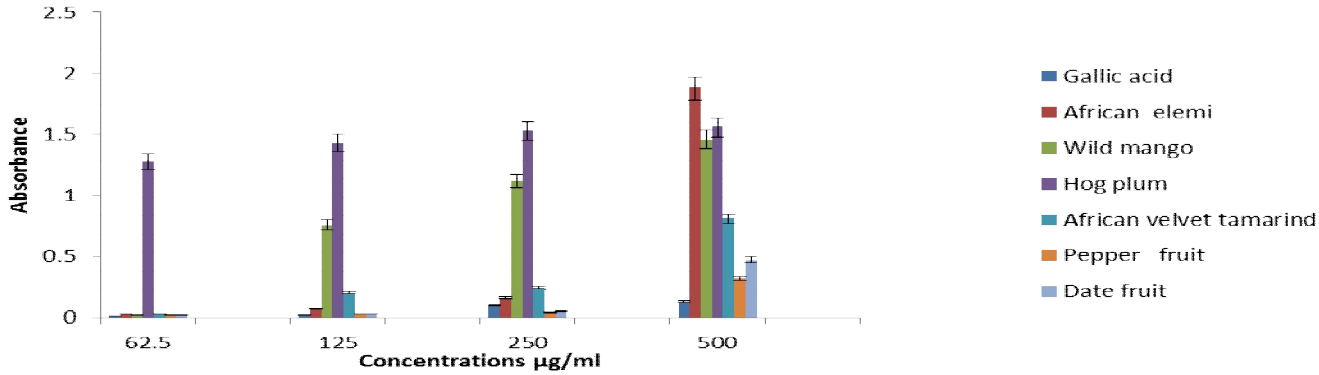


Values are mean ± standard deviation of triplicate determinations.

Figure 1.3: Hydroxyl (OH^-) Radical Scavenging Assay of the Selected Wild Fruits

4.2.3 Ferric Reducing Antioxidant Power (FRAP) of Selected Wild Fruits

The result in Figure 1.4, showed that Gallic acid reduced ferric ion to ferrous ion more than other extracts used in this study. The selected wild fruits reduced ferric ion to ferrous form in a concentration dependent manner which is an indication of antioxidant capacity.

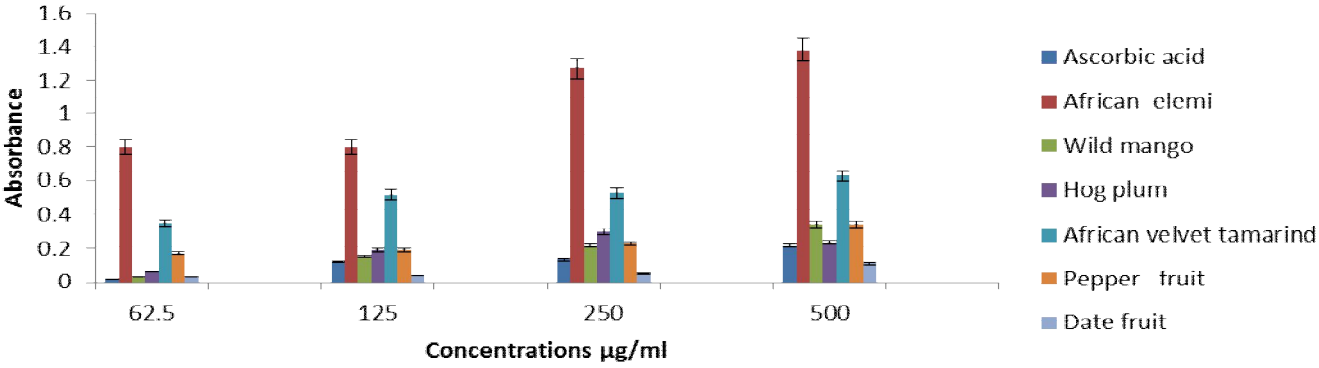


Values are mean ± standard deviation of triplicate determinations

Figure 1.3: Ferric Reducing Antioxidant Power (FRAP) Assay of Selected Wild Fruits

4.2.4 Total Antioxidant Capacity (TAC) Assay of Selected Wild Fruits

The result in Figure1.4 showed that date fruit (0.11 ±0.00 µg/ml) had more of the antioxidant compounds in this study compared to ascorbic acid (0.22 ± 0.01µg/ml) and other fruits. The differences could be as a result of different concentration of polyphenolic compounds present in the extracts.



Values are mean ± standard deviation of triplicate determinations

Figure: 1.4: Total Antioxidant Capacity (TAC) Assay of Selected Wild Fruits.

5.0 Discussion

Phytochemicals are plant-derived chemical compounds that thrive or thwart predators, competitors, or pathogens [16]. The major phytochemicals found in fruits studied are flavonoids, alkaloids, tannins, phenol,

saponins, glycosides, oxalates, and phytate. Flavonoids are prevalent plant secondary metabolites which include flavones, flavonols and condensed tannins that modulate lipid peroxidation [17]. They occur as glycosides and contain several phenolic hydroxyl groups on their ring structure.

The result of this study in Fig 1.0 showed that African velvet tamarind has the highest amount of flavonoids followed by hog plum and least in wild mango. This data appears to be lower than that of the previous study done by [18]. This could indicate that African velvet tamarind has high antioxidant potential. Previous studies have shown that flavonoids possess antibacterial, antiviral, anti-inflammatory, anticancer and anti-allergic abilities [19,20].

The alkaloid content was highest in hog plum followed by pepper fruit and least in date and African velvet tamarind. Alkaloids are nitrogen containing naturally occurring compounds found to have antimicrobial properties due to their ability to intercalate with DNA of the microorganisms [21].Figure 1.2, showed that the alkaloids content was highest in hog plum followed by pepper fruit and least in date fruit and African velvet tamarind. The high level of alkaloid in hog plum was in agreement with that of the previous research done by [22]. Alkaloids are beneficial chemicals to plants. They help in repelling predators and parasites. However, when ingested by animals, they affect glucagon, thyroid stimulating hormone and inhibit certain mammalian enzymatic activities [23]. Fruits normally store phytochemicals such as alkaloids to protect them from infections and insects. When alkaloids found in fruits began to reduce, their concentration weakens as the fruits ripen.

The above results Fig 1.2 showed the presence of saponin, oxalate and steroids in the highest quantity in wild mango than the other six selected wild fruits used in this study. These compounds are known to be biologically active and therefore aid in the microbial activities [24]. The glycosides were highest in date fruit and African velvet tamarind followed by pepper fruit and least in hog plum. Glycoside has anticancer effect and helps to increase the tone, excitability and contractility of the cardiac muscles, and also helps to exert diuretic effect due to increased renal circulation [18]. African velvet tamarind was highest in glycoside followed by pepper fruit and least in hog plum. The level of glycosides in African velvet tamarind in this study appears to be lower than that of the previous study done by [18] which may be due to variations in environmental, climate, soil, etc. Tannins are significant water soluble plant secondary metabolites gotten by the condensation of simple phenolics resulting to condensed tannins which are divided into hydrolysable and condensed proantho-cyanidins[25]. They also have powerful biological activities and nutritional effects. Figure 1.2 showed that tannin was highest in African elemi followed hog plum and least in wild mango. This result finding was higher than that of the previous study done by [26]. The higher tannin level found in this selected fruit may be partly responsible for the bitter principle associated with the fruits [20]. Researchers

around the world are in search of potent molecules from plants for developing useful drugs with least or no side effect and cytotoxicity [27]. Tannic acid present in these six selected wild fruits can thus be of immense use in endeavors of drugs development from natural sources. Phenol was highest in pepper fruit, followed by date fruit and least African velvet tamarind. The differences in the mean values of various phytochemicals studied were significantly different from each other at $p < 0.05$. From the result above in Figure 1.2 which indicated that the high level of phenol in pepper fruit was slightly higher than that of the previous study done by[27] which may be due to the physiological changes which must have cause an increase in the total phenol content [28,29]. It also indicated that the high phenol present especially in pepper fruit are rich source of antioxidants because studies have shown that antioxidants capacity of plants is tightly correlated with phenolic compounds [30,31].

5.1 In *Vitro* Antioxidant Assay

Owing to the complication of the oxidation, antioxidation processes and to the diverse nature and function of antioxidant components found in extracts, it is evident that one test is unable to provide a clear data of the antioxidant capacity of some samples [32]. However, in this study, four methods have been applied to evaluate the antioxidant potentials of the different selected wild fruits.

5.1.1 DPPH (2-2 Diphenyl-1-Picrylhydrazyl) Radical Scavenging potentials of selected wild fruits

African elemi and African velvet tamarind with EC_{50} 1.68 ± 0.03 and 1.81 ± 0.01 µg/ml respectively scavenged DPPH radical more than other extracts compared to ascorbic acid EC_{50} 1.25 ± 0.02. This result could be as a result of the antioxidant compounds present in the extracts neutralizing the radical nature of DPPH either by transferring electron or single hydrogen atom to DPPH·[33]

5.1.2 Hydroxyl (OH^-) Radical Scavenging potentials of Selected Wild Fruits

The hydroxyl radical ($^{\cdot}OH^-$) is the most reactive radical known to initiate lipid peroxidation and damage of biomolecules [34]. Wild mango, pepper fruit and date fruit with EC_{50} 0.98 ± 0.01, 1.75 ± 0.02 and 1.88 ± 0.01 µg/ml respectively scavenged (OH^-) more than other extracts compared to ascorbic acid. The antioxidants in the extracts could be acting as Fe^{3+}chelator in the system or donating hydrogen atom and accelerating the conversion of H_2O_2 to H_2O [35].

5.1.3 Ferric Reducing Antioxidant Power (FRAP) of Selected Wild Fruits

The ferric reducing antioxidant power (FRAP) assay was used to study the capability of extracts to reduce the ferricyanide (Fe^{3+}) complex to their ferrous form (Fe^{2+}) by donating an electron. The result showed that

Gallic acid (0.13 ± 0.01 µg/ml) reduced ferric ion to ferrous ion more than other extracts used in this study. The selected wild fruits reduced ferric ion to ferrous form in a concentration dependent manner which is an indication of antioxidant capacity. The differences in reducing capacities between the samples may be related to difference in concentrations of the bioactive compounds in the extracts.

5.1.4 Total Antioxidant Capacity (TAC) Assay of Selected Wild Fruits

Total antioxidant capacity (TAC) of the phosphomolybdenum model evaluated both water-soluble and fat-soluble antioxidant capacity [36]. It is a quantitative assay that is based on the reduction of Mo^{6+} to Mo^{5+} by the formation of a green phosphate/Mo (V) complex at acidic pH. The result showed that date fruit (0.11 ±0.00 µg/ml) had more of the antioxidant compounds in this study compared to ascorbic acid (0.22 ± 0.01µg/ml). The differences could be as a result of different concentration of polyphenolic compounds present in the extracts.

6.0 CONCLUSION

This study has shown that the six selected wild fruits grown in South Eastern, Nigeria are rich in, phytochemicals and possess *in vitro* antioxidant properties. This showed that the use of these fruits is quite beneficial for healthy living. The world is endowed with a variety of wild fruits which can be exploited independently throughout the year, however the major challenges to their inclusion in the diet remain acceptability and accessibility and in general, lack of interest and neglect by individuals across the world. These challenges can be overcome by educating people on their health benefits, to create an interest in them and eventual inclusion in the daily diet.

Declaration of Competing Interest

No competing interests are declared by authors

Author's contributions

Maryann Chinenye Maduako: Conceptualization and methodology. Kizito Ifeanyi Amaefule: Writing (original draft). Review and editing. Jonathan Chinenye Ifemeje: Formal analysis. All authors have read and approved the final article.

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APPENDIX

Plate 1.1 Hog plum (*Spondiasmombin)*

A. African velvet tamarind with the skin B. African velvet tamarind without the skin

Plate 2.2 African Velvet Tamarind (*Dialiumguineense).*

Plate 1.3 Wild Mango (*Irvingiagabonensis).*

A. Date fruit with the nut. B. Date fruits without the nut.

Plate 1.4 Date Fruit (*Phoenix dactylifera L.*).

A. African Elemi with the nuts. B. African elemi without the nuts.

Plate 1.5 African Elemi (*Canariumschweinfurthii*).

Plate 1.6 Pepper fruit (*Dennettiatripetala). Source:* Maduako, 2021.