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QUALITATIVE PHYTOCHEMICAL ANALYSIS AND SELECTED *IN VITRO* ANTIOXIDANT ACTIVITIES OF PARTLY PROCESSED SEED OF *TRECULIA AFRICANA*

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ABSTRACT
Treculia africana belongs to the *Moraceae* family, and grows in dense forests in Africa. The seeds of the plant have medicinal properties. The present study investigated the phytochemical analysis and the *in vitro* antioxidant activities of aqueous seeds extract of *Treculia africana*. Aqueous extract of *T. africana* seeds were prepared using standard method. The quantitative phytochemical and *in vitro* antioxidant activities of the extract were also determined using standard methods. The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity and total antioxidant capacity (TAC) of the ascorbic acid were significantly higher than those of aqueous extract ($p < 0.05$). However, there were no significant differences in their nitric oxide (NO) scavenging activity, thiobarbituric acid (TBARS), and reductive potential($p > 0.05$).The ferric reducing antioxidant power (FRAP) of the aqueous extract was significantly higher than those of aqueous extract, ascorbic acid and of gallic acid ($p < 0.05$). These results suggest that phenolics present in the seeds extract may play a role in free radical scavenging activities of the medicinal seeds. In essence, the partly processed seed sample of *Treculia africana* used, gave results that show that *Treculia africana* seeds are rich in phytochemicals, and have free radical scavenging ability to a good extent.

Keywords: Phytochemicals; Antioxidants; *Treculiaafricana*; 2,2-diphenyl-1-picryl-hydrazyl; Free radical

1.0 Introduction:

The use of herbal medicinal plant products and phytonutrients had been increased in the last decade; the public interest and acceptance of their use as alternative and complementary medicine is not only in developing countries but also in developed ones (Liviac *et al*., 2019). The practice of traditional medicine is as old as the existence of man. The use of plants in traditional medicine, called herbalism, or simply botanical medicine, falls outside the mainstream of Western or Orthodox medicine. It is estimated that two-thirds of the world's population (mostly in the developing countries) depend on traditional medicine as their primary form of health care (Parker *et al*., 2016).

The use of traditional medicine cannot disappear in the treatment and management of diseases in the African continent and this is attributable to socio-cultural and socio-economic lifestyles; lack of basic health care and skilled personnel (Elujoba *et al*., 2005). Plant seeds contain active components such as flavonoids, glycosides, saponins, tannins, etc. which possess medicinal properties that are used for the treatment of various diseases (Feher and Schmidt, 2003). The active ingredients of many pharmaceutical-derived drugs contain components derived from

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phytochemicals in the seeds (Nwodo *et al*., 2016). These substances which contain the healing property are known as the active ingredients and it has been found that they vary from seed to seed. Among these seeds are pumpkins whose parts are eaten as a support food or main dish and can be aromatic, bitter or tasteless (Galm and Shen, 2007).

The plant kingdom offers a variety of natural antioxidants and medicinal values (Okojoh *et al*., 2014). Dietary plant seeds with proven antioxidant properties can act as a direct anti-radical chain breaker of free radical propagation, interaction with transition metals and inhibition of enzymes that generate reactive oxygen species (ROS). *Treculia africana* is one of those medicinal seeds whose medicinal values has stood the test of time (Osabor *et al*., 2009). The present study investigated the phytochemical analysis and the *in vitro* free radical scavenging activities of aqueous seeds extract of *Treculia africana* so as to possibly justify its local uses in Nigeria.

2.0 Literature Review

Treculia africana is a species of forest tree commonly known in English as the African breadfruit (in Tanzania, Zambia, Uganda, and USA), breadfruit (in Nigeria), wild jackfruit (in Tanzania and Uganda), and African boxwood (in Malawi). It belongs to the *Moraceae* family, a diverse and economically important family of flowering plants. African breadfruit is identified by different names among the tribes in Nigeria, such as "ukwa" (Igbo), "afon" (Yoruba), "barafuta" (Hausa), "Ize" (Benin), "eyo" (Igala) and "edikang" (Efik). It is called 'ulu' in Hawaii (Okonkwo and Ubani, 2012).

The *Treculia africana* Decne treeis a large tall tree (with a maximum total height of about 50 m, and a mean total height of the matured tree between 20 and 30 m) which is common in tropical regions of Africa (Nuga and Ofodile, 2010) and other sub-tropical countries (Agu*et al*., 2007). *Treculia africana* is geographically distributed in West and Central Africa and can grow below 1,500 meters above sea level (Bingham, 2017). In Nigeria, African breadfruit is more abundant in the Southern parts (Oyetayo and Omenwa, 2006). It belongs to the *Moraceae* family and the stem can grow up to 6 meters in length (Nutrecul Agroforestry Company (NAC), 2013).

A mature African breadfruit tree has a broadly spread, round and dense crown, making it a shade tree species. The African breadfruit tree has a grey bark, large leaves (dark green above and lighter below) and discharges a cream latex. It could either be dioecious (sexes on separate trees) or monoecious (Bingham, 2017). It is typically evergreen and is usually in season between November and April (Osuji and Owei, 2010). It is the above described tree that yields the African breadfruit. The genus name '*Treculia*' was given after the name of a nineteenth century French botanist, Auguste Trécul; while the common name "African breadfruit" was given because of the largeness of the fruit (over 20 cm in diameter and 60 cm in longitudinal circumference) and edibility of the seeds, which can be cooked and consumed in a similar way as the actual breadfruit (several species of the genus *Artocarpus*) (Nuga and Ofodile, 2010).

A single African breadfruit tree can produce up to 100 fruits per season. The female flower (pistillate) lines the outer surface of a large receptacle which is the growing breadfruit; and the flowering period is from October to February. The African breadfruit is typically hard and fibrous (spongy when ripe) with a spiky skin surface. It is about the shape (round) and size of a volleyball, has a round surface, and can weigh up to 8.5 kg. Unripe fruits are green, while ripe ones are greenish yellow in color. The mesocarp of the fruit is about 5cm thick and is pinkish white in color. It contains many seeds, which are the edible part of the fruit. These seeds are dicotyledonous, and vary in sizes (Akubor and Badifu, 2004)

A mature seed consists of an outer covering or seed coat and an inner endosperm which is the edible part. The seed integument (shell) is brown in color and the seed can be about 8.5 mm long. The husk is coated with a thin, viscous, highly

hydrated layer or mesocarp, similar to coffee bean mucilage (Onweluzo and Odume, 2007).
The African breadfruit contains polyphenols (Ogbonnia *et al*., 2008). The seeds are rarely eaten raw but are usually baked, boiled, roasted or fried before consumption. They can also be ground into flour which can be used to substitute for wheat flour in bakery products (Ijeh *et al*., 2010). The seeds, which are legumes, are utilized to produce several traditional foods (Olapade and Umeonuorah, 2014).

3.0 Materials and Methods

3.1 Plant Sample Collection and Preparation

The plant seeds of *Treculia africana* were obtained from Nkwo-Okpu Market, Isiala-Ngwa South LGA, Abia State, Nigeria. They were identified at Bioresources Development and Conservation Program (BDCP) Research Centre, Nsukka, Enugu State, Nigeria, after which the seeds were washed and sorted to remove any broken and possible contaminants. Preparation and extraction was carried out using the method of Abu *et al*. (2015). They were then dried using an oven, and dry roasted using a steel frying pot, over moderate heat. They were peeled manually by breaking them between two hard surfaces and the chaffs (husk) was separated from the endosperm. The peeled sample seeds were then grinded to powder, and stored in a plastic container for extraction and other subsequent works (Fasasi *et al*., 2003).

3.2 Qualitative Phytochemical Analysis of Aqueous Seeds Extract of *Treculia africana*

Qualitative phytochemical analysis of the aqueous seeds extract of *T. africana* were carried out according to the method of Harborne (1989) and Trease and Evans (1989) to identify its active constituents.

3.3 DPPH Radical Scavenging Assay

The free radical scavenging capacity of the plant extracts against the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH) was determined using a slightly modified method of Brand-Williams *et al*. (1995). Briefly, 0.5 ml of 0.3 mM solution of DPPH in methanol was added to 2 ml of various concentrations of the extracts (0.2 - 1.0 mg/ml). The test tubes were shaken and incubated for 15 minutes at room temperature in the dark and the absorbance was read at 517 nm. All tests were performed in triplicate. Ascorbic acid (vitamin C) was used as a control at concentrations similar to those of the test samples. A blank containing 0.5 ml of 0.3 mM DPPH and 2 ml of methanol was prepared and treated as a test sample. The radical scavenging activity was calculated as shown in equation 1:
DPPH radical scavenging activity (%) =

$$\frac{Ao - A1\ X\ 100}{Ao} \qquad (1)$$

Where A_o was the absorbance of DPPH radical + methanol;
A_1 was the absorbance of DPPH radical + sample extract or standard. The EC_{50} value represented the sample concentration that resulted in a 50% reduction in the initial DPPH concentration.

3.4 Total Antioxidant Capacity (TAC)

The TAC of the extract was evaluated by the phosphomolybdenum method based on the procedure described by Prieto *et al*. (1999). The assay was carried out by the phosphomolybdenum method. A 0.1 milliliter (0.1ml) aliquot of different concentrations (25, 50, 100, 200, 250, 300 and 1000 mg/L) of the extract and ascorbic acid was mixed with 1ml of reagents solution (600mM sulfuric acid 28mM sodium phosphate and 4mM ammonium molybdate, 1:1:1).

The test tube were covered with aluminum foil and incubated in the water bath at 95°C for 90 minutes. After the extract was cooled to room temperature, the absorbance of the mixture was determined at 765nm against a blank containing 1 ml of the reagent solution. Ascorbic acid was used as a standard. The assay was performed in triplicate. The TAC was expressed as milligram equivalents of ascorbic acid and calculated as show in equation 2:

$$\text{TAC (mg AAE/g extract)} = \frac{C\ x\ V}{m} \qquad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where C = concentration of ascorbic acid in mg/ml extrapolated from the standard calibration

curve; V = volume of extract in ml; and m = weight of crude plant extract in grams.

3.5 Nitric Oxide (NO) Radical Scavenging Capacity

The method described by Makhija *et al.* (2011) was used. Briefly, 1 ml of 10 mM sodium nitroprusside was mixed with 1 ml of extract prepared in phosphate buffer. The mixture was incubated at 25°C for 150 minutes. 1 ml of Griess' reagent was added to 1 ml of incubated solution. Then the absorbance was read at 546 nm. The percentage (%) inhibition of nitric oxide radical was calculated as shown in equation 3:

$$\text{Nitric oxide scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of extract } X\ 100}{\text{Absorbance of control}} \quad (3)$$

3.6 Ferric Reducing Antioxidant Power (FRAP)

A modified method of Benzie and Strain (1996) was used for the FRAP assay. The principle of this assay is the ability of the sample to reduce the ferric tripyridyltriazine (Fe (III) - TPTZ) complex to ferrous tripyridyltriazine (Fe (II) - TPTZ), which at low pH produces an intense blue colour which can be read at 593 nm. Briefly, 1.5 ml of freshly prepared FRAP solution (25 ml of 300 mM acetate buffer pH 3.6, 2.5 ml of 10 mM of 2,4,6-tripyridylstriazine (TPTZ) in 40 mMHCl and 2.5 ml of 20 mM ferric chloride solution ($FeCl_3 \cdot 6H_2O$) was mixed with 1 ml of varied concentrations of the extract (0.2 - 1.0 mg/ml). The reaction mixtures were incubated for 30 minutes at 37°C and the absorbance was read at 593 nm. Gallic acid acted as a control while $FeSO_4$ was used for calibration and values expressed as mmol $FeSO_4$ equivalent per gram of sample.

3.7 Estimation of Thiobarbituric Acid Reactive Substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) were estimated according to the method described by Ohkawa *et al.* (1979). Egg yolk homogenate (0.5 ml of 10% v/v) and 0.1 ml of extract were mixed in a test tube and made up to 1 ml with distilled water. Then 50 µL of $FeSO_4$ (0.07 M) was added to induce lipid peroxidation and incubated for 30 minutes. This was followed by the addition of 1.5 ml of 0.8% TBA in 1.1% sodium dodecyl sulfate (SDS) and 50 µL 20% TCA and vortex. The resultant mixture was heated at 95°C for 60 minutes. The absorbance of the sample was read at 532 nm. Lipid peroxidation inhibition (%) was calculated as shown in equation 4:

$$\text{Inhibition of lipid peroxidation (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of extract } X\ 100}{\text{Absorbance of control}} \quad (4)$$

3.8 Statistical Analysis

The data obtained were analyzed using Statistical Product and Service Solutions (SPSS) version 20.1 and the results were expressed as the mean ± standard error of the mean. Significant differences of the result were identified by one-way analysis of variance (ANOVA) and the acceptance significance level was $p \leq 0.05$ for all the results.

4.0 RESULTS AND DISCUSSION

4.1 The Qualitative Phytochemical Contents of Aqueous Extract of *Treculia africana* Seeds

Table 1 shows the results of the qualitative content of the samples. The result shows that aqueous extracts of *Treculia africana* seeds contain reducing sugars, steroids, tannins, alkaloids, flavonoids, glycosides, phenols and terpenoids while saponins were not detected.

4.2 *In Vitro* Antioxidant Activities of *Treculia africana* Seed Extracts

The scavenging activity of the DPPH radicals and the TAC of ascorbic acid were significantly higher than those of the aqueous extract ($p < 0.05$). However, there were no significant differences in the NO scavenging activity of the ascorbic acid and TBARS of the butylated hydroxytoluene ($p > 0.05$). The FRAP of aqueous extract was significantly higher than that of gallic acid ($p < 0.05$). These results are shown in Figures 1 to 5.

Table 1: Results of the Qualitative Phytochemicals of Aqueous Extracts of *Treculia africana* Seeds

Phytochemicals	***Aqueous Seed extract***	***Interpretation***
Reducing sugars	+++	*Present in high concentration*
Steroids	++	*Present in moderate concentration*
Saponins	-	*Not detected*
Tannins	++	*Present in moderate concentration*
Alkaloids	+++	*Present in high concentration*
Flavonoids	+	*Present in low concentration*
Glycosides	+++	*Present in high concentration*
Phenols	++	*Present in moderate concentration*
Terpenoids	++	*Present in moderate concentration*

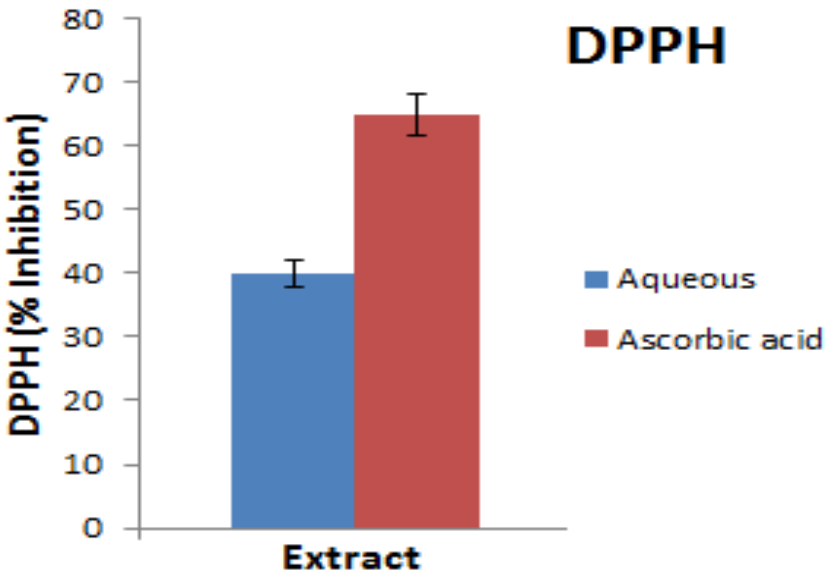

Figure 1: Bar chart showing the DPPH scavenging activity of aqueous extract of *Treculia africana* seeds. P<0.05, when compared with ascorbic acid.

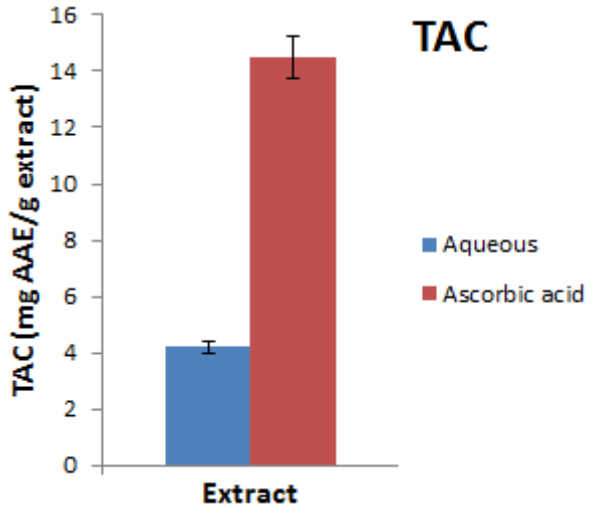

Figure 2: Bar chart showing the total antioxidant capacity (TAC) of aqueous extract of *Treculia africana* seeds. P<0.05, when compared with ascorbic acid.

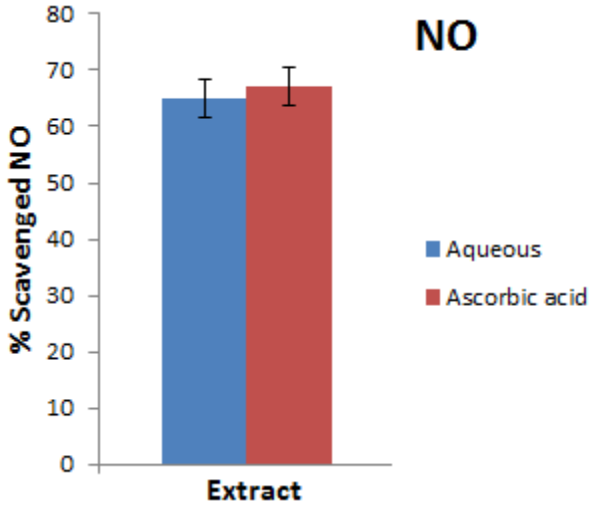

Figure 3: Bar chart showing the nitric oxide (NO) scavenging potentials of aqueous extract of *Treculia africana* seeds. P>0.05, when compared with ascorbic acid.

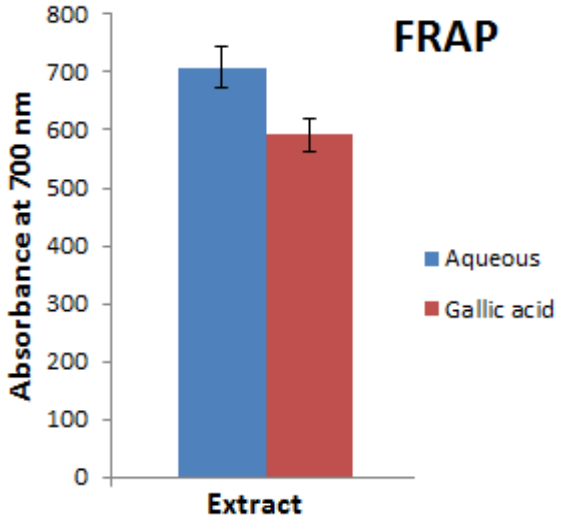

Figure 4: Bar chart showing the ferric reducing antioxidant potential (FRAP) of aqueous extract of *Treculia africana* seeds P<0.05, when compared with gallic acid.

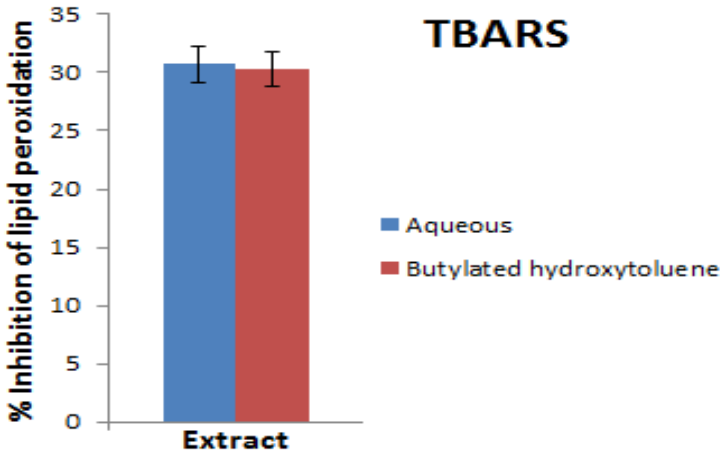

Figure 5: Bar chart showing the thiobarbituric acid reactive substances (TBARS) of extract of *Treculia africana* seeds. P>0.05, when compared with butylatedhydroxytoluene.

4.3 Discussion

The study showed the results of research of qualitative phytochemical analysis and *in vitro* antioxidant potential of partly processed seeds aqueous extract of *Treculia africana*. These results revealed the presence of reducing sugars, steroids, tannins, alkaloids, flavonoids, glycosides, phenols and terpenoids in the aqueous seeds extract, while saponins was not detected (as in Table 1). The high levels of phytochemicals observed in the aqueous extract of *T. africana* from qualitative phytochemical analyses were indication of its richness in important phytochemicals that could provide a useful health benefit to human when consumed in the right proportion at the right time. The presence of tannins, alkaloids and flavonoids in the aqueous seeds extract was consistently in line with that of Mbagwu *et al*. (2010) reports. Plant parts are still screened to discover potential antioxidant properties, although synthetic free radical scavengers such asbutylhydroxyanisole (BHA) and butylhydroxytoulene (BHT) exist, but concerns over possible side effects necessitate the continued screening of natural plant parts for potential antioxidant properties.

Antioxidants help prevent tissue damage by neutralizing the effects of free radicals. They act as scavengers. Dietary antioxidants play a key role in replenishing molecules and antioxidant enzymes *in vivo* in the fight against free radicals (Du *et al*., 2009). Phenols and flavonoids represent phytochemicals whose relative abundance in plant extracts has been linked to an antioxidant effect (Padmanabhan and Jangle, 2012).

The DPPH radical can accept an electron or a hydrogen ion to become a stable molecule (Du *et al*., 2009). DPPH radical scavenging is a widely used method to evaluate the free radical scavenging capacity of plants or chemical materials (Lee *et al*., 2003). The DPPH method is rapid, sensitive, reproducible, and requires simple conventional laboratory equipment to access the antioxidant activity of the samples (Du *et al*., 2009). Reactive nitrogen species (RNS) are free radicals that result from the interaction of NO with oxygen or reactive oxygen species (ROS). Nitric oxide is classified as a free radical due to its unpaired electron and exhibits significant reactivity with certain types of proteins and other free radicals such as superoxide anion. It is synthesized by three isoforms of the enzyme nitric oxide synthase (NOS): endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) (Abu *et al*., 2020).

Nitric oxide (NO) is produced from the amino acid L-arginine by the enzymes in the vascular endothelial cells, some neuronal cells and phagocytes (Nagmoti *et al*., 2011). Low concentrations of NO are sufficient in most cases to ensure the physiological functions of the radical. It is a diffusible free radical that plays many roles as an effector molecule in various biological systems, including neuronal messenger, vasodilation, and antimicrobial and antitumor activities (Bhaskar and Balakrishnan, 2009). Chronic exposure to the NO radical has been associated with several carcinomas and inflammatory conditions such as juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis. The toxicity of NO increases considerably when it reacts with the superoxide radical to form the highly reactive peroxynitrite anion ($ONOO^-$). Nitric oxide has been shown to be directly absorbed by flavonoids scavengers (Lakhanpal and Rai, 2007). Due to the reactivity of TBA with various reactive substances in a biological sample, a more widely accepted terminology called TBARS is now commonly used. Thiobarbituric acid reactive substances (TBARS) are now considered as a standard marker for oxidative stress induced by lipid peroxidation (Tsai and Huang, 2015).

Phenolic compounds are antioxidant agents which act as free radical terminators. The antioxidant potential of phenols is believed to be conferred on them by their hydroxyl group ($^-$OH), which is bond to an aromatic hydrocarbon (phenyl) ring. For this reason, they readily donate electrons to free radicals in search of electrons, reducing their threat to living cells (Uyoh *et al*., 2013). Studies have revealed a direct relationship between total phenol content and antioxidant effect in different plants.

High phenolic content-containing plant materials have high radical scavenging abilities (Hegazy and Ibrahim, 2012). The EC_{50} is the amount of antioxidant needed to reduce the concentration of the DPPH radical by 50%. It is inversely proportional to the antioxidant potential and therefore a lower EC_{50} corresponds to a higher antioxidant potential (Chanda *et al*., 2011). The results obtained in this study indicate that aqueous extract of *Treculia africana* show great promise to act as important antioxidant molecules. This means that African breadfruit seeds are rich in antioxidants, or free radical scavengers, making them a healthy choice of carbohydrate source to prevent diseases (such as cancer, diabetes, etc.) caused by the action of free radicals in the body system.

Again, since some phytochemicals could be useful to humans when consumed, for protection against degenerative diseases (Dandjesso *et al*., 2012), African breadfruit seeds are a good source of certain phytochemicals that may contribute to human health (eg steroids, tannins, alkaloids, glycosides, phenols and terpenoids).

5.0 Conclusion

The results of this study indicate that the aqueous extract of partially processed seeds of Treculia *africana* contains a large number of phytochemicals, and could be considered a healthy carbohydrate source as it also contains some number of antioxidants. In essence, the partly processed seeds sample of *Treculia africana* used, gave results which shows that *Treculia africana* seeds are rich in phytochemicals and have high free radical scavenging abilities. However, the phenols also present in the partially processed seeds extract may play a role in the elimination of free radical scavenging activities of the medicinal plant.

Authors' contributions

This experiments was carried out in collaboration between all authors. NAPC, IVE, and AAC designed the experiments; NAPC, SNDN and MCN performed experiments and collected data; NAPC, IVE, SNDN, MCN and AAC discussed the results and strategy; NAPC supervised, directed and managed the study; NAPC, IVE, SNDN, MCN and AAC read and approved the final manuscript (NAPC = Nweje-Anyalowu Paul Chukwuemeka, IVE = Ihuomah Victor Emeka, SNDN = Solomon-Nwaejike Deborah Nzubechi, MCN = Madukoma Charles NnamdI, AAC = Ako Alwell Chinonso)

Conflict of Interest

The authors declare no conflicts of interest.

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