Comparative antioxidant activity of selected plants possessing characteristic colours

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Abstract

A wide variety of plants with economic values are rich in natural colours. These coloured plants contain phytochemicals that are believed to have many pharmacological activities including antioxidant properties. In this study, five plants with known characteristic colours in any of their parts were selected for comparison of their antioxidant potentials. Rhizomes of turmeric, tubers of beetroot, leaves of star cucumber, barks of camwood, and flowers of hibiscus were collected and carefully dried, powdered, extracted and subjected to qualitative and quantitative phytochemical studies, as well as *in vitro* antioxidant assessments using FRAP and DPPH scavenging activities protocols. The best performing coloured extract (turmeric) was thereafter subjected to *in vivo* antioxidant studies employing catalase and lipid peroxidase (MDA) protocols and using jackfruit concentrate in various proportions as vehicle for oral delivery. The phytochemical analyses revealed the presence of various valuable secondary and primary metabolites, including alkaloids, tannins, flavonoids, terpenoids, cardiac glycosides, reducing sugars, carbohydrates, and proteins. The antioxidant activity/reactive oxygen scavenging abilities comparison of plant extracts showed that turmeric on the overall, exhibited the best scavenging activity. The admixture of the jackfruit concentrate and turmeric extract of 8:2 gave the best antioxidant performance which is comparable to the positive control (ascorbic acid). Significantly, the jackfruit concentrate used as vehicle, gave very promising *in vivo* antioxidant activity. Invariably, an 8 : 2 admixture of jackfruit concentrate and turmeric extract will serve as a promising extemporaneous nutraceutical for reactive oxygen scavenging.

Keywords: Plants with coloured part, phytochemcals, *in vitro* antioxidant activity, *in vivo* antioxidant activity.

Introduction

Plants are common sources of natural products that are often used as medicines in the treatment of various illnesses (Tayade *et al.*, 2018). Of particular interest among these plants are those with colourd parts; leaves, flowers, fruits, tubers, rhizomes etc. The coloured pigments found in plants have been attributed to the presence of these secondary metabilites: anthocyanins, carotenoids, betalains, crocins, anthraquinones, and many others (Ruslan *et al.*, 2017; Gohari *et*

al., 2013;). These coloured pigments are eco-friendly, biodegradable, and mostly non-toxic (Rahayuningsih *et al*, 2020). The extracts of these coloured plant parts serve as sources of dyes for colouring foods, beverages, pharmaceuticals, and textile, as people seek to improve the aesthetic appeal of these products. Medicinally, it has been shown that the pharmacological activities associated with these plants are due to the presence of these pigments. For example, the antioxidant and anti-inflammatory activity (Hewlings *et al*, 2017), antimutagenic, and antimicrobial activity (Okezie *et al*., 2017) and anticancer properties (Wright *et a*l, 2017) of turmeric are due to the orange pigment cucumin. It has been speculated that coloured pigments mostly found in plants possess antioxidant properties due to ease of sequestration of the reactive free radicals (oxidants) in the body by these pigment molecules.

In this research therefore the pigments found in these plants: *Curcuma longa* (Tumeric), *Beta vulgaris* (Beetroot), *Hibiscus sabdariffa* (Roselle), *Baphia nitida* (Camwood) and *Sicyos alba* (Star cucumber) were extracted and their antioxidant properties compared for possible inclusion into formulations.

Materials and Methods

Plants collection and Extraction

Fresh rhizomes of tumeric (*Curcuma longa*), fresh tubers of Beetroot (*Beta vulgaris)*, dried leaves of Roselle (*Hibiscus sabdariffa)* and bark of Camwood (*Baphia nitida)* were purchased from Eke Awka Market in Awka Anambra State Nigeria. The fresh leaves of Star cucumber (*Sicyos alba*) were collected from Umuohi Ihembosi in Ekwusigo LGA Anambra State, Nigeria. The plant samples were authenticated at the Department of Pharmacognosy and traditional Medicine, Nnamdi Azikiwe University, Awka, Anambra State Nigeria. The voucher specimen of the plants were deposited at the herbarium of the Department with herbarium numbers PCG 474/Z/024, PCG 474/A/002, PCG 474/F/030, PCG 474/M/031 and PCG474/C/026 respectively. The plants (except Cam-wood) were washed and cut into tiny pieces. They were dried on a hot air circulated oven,with door kept ajar and at a temperature of 40 ^{O}C until well dried. The samples were milled using a kitchen blender.

The powdered samples were individually subjected to Soxhlet extraction using absolute ethanol. The extracts were evaporated using an open door hot air circulated oven at 40^{o}C. The extracts were stored in amber bottle until ready for use.

Qualitative phytochemical screening

Phytochemical tests were carried out on the ethanolic extracts using standard procedures to identify these constituents: tannins, saponins, flavonoids, steroids, tepenoids, cardiac glycosides, proteins, carbohydrates, reducing sugars, and alkaloids (Sofowara, 1993; Trease and Evan, 1989; Harborne, 1973).

Quantitative phytochemical Constituents.

The various phytochemicals were quantified following standard methods: Saponin (Obadoni and Ochuko, 2021), Alkaloids and Flavonoids (Harborne, 1973), Total ash, Acid Insoluble Ash, Water soluble ash and Moisture content (Odoh and Schreckenbach 2011).

In-vitro anti-oxidation evaluation

DPPH essay

The *in vitro* antioxidant activity was determined using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) according to the method described by Erhirihie *et al,* (2020). A solution of 1,1 –diphenyl-2-pieryllydrazil solution (0.6mM) was freshly prepared using methanol. The reaction mixtures which contains 0.25 ml of various concentration of the samples (31.25-, 62.5-, 125-, 250-, 500- and 100 ug/ml) were reconstituted. For ascorbic acid, 1.96-, 3.91-, 7.82-, 15.63-, 31.25-, 62.5-, 125-, 250-, 500- and 1000 ug/ml were employed.

Samples/or ascorbic acid were mixed with 0.25 ml of 0.6 mMol DPPH and 2 ml of ethanol and was incubated in the dark for thirty minutes at room temperature. Thereafter, the absorbances of the samples were measured at 517nm using a UV-visible Spectrophotometer (Peace spec, model 721). A tube containing 0.25 ml of DPPH solution and 2.25ml of ethanol served as a control. Assays were carried out in triplicates. Free radical scavenging activities of samples were obtained using the formula below:

DPPH scavenging activity = 100 x (AC- AS)/AC)

AC = Absorbance of control

AS = Absorbance of sample

A graph of percentage inhibition against concentration was plotted and the concentration was then plotted.

FRAP assay

Ferric reducing antioxidant power **(**FRAP) assay was carried out following the method described by Habibur *et al* (2013). A 0.25 ml of various concentration (31.25-, 62.5-, 125-, 250-,500- and 1000 ug/ml) of samples or ascorbic acid (1.96-, 3.91-, 7.82-, 15.63-, 31.25-, 62.5-, 125-, 250-, 500- and 1000 ug/ml) were mixed with 0.625 ml of phosphate buffer and 0.625 ml of 1% potassium ferricyanide [K_3FeCN_6]. The mixtures were heated at 50 ^oC for twenty minutes. Then, 0.625 ml of 10% trichloro acetic acid (TCA) was added and the mixtures centrifuged at 3000 rpm for 5 minutes. From the upper layer, 0.625 ml was pipetted and mixed with 0.625 ml of distilled water and 0.125 ml of 0.1% (w/v) ferric chloride ($FeCl_3$) solution. Absorbance of the mixture was measured at 700 nm against air using a UV-VIS spectrophotometer (Peace Sky). Test were performed in triplicates. Percentage inhibition was calculated using the formula below

% inhibition = (Absorbance of sample – Absorbance of Blank) x100

A graph of the percentage inhibition against concentration was thereafter plotted.

***In-vivo* anti-oxidation activity evaluation**

The turmeric extract was reconstituted using jackfruit concentrate prepared in our laboratory. The jackfruit mesophyll enclosing the seed were blended and sieved through a filter cloth. The resulting free flowing slurry was heated to boiling for about 30 min. Thereafter, the resulting jackfruit concentrate and the turmeric extract were reconstituted and administered to the animals as shown in the table below:

Treatment group	Composite ratio (jackfruit : Turmeric extract)	Dosage
1 (T1)	90:10	500mg/70kg
2 (T2)	80:20	500mg/70kg
3 (T3)	70:30	500mg/70kg
4 (T4)	60:40	500mg/70kg
5	Turmeric	500mg/70kg
6	Jackfruit	500mg/70kg
7	Ascorbic acid	1000mg/70kg
8	Distilled water	10mg/kg

Table 1: Formula for the reconstitution of turmeric extract and jackfruit concentrate and their dosage

The *in vivo* antioxidant evaluation of turmeric extract (*Curcuma longa)* with jackfruit (*Artocarpus heterophyllus*) concentrate serving as vehicle was carried out using animal model as was described by Patro (2016). Twenty four adult albino rats were grouped into eight groups of three (3) rats each. The composition of the preparation as shown in table 1 were administered orally once daily for a period of fourteen days to the respective group. Blood samples were collected through ocular-puncture and processed into sera. The sera were assayed for Catalase and MDA activity.

Determination of Catalase activity

Catalase activity was determined according to Sinha (1972). It was assayed colorimetrically at 620nm and expressed as micromoles of H_2O_2 consumed /min/mg protein at 25 ^{o}C.

The reaction mixture contained 0.1ml of serum, 1.0ml of 0.01 phoshate, buffer (PH 7.0) and 0.4ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0ml of dichromate acetic acid reagent (5% $K2Cr_20_2$ and glacial acetic acid mixed in the ratio of 1:3 respectively)

Analysis of Lipid Peroxidation

Malondilaldehyde (MAD), an index of Lipid peroxide was reacted with thiobabarturic acid (TBA) to give a complex pink color. This was used to assess lipid peroxidation using the method of Buege and Aust (1978). An aliquot (1.0 ml) of the diluted serum in normal saline was added to 2.0 ml of the reconstituted reagent: 15 % Trichloro acetic acid, 0.3 % thiobabartuic acid and 0.2 mM hydrochloric acid in the ratio of 1:1:1 and boiled at 100^{o}C for 15min and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against the blank. Malondialdehyde (in μM) was calculated using the molar extinction coefficient MDA-TBA complex of 156 x 10^{-5} $M^{-1}CM^{-1}$

Statistical Analysis

Simple statistics of mean, standard deviation and chi square were employed in the data analysis.

Results

Qualitative Phytochemical Screening

The results of the qualitative phytochemical screening conducted on the extracted colour pigments are presented in table 2. It is evident that most of these extracts contain the following secondary metabolites; alkaloids, tannins, flavonoids, terpenoids and cardiac glycosides but lack the presence of steroids.

Table 2: Phytochemical composition of selected plant parts with characteristic colours

Metabolite Plant	Turmeric	Camwood	Hibiscus	Beetroot	Star cucumber
Alkaloids	+	+	+	+	+
Saponins	+	+	+	+	+
Tannins	+	-	-	+	+
Flavonoids	+	+	+	+	+
Steroids	-	-	-	-	-
Terpenoids	+	+	+	+	-
Cardiac glycosides	+	+	-	+	+
Carbohydrates	+	+	+	+	+
Proteins	+	-	+	-	-

Reducing sugar	-	+	+	-	+

+ = presence, - =absence

Quantitative Phytochemical Screening

The results of the quantitative phytochemical screening are presented in table 3. The highest percentage of alkaloids and flavonoids occurred in the turmeric with values of 4.36 % and 4.76 % respectively while the lowest values of the alkaloids and flavonoids occurred in the star cucumber with the values of 3.58 % and 2.8 %respectively.

Table 3: Quantitative phytochemical screening of selected metabolites and proximate analysis of the plants

Plant Metabolite	Saponin (%)	Alkaloid (%)	Flavonoid (%)	Total ash (%)	Acid insoluble ash (%)	Water soluble ash (%)
Turmeric	3.2	4.36	4.76	11.30	6.15	9.50
Camwood	1.9	3.86	3.34	13.50	4.55	5.05
Hibiscus	3.2	4.04	2.82	11.00	5.45	4.80
Beetroot	1.45	3.78	4.38	11.10	5.95	5.45
Star cucumber	4.65	3.58	2.8	10.07	3.65	5.15

***In-vitro* antioxidant activity evaluation**

The results of the DPPH scavenging ability of the extracts as compared with Ascorbic acid used as standard are presented in figure 1 while that of FRAP are presented in figure 2.

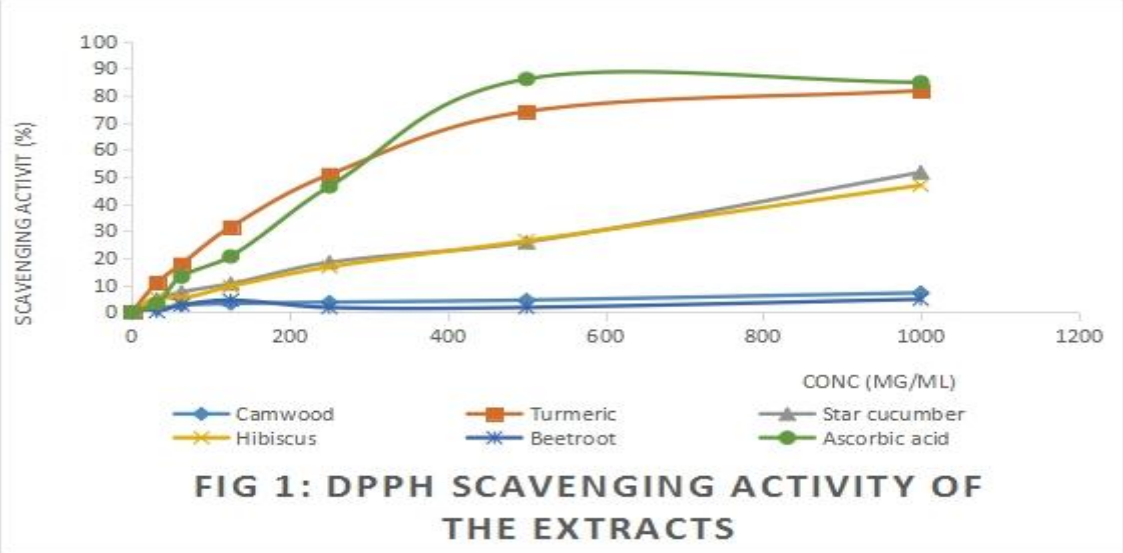


FIG 1: DPPH SCAVENGING ACTIVITY OF THE EXTRACTS

From the DPPH scavenging activity, the turmeric consistently performs better than other pigments except for the positive control (ascorbic acids) with which there is no significant difference when compared using Qui-square test statistics (p = 0.5) In fact, in the FRAP scavenging activity evaluation, turmeric displayed better scavenging activity than the Ascorbic and all other pigment extracts as shown in figure 2.

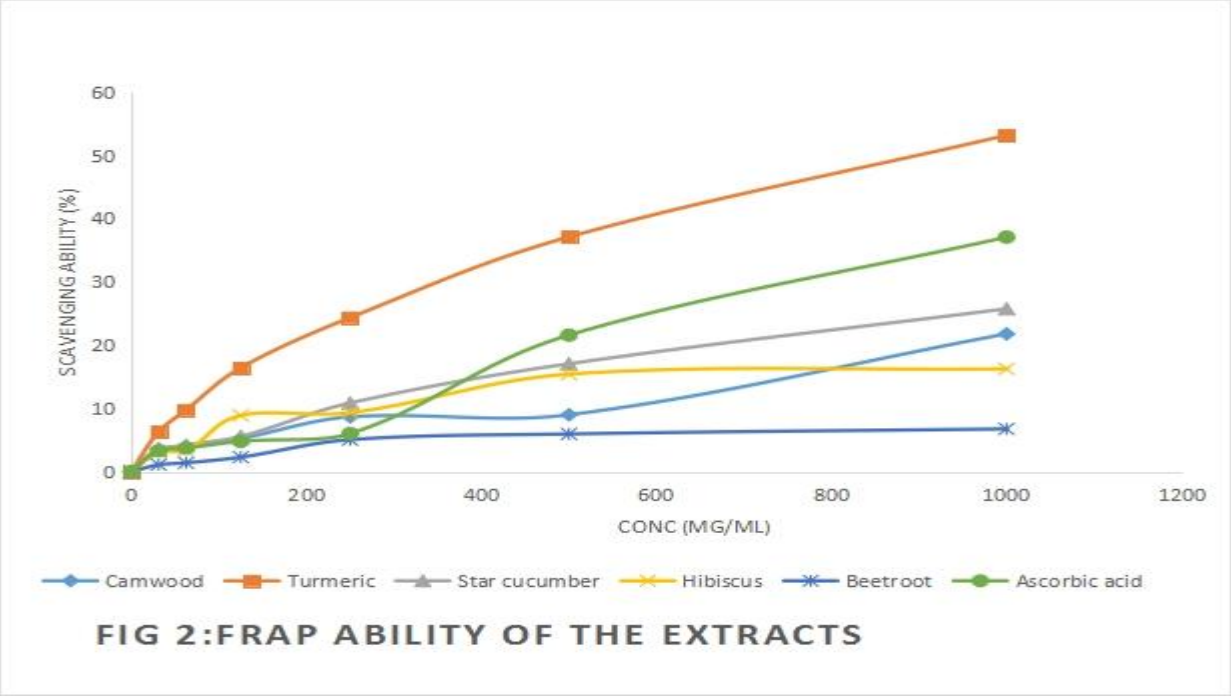


FIG 2:FRAP ABILITY OF THE EXTRACTS

***In-vivo* anti-oxidation evaluation of turmeric**

The ability of the turmeric pigment in preventing the oxidative stresses were assessed by administering turmeric pigment powder reconstituted using jackfruit concentrate which serves as the vehicle. The reconstitution formula as well as the administration dose levels are shown in table 1. The results of such *in-vivo* investigation are shown in figure 3.

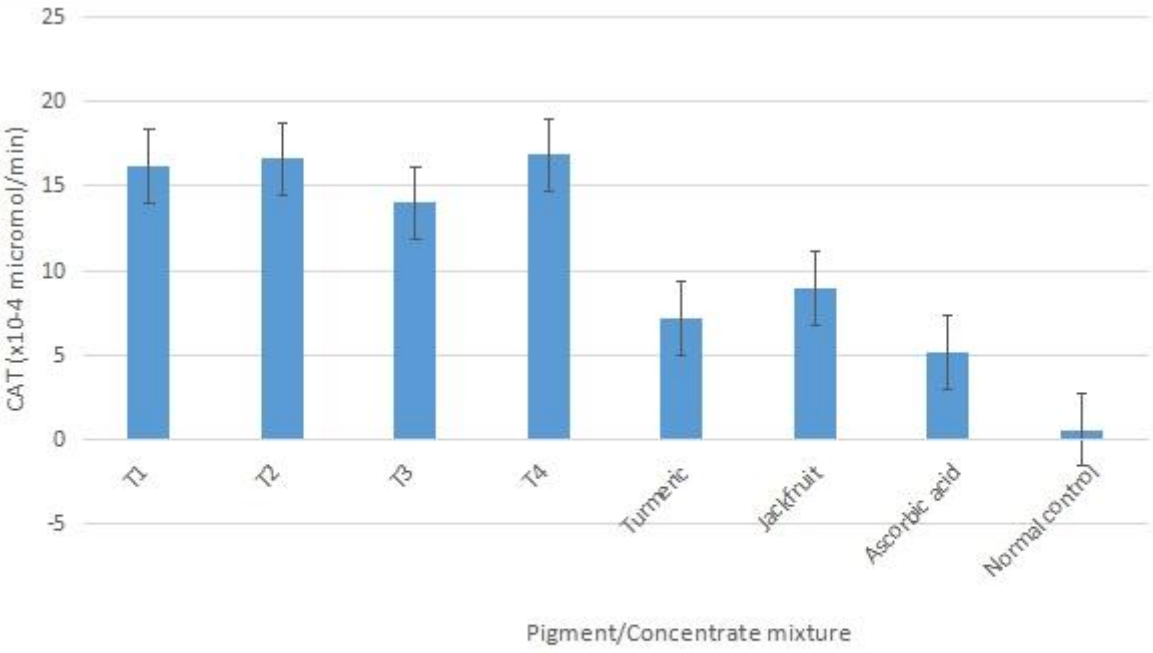


Fig 3: Anti-oxidant activity of the turmeric and its admixture using CAT protocol

From the figure 3, it is evidently clear that the turmeric extract performs better that the ascorbic acid used as positive control when CAT protocol is used.

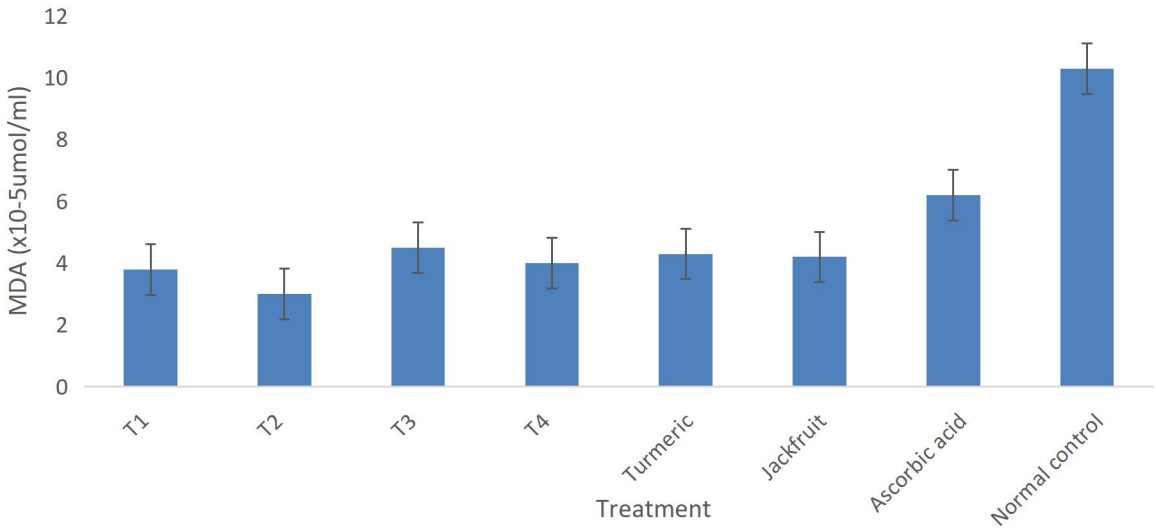


Fig 4: Antioxidant activity of turmeric and its admixture using MDA protocol

More so, figure 4 showed that the turmeric - jackfruit admixture T2 (80:20) was able to provide a better protection from the oxidative stress as measured using the MDA protocol.

Discussion

All the plant parts tested positive for at least 4 of alkaloids, saponins, flavonoids, cardiac glycosides and terpenoids. Beetroot and turmeric each showed positive tests for at least 6 of the 7 secondary metabolites. Alkaloids, saponins, flavonoids, cardiac glycosides and terpenoids are often very conjugated natural products which means they absorb energy at the visible light range. This explains why plants possessing them have characteristic colours. Phytochemicals exhibit a wide range of pharmacological activities that are of numerous benefits to humans and animals. Some of these properties are executed through their antioxidant properties which counteracts oxidative stress. Oxidative stress is caused by the circulating reactive radicals and have been associated with diseases such as cancer, inflammation and many other neurodegenerative diseases (Kim *et al*., 1994; Jones, 2008).

In vitro antioxidant activity tests on these plant parts using DPPH and FRAP assay showed that turmeric had the highest antioxidant activity in both tests.

Tumeric as could be seen from table 3 has the highest concentration of flavonoids, alkaloids and saponins. These secondary metabolites, especially flavonoids, are known to have high antioxidant properties (Panche *et al*, 2016). It could be seen from table 3 that turmeric is rich in flavonoids with a value of 4.76 % compared to other coloured plants used. Flavonoids are a class of polyphenols and polyphenols are known to participate in the adaptation of plants in stress conditions (Zhao, 2015).

These explains why Tumeric showed the highest antioxidant properties in all the in vitro antioxidant experiments carried out.

Turmeric showed better activity than ascorbic acid (standard) in FRAP assay and it compared favourably with ascorbic acid in DPPH assay.

Curcumin (diferuloylmethane), a flavonoid and the main active ingredient of turmeric (*C. longa*) known for its antioxidant/reactive radical scavenging activity may have contributed much of the 4.76% of flavonoids observed in this study.

The *in vivo* antioxidant assay also showed that turmeric performed better than ascorbic acid as could be seen from Fig. 3. However, jackfruit which was used as a vehicle to administer turmeric showed a better antioxidant activity than turmeric as well as ascorbic acid.

Furthermore, results from catalase assay showed a synergistic interaction between the turmeric extract and the jackfruit concentrate as could be seen in the catalase activities of different combinations of tumeric and jackfruit concentrates.

The MDA assay further showed the superiority of turmeric extract alone and in combination with jackfruit concentrate over ascorbic acid
The following order of better oxidative stress control can be observed from figure 4: T2 > T4 > turmeric ≈ jackfruit > T1 > T3 > Ascorbic acid.

These turmeric - jackfruit concentrate combinations can be employed as extemporaneous preparations in the management of disease conditions predisposed by existence of free radicals in the physiological systems including cancer.

Conclusion

The extracts of *Curcuma longa* (Tumeric), *Beta vulgaris* (Beetroot), *Hibiscus sabdariffa* (Roselle) and *Baphia nitida* (Camwood) have been shown to possess antioxidant activity to varying degrees following DPPH radical scavenging activity and FRAP assay. The turmeric displaying the highest antioxidant activity was thereafter selected and combined with jackfruit concentrate in varying ratios to serve as vehicle for oral administration. The jackfruit, turmeric and their various

combinations performed better than the positive control in the *in vivo* catalase essay while the order of performance in the MDA protocol is T2 > T4 > turmeric ≈ jackfruit > T1 > T3 > Ascorbic acid.

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