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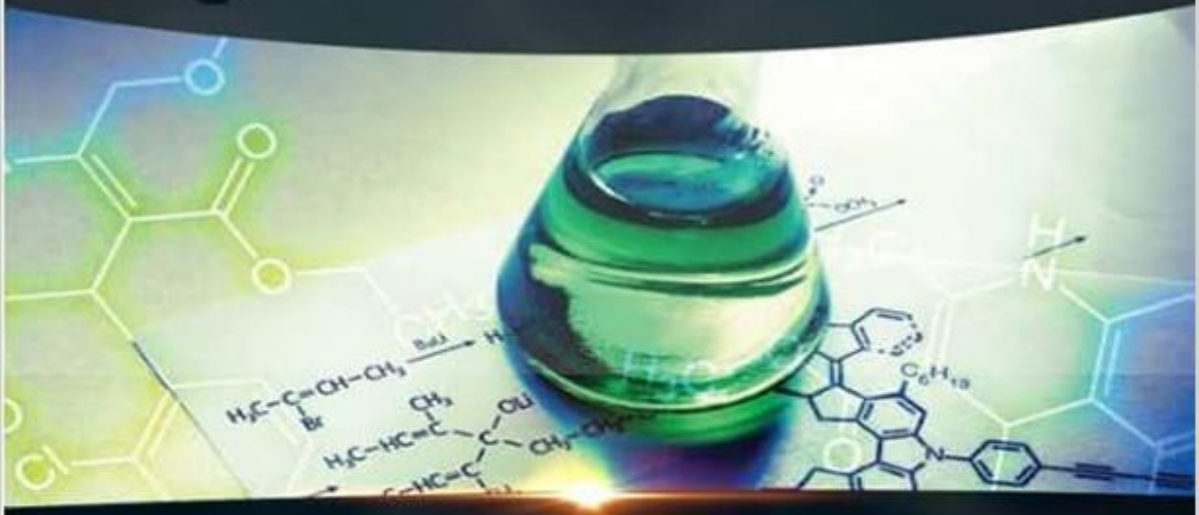

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In vitro antioxidant properties of free and bound phenolic extract of *Celosia argentea, Corchorus olitorius, Amaranthus hydridus* and *Jatropha tajorensis*

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ABSTRACT

Polyphenols have attracted a lot of interest recently due to their antioxidant property. The present study is aimed at investigating the antioxidant properties of free and bound phenols of methanol leaf extracts of *Celosia argentea, Corchorus olitorius, Amaranthus hydridus* and *Jatropha tajorensis*. Total flavonoids and phenolic contents of the extracts was determined using spectrophotometric method while the antioxidant activity of crude extract, free and bound phenols (FP and BP) was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The flavonoid contents ranged between 35.90±4.89 mg/100g in *C. olitorius* to 292.67±8.39 mg/100g in *A. hydride* while the phenolic contents ranged from 264.43±4.47 mg/100g in *J. tajorensis* to 431.98±4.90 mg/100g in *C. argentea*. The DPPH scavenging activity of the crude, free and bound phenolic extracts of the extratcs gave an IC_{50} of 383.52±1.05, 114.66±3.24 and 279.06±4.51 µg/mL for crude, FP and BP for *J. tajorensis* respectively, 331.29±1.33, 109.74±3.86 and 195.89±5.12 µg/mL for crude, FP and BP of *C. argentea* respectively, 379.46±3.11, 180.34±3.12 and 227.50±4.34 µg/mL for crude, FP and BP of *C. olitorius* respectively and 136.34±2.05, 135.47±1.88, 193.95±3.56 µg/mL for *A. hydridus* respectively which are significantly higher ($p<0.05$) with the standard (Ascorbic acid) with an IC_{50} of 12.66±3.33 µg/mL. From the result obtained, it is rational to attribute the wide usage of these vegetables in folkloric medicine to its high phenolic content. Hence, information from this study could be exploited in the global fight against degenerative diseases, whose etiology has been linked to oxidative stress.

Keywords: Polyphenols, antioxidants, Free phenols, Bound phenols, Degenerative diseases

INTRODUCTION

Polyphenols are a wide and complex group of secondary plant metabolites essential for the physiology of plants, having functions in growth, structure, pigmentation, pollination, allelopathy, and resistance for pathogens and predators (Manach *et al.,* 2004). Polyphenols have attracted the interest of researchers because of their antioxidant capacities. They have long been recognized to possess anti-allergic, anti-inflammatory, antiviral and anti-proliferative, anticarcinogenic and antioxidant properties (Atansuyi *et al.,* 2012). Reports have shown that

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there is an inverse relationship between the intake of flavonoids and the risk of coronary heart disease, lung cancer (Atansuyi *et al.,* 2012) and stomach cancer (Garcia-Closas *et al.,* 1999).

According to Nathan AND Ding (2010), free radicals and other small reactive molecules are important regulators of many physiological and pathological processes. Increased levels of these reactive molecules can cause oxidative damage to biological macromolecules and disrupt the cellular reduction–oxidation (redox) balance (Dowling and Simmons, 2009). Yoshihara *et al.,* 2010 have reported that oxidative stress caused by the accumulation of free radicals in the body has been responsible for many illnesses including cardiovascular diseases, cancer, neurodegenerative disorders, and aging.

In view of the recognition of the potent antioxidant properties of polyphenols, researchers have been tailoring their efforts towards identifying plants polyphenols with potent antioxidant properties that could be exploited for the management of degenerative diseases.

Green leafy vegetables (GLVs) are micronutrient dense nature's gift to mankind that provide more vitamins per mouthful than any other food (Anchalsingh, and Andnitisha, 2014). They are said to be rich sources of calcium, iron, β-carotene, vitamin C, dietary fibre and many trace minerals (Anchalsingh, and Andnitisha, 2014). It has been reported that some GLV contain immense varieties of bioactive non-nutritive health enhancing factors such as antioxidants, phytochemicals and essential fatty acids (Anchalsingh, and Andnitisha, 2014). This according to ethnomedicinal usage is increasing their recognition of the roles which their phytonutrients content play in the prevention of non-communicable diseases (Anchalsingh, and Andnitisha, 2014; Doughari *et al.,* 2009). Various studies reported the presence of abundant phenolic compounds such as quercetin, kaempferol and acacetin in GLVs (Anchalsingh, and Andnitisha, 2014).

Amaranth hybridus is a gluten-free pseudocereal thrives in all temperate-tropical areas of the world particularly in Mexico and South America (Rastogi *et al*., 2013). Moreover, in certain regions of the world, such as eastern Africa, amaranth leaves are consumed as a vegetable because it is a fast-growing plant available most of the year (Karamac *et al*., 2019). There has been an increase renewed interest in this ancient and highly nutritious food crop due to the excellent nutritional value of seed and leaves (Venskutonis *et al.,* 2013). Amaranth proteins have a well-balanced amino acid composition (Karamac *et al*., 2019), high bioavailability (Venskutonis *et al.,* 2013), and good functional properties (Lopez *et al*., 2019). Dietary fiber, vitamins and precursors of vitamins (ascorbic acid, riboflavin, tocols, carotenoids), as well as minerals (Ca, Fe, Mg, K, Cu, Zn, and Mn) are other important nutrients present in seeds and leaves of amaranth (Karamac *et al.,* 2019). Their contents are high compared to these in some cereals and green leafy vegetables (Karamac *et al*., 2019).

Celosia argentea L. is an herbaceous plant which belongs to the family *Amaranthacaceae* and one of the leading leaf vegetables in south-western Nigeria, where it is known as sokoyòkòtò' in Yoruba language meaning make husbands fat and happy (Kanu *et al.,* 2017). It is also known as red soko 'because it has red pigment on the leaves which differentiates it from the green soko'(Malomo *et al.,* 2011). *C. argentea* impact the anthocyanin- red colour into soup, making it less popular than green (Kanu *et al.,* 2017). The

entire plant is used in treatment of ulcers, piles, bleeding nose, inflammation, gynecologic disorders, mouth sores, eye diseases, glandular swellings, eczema, constipation and as an aphrodisiac (Rub *et al.,* 2015). The seeds are used in the treatment of blood diseases, diarrhea and the roots are well known for their anti-diabetic activity (Kanu *et al.,* 2017). The in vitro and in vivo antioxidant activity of the plant is reported to be due to abundance of phenolics making *Celosia argentea* as a potential source of cheap, natural antioxidants (Rub *et al.,* 2015).

Jatropha tanjorensis is a perennial herb, a member of the *Euphorbiaceae* family, commonly called 'hospital too far', catholic vegetable, Jatropha. It is used locally in soups as well as medicinally in treating anaemia, skin disease, malaria fever (Oduola *et al*., 2005; Omoregie and Osagie, 2011). Meanwhile, *J. tanjorensis* has received a lot of attention due to its potential health benefits, availability and affordability (Omoregie and Osagie, 2007; Omobuwajo *et al*., 2011). *J. tanjorensis* have also been shown to exhibit antibacterial activity (Iwalewa et al., 2005). In fact, earlier reports have shown that *J. tanjorensis* is rich in antioxidant nutrients like phosphorus, selenium, zinc and vitamins C and E (Omobuwajo *et al.,* 2011).

Corchorus olitorius (malvaceae), is a plant native to both tropical and subtropical regions throughout the world with mallow leaves commonly consumed as a leafy vegetable (Adedosu *et al.,* 2015). The leaves are used in ethnomedical practices to treat ache and pain, dysentery, malaria, enteritis, fever, gonorrhea, pectoral pains and tumors (Abdul Sadat *et al.,* 2017). Hence this research is aim at investigating the antioxidant activity of free and bound phenols in methanol extract of *Celosia argentea, Corchorus olitorius, Amaranthus hydridus* and *Jatropha tajorensis*.

Materials and Methods

Materials

Fresh leaves of *Amaranthus hybridus, Celosia argentea, Jatropha tajorensis* and *Corchorus olitorius* were obtained from Kure market in Minna, Niger State, Nigeria. The leaves were washed, and dried at room temperature for 7 days at the Biochemistry Laboratory of Federal University of Technology, Minna, Niger State**.** All chemicals and reagents used in this study are of analytical grade and were obtained from NAHSON Chemicals, Minna, Niger State.

Plant Processing and Extraction

The dried leaves were pulverized into powder using kitchen type blender to obtained a fine powder. Fifty grams (50 g) each of the powdered sample was extracted exhaustively with 20 mL of methanol for 2 hours at 45^0C using reflux extractor. The extract was filtered using muslin cloth and further filtration with filter paper to obtain a fine filtrate (Kabiru *et al.,* 2012). The filtrate was dried at a reduced temperature of 40^0C using water bath to obtain a semi-solid paste. The yield of the extract was calculated using the formula below:

$$\%\, Yield = \frac{Weight\ of\ extract}{Weight\ of\ dry\ sample}\ X\ 100$$

…… Equation (1)

Extraction of Free and Bound Phenols (Polyphenols)

The extraction of the soluble free phenols (FP) was carried as reported previously by Chu *et al.*, 2002 and reported by Atansuyi *et al.,* 2012 with minor modifications. Four grams (4 g) of the methanol extract was solubilized in methanol-water (80:20, v/v), sonicated and homogenized

at room temperature for 1 h 30 min. The solution was filtered through Whatman filter paper, using a Buchner funnel under vacuum. The filtrate was then evaporated using a rotary evaporator under vacuum at 40°C to obtain the FP extract. On the other hand, bound phenols (BP) was extracted according to the method of Krygier *et al.,* 1982 as reported by Atansuyi *et al.*, 2012 with slight modifications. Briefly, residues recovered from the extraction of FP was dried and hydrolyzed with 4 M NaOH at room temperature under shaking. The mixture was acidified to pH 2 with concentrated HCl, extracted four times with ethyl acetate, pooled together and evaporated at 40°C to dryness under vacuum to yield BP extract. The yield of extraction was calculated using equation (1) above.

Determination of total phenol content

The total phenol content of the extracts was determined according to Singleton *et al.,* (1999) using gallic acid as standard. Zero-point five milliliter (0.5 mL) of 1 mg/mL of crude methanol extract, FP and BP were oxidized with 2.5 ml of 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm. The amount of phenol present in the extracts was expressed as gallic acid equivalents (GAE).

Determination of total flavonoid content

The total flavonoid content of the extracts were determined as reported by Meda *et al.* (2005) with slight modification. Zero-point five milliliter (0.5 mL) of 1 mg/mL of crude methanol extract, FP and BP were mixed each with 0.5 mL methanol, 50 µL of 10% $AlCl_3$, 50 µL of 1 M potassium acetate and 1.4 mL water and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm and the total flavonoid was calculated using quercetin as standard, and expressed as quercetin equivalent (QE).

Antioxidant assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH radical was evaluated as described by Gyamfi *et al.* (1999). Briefly, an appropriate dilution (50 – 250 µg/mL) of the crude, FP and BP (1 ml) was mixed with 1 mL of 0.4 mM methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm. The DPPH free radical scavenging ability was subsequently calculated with respect to the reference (which contains all the reagents without the test sample).

RESULTS AND DISCUSSION

Results

Table 1: Percentage yield of crude methanol extract of the vegetables

Extracts	% yield
C. argentea	20.40
C. olitorius	18.00
A. hybridus	15.00
J. tajorensis	18.40

Table 2: Total flavonoids and phenolics contents (mg/100g) of plant extracts.

Samples	Phenols	Flavonoids
C. argentea	431.98±4.90^{d}	159.75±1.41^{b}
C. olitorius	269.68±11.34^{a}	35.90±4.89^{a}
A. hybridus	407.05±3.41^{b}	292.67±8.39^{c}
J. tajorensis	264.43±4.47^{a}	165.40±7.06^{b}

Values are expressed in mean ± standard error of mean of triplicate determination
Values are present in mean ± standard error of mean. Values with same superscript on the same column have no significant difference at $p<0.05$

Table 3: IC_{50} of methanol, free and bound phenolic extracts of the four selected vegetables

Extracts	MeOH Extracts	Free Phenols	Bound Phenols
C. argentea	331.29±1.33^{c}	109.74±3.86^{a}	195.89±5.15^{b}
C. olitoius	379.46±3.11^{c}	180.34±3.12^{a}	227.50±4.34^{c}
A. hybridus	136.34±2.05^{a}	135.47±1.88^{a}	193.95±3.56^{b}
J. tajorensis	383.52±1.05^{c}	114.66±3.24^{a}	279.06±4.51^{b}
Ascorbic acid	12.66±3.33*		

Key: MeOH = Methanol
Values are present in mean ± standard error of mean. Values with same superscript on the same row have no significant difference at $p<0.05$

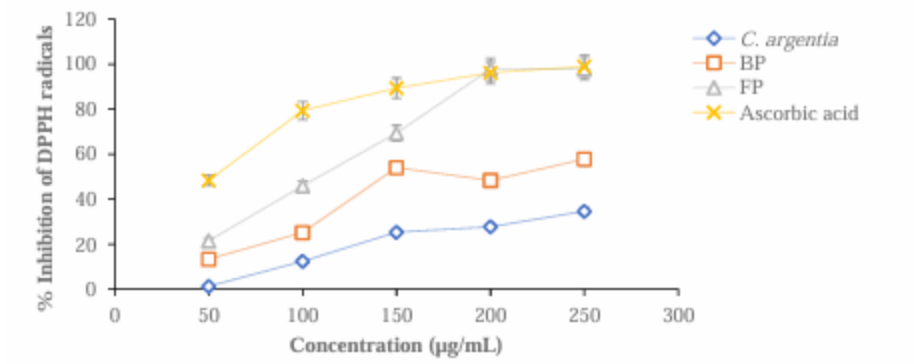

Figure 1: DPPH radical scavenging activity of free phenol (FB) and bound phenol (BP) of methanol extract of *C. argentea.*

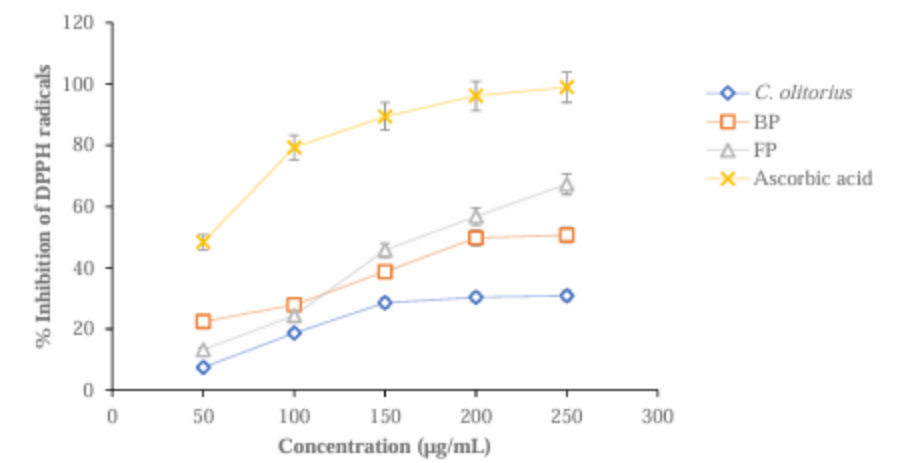

Figure 2: DPPH scavenging activity of free phenol (FB) and bound phenol (BP) of methanol extract of *C. olitorius*

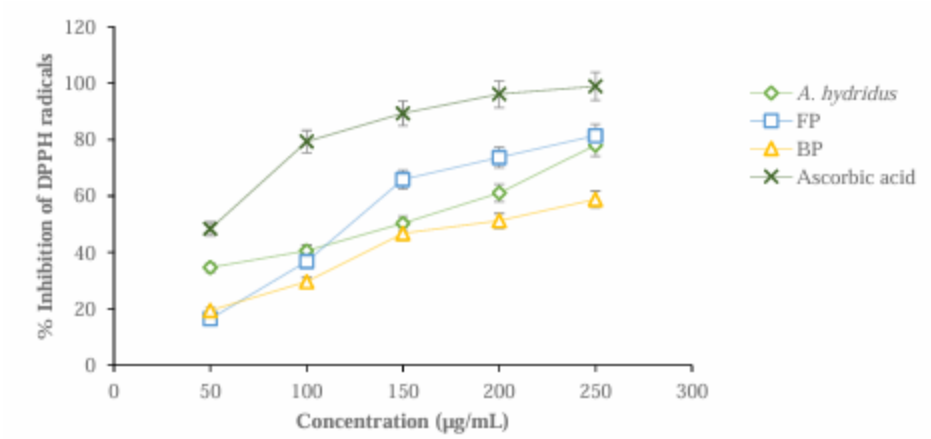

Figure 3: DPPH scavenging activity of free phenol (FB) and bound phenol (BP) of methanol extract of *A. hybridus*

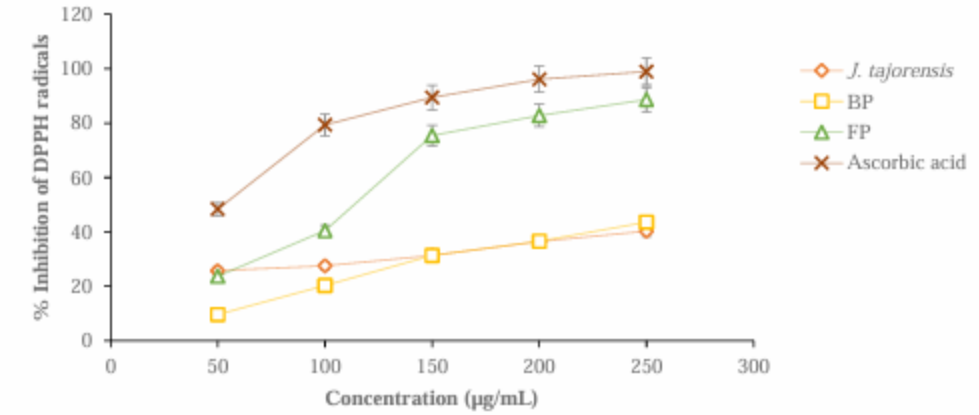

Figure 4: DPPH scavenging activity of free phenol (FB) and bound phenol (BP) of methanol extract of *J. tajorensis*

DISCUSSION

Globally, interest in natural product that could ameliorate the menace of oxidative stress has been on the rise due to cost and unavailability of conventional synthetic drugs (Manach *et al.,* 2004). Hence, efforts are now being tailored at discovering plants with potent antioxidant properties which could be harnessed and exploited for therapeutic purposes. Antioxidant properties of plants are intricately related to their phytochemicals (Atansuyi *et al.,* 2012; Doughari *et al.,* 2009). Recently, these bioactive substances, especially the polyphenols, have been found to be responsible for the antioxidant properties of plants (Omobuwajo *et al.,* 2011). Hence, an increased interest in the isolation of the polyphenolic components of plants that could be used in the management of degenerative diseases.

The % yield of methanol extract of *C. argentea, C. olitorius, A. hybridus* and *J. tajorensis*. Highest yield (20.40 %) was recorded in *C. argentea* followed closely by *C. olitorius* and *J. tajorensis* with 18.00 and 18.40 % respectively while *A. hybridus* recorded the least yield (15.00 %) (Table 1). Yield of extract play a major role in the choice of plant and is affected by factors such as time/season of collection, place of collection, and the species of plant. Hence the reason for the variation in the yield recorded above.

Table 2 shows the phenolics and flavonoids contents of the extract. *C. argentea* contain the highest amount of phenols (4.31.98±4.90 mg/100) followed by 407.05±3.41, 269.68±11.34 and 264.43±4.47 mg/100g for *A. hybridus, C. olitorius* and *J. tajorensis* respectively. High flavonoids content (292.67±8.39 mg/100g) was also recorded in *A. hybridus* followed by *J. tajorensis, C. argentea* and *C. olitorius* with flavonoids content of 165.40±7.06, 159.75±1.41 and 35.90±4.89 mg/100g respectively. The amount of phenols and flavonoids content of these plants justify their wide usage particularly among the Yoruba's of the South-Western part of Nigeria (Alegbejo, 2013). This may also explain the rationale behind their widespread usage in folkloric medicines for the treatment of ailments. However, it is not enough to know

what is responsible for the pharmacopotency of these leaves, without unraveling the mechanism involved in their therapeutic effects. Hence, we tested the *in vitro* antioxidant properties of free phenols (FP) and bound phenols (BP) of extracts of *Amaranthus hybridus, Celosia argentea, Jatropha tajorensis* and *Corchorus olitorius* with a view of gaining an insight into the mechanism(s) involved in their antioxidant action.

The DPPH radical scavenging activity has been extensively used for screening antioxidants ranging from fruits, cereals and vegetable juices or extracts (Ayoola *et al.,* 2006). Therefore, the ability of the FP and BP extracts to scavenge DPPH radicals were investigated and presented in Table 3 and Figure 1–4. DPPH is an unstable diamagnetic molecule that attains stability through protonation. This stability is visually noticeable by an abrupt discoloration from purple to golden yellow. Antioxidant potentials of plant extracts is judged based on their median inhibition concentration (IC_{50}). According to Blois, 1958 classification of IC_{50} as reported by (Fidrianny *et al.,* 2014), extracts with an IC_{50} <50 µg/mL is said to be a very strong antioxidant, 50-100 µg/mL as strong antioxidant, 101-150 µg/mL as medium antioxidant, while extracts with IC_{50} >150 µg/mL as a weak antioxidant (Fidrianny *et al.,* 2014). Based on this classification, free phenolic extract of *C. argentea, A. hybridus* and *J. tajorensis* are classified as medium antioxidant with an IC_{50} of 109.74±3.86, 135.47±1.88 and 114.66±3.24 µg/mL respectively while free phenolic extract *C. olitoius* is a weak antioxidant with IC_{50} of 180.34±3.12 µg/mL. Methanol and bound phenolic extracts of the plant shows a weak inhibition of the DPPH radicals with IC_{50} value > 150 µg/mL (Table 3). Although the reason behind this observation is not completely understood, it is logical to speculate that the amount of phenols and flavonoids present in these extracts maybe responsible for their antioxidant activity which have been reported to be a natural antioxidant in plants (Doughari *et al.,* 2009; Fidrianny *et al.,* 2014). Furthermore, the observation further justifies the widely speculated report that one of the mechanisms of antioxidant activity of polyphenols is through radical scavenging (Ayoola *et al.,* 2006). Phytochemicals can act as antioxidants by protecting cell membrane and cellular oxidative processes from damages that may give rise to diseases (Atansuyi *et al.,* 2012). Hence, antioxidants are usually assessed by their ability to offer protective shields to lipids intentionally assaulted with peroxidants.

CONCLUSION

Phytochemical screening of methanol extracts of *C. argentea, C. olitorius, A. hybridus* and *J. tajorensis* shows that phenols was higher in all the extract than flavonoids. The antioxidant activity also shows that free phenols have lower IC_{50} than the bound phenols hence high antioxidant capacity. The extracts of these plants can be a good source of antioxidants for the management of oxidative stress and other related diseases.

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