Available online at http://www.ajol.info/index.php/njbas/index
Nigerian Journal of Basic and Applied Science (June, 2017), 25(1): 17-22
DOI: http://dx.doi.org/10.4314/njbas.v25i1.3
ISSN 0794-5698

Full Length Research Article

In vitro Antioxidant Activity of *Chrysophyllum albidum* Fruit

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ABSTRACT
Chrysophyllum albidum (African star apple) is a tropical plant commonly found in Nigeria. It has acquired a reputation in folklore as a formidable therapeutic agent against yellow fever, malaria, diarrhea, vaginal and dermatological infections. This study was carried out to investigate the *in vitro* antioxidant activity of three extracts [aqueous (AE), methanol (ME) and petroleum ether (PE)] of the plant fruit using two antioxidant tests. The ferric ion reductive power and % H_2O_2 inhibition of the fruit extracts at 1 mg/ml concentration were evaluated. The result followed the pattern AE > ME > PE showing the strongest to the least strong antioxidant activity. However, the % H_2O_2 inhibition between the aqueous and methanolic extracts has no significant ($p > 0.05$) difference. A Comparative study reveals that the reductive power of ascorbic acid (1 mg/ml) is significantly ($p < 0.05$) higher than that of the fruit extracts except of aqueous extract. Though the % H_2O_2 inhibition of ascorbic acid was greater than all the extracts, a statistical difference ($p < 0.05$) was only observed in petroleum ether extracts of the fruit. This finding suggests that African star Apple has the potential to prevent lipid peroxidation and radical chain reactions. Hence, relishing the fruit as part of dietary intake and further exploitation as a therapeutic agent should be encouraged.
Keywords: *Chrysophyllum albidum*, antioxidant activity, reducing power, fruit extracts.

INTRODUCTION
It is a well-established fact that fruits, herbs and spices rich diets are associated with low risks of many ailments (McClemets and Decker, 2000). In Nigeria, *Chrysophyllum albidum* has been adjudged as one of the most auspicious plants with diverse ethnobotanical uses (Amusa *et al.,* 2003). The tropical tree belongs to the family *Sapotaceae* and is commonly known as African Star Apple, it is widely distributed in Nigeria, Uganda, Niger, Cameroun and Cote d' Ivoire (Duyilemi & Lawal, 2009; Adebayo *et al*., 2011). Local names of *C. albidum* in South-West and South-East regions of Nigeria are "agbalumo" and "udara" respectively (Idowu *et al.,* 2006). In recent years, the plant has attracted attention of explorers and is being studied for it commercial benefits. The fruit was found to be a rich source of resin and contains ample amount of anacardic acid that can be utilized industrially for wood protection (Oboh *et al.,* 2009). The fleshy pulp of the fruit is eaten as a snack (Amusa *et al*., 2003), it can also be exploited for the production of soft drinks or fermented for alcohol production (Ajewole and Adeyeye, 1991). More so, its seeds are source of oil, which is utilized for several purposes (Ugbogu and Akukwe, 2008).

Therapeutically, the tree bark is used as a remedy for malaria and yellow fever, while the leaves are used as palliatives for the treatment of dermatological problems, stomachache and diarrhea (Idowu *et al*., 2006). The cotyledons are used as unguents for the treatment of vaginal infections (Akubugwo and Ugbogu, 2007) and as hypoglycemic and hypolipidemic agent (Olorunnisola *et al*., 2008). The antimicrobial activity of *C. albidum* has been well studied; eleagnine was successfully isolated and was shown to be responsible for bacterial growth inhibition (Idowu *et al*., 2003; Duyilemi & Lawal, 2009). This potent compound was further shown to demonstrate anti-inflammatory, anti-nociceptive and anti-oxidant activities (Idowu *et al*., 2006). Recently, the effect of *C. albidum* leaf extracts on biochemical and hematological parameters of albino rats was demonstrated and a myricetin rhamnoside with antioxidant activity and excellent radical scavenging activity was isolated (Adebayo *et al*., 2010; Adebayo *et al.,* 2011).

Despite the aforementioned studies/ findings, little attention has been given to investigating the antioxidant activity of the edible fruit of *C. albidum* which is widely relished. Antioxidants are agents that inhibit the production or counteract the damaging effect of free radicals such as reactive oxygen species (ROS) in the biological system. Therefore, this study was aimed at investigating the *in vitro* antioxidant potentials of different extracts of *C. albidum* fruit.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

In April 2014, fruits of *C. albidum* plant were obtained from Sokoto South Local Government Area of Sokoto State, Nigeria. The botanical identity of the fruit was further confirmed at the Herbarium of the Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto. The fresh fruits obtained were washed with distilled water to remove dirt, and then the exocarp was gently removed. The exocarp and fleshy pulp were air dried at room temperature, mashed into a fine powder and stored in air-tight containers until needed for the experiment.

Preparation of Extracts

The procedure of Dandare *et al*. (2014) was adopted for extract preparation. Fifty (50) grams of each sample was exhaustively extracted with 95% ethanol for 24 hrs. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was evaporated at 40 ^{0}C for 72 hrs. The residue was dissolved in 50 ml deionized water and successively extracted with methanol and petroleum ether. These fractions were evaporated and subsequently screened for their antioxidant activity.

Hydrogen Peroxide Scavenging Activity

The extracts capability to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*. (1989). A 2mM hydrogen peroxide solution was prepared in 50mM phosphate buffer (pH 7.4). Aliquots (0.1 ml) of the different fractions were transferred into test tubes, and their volumes were made up to 0.4 ml with 50mM phosphate buffer. 0.6 ml hydrogen peroxide solution was added after which the tubes were vortexed, and the absorbance of hydrogen peroxide was measured at 230 nm after ten (10) minutes against a blank solution containing only the phosphate buffer. The percentage of hydrogen peroxide inhibition was calculated using the following equation:

$$\%\,\text{Scavenged}\ [H_2O_2] = [(A_0 - A_1)/A_0] \times 100$$

Where: A_0 = Absorbance of the control

A_1 = Absorbance of the extract or standard

Ferric Ion Reducing Power

The method described by Oyaizu (1986) was employed for determination of *C. albidum* reducing power. Briefly, 1.0 ml of the extracts and ascorbic acid (standard) were prepared in distilled water. Each extract was mixed individually with 0.5 ml of 0.2 M phosphate buffer (pH 6.6) and 0.5 ml potassium ferricyanide (1% w/v). The resulting mixtures were incubated at 50 ^{0}C for 20 minutes and 2.5 ml of 10% trichloroacetic acid added to each of them. The entire mixture was centrifuged for ten (10) minutes at 3000 rpm. A mixture of 2.5 ml of the supernatant and 2.5 ml of distilled water was made, 0.5 ml of ferric chloride (0.1%) was then added, and the absorbance read at 700 nm.

Statistical Analysis

All values were expressed as mean ± standard deviation of triplicate determination and one-way analysis of variance (ANOVA) was done to analyze significant difference using the statistical analysis software package SPSS (version 16.0). Values with $P < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

Numerous methods exist for the evaluation of antioxidant activity. The most commonly used ones are total antioxidant activity, reducing power, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, metal chelating, active oxygen species

(such as H_2O_2, O_2^{-} and OH) and quenching assays (Mitsuda *et al*., 1996). Since the antioxidant activities of an oxidant cannot be evaluated by using a single method due to differences in oxidative processes (Gülçin *et al*., 2005), we therefore employed two methods namely; ferric ion reducing power and hydrogen peroxide scavenging capacity for the evaluation of antioxidant ability of *C. albidum* fruit.

TABLE 1: Ferric Reducing Antioxidant Power (FRAP) of different extracts of *C. albidum* fruit and ascorbic acid

Extract	FRAP of Fruit (%)	
	Pulp	Exocarp
Methanol	79.67±0.04[a]	72.67±0.16[d]
Petroleum ether	70.33±0.12[b]	62.67±1.09[e]
Aqueous	96.00±1.28[c]	84.33±1.20[f]
Ascorbic acid (Standard)	97.00±0.68[c]	97.00±0.68[c]

Values represent Mean ± SD of triplicate measurement. Values with different superscripts differ significantly at $p < 0.05$.

Exogenous chemicals and endogenous metabolic processes might produce highly reactive oxygen species (ROS) which can be degraded by all aerobic organisms. However, ROS have the capacity to react with most biomolecules (protein, lipids, DNA) and cause oxidative damages which play significant pathological role in human diseases (Nordberg *et al*., 2001; Gülçin *et al*., 2002). Antioxidants can counteract the oxidation process via various mechanisms which include reacting with free radicals, chelating catalytic metals, oxygen scavenging, decomposition of peroxides and prevention of chain initiation (Yildrim *et al*., 2000; Büyükokurog˘lu *et al*., 2001; Gülçin *et al*., 2003). It is a well-established fact that reducing power is firmly related to antioxidant potential and it correlates with the phenolic constituent in various foods. Hence, the reducing capacity of a substance may serve as a significant indicator of its potential antioxidant activity (Gülçin *et al*., 2005; Oloyede and Oloyede, 2014).

Table 1 shows the reductive capability of different extracts of *C. albidum* fruit and ascorbic acid. All extracts showed very high (62-96%) activities, with the aqueous extract (AE) having the highest activity followed by methanolic extract (ME) then petroleum ether extract (PE). In each case, extract from the pulp showed significantly ($p < 0.05$) higher reductive capability than its corresponding skin extract. Comparison of the reducing power of ascorbic acid with all *C. albidum* extracts showed that ascorbic acid was significantly higher ($p < 0.05$). Thus, the reducing power of ascorbic acid and *C. albidum* extracts followed the order: Ascorbic acid>AE>ME>PE. This finding supports a recent report by Oloyede and Oloyede (2014), which showed that the antioxidant activity of *C. albidum* fruit is very high (92.5%), though the food value was found to be low. The antioxidant activity was attributed to the high amount of phenolic compounds present in the fruit. Another study indicated that the antioxidant activity of the exocarp of *C. albidum* is concentration dependent and ~ 55% activity was found at a concentration of 1 mg/ml (Orijajogun *et al*., 2013), which correlates well with our finding. Similar studies that focused on the leaves of the plant also showed comparable significant antioxidant and free radical scavenging capacities (Adebayo *et al*., 2011; Oguntoyinbo *et al*., 2015).

The percentage hydrogen peroxide inhibition of different extracts of *C. albidum* fruit and ascorbic acid is presented in Table 2. Hydrogen peroxide (H_2O_2) is not very reactive but is very important due to its ability to penetrate biological membranes and produce hydroxyl radical. Thus, removing it is paramount for the protection of the biological system (Gülçin *et al*., 2005).

TABLE 2: Percentage Hydrogen peroxide inhibition of different extracts of *C. albidum* fruit and ascorbic acid

Extract	% H_2O_2 Inhibition	
	Pulp	Exocarp
Methanol	98.14±0.68[a]	98.67±0.64[a]
Petroleum ether	86.86±0.17[b]	85.96±0.13[b]
Aqueous	99.21±0.59[a]	99.22±0.48[a]
Ascorbic acid (Standard)	99.48±0.51[a]	48±0.51[a]

Values represent mean ± SD of triplicate measurement. Values with different superscripts differ significantly at $p < 0.05$.

Comparative analysis using *C. albidum* extracts and ascorbic acid to scavenge H_2O_2 revealed that ascorbic acid had the strongest H_2O_2 scavenging activity. However, statistical significance ($p < 0.05$) was only evident with the petroleum ether extracts. The percentage H_2O_2 inhibition was greater than 85% for PE, 98% for ME and 99% for both AE and ascorbic acid. Adebayo *et al*., 2011 also showed that the PE extract of *C. albidum* leaves had the least radical scavenging activity in comparison with other solvents (ethanol, butanol, and ethyl acetate) extracts. This may be due to the polarity of the major bioactive constituents of the plant. Lower free radical scavenging activity (76% at 5 mg/ml) by ethyl acetate extract of *C. albidum's* exocarp using the DPPH method has also been reported (Orijajogun et al., 2013). The ability of *C. albidum* extracts to scavenge H_2O_2 may be attributed to the presence of phenolic groups that could donate electrons to H_2O_2, thereby neutralizing it into water.

CONCLUSION

The present investigation shows that both the pulp and exocarp of the fruit of *C. albidum* have antioxidant activities by virtue of their ability to serve as reducing agents and free radical scavengers. Amongst all the solvent extracts of *C. albidum*, the aqueous extract possessed the greatest antioxidant activity. Therefore, while encouraging the use of the fruit as part of our diet and exploitation as a therapeutic agent, there is need for further investigation of the active compounds responsible for *C. albidum's* power and it's *in vitro* effect on free radicals/ oxidants.

ACKNOWLEDGEMENT

The authors wish to acknowledge Mallam Anas Isah, a technician of the department of biochemistry, Usmanu Danfodiyo University, Sokoto for helping with generating some of the laboratory results.

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