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ANTIOXIDANT ACTIVITIES OF CHRYSOPHYLLUM ALBIDUM LEAVES, FRUITS, AND SEEDS

George Olajide A[1], Adenipekun Eyitayo O [2], Fasogbon Samuel Ayobami[3], Oparanozie Jude A[3].

[1]*Medical Laboratory Science Council of Nigeria, South-South Zonal office, Benin city, Nigeria*
[2]*Department of Medical Laboratory Science, College of Medicine, University of Lagos, Nigeria*
[3]*Public Health In-vitro Diagnostic Control Laboratory, Medical Laboratory Science Council of Nigeria, Yaba-Lagos, Nigeria*

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ABSTRACT: BACKGROUND: *Chrysophyllum albidum* is a medicinal plant that belong to the Sapotaceae family. The study was carried out to determine the antioxidant activities on the leaves, seed, and fruits of *Chrysophyllum albidum.* MATERIALS AND METHOD: The fruits, leaves and seed of *Chrysophyllum albidum* were extracted with distilled water, Seaman's Schnapps, Methanol and Petroleum ether using cold extraction. The antioxidant screening of the leaf extracts was done with 1-1-diphenyl- 1-picryl-hydrazyl (DPPH). RESULT and DISCUSSION: Antioxidant screening of the *Chrysophyllum albidum* with DPPH was positive indicating the presence of free radical scavenging molecules. CONCLUSION: *Chrysophyllum albidum* aqueous leaves extract can be developed and use as an antioxidant.

KEYWORDS: *Chrysophyllum albidum*, Antioxidant, DPPH, Extracts.

INTRODUCTION:

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables; they are also available as dietary supplements[1]. Antioxidants interact with and stabilize free radicals, they are used as important additives in gasoline, it prevent oxidative stress that lead to cells and tissue damage, it is used in dietary supplements and have been investigated for the prevention of diseases such as cancer [2], coronary heart disease and have many industrial uses such as preservatives in food and cosmetics [3]. Example of antioxidants includes beta-carotene, lycopene, vitamin C, E, A, Lutein, and Selenium [1,4]. There is good evidence that eating a diet with lots of vegetables and fruits is healthy and lowers risks of certain diseases[1]. Chrysophyllum albidumis a medicinal plant that belong to the Sapotaceae family which has up to 800 species and make up almost half of the order Ericales [5].

Corresponding Author:
George, Olajide Akintunde
Medical Laboratory Science of Nigeria73, Muritala Mohammed Way, Benin City, Edo State, Nigeria.

Chrysophyllum is a genus of about 70-80 species of tropical trees growing rapidly to 10-20m or more in height. The generic name is derived from the Greek word Chrysos meaning 'gold' and Phyllos meaning 'leaf [6].The genus is native to tropical regions throughout the world, with the greatest number of species in northern South America. Chrysophyllum albidum is a dominant canopy tree of lowland mixed rain forest, sometimes riverine. It is widely distributed from West Africa to the Sudan with an eastern limit in Kakamega forest, Kenya[6]. Chrysophyllum albidum, is distributed throughout the Southern part of Nigeria where it is called 'Agbalumo' (Yoruba) ; 'Udara' (Igbo), while in the Northern Nigeria, it is called 'Khada'(Hausa) [7,8]. Tannins, flavonoids, terpenoids, protein, carbohydrates and resins are the phytochemicals that have been reported in Chrysophyllum albidum[9]. Chrysophyllum albidum has antioxidant properties by scavenging free radicals, decreasing lipid peroxidation and increasing the endogenous blood antioxidant enzymes level[10].The leaves of Chrysophyllum albidum was shown to reveal the presence of Alkaloids, Cardiac glycoside, Anthraquinones, Flavonoids, Terpenoids, and Steriods [11], which are useful substances that have medicinal and physiological activities[9]. Antioxidants have been used in reduction of oxidative stress and reduced carcinogens in the human body system, however, few data exists on determining antioxidant properties of medicinal plants [12], however, this study will determine the antioxidant properties of Chrysophyllum albidum. Recently, many natural and synthetic free radical scavengers and antioxidant have been employed in protecting bio-molecules against free radical mediated damages.

Plate 1.Chrysophyllum albidum Leaves

Plate 2.Chrysophyllum albidum Fruits

MATERIALS AND METHODS:

Area of Study

This study was carried out at Department of Medical Microbiology, College of Medicine University of Lagos, Lagos State, Nigeria.

Collection and Preparation of Plant Extract

The plants was identified and authenticated at the herbarium unit and Pharmacognosy Laboratory, College of Medicine, University of Lagos. The leaves and fruits of *Chrysophyllum albidum (Agbalumo-Yoruba, Udara-Igbo and Khada-Hausa)* were purchased at the Mushin Market in Lagos, Nigeria. The seed were obtained from the fruit pulp.

Processing of Plant Materials

The fresh leaves and fruits were properly washed in tap water and the seed were removed manually and washed in tap water. The plant leaves, fruits and seeds were allowed to dry for two weeks. The plants materials were pulverized into powder using an electric blender.

Extraction of Plant Materials

Distilled water, Seaman's Schnapps, Methanol and petroleum ether were used for the extraction of plant extracts. 100 grams of the dried and grounded leaves were suspended in 500ml of distilled water, Seaman's Schnapps and Methanol for extraction. 100 grams of the dried and grounded fruits were suspended in 500ml of Distilled water, Seaman's Schnapps and Methanol for extraction while 80 grams of the dried and grounded seed was soaked in distilled water and Petroleum ether. This was left to soak at room temperature for 72hours with agitation at intervals. The extracts were filtered with Whatman filter paper No. 42 (125mm). The filtrate were concentrated using the rotary evaporator at 45^0C.The concentrated extracts from each solvent were taken for lyophilization at the Department of Biochemistry, College of Medicine, University of Lagos. Each solid extract/ paste obtained after lyophilization was reconstituted in their respective solvents to obtain a stock solution of 512µg/ml [13, 14]. The stock solutions were stored in sterile capped bottles and labeled as follows:

1. Aqueous Leave Extract (ALE)
2. Aqueous Fruit Extract (AFE)
3. Aqueous Seed Extract (ASE)
4. Seaman's Schnapps Leave Extract(SSLE)
5. Seaman's Schnapps Fruit Extract (SSFE)
6. Methanolic Leave Extract (MLE)
7. Methanolic Fruit Extract (MFE)
8. Petroleum ether Seed Extract (PSE)

The stock solutions were stored at 4 ^{0}C - 8^0C.

Determination of Antioxidant Activity

- **Rapid Screening for free Radical Scavenging Activity:** Rapid thin layer chromatography screening for antioxidant activity was carried out by spotting concentrated methanolic solution of each extract on silica gel plate. The plates were sprayed with w/v DPPH in methanol. The plants were visualized for the presence of yellowish spots [15].
- **DPPH'S Radical Scavenging Activity;** The radical scavenging activity of the plant extracts against 1, 1-diphenyl-1-picryl-hydrazyl (DPPH) -Sigma Aldrich) radical was determined by measuring UV absorbance at 517nm. Radical Scavenging activities were measured by a slightly modified method of [16].

Principle

DPPH method is based on the reduction of methanolic solution of the colour free radical, DPPH, by a free radical scavenger. DPPH, a protonated radical, absorbs maximally at 517nm (absorbance maxima) in visible spectroscopy and this decreases with the scavenging of the proton radical, the characteristics which has been widely utilized in evaluating free radical scavenging effect of natural antioxidant [17].
The following concentration of extracts were prepared; 0.02,0.04,0.06,0.08 and 0.1 mg/ml.Ascorbic acid and alpha-tocophenol were used as standards, and the same concentrations were prepared as the test solutions. All the solutions were prepared with methanol. 2ml of each prepared concentrations were placed into test tube. 0-5ml of 1mM DPPH solution in methanol was added thereafter, the mixture was shaken and the test tubes were incubated for 15 minutes at room temperature, and the absorbance read at 517nm.The experiment were carried out in triplicates.

A blank solution was prepared and measured containing the same amount of methanol and DPPH. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The ability to scavenge DPPH radicals was calculated using this equation:

DPPH scavenging effect (%) = **Ao-A/Ao x 100**
Where Ao= Absorbance of blank sample
A1=Absorbance of test extract or standard.

- **Total Phenolic Content:** The total phenolic content of the leaves and fruit extracts were determined according to the [18]. Calibration curve was prepared by mixing ethanolic solution of gallic acid (1ml,0.025 to 0.4mg/ml) with 5ml Folin-Ciocalteu reagent (diluted ten fold) and Sodium Carbonate-Na_2CO_3 (4ml,0.7M). Absorbance was measured at 765nm and the calibration curve drawn. 1ml of ethanolic plant extract (5mg/ml) was also mixed with the reagents above and after 2 hours, the absorbance was measured to determine the total phenolic contents. All determinations were carried out in triplicates. The total phenolic content in the extract in garlic acid equivalents was calculated by the formula: **T=C.V/M**

  T=total content of phenolic compound (mg/g) plant extract in GAE (garlic acid equivalent).
  C=concentration of garlic acid established from the calibration curve (mg/ml).
  V=volume of extract (ml)
  W=weight of plant extract (g)

- **Total Flavonoid Content:** The total flavonoid content was determined using a method of [19]. To 2ml sample was added 2ml of 2% Aluminum chloride ($AlCl_3$) in ethanol. The absorbance was measured at 420nm after 1 hour at room temperature. Concentration of 0.1mg/ml and 1mg/ml of the extract in methanol were used, while rutine concentrations of 0.01, 0.02, 0.04, 0.08 and 0.10 mg/ml were used to obtain the calibration curve. Solutions were prepared in methanol. The total flavonoid content (RE) in mg/g using the following equation based on the calibration curve.

RESULTS:

The rapid screening for free radical scavenging activity showed positive activity for all the extracts; since they showed yellowish spot indicating the presence of antioxidant on the plant parts. Seaman's Schnapps and methanolic leaf extracts had high content of antioxidant activity almost competing with vitamin C and E, while Seaman's Schnapps fruit extract and methanolic fruit extract showed certain degree of radical scavenging activity (Table 1).

The total phenolic content of the extracts are: Seaman's Schnapps leaf was 88.2mg/g ,Methanolic leaf was 89.4mg/g ,Ethanolic fruit was 25.2mg/g and Methanolic fruit was 29.6mg/g. The scavenging effect order of the four extracts and the standards in decreasing order: Vitamin C {Ascorbic acid} > Vitamin E {alpha-tocopherol} > Seaman's Schnapps leave extract -SSLE> Methanolic leave extract –MLE> Seaman's Schnapps fruit extract –SSFE>Methanolic fruit extract –MFE.

The total flavonoid contents of the extracts were: Seaman's Schnapps leaf was 607mg/g, Methanolic leaf was 876mg/g , Seaman's Schnapps fruit was 32mg/g and Methanolic fruit was 26mg/g.

TABLE 1: DPPH SCAVENGING EFFECT (%) OF CHRYSOPHYLLUM ALBIDUM

EXTRACTS	CONCENTRATIONS WITH DPPH SCAVENGING EFFECT				
	20µg	40µg	60µg	80µg	100µg
Vitamin C	96.62	96.69	96.32	95.95	96.62
Vitamin E	60.77	82.60	95.27	95.42	96.02
SSLE	43.21	57.84	69.32	89.20	92.12
MLE	32.41	51.84	63.62	79.89	85.07
SSFE	15.53	20.11	18.53	28.06	29.71
MFE	9.68	19.13	25.36	26.86	32.56

KEY:
SSLE=Seaman's schnapps leave extract,
MLE=Methanolic leave extract,
SSFE=Seaman's schnapps fruit extract,
MFE=Methanolic fruit extract

TABLE 2: PHENOLIC CONTENT OF CHRYSOPHYLLUM ALBIDUM SHOWING CONCENTRATION AGAINST ABSORBANCE

EXTRACTS	CONCENTRATION OF CHRYSOPHYLLUM ALBIDUM					
	5mg/ml	25µg/ml	50µg/ml	100µg/ml	200µg/ml	400µg/ml
Blank	0.0790	-	-	-	-	-
Galic acid	-	0.273	0.639	1.385	2.244	3.241
SSLE	3.971	-	-	-	-	-
MLE	4.025	-	-	-	-	-
SSFE	1.138	-	-	-	-	-
MFE	1.339	-	-	-	-	-

KEY:
SSLE=Seaman's schnapps leave extract,
MLE=Methanolic leave extract,
SSFE=Seaman's schnapps fruit extract,
MFE=Methanolic fruit extract

TABLE 3: FLAVONOID CONTENT OF CHRYSOPHYLLUM ALBIDUM SHOWING CONCENTRATION AGAINST ABSORBANCE

EXTRACTS	CONCENTRATION OF CHRYSOPHYLLUM ALBIDUM					
	10µg	20µg	40µg	80µg	100µg	1000µg
Blank	-	-	-	-	-	0.003
Rutin Hydrate	0.015	0.03	0.05	0.1	0.12	-
SSLE	-	-	-	-	0.064	0.607
MLE	-	-	-	-	0.080	0.876
SSFE	-	-	-	-	0.006	0.032
MFE	-	-	-	-	0.005	0.026

KEY:
SSLE=Seaman's schnapps leave extract,
MLE=Methanolic leave extract,
SSFE=Seaman's schnapps fruit extract,
MFE=Methanolicfruit extrat.

DISCUSSION:

The DPPH test showed the ability of the Seaman's Schnapps leaves and fruits extracts (SSLE and SSFE) and methanolic leaves and fruits extracts(MLE and MFE), to act as a free radical scavenger by mopping up the free radical which can cause cell damages. This study supported the work reported by[15] in which the extract of Tetracarpidium conophorum leaves exhibited good free radical scavenging activity.

All the four extracts showed considerable radical scavenging activity in a concentration dependent manner. The Seaman's Schnapps leaf and fruit extracts and the methanolic leaf and fruit extracts of C.albidum exhibited a good potential to act as a free radical scavenger in comparison to that of Vitamin C and Vitamin E reference standard which are widely recognized as free radical scavengers. Seaman's Schnapps and methanolic leaf extracts (SSLE and MLE) had high antioxidant properties similar to vitamin C and E; while Seaman's Schnapps and methanolic fruit extracts (SSFE and MFE) also showed certain degree of radical scavenging activity. It is well established that the free radical scavenging activity is concentration dependent, as the rate of scavenging increased with rise in concentration. Hence, a bit higher concentration of Seaman's Schnapps and methanolic leave extracts would be required to achieve maximal inhibition of DPPH compared to Vitamins C and E, while a much more higher concentration of Seaman's Schnapps and methanolic fruit extracts will be required to achieve same maximal inhibition of DPPH compared to Vitamin C [15]. The phenolics are major group of compounds acting as primary antioxidants or free radical scavenger [20].

The phenolic content in the plant extracts SSLE,MLE,SSFE, and MFE was determined with respect to the calibration curve of Garlic Acid Extract {GAE} which is known to be very rich source of phenolic. The total phenolic content of Seaman's Schnapps leave extract of Chyrsophyllum albidum was greater than that of Tetracarpidium conophorum as reported by [15].

These results showed almost similar trend to that of DPPH inhibition. The value of the four extract were considerably high which showed that they were very rich in phenolic content which had been noted to be an antimicrobial agent [21, 22] thereby authenticating its antimicrobial activity in vitro on Multidrug Resistant organisms.

Many researchers have reported positive correlation between free radical scavenging activity and total phenolic content. Phenolic and flavonoids have been shown to have antibacterial, antiviral, antineoplastic, anti-inflammatory, anti-allergic, antithrombotic and vasodilatory activities [23, 24]. The phenolic and flavonoid activities may have confer the ability to inhibit the organisms tested in this study on the Chyrsophyllum albidum extracts. The plant extracts which showed high activities of DPPH radicals, phenolic and flavonoid contents as antioxidant will be useful in mopping up the free radicals that may cause cellular degeneration and death as reported by [15].

Conclusion

The extract of Chyrsophyllum albidum leaves exhibited high antioxidant activities more than the fruit extract. The plant extracts of the leaves and fruits were also rich in phenolic and flavonoid content. Therefore it is recommended that Chyrsophyllum albidum can be developed and be use as an antioxidant

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