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(RESEARCH ARTICLE)

Study on *in vitro* antioxidant activities of *Chrysophyllum albidum* G. Don stem-bark

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Abstract

Chrysophyllum albidum G.Don is commonly known as African star apple. It possesses medicinal properties and used in folk medicine for the treatment of coronary heart diseases, stroke, anti-inflammatory, diabetes, cancers and diarrhea. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress. The present study was carried out to investigate the phytochemicals and antioxidant activities of ethanol extract *of Chrysophyllum albidum* stem-bark. The phytochemical tests reviewed the presence of alkaloids (53%), tannins (6%), saponins (21%), flavonoids (10.25%), cardiac glycosides (21.36%), steroids (7.58%) and anthraquinone (8.72%). The overall antioxidant activities observed in the stem-bark of *Chrysophyllum albidum* showed strong free radical scavenging activity with DPPH (81.88±0.83 at 50 mg/ml) when compared with the reference chemical (TROLOX). The present study validates the traditional use, demonstrating that the stem-bark of *Chrysophyllum albidum* possesses concentration–dependent antioxidant activity.

Keywords: *Chrysophyllum albidum*; Folk medicine; Antioxidant; DPPH.

1. Introduction

An antioxidant is a molecule capable of showing or preventing the oxidation of other molecules.Oxidation is a chemical reaction that tranfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by oxidizing it. Therefore, the main characteristic of an antioxidant is its ability to trap or scavenge free radicals generated as part of body's normal metabolic processes, thus inhibiting the oxidative mechanisms that lead to degenerative diseases or pathological disorder such as ischemia, cancer, gastrointestinal inflammation, asthma, cataracts, cardiovascular disease, diabetes mellitus, aging process, neurological degeneration and other inflammatory processes [1]. Antioxidant-based drugs/formulations for the prevention and treatment of these diseases have appeared during the last 3 decades [2]. This has attracted a great deal of research interest in natural antioxidants. Subsequently, a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables has increased [3, 4, 5]. Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger, and several medicinal plant extracts [6, 7].

Chrysophyllum albidum, commonly known as Africa star apple, is a wildly grown plant which belongs to the family of trees known as Sapotaceae which has about 800 species [8. 9]. It is a common plant among many locals in Nigeria (particularly in Ondo State) where the fleshy pulp of the fruit is eaten chiefly as snack and its fruit has been found to have the highest content of ascorbic acid with 1000 to 3,330 mg of ascorbic acid per 100g of edible fruit or about 100 times that of oranges and 10 times of that of guava or cashew [1, 3]. It is also reported as an excellent source of vitamins, irons, and flavors to diets. Preceding studies in Western Africa have shown that the roots, barks and leaves of

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Chrysophyllum albidum have been employed in folk medicine for the treatment of various diseases such as skin eruption, stomach ache, diarrhea, yellow fever, malaria [11, 12], heart diseases, stroke, diabetes and cancers [2]. In spite of the rich component and vast local use of *Chrysophyllum albidum* stem-bark, there is dearth of information on its' antioxidant properties. Thus, the present study was aimed to investigate the *in vitro* antioxidant activity of ethanol extract of *Chrysophyllum albidum* stem-bark.

2. Material and methods

2.1. Plant material and extraction

Plant specimen (stem-bark) was collected locally or obtained from Apex Academy premises, Ibaka Akungba Akoko, Ondo State. Nigeria. The plant was identified and authenticated at Forest Research Institute of Nigeria (FRIN) Ibadan, Oyo State. The voucher specimen was deposited in Forest Herbarium, Ibadan (FHI) at FRIN as 110130 and also deposited at the Department of Plant Science and Biotechnology Herbarium, Adekunle Ajasin University, Akungba akoko, Ondo State. Nigeria. About 400 g of dried, powdered plant material was macerated in ethanol for 7 days. The final extract was passed through No.1 Whatman filter paper (Whatman Ltd., England). The filtrate obtained was concentrated under vacuum on a rotary evaporator at 40°C and stored at 4 °C for further use.

2.2. Quantitative and qualitative phytochemical analyses

The screenings of the sample were carried out for the determination of alkaloid, saponins, tannins, anthraquinones, cardiac glycoside, flavonoids and steroids [13, 14].

3. *In vitro* studies

3.1. DPPH scavenging activity

The free radical scavenging ability of the extract was determined by DPPH (1,1-diphenyl-2-picryhydrazyl) [15]. 1 ml of the extract or standard (Trolox) was mixed with 1ml of the 0.4mM methanol solution of the DPPH. The mixture was left in the dark for 30 min before measuring the absorbance at 516 nm and the scavenging ability of the extract was calculated as:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(\text{Abs control} - \text{Abs sample})]}{(\text{Abs control})}$$

Where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance.

3.2. Estimation of total phenolic content

The total phenolic content of the extract was estimated by mixing 0.2 ml of the extract thoroughly with 2.5 ml of 10% Folin ciocalteau's reagent and 2 ml of 7.5% Sodium carbonate. The reaction mixture was subsequently incubated at 45°C for 40mins, and the absorbance was measured at 700nm in the spectrophotometer. Garlic acid was used as standard phenol.

3.3. Estimation of total flavonoids

The total flavonoid content of the extract was determined using a colorimeter assay developed by [16]. 0.2ml of the extract was added to 0.3ml of 5% $NaNO_3$ at zero time. After 5 minutes, 0.6ml of 10% $AlCl_3$ was added and after 6 minutes, 2ml of 1M NaOH was added to the mixture followed by the addition of 2.1ml of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

3.4. ABTS scavenging activity

The ABTS (2,2–azino-bis(3-ethylbenthiazoline-6-sulphonic acid) scavenging ability of the extract was determined by [17]. The ABTS was generated by reacting 7mM ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM/l, final conc.) in the dark for 16 hours and adjusting the absorbance at 734nm to 0.700 with ethanol. 0.2 ml of the appropriate dilution of the extract was then added to 2.0 ml of ABTS solution and the absorbance was read at 732nm after 15 minutes. The TROLOX (6-hydroxyl-2,5,7,8-tetramethyl chroman-2-carboxylic acid) equivalent antioxidant capacity was subsequently calculated.

3.5. Superoxide (S0) anion scavenging activity assay

The superoxide anion radicals were produced in 2 ml of phosphate buffer (100 mM, pH 7.4) with 78 µM β- nicotinamide adenine dinucleotide (NADH), 50µM nitro blue tetrazoliumchloride (NBT) and test samples at different concentrations. The reaction mixture was kept for incubation at room temperature for 5 min. It was then added with 5-methylphenazinium methosulphate (PMS) (10 µM) to initiate the reaction and incubated for 5 min at room temperature. The colour reaction between superoxide anion radical and NBT was read at 560 nm. Vitamin C was used as a positive control agent for comparative analysis. The reaction mixture without test sample was used as control and without PMS ($C_4H_{12}N_2S0_4$) was used as blank [18]. The percentage inhibition of superoxide anion radical was calculated as:

$$\text{Superoxide anion radical scavenging activity (\%)} = \frac{[(\text{Abs control} - \text{Abs sample})]}{(\text{Abs control})} \times 100$$

Where Abs control is the absorbance of superoxide anion radicals, Abs sample is the absorbance of superoxide anion radical + plant extract.

3.6. Hydroxyl (OH) Radical Scavenging Activity

The ability of the extract to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose was carried out using modified method [19]. The extract (100µl) was added to a reaction mixture containing 120µl, 20mM deoxyribose, 400µl, 0.1M phosphate buffer pH 7.4, 40µl, 20mM hydrogen peroxide and 40µl, 500µM $FeSO_4$, and the volume was made to 800µl with distilled water. The reaction mixture was incubated at 37°C for 30 min and the reaction was stopped by the addition of 0.5ml of 2.8% TCA, this was followed by the addition of 0.4ml of 0.6% TBA solution. The reaction mixture was subsequently incubated in boiling water for 20min. The absorbance was measured at 532nm in spectrophotometer.

$$\% \text{ inhibition} = \frac{[(A_o - A_1)]}{[A_o]} \times 100$$

Where A_o is the absorbance of the control, A_1 is the absorbance in the presence of plant extract and known standard.

3.7. Statistical analysis

Each test was performed in duplicate and the results were expressed as mean ± standard deviation. The results were statistically assessed by one-way analysis of variance (ANOVA). $P<0.05$ was considered significant. One way ANOVA diagnosis of the data was done using SPSS software.

4. Results and discussion

Percentage yield of the ethanol extract of powdered stem-bark of *Chrysophyllum albidum* was 26.09%, the yield was low when compared to results of extract yield from plant sources of other authors [20, 21]. Basically, extraction of bioactive components from medicinal plant is known to permit the demonstration of their physiological activity as well as to facilitate pharmacological studies on the plant leading to synthesis and discovery of pure and potent compounds that have low toxicity when used as drugs. The results of phytochemical screening showed presence of Alkaloids, Tannins, Steroids, Flavonoids, Saponins, Cardiac glycosides and Anthraquinone in the ethanol extract of *C. albidum* stem-bark screened for secondary metabolites (Table 1). These secondary metabolites were known to show medicinal activities as well as exhibiting physiological activities [22]. The quantitative analysis results (Table 2) showed that Alkaloids had the highest percentage (53.00%) while Tannins had the least percentage (6.33%). Alkaloids often have pharmacological effects and are used as medications and recreational drugs such as antimalarial agents, analgesics and can act as stimulants. Glycoside moieties such as saponins, anthraquinones, cardiac glycosides and flavonoids can inhibit tumor growth, act as an antiparasitic agent, and can be used as an antidepressant. The antioxidant activity of the ethanol extract of *Chrysophyllum albidum* stem-bark was investigated against *in vitro* models. Since, free radicals are of different chemical entities, it is essential to test the extract against many free radicals to prove its antioxidant activity. Hence, a large number of *in vitro* methods were used for the screening. Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms [23]. Herbal drugs containing free radical scavengers are gaining importance in treating diseases. The antioxidant activities of ethanol extract of *C. albidum* stem-bark were evaluated by DPPH radical scavenging activity, Hydroxyl radical scavenging activity (OH), Total phenolic content, Total flavonoids, ABTS scavenging activity and

Superoxide (SO) radical scavenging activity. The anti-radical and antioxidant activities of the extract using different methods showed that these activities vary, depending on their concentrations. The $IC_{50,100,200\ \&300}$ of the extract on Phenol, Flavonoid, OH, ABTS, DPPH and SO varied from 8.30 to 31.14mg/ml, 8.30 to 27.40mg/ml, 17.76 to 40.48mg/ml, 21.47 to 46.46mg/ml and 62.54 to 84.14mg/ml respectively (Table 3). The noticeable increase in the antioxidant properties of this plant could serve as a significant indicator of its antioxidant potential when compared with the reference chemicals (Table 4). The result showed that DPPH had the highest activity when compared with all other parameters investigated The DPPH is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants. A high proportion of antioxidants in our diet prevent the body from any oxidative damage in order to maintain a better healthy condition and also to slow down aging process. Numerous studies have shown that antioxidants have protective effects on health problems. It was reported that antioxidant prevents the occurrence of diseases, like cancer, aging and that they can also interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavenger [24].

Table 1 Preliminary qualitative phytochemical result of *C. albidum* stem-bark

TEST	OBSERVATION	INFERENCE
Alkaloids		
Dragendorff's reagent	Yellow precipitate.	++
Mayer's test	Dark yellowish precipitate.	++
Saponins		
Frothing test	Frothing persist.	++
Benedict test	Blue-Black precipitate.	++
Tannins		
2ml of extract + 2ml $Fecl_3$.	Brownish-Green.	+++
2ml of extract + 2ml bromine.	Blue-Black precipitate.	+++
Flavonoids		
2ml of extract +2ml of 10% Lead acetate.	Yellowish precipitate.	++
2ml of extract + 2ml dil.NaOH.	Golden precipitate.	++
Cardiac Glycoside		
Keller-Kiliani test	Brownish ring.	++
Kedde test	Reddish-Brown ring.	++
Steroids		
2ml of extract + 4ml of acetate anhydride + 2ml of H_2SO_4	Violet coloration.	+
Anthraquinone		
Borntrager's test	Violet coloration	++

KEY NOTE: +++ HIGHLY POSITIVE, ++ MODERATETY POSITIVE, + POSITIVE

Table 2 Results of quantitative analysis of C*. albidum*

S/N	Phytochemicals	Mean ± standard deviation
	Alkaloid	53.00 ± 0.5
	Saponin	21.18 ± 0.64
	Tannin	6.33 ± 0.38
	Flavonoid	10.25 ± 0.01
	Cardiac glycoside	21.36 ± 0.20
	Steroids	7.58 ± 0.04
	Anthraquinone	8.72 ± 0.08

Values are the average of duplicate experiment and represented as mean ± standard deviation.

Table 3 Results of the antioxidant properties of *C. albidum* stem-bark

Concentrations(mg/ml)	50	100	200	300
Phenol	8.30 ±0.15	11.66±0.15	21.41±0.01	31.14±0.15
Flavonoid	8.3±0.02	6.62±0.03	17.13±0.02	27.40±0.31
OH	17.76±0.58	20.03±0.58	30.54±0.59	40.48±0.15
DPPH	81.88±0.83	84.66±0.35	95.66±0.35	98.60±0.36
ABTS	21.47±0.05	24.49±0.65	35.45±0.05	46.46±0.65
SO	62.54±0.48	64.17±0.22	74.51±0.48	84.14±0.48

Values are represented as mean ± standard deviation.

Table 4 Reference chemicals against antioxidant parameters

Reference chemical	Galic	Rutin	Vitamin C	Trolox
Phenol	0.07±0.02			
Flavonoid		0.05±0.12		
OH			0.09±0.45	
DPPH				0.09±0.23
ABTS				0.09±1.03
SO			0.09±0.24	

Values (300mg/ml) are represented as mean ± standard deviation.

5. Conclusion

This study showed that the stem-bark of *C. albidum* had significant number of useful bioactive components and also a lot of potentials as an antioxidant agent. These observed activities provided the basis for their folkloric uses, as cure for some human ailments like skin infection, diarrhea and stomach-ache, which are as a result of infections and inflammatory reaction. The results of this study justified the traditional uses of the stem-bark of *C. albidum* for therapeutic purposes.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declared no conflict of interest.

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