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EVALUATION OF THE PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIALPROPERTIES OF EXTRACTS FROM *CHRYSOPHYLLUM ALBIDUM (*AFRICAN STAR APPLE) LEAF

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ABSTRACT

Phytochemical, antioxidant and antimicrobial properties of n-butanol, petroleum ether and ethanol extracts of the leaf of Chrysophyllumalbidum were investigated. Antioxidant activity was carried out using various tests which include; 2, 2-diphenyl-picrylhydrazyl (DPPH) scavenging activity, total antioxidant activity, ferric reducing antioxidant power (FRAP), total flavonoid content and the total phenolic content. The antimicrobial test was conducted on the following micro-organisms; Salmonella dysenteriae, Bacillus cereus, Salmonella typhi, Acinetobacter spp., Escherichia coli, Klebsiellapneumoniae, Penicillum spp., Aspergillusflavus, Fusariumverticoides, Aspergillus tamari and Aspergillusparasiticus using the agar well diffusion method. Results revealed that petroleum ether gave the highest extract yield (16.8%) while butanolic extract had the least yield (4.6%). At 250 µg/ml extract concentration, petroleum ether extract demonstrated the highest (73.57%) DPPH scavenging activity followed by ethanol extract (59.32%) while butanolic extract had the least scavenging activity of 32.02%, and these were significantly different (P<0.05). Total antioxidant activity (ascorbic acid equivalent) of C. albidum ranged from 42.33AAE in butanol extract to 50.6AAE in ethanol extract. Furthermore, ferric reducing antioxidant power (FRAP) of the leaf extracts revealed that at 250 µg/ml extract concentration, ethanol extract had the highest (0.39µmolFe(ii)/g) reduction potential, followed by petroleum ether extract (0.33µmolFe(ii)/g) and butanol extract (0.29 µmolFe(ii)/g). The total phenol content of the extracts ranged from 0.02µg/mlTAE–0.09µg/mlTAE at 1000µg/ml extract concentration. The total flavonoid content ranged from 0.47mg/gQE in petroleum ether extract to 12.74mg/gQE in butanol extract . The antimicrobial activities of the extracts of the leaf of C. albidum showed zone of inhibition ranging from 9.7mm to 31.0mm. The result showed that butanolic extract demonstrated a broad spectrum antimicrobial activity by inhibiting all the microorganisms tested while petroleum ether extract possessed no antimicrobial property. This result may be an indication that C. albidumleaf extracts could be used as an easily accessible source of natural antioxidant and antimicrobial agent.

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Keywords: *C. Albidum*, Phytochemical, Antioxidant and antimicrobial.

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Contribution/ Originality

This study documents the phytochemical, antioxidant properties and antimicrobial potential of the extracts of the leaves of *Chrysophyllum albidum* using different organic solvents (Petroleum ether, ethanol and butanol). This comparative evaluation in a single research work is scarce and was therefore investigated in this paper.

1. INTRODUCTION

African star apple (*Chrysophyllumalbidum* G. Don) is a tropical edible fruit. It belongs to the family of Sapotaceae which has up to 800 species and make up almost half of the order (Ehiagbonare *et al*., 2008). It is primarily a forest tree and its natural occurrences have been reported in diverse zones in Nigeria, Uganda, Niger Republic, Cameroon and Cote d'evoire (Bada, 1997).

The plant has in recent times become a crop of commercial value in Nigeria (Oboh *et al*., 2009). The fleshy pulp of the fruit is eaten especially as snacks and its fruit has been found to have higher content of ascorbic acid than oranges and guava (Amusa *et al*., 2003). It is also reported as an excellent source of vitamins, iron and flavours to diets (Adisa, 2000). The seed are either used for local game or discarded (Bada, 1997). *C. albidum* fruit is common in both urban and rural centres especially during the months of December to April. The fruits are not usually harvested from the tress, but left to drop naturally to the forest floor where they are picked up (Amusa *et al*., 2003; Abiodun *et al*., 2011).

The leaves are used as emollient and for the treatment of skin eruptions, diarrhoea and stomachaches, which occur as a result of infections and inflammatory reactions (Adisa, 2000). The people of South Western Nigeria have been using *C. albidum* leaves for the management of infections.

This research work therefore aim to examine the effect of various solvents used on the phytochemicals present in leaf extracts of African star apple and the ability of the extracts to act as antioxidant and antimicrobial agents.

2. MATERIAL AND METHODS

2.1. Sample Collection and Identification

Fresh leaves of the *Chrysophyllumalbidum* were collected from Odo-nla area of Ikorodu town in Lagos state. The authentication of the plants was done at the Department of Taxonomy/Botany of the Forest Research Institute of Nigeria (FRIN), Jericho, Ibadan, Oyo State, Nigeria.

2.2. Sample Preparation

The plant samples were air-dried under shade and ground into powder using Kenwood blender (Model:09773) The powdered samples were weighed on a weighing balance (Ohaus, USA) and further stored at room temperature.

2.3. Sample Extraction

The solvent extraction of each plant sample was prepared by soaking 50g of the plant sample in 100ml of each ethanol, petroleum ether and n-butanol respectively. These were properly mixed with shaker for homogeneity and thereafter left for 72 hours (3 days) at ambient temperature. The extracts were obtained by filtration using nest-cloth and the residues were discarded. Each filtrate was then concentrated at reduced pressure using a Resona rotary evaporator (Type: SW 2000).The concentrated extracts were then freeze-dried using an Edward freeze dryer.

2.4. Phytochemical Screening

Chemical tests were carried out on the ethanol, petroleum ether (pet ether) and n-butanol extracts using standard procedures to identify the constituents as described by (Harbone, 1973; Sofowara, 1993); (Trease and Evans, 2002) with slight modifications.

2.5. Assessment of Antioxidant Activity in Crude Extracts

This was done using DPPH Photometric Assay (Scavenging activity) as modifiedby Mensor *et al.* (2001) while total flavonoids was determined using the method of Kim *et al.* (2005). The method of Cai *et al.* (2004) was used for total phenolic content using Folin-Ciocalteu reagent. The antioxidant capacity of the extract was evaluated according to procedure of (Schuier *et al.*, 2005; Abiodun *et al.*, 2011). Total antioxidant activities was determined using a modified method of the FRAP ASSAY. A fresh working (FRAP) solution was prepared by mixing 25ml acetate buffer, 2.5ml TPTZ and 2.5ml $FeCl_3.6H_2O$. the temperature of the solution was raised to 37°C prior to use. Plant extract (150µL) was allowed to react with 2850µl of the FRAP solution for 30min in the dark condition. Reading of the coloured product (ferrous tripyridyltriazine complex) was done at 593nm. The standard curve was linear between 200 and 1000µm $FeSO_4$. Results were expressed in µmFe(II)/g dry mass and compared with that of BHT and quercetin.

2.6. Determination of Antimicrobial Activity of the Extracts

The bacteria used for this study were *Escherichia coli*, *Salmonella dysenteriae*, *Acinetobacter*, *Salmonella typhi*, *Bacillus cereus*, *klebsiliapeneumonia*while the fungi used were *Aspergillusniger*, *Aspergillusflavus*, *Aspergillus tamari*, *Aspergillusparasiticus*, *Pencilliumspp and Fusariumverticiloides* The fungi were maintained on Potato Agar Slant, at 30°C and incubated for 72hours (Ifesan *et al.*, 2010).

The bacteria suspension (10^8) was compared with MacFarland standard. For fungi 10^8 spore suspension was prepared of which 1ml of the suspension was dissolved in 20mls of Potato

Dextrose Agar and poured in the petri dish to solidify before boring the wells as described by Memnune *et al.* (2009).

2.7. Inoculation Procedure

After the adjustment of the turbidity, a sterile cotton bud was dipped into the bacteria suspension. Pressing firmly against the inside wall of the tube just above the fluid level it was rotated to remove excess fluid. The cotton bud was streaked on the surface of the solidified medium (Nutrient agar), rotating the plate approximately 60 degrees after each application to ensure even distribution and proper seeding of the inoculum on the agar.

2.8. Antimicrobial Susceptibility Test

The antimicrobial analysis was done using the agar well diffusion assay. The swabbed surface of the solidified nutrient agar was bored using sterile cork borer while each of the solvents was used as control. About 0.4ml of the extracts (0.5mg) was pipetted into each well. This was carefully done to prevent splashing on the nutrient agar and surrounding wells. The plates were incubated at 35°C for 18 hours. After incubation, the diameter of the zones of inhibition was measured and recorded in millimeters.

2.9. Statistical Analysis

Data were subjected to analysis of variance (ANOVA). Comparisonof means was carried out by Duncan's multiple range test (Steel and Torie, 1980). Statistical analysis was performed using the Statistical Package for Social Sciences. Each determination was replicated thrice.

3. RESULT AND DISCUSSION

Table-1. Percentage yield of extract of *Chrysophllumalbidum*

Fraction	Weight of powdered sample(g)	Weight of sample extract(g)	% yield
Butanolic	50g	2.3g	4.6%
Ethanolic extract	50g	4.3g	8.6%
Pet ether extract	50g	8.4g	16.8%

Table-2. Phytochemical screening of *chrysophyllumalbidum*leaf extracts

Phytochemical	Pet ether extract	Ethanolic extract	Butanolic extract
Flavonoid	+	+	+
Saponin	_	++	+
Tannin	++	+	__
Phlobatannin	__	__	__
Anthraquinones	__	__	__
Steroid	__	__	__
Terpenoids	+	__	__
Cardiac glycoside	+	__	__

CONCENTRATE ++
TRACE +
ABSENT -

Table 1 shows the percentage yield of extract obtained from each organic solvents used to extract the leaf of *C. albidum*. Pet ether extract gave the highest yield (16.8%), followed by ethanol (8.6%) while the lowest yield was obtained from n-butanol (4.6%) extract.

Phytochemical screening of the leaf extract using different solvent is shown on Table 2. From the result pet-ether extract showed trace amount of flavonoid, terpenoid and cardiac glycoside but a concentrate of tannin. while flavonoid and tannin occurred in trace quantity in the ethanolic extract. The butanolic extract however, showed only a trace quantity of flavonoid and saponin. The presence of tannin in leaves may have anti-inflammatory effect which helps control all indication of gastritis, oesophagitis, enterits, and irritating bowel disorder (Hayashi *et al*., 1993; Dharmanada, 2001). It has been observed that tannin is responsible for anti-diarrhoeal activity (Enzo, 2007) and saponin used as dietary supplements, expectorant and anti-inflammatory agent (Xu *et al*., 1996). The high content of saponin in ethanolic extract of the leaves may be responsible for the use of the extract to control human cardiovascular disease and blood cholesterol (Aletor, 1993).

Flavonoids are potent water soluble super antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anti cancer activity (Stauth, 1993). Flavonoid in intestinal tracts lower the risk of heart disease (Okwu and Emenike, 2006; Orijajogun *et al*., 2013). The biological function of flavonoids include protection against allergies, inflammatory, free radical scavenging, and platelets aggregation (Okwu and Omodamiro, 2005; Okwu and Emenike, 2006). This may account for the natural antioxidant properties of the flavonoids by acting against oxidative stress related diseases such as diabetics, cancer and coronary heart disease (Bunt and Bucar, 2000). Hence, people that are prone to such diseases can feed on *C. albidun* plant as source of natural antioxidants.The presence of phenolic compounds in the plant part indicates that *C. albidum* contain antimicrobial agents. Cardiac glycosides have been found to be effective in congestive heart failure (Aboaba *et al*., 2006). The presence of the above metabolites in plant confirmed the use of this plant in the treatment of various diseases conditions due to their antimicrobial, anti-inflamatory, and anti-carcinogenic effect. The presence of alkaloids in the leaves of this plant may be responsible for its antimalaria effect, possession of analgesic properties and its use in treatment of stomach disorder, A view supported by Okwu and Emenike (2006). Hence the presence of these metabolites in *C. albidum* leaves tend to support its medical use.

Table-3. Scavenging activity (%) of *chrysophyllumalbidum*leafextracts

Concentration (µg/ml)	%Scavenging activity of butanolic extract	% Scavenging activity of ethanolic extract	% Scavenging activity of pet ether
50	5.30 ± 0.58^{a}	15.77 ± 0.98^{c}	13.90 ± 0.74^{b}
100	11.86 ± 0.47^{a}	28.72 ± 1.01^{c}	24.98 ± 1.21^{b}
250	32.029 ± 0.98^{a}	59.32 ± 0.79^{b}	73.57 ± 3.25^{c}

Mean values with similar superscript in a column are not significantly different (P> 0.05)

Table 3 reveals the percent scavenging activity of the *C. albidum* leaf extract. The scavenging abilities of extracts range from 5.30% in butanolic extract to 73.57%, in pet ether extract The result shows that the ability of the pet ether extract of *C.albidum* leaf to quench free radicals is similar to that reported for Uzazi leaf extract which range from 43.99 – 73.87 (Dada, 2010). As antioxidant donates proton to these radicals, the absorption decrease And this decrease in absorption is taken as a measure of the extent of radical scavenging (Turkoglu *et al*., 2007; Orijajogun *et al*., 2013). Thus the degree of discolouration of the solution indicates the scavenging efficiency of the added substance. This result may be explained that the consumption of the leaf extract may fight against the free radicals present in the body which are produced during respiration and other body metabolism.

Table-4. Total antioxidant activity of *chrysophyllumalbidum*extracts TAAE (ascorbic acid equivalent) mg/100gTAAE

Concentration (µg/ml)	Butanolic extract	Pet-ether extracts	ethanolic extracts
5	21.33± 1.52[a]	24.51.±3.21[a]	32.0±1.15[b]
25	28.33± 1.52[a]	29.66±2.00[a]	37.66±1.52[b]
50	34.00±4.00[a]	35.00±1.00[a]	40.66±1.15[b]
100	38.66±1.52[a]	37.33±1.52[a]	44.66±2.31[b]
250	42.33±1.15[a]	46.66±0.57[b]	50.6±1.15[c]

Mean values with similar superscript in a column are not significantly different (P> 0.05)

Total antioxidant activity of the leaf extracts (5-250µg/ml) ranged from 21.33 to 50.6 ascorbic acid equivalent(Table 5). Ethanolic extract exhibited the highest total antioxidant activity while the least was recorded in butanolic extract. The total antioxidant activity obtained in this study can be compared with extract of *Cynarascolymus* (*Cynarae folium*) which ranged from 18.17 to 50.38 mg/100gTAAE. It was observed that antioxidant capacity increases as the concentration of the extract increases. There was no significant difference (p>0.05) in the antioxidant activity of the butanolic extract irrespective of the concentrations used while significant difference only exist in the pet ether extract at a concentration of 250µg/ml Total antioxidant activity of a plant extracts explains its action against the free radicals. This shows that the leaf extracts possess antioxidant properties that helps to stabilize the integrity of cell membrane (Abiodun *et al*., 2011; Tawheed and Monika, 2014).

Table 5 reveals the ferric reducing antioxidant power (FRAP)of the extracts of *C. albidum* leaf extract using different solvents with different concentration.The values ranged from 0.243µmolFe(ii)/g in butanolic extract to 0.392µmolFe(ii)/g in ethanolicextracct. The ferric reducing antioxidant content (FRAP) of *C.albidum* was quite low compared to the value of 0.88 µmolFe(ii)/g to 3.36 µmolFe(ii)/g reported for *Cynarascolumus* (Pracheta *et al*., 2010; Orijajogun *et al*., 2013). The antioxidant potential of the extracts of the leaf of *C. albidum* were estimated from

their ability to reduce TPRZ Fe(III) complex to TPTZ – Fe(II) at 593nm and its antioxidant activity increased proportionally with the polyphenol content. Thus leaf extracts act as free radical scavenger, capable of transforming reactive free radical species into stable non radical products.

Table-5. Ferric reducing antioxidants power (FRAP) of *chrysophyllumalbidum* leaf extracts (µmolFe(ii)/g)

Concentration µg/ml	Butanolic extract	Pet ether extract	Ethanolic extract
5	^{a}0.24±0.00	^{a}0.25±0.00	^{b}0.31±0.00
25	^{a}0.25±0.00	^{a}0.25±0.00	^{b}0.34±0.01
50	^{a}0.25±0.00	^{b}0.28±0.00	^{c}0.35±0.00
100	^{a}0.26±0.00	^{b}0.32±0.00	^{c}0.36±0.01
250	^{a}0.29±0.00	^{b}0.33±0.01	^{c}0.39±0.01

Mean values with similar super script in a column are not significantly different (P> 0.05)

Table-6. Total flavonoid content and total phenolic content of *chrysophyllumalbidum*leaf extracts.

Extracts	Total flavonoid (mg/gqe)		Total phenolic (µg/mltae)	
	500mg/g	1000mg/g	500µg/ml	1000µg/ml
PET ETHER	^{a}-0.43±0.69	^{a}0.47±0.69	^{b}0.04±0.00	^{b}0.07±0.00
ETHANOL	^{b}2.40±0.68	^{b}6.35±1.07	^{c}0.07±0.04	^{c}0.09±0.01
BUTANOL	^{c}5.34±1.15	^{c}12.74±1.74	^{a}0.01±0.00	^{a}0.02±0.00

Mean values with similar super script in a column are not significantly different (P> 0.05)

Total flavonoid and total phenolic content of *C. albidum* leaf extract using different solvent ethanol, butanol and petroleum-ether at different concentration is shown in Table 6. The results reveal that petroleum ether possessed the least value at 1000mg/g extract concentration (0.47 mg/gQE), followed by ethanol extract (6.35mg/gQE) and the highest was with n-butanol extract (12.47 mg/gQE). Total flavonoid content of *C. albidum* leaf extract was quite high compared to 1.79 mg/gQE reported for extract of *Euphorbia nerufolia* leaf extract (Pracheta *et al.*, 2010). Flavonoid are water soluble super antioxidants and free scavenging radicals which prevent oxidative cell damage, have strong anticancer activity and inhibits tumor growth (Stauth, 1993; Tawheed and Monika, 2014). Total phenolic content of *C. albidum* leaf extracts (0.01µg/mlTAE-0.09µg/mlTAE) is within the same range with that reported for extracts of *E.nernfolia* leaf extract. The presence of phenolic compounds in the plant part may indicates that *C. albidum* contains antimicrobial agents. According to Memnune *et al.* (2009), the extracts may possibly contain different type of phenolic compounds, which have different antioxidant capacities . Phenolic compounds contribute to modification of colour, taste, aroma and flavor and also help in providing health beneficial effects. They also serve in plant mechanisms to counteract reactive oxygen species (ROS) in order to prevent molecular damage (Abiodun *et al.*, 2011).

Table-7. Antibacterial activities (mm) of *chrysophyllum albidum* leaf extracts

Organisms	Ethanolic extract	Butanolic extract	Pet ether extract
Salmonella dysenteriae	9.7±1.09	14.0±0.91	_
Bacillus cereus	_	31.0±0.56	_
Salmonella typhi	_	_	_
Acetobacter spp.	_	26.0±0.83	_
Escherichia coli	_	28.0±0.90	_
Klebsillapneumoniae	_	14.0±0.91	_

Values are mean of the three replicate ± standard deviation

Table-8. Antifungal activities (mm) of *chysophyllumalbidum*leaf extract

Organisms	Ethanolic extract	Butanolic extract	Pet ether extract
Penicillum spp.	_	21.0±0.81	_
Aspergillusniger	_	19.0±0.91	_
Aspergillusflavus	_	18.0±0.91	_
Fusariumverticiloides	_	13.0±0.91	_
Aspergillus tamari	_	15.0±0.00	_
Aspergillusparasiticus	_	17.0±0.91	_

Values are mean of the three replicate ± standard deviation

Antibacterial activities of *C. albidum* leaf extract is as shown in Table 8. Ethanol extract demonstrated inhibition against *Salmonella dysenteriae* (9.7mm) but could not inhibit other bacteria tested while pet ether extract failed to inhibit any of the bacteria isolates studied. Butanolic extracts on the other hand showed inhibition against all the test bacteria except *S.typhi*The ability of butanolic extract to inhibit the test bacteria may be as a result of the high total flavonoid content recorded for butanol extract. In addition, it is possible that butanol is able to extract the bioactive substances present in the leaf making it a suitable solvent for the study of antimicrobial activity (Makasci *et al*., 2010). The antifungal activity of extracts of the leaf of *C. albidum* again revealed that Butanolic extract showed inhibition against all the fungi, producing inhibition zones which range from 13.0mm in Fusariumverticilliodes to 21.0 mm in Penicilliumspp, while the ethanolic and pet-ether extracts produced no inhibition. Possible synergistic and anatagonistic effect of compounds also play an important role in fungal inhibition (Farah *et al*., 2008).

4. CONCLUSION

Crude extracts of the leaf of *Chrysophyllumalbidum*were found to possess radical scavenging abilities and antimicrobial properties. We may conclude that ethanol and petroleum ether extracts exhibited better antioxidant activities compared to butanolic extract. However, butanolic extract possesses antimicrobial properties which may be referred to as broad spectrum based since it inhibited the growth of both Gram negative and Gram positive bacteria as well as fungi investigated in this study. These results indicate that *Chrysophyllumalbidum*leaf extract could be used as an easy and accessible source of natural antioxidant and antibacterial agent.

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