

Research Article

A Comparative Study on the Phytochemical Screening and *in vitro* Antioxidant Activity of Methanol Leaf Extracts of *Chrysophyllum albidum* and *Irvingia gabonensis*

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Abstract: *Chrysophyllum albidum* and *Irvingia gabonensis* have been reported to possess several medicinal values. Most studies on these plants have focused on the fruits rather than the leaves. The present study therefore, evaluated the phytochemical constituents and *in vitro* antioxidant activity of methanol leaf extracts of *Chrysophyllum albidum* and *Irvingia gabonensis*. Photochemical screening was carried on the plant samples using established protocols. The results of the phytochemical screening of both extracts revealed the presence of saponins, flavonoids, alkaloids, steroids and tannins. Terpenoids and cardiac glycosides were below detectable levels in both extracts. *Irvingia gabonensis* extract showed significantly higher ($p < 0.05$) levels of total phenol, total flavonoid and total tannin than the *Chrysophyllum albidum* extract. However, there was no significant difference ($p > 0.05$) in the proanthocyanidin content of both plant extracts. *Irvingia gabonensis* extract had a significantly higher ($p < 0.05$) ferric reducing antioxidant potential (FRAP) value and was a better 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenger than *Chrysophyllum albidum* extract. The results of the study revealed that extract of *Irvingia gabonensis* had more phytochemicals and consequently, possessed higher antioxidant activity than *Chrysophyllum albidum* extract.

Keywords: *Chrysophyllum albidum*, *Irvingia gabonensis*, antioxidant, phytochemical, flavonoids

INTRODUCTION

Reactive oxygen species (ROS) are not only produced naturally in cell following stress or respiration but also have been reported to be produced by radiation, bacterial and viral toxin, smoking and alcohol. Overproduction of ROS or inadequate antioxidants has been implicated in the pathogenesis and complications of some disease conditions like diabetes, Alzheimer's disease, cancer, atherosclerosis, arthritis, neurodegenerative disease, and aging process [1]. Antioxidants act as a defense mechanism that protect against deleterious effects of oxidative reaction produced by reactive oxygen species (ROS) in a biological system; thus, they are absolutely critical for maintaining optimal cellular as well as systemic health, and well-being [2]. Recently, there has been an upsurge of interest in the therapeutic potential of plants as antioxidants in reducing oxidative tissue injuries [3]. Plants, rich in phenolic compounds such as flavonoids, have been demonstrated to have anti-inflammatory, antiallergenic, antiviral, anti-aging, and anti-carcinogenic activities which can be attributed to their antioxidant properties [4].

Chrysophyllum albidum, commonly called white star apple is a very useful medicinal plant common in the tropical and sub-tropical regions of the world [5]. Extracts of *Chrysophyllum albidum* have been reported to possess hepatoprotective [6], anti-plasmodial [7], and anti-bacterial activities [8]. Similarly, *Irvingia gabonensis* (African bush mango) has several medicinal uses. The shavings of the stem bark are consumed by mouth to treat hernias, yellow fever, and dysentery, and to reduce the effects of poison in French Equatorial Africa [9]. The antibiotic properties of the bark of *Irvingia gabonensis* help heal scabby skin, and the boiled bark relieves tooth pain [10]. Most studies on the antioxidant property of *Chrysophyllum albidum* have focused on the fruit [11], [12]. Similarly, most studies on the antioxidant property of *Irvingia gabonensis* have been focused on the seeds [13]. Therefore, this study evaluated the phytochemical constituents and *in vitro* antioxidant activity of methanol leaf extracts of *Chrysophyllum albidum* and *Irvingia gabonensis*. Results from this study will be

useful in ascertaining the probable medicinal properties of the plants.

MATERIALS AND METHODS

Collection of Plant Leaves

Leaves of *Chrysophyllum albidum* and *Irvingia gabonensis* were collected from a private farm in Benin City, Edo State, Nigeria. The leaves were identified in the Department of Plant Biology and Biotechnology, University of Benin. The authenticated leaves were washed and air dried at room temperature in the laboratory.

Preparation of Extracts

The dried leaves were pulverized and 200g of the pulverized leaves were soaked in absolute methanol (2500ml) for 72 hours with occasional stirring. The extracts were filtered using double layered muslin cloth and the filtrate concentrated to dryness by means of a rotary evaporator at reduced pressure. The dried extracts were stored at 4°C until use.

Phytochemical Screening

Phytochemical screening was carried out on the plant samples using established protocols as described by Harbone [14], Sofowora [15], Trease and Evans [16].

Determination of Total Phenolic Content

The total phenolic content was determined using the Folin - Ciocalteu method as described by Cicco *et al.*, 2009 [17]. Concentrations of gallic acid, ranging from 0.2 - 1 mg/ml or extracts (1mg/ml), were prepared in methanol. Then, 4.5ml of distilled water was added to 0.5 ml of the extract and mixed with 0.5 ml of a ten-fold diluted Folin- Ciocalteu reagent. Five ml of 7% sodium carbonate was then added to the tubes and another 2ml of distilled water was added. The mixture was allowed to stand for 90 min at room temperature; absorbance was then read at 760 nm. All determinations were performed in triplicates with gallic acid utilized as the positive control. The total phenolic content was expressed as Gallic Acid Equivalent (GAE).

Determination of Total Flavonoid Content

The total flavonoid content was determined using the method of Miliuskas *et al.* 2004 [18]. Two milliliters of 2% AlCl₃ in ethanol was mixed with 2ml of the extracts (1mg/ml), in methanol. The absorbance was measured at 420 nm after one hour incubation at room temperature. Similar concentrations of quercetin, the positive control were used. The total flavonoid content was calculated as mg quercetin equivalent /g of extract.

Determination of Total Tannin Content

The total tannin content was determined by Folin Denis method as described by Polshettiwar *et al.*, 2007 [19]. Briefly, 1ml of the extract (1mg/ml) and

standard solution of tannic acid (0.1-1.0mg/ml) was made up to 7.5ml with distilled water. Then 0.5ml Folin Denis reagent and 1ml Na₂CO₃ solution were added. The volume was made up to 10ml with distilled water and absorbance was read at 700nm. The total tannin content was expressed as mg of tannic acid equivalent per gram of extract.

Determination of Proanthocyanidin Content

The determination of proanthocyanidin was carried out according to the method of Sun *et al.* 1998 [20]. To 0.5ml of 1.0mg/ml of each extract was added 1ml of 4 % methanol solution and 0.75ml of concentrated hydrochloric acid. The mixture was left undisturbed for 15 minutes and the absorbance was read at 500nm. Ascorbic acid was used as standard.

In vitro Antioxidant assays

The in vitro antioxidant assays carried out on the plant extracts were; Ferric Reducing Antioxidant Power (FRAP), Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity and Thiobarbituric acid reactive substances (TBARS).

Ferric Reducing Antioxidant Power (FRAP) Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out using a modified method of Benzie and Strain 1996 [21]. To 1.5 ml of freshly prepared FRAP solution (25 ml of 300 mM acetate buffer pH 3.6, 2.5 ml of 10mM 2,4,6-tripyridyls-triazine (TPTZ) in 40mM HCl, and 2.5 ml of 20 mM ferric chloride (FeCl₃.6H₂O) solution) was added to 1ml of the extracts (1mg/ml). The reaction mixtures were incubated at 37°C for 30 min and the increase in absorbance at 593nm was measured. FeSO₄ was used for the calibration curve and ascorbic acid served as the positive control. FRAP values (expressed as mg Fe (II)/g of the extract) for the extracts were then extrapolated from the standard curve.

Estimation of Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging capacity of the leaf extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by a slightly modified method of Brand-Williams *et al.*, 1995 [22]. Briefly, 0.5 ml of 0.3 mM DPPH solution in methanol was added to 2ml of various concentrations (0.01 – 0.2 mg/ml) of the extracts. The reaction tubes were shaken and incubated for 15 min at room temperature in the dark; absorbance read at 517 nm. All tests were performed in triplicates. Ascorbic acid was used as standard control, with similar concentrations as the test samples prepared. A blank containing 0.5ml of 0.3 mM DPPH and 2ml methanol was prepared and treated as the test samples.

The radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_0 - A_1) / (A_0)] \times 100$,

Where, A_0 was the absorbance of DPPH radical + methanol; A_1 was the absorbance of DPPH radical + sample extract or standard. The 50% inhibitory concentration value (IC_{50}) was calculated as the effective concentration of the extract that is required to scavenge 50% of the DPPH free radicals.

Estimation of Thiobarbituric Acid Reactive Substances (TBARS)

TBARS was estimated according to the method of Ohkawa *et al.*, 1979 [23]. Egg yolk homogenate (0.5 ml of 10% v/v) and 0.1 ml of extract (1mg/ml) were added to a test tube and made up to 1ml with distilled water. 0.05ml of $FeSO_4$ (0.07 M) was added to induce lipid peroxidation and incubated for 30 min. Then 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 ml of 0.8% (w/v) TBA in 1.1% sodium dodecyl sulphate and 0.05 ml 20% TCA were added and the resulting mixture was vortex; it was then heated at 95°C for 60 min. The generated colour was measured at 532 nm.

Inhibition of lipid peroxidation (%) was calculated with the formula:

$$(C-E)/C \times 100\%;$$

Where C is the absorbance value of the fully oxidized control and E is ($Ab_{532+TBA} - Ab_{532-TBA}$).

STATISTICAL ANALYSIS

The experimental results were expressed as mean \pm standard error of mean (SEM) of three replicates and were subjected to student's t-test. p values < 0.05 were regarded as significant.

RESULTS

The result of the phytochemical screening of methanol leaf extracts of *Chrysophyllum albidum* and *Irvingia gabonensis* are presented in Table 1. The result

revealed the presence of several phytochemicals specifically saponins, flavonoids, alkaloids, phenols, steroids and tannins. Phenols, flavonoids, tannins, and steroids were higher in *Irvingia gabonensis* extract than *Chrysophyllum albidum* extract. Only saponins were higher in *Chrysophyllum albidum* extract than *Irvingia gabonensis* extract. However, terpenoids and cardiac glycosides were not detected in both extracts.

Figure 1 shows total phenol, total flavonoid, total tannin and proanthocyanidin contents of methanol leaf extracts of *Chrysophyllum albidum* and *Irvingia gabonensis*. Methanol leaf extract of *Irvingia gabonensis* had significant higher ($p < 0.05$) levels of total phenol, total flavonoid and total tannin than methanol leaf extract of *Chrysophyllum albidum*. However, there was no significant difference ($p < 0.05$) in the proanthocyanidin content of the two extracts.

The Ferric Reducing Antioxidant Potential (FRAP) results are presented in Figure 2. The results revealed that *Irvingia gabonensis* extract had a significantly higher ($p < 0.05$) FRAP value (7.95 ± 0.18 mg Fe(II)/g extract) than *Chrysophyllum albidum* extract (2.73 ± 0.11 mg Fe(II)/g extract).

The results of the DPPH radical scavenging activities of *C. albidum* and *I. gabonensis* extracts leaf extracts are shown in Figure 3 with the IC_{50} values in Table 2. The results show that *I. gabonensis* extract was a better inhibitor of the DPPH radical than the *C. albidum* extract. The IC_{50} values (Table 2) were 147.70 ± 1.15 μ g/ml for the *C. albidum* extract and 111.70 ± 2.41 μ g/ml for the *I. gabonensis* extract in contrast to that of ascorbic acid (69.24 ± 4.24 μ g/ml)

Figure 4 shows the thiobarbituric acid reactive substances (TBARS) assay results of the *C. albidum* and *I. gabonensis* leaf extracts. The percentage inhibition of lipid peroxidation was significantly higher ($p < 0.05$) in the *I. gabonensis* extract ($5.95 \pm 0.15\%$) than in the *C. albidum* extract ($4.53 \pm 0.17\%$).

Table 1: Phytochemical constituents of methanol leaf extracts of *Chrysophyllum albidum* and *Irvingia gabonensis*

Phytochemical	<i>Chrysophyllum albidum</i>	<i>Irvingia gabonensis</i>
Saponins	++	+
Alkaloids	+	+
Phenols	+	++
Tannins	+	+++
Flavonoids	+	++
Steroids	+	+++
Cardiac glycosides	-	-
Terpenoids	-	-

KEY: - absent, +: trace amounts, ++: moderate amount, +++: high amounts

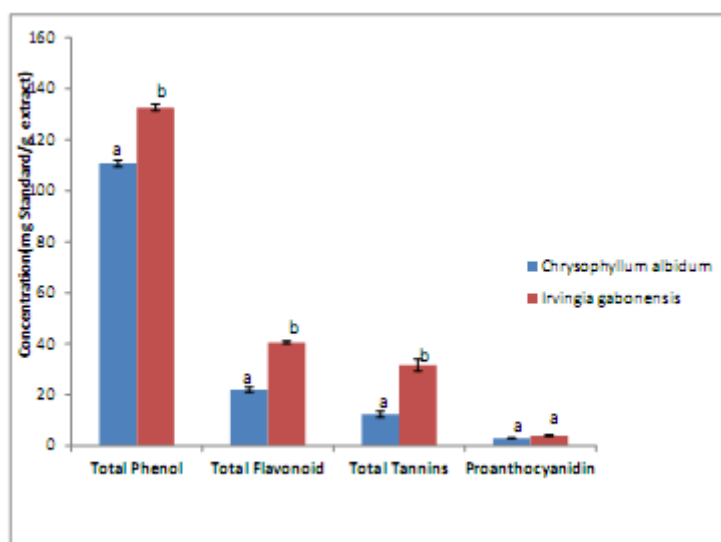


Fig-1: Total phenol, total flavonoid, total tannin and proanthocyanidin content of extracts of *C. albidum* and *I. gabonensis* leaves. Total phenol is expressed as mg Gallic Acid Equivalent / g extract, Total flavonoid is expressed as mg Quercetin Equivalent / g extract, Total tannin is expressed as mg Tannic acid Equivalent / g extract and Proanthocyanidin content is expressed as mg Ascorbic acid Equivalent / g extract. Values are expressed as mean± SEM, $n = 3/\text{group}$. Different lowercase letters for each phytochemical represent significant difference between means at $p < 0.05$.

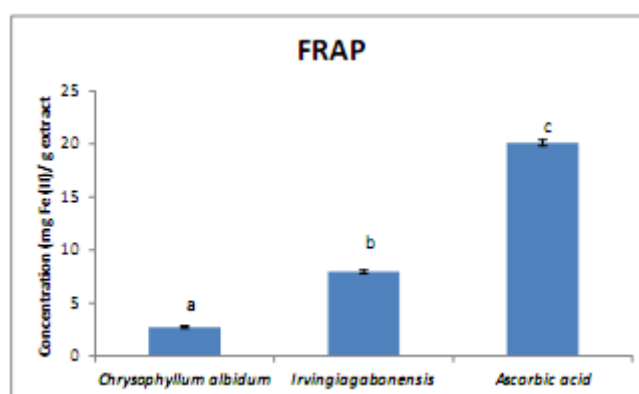


Fig-2: Ferric acid reducing antioxidant potential (FRAP) assessment of methanol leaf extracts of *C. albidum* and *I. gabonensis*. Values are expressed as mean± SEM, $n = 3/\text{group}$. Different lowercase letters represent significant difference between means at $P < 0.05$

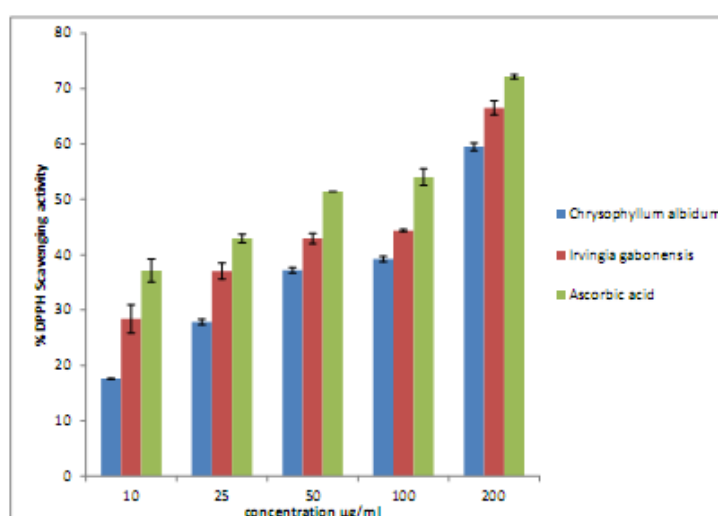


Fig-3: DPPH's radical scavenging activity of methanol leaf extracts of *C. albidum* and *I. gabonensis*. Values are expressed as mean± SEM, $n = 3/\text{group}$

Table 2: IC₅₀ of methanol leaf extracts of *C. albidum* and *I. gabonensis*

Extract	IC ₅₀ (µg/ml)
Ascorbic acid	69.24 ± 4.24 ^a
<i>C. albidum</i>	147.70 ± 1.15 ^b
<i>I. gabonensis</i>	111.70 ± 2.41 ^c

Data represent Mean ± SEM of triplicate analysis. Values with different lowercase letters within the column indicate significant difference at $p < 0.05$

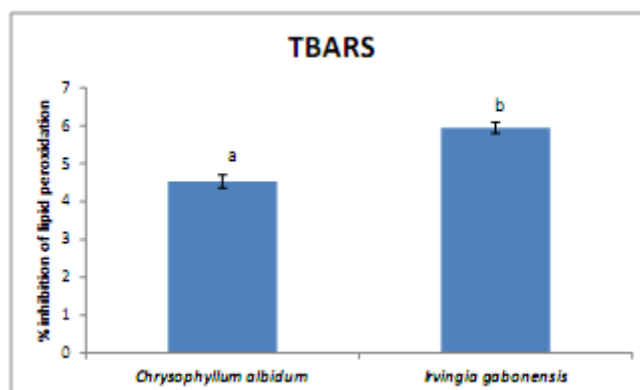


Fig-4: Result of Thiobarbutaric Acid Reactive Substances (TBARS) assay of *C. albidum* and *I. gabonensis* extracts. Values are expressed as mean ± SEM, $n = 3$ /group. Different lowercase letters represent significant difference between means at $p < 0.05$

DISCUSSION

Free radicals play a major role in bringing up a number of disorders in human, including arthritis, gastritis, ageing, respiratory diseases, e.t.c. [24]. The use of synthetic antioxidant possesses serious threats such as carcinogenicity, thus researchers have turned towards the use of herbal plants with effective antioxidant property that is capable of protecting the cells against the damaging effects of free radicals. Phytochemicals are potent antioxidants, metal chelators or free radical scavengers, thus they possess health promoting properties [25]. The qualitative phytochemical screening of methanol leaf extract of *Chrysophyllum albidum* revealed the presence of saponins, flavonoids, alkaloids, phenols, steroids and tannins. These findings corroborate with those of previous researchers [26], [27], who also detected alkaloids, tannins, saponins, flavonoids and steroids in leaves of *Chrysophyllum albidum*. Similarly, qualitative phytochemical screening of methanol leaf extract of *Irvingia gabonensis* revealed the presence of saponins, flavonoids, alkaloids, phenols, steroids and tannins. Previous researchers have reported the presence of saponins, alkaloids, tannins, flavonoids in leaf extracts of *Irvingia gabonensis* [28], [29]. In this study, phenols, flavonoids, tannins and steroids were higher in *Irvingia gabonensis* extract than *Chrysophyllum albidum* extract. Only saponins were higher *Chrysophyllum albidum* extract than *Irvingia gabonensis* extract. However terpenoids and cardiac glycosides were not detected in both extracts.

Irvingia gabonensis extract had higher total phenolic, total flavonoid, total tannin and proanthocyanidin content than *Chrysophyllum albidum*

extract. The higher quantification of these phytochemicals in *Irvingia gabonensis* extract than in the *Chrysophyllum albidum* extract is consistent with the result of the qualitative phytochemical screening.

Antioxidants can deactivate radicals by three major mechanisms, namely via Hydrogen Atom Transfer (HAT), Electron Transfer (ET), and combination of both HAT and ET [30]. HAT measures the ability of an antioxidant to quench free radicals by hydrogen donation, while ET detects the ability of antioxidant to transfer one electron to reduce radicals, metals and carbonyls. Ferric Reducing Antioxidant Power (FRAP) is an ET assay while DPPH assay combines both HAT and ET mechanisms [30]. As a rapid and simple measure of antioxidant activity, the DPPH radical scavenging capacity is based on the reduction of the stable radical DPPH to yellow colored diphenylpicrylhydrazine in the presence of a hydrogen donor [30]. The results of the DPPH radical scavenging activities of *C. albidum* and *I. gabonensis* extracts leaf extracts are shown in Figure 3 with the IC₅₀ values in Table 2. The results showed that *I. gabonensis* extract was a better inhibitor of the DPPH radical than the *C. albidum* extract.

The ferric reducing antioxidant potential test is based on the ability of antioxidants present in the test extracts to reduce Fe^{3+} to Fe^{2+} . The *I. gabonensis* extract was observed to have a significantly higher ($p < 0.05$) FRAP value than the *C. albidum* extract. Both extracts however have significantly lower ($p < 0.05$) FRAP values than the standard antioxidant (ascorbic acid).

Thiobarbituric acid reactive substances (TBARS) assay is a well established method for screening and monitoring lipid peroxidation [31]. The percentage inhibition of lipid peroxidation, estimated by TBARS assay was significantly higher ($p < 0.05$) in the *I. gabonensis* extract than in the *C. albidum* extract.

Overall results showed that *I. gabonensis* extract is a better inhibitor of lipid peroxidation and a more powerful antioxidant than *C. albidum* extract. Polyphenols are strong natural antioxidants that possess free radical scavenging activity [32]. This may explain why the *I. gabonensis* extract, which was observed to contain a higher total phenolic content, is a better inhibitor of lipid peroxidation and a better antioxidant than *C. albidum* extract.

CONCLUSION

The study revealed that methanol leaf extract of *Irvingia gabonensis* had more phytochemicals and therefore, possessed higher antioxidant activity than methanol leaf extract of *Chrysophyllum albidum*. Future research will entail the isolation and characterization of the most active ingredients in these plants as well as their potentials to serve as therapeutic agents.

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