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IN VITRO ANTIOXIDANT ACTIVITY, DIURETIC AND HYPOGLYCEMIC ACTIVITIES OF ETHANOL EXTRACT FROM LEAF OF CHRYSOPHYLLUM ALBIDUM G. IN WISTAR ABINO RATS

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ABSTRACT

Chrysophyllum albium G. is generally regarded as a plant with many ethno-medicinal uses. The study was aimed at evaluating the in vitro antioxidant, diuretic, and hypoglycemic properties of the ethanol extract of leaves of C. albium Male Wistar albino rats weighing between 150g and 200g were used for the experiment. The DPPH scavenging activity was determined using DPPH assay, inhibition of lipid peroxidation was carried out using the reactions of peroxides with thiobarbituric acid, while nitric oxide was conducted based on the Greiss reaction. Diuretic activity was carried out using the method of Lipschitz with slight modifications. Alloxan (140 mg/kg) was used to induce diabetes in the rats. The extract showed a concentration-dependent increase in DPPH radical scavenging activity, anti-lipid peroxidation activity, and nitric oxide inhibition activity maximal at 200 mg/ml (84.99±7.97, 79.82±6.10, 69.99±2.22 respectively) and this was significant (p<0.05) when compared with that of 100 mg/ml of vitamin C (97.68±0.84, 99.05±0.24 and 98.15±0.29 respectively) which served as the control. The extract also significantly (p<0.05) increased; urine volume, urinary sodium, and urinary chloride particularly at 2000 mg/kg (1.55±0.07, 109.60±0.57, and 106.00±1.41 respectively) when compared with the standard drug (1.85±0.07, 115.20±0.57, and 112.85±0.49 respectively). The increase in potassium excretion was also significant (p<0.05) particularly at 2000 mg/kg (13.70±0.28) but less than those of sodium (109.60±0.57) and chloride (106.00±1.41) at the same concentration. There was no significant (p>0.05) increase in urinary bicarbonate the maximum being at 250 mg/kg (22.55±0.78). The extract caused a non-significant (p>0.05) decrease in the fasting blood glucose level of the rats. It was concluded that the leaves of C. albium could be used as a source of natural antioxidant boosters and also a diuretic.

Keywords: Chrysophyllum albium, Diuretic, Antioxidant, Hypoglycemic, Wistar Albino rat

INTRODUCTION

Plants have been used for medicinal purposes long before recorded in history. Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [1]. The use of medicinal plants constitutes an important part of African heritage [17]. Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various purposes [26]. Although oxidation reactions are crucial for life, they can also be damaging. Oxidation reactions can produce free radicals, which start chain reactions that damage cells [11]. In recent years, antioxidants derived from natural sources mainly plants have been extensively used to treat oxidative damage because of its advantages over the synthetic ones; as they are easily obtained, economical and have negligible side effects [20]. Diuresis is the process by which urine production by the kidneys is increased usually, with loss of electrolytes [25]. Diuretics are drugs that cause diuresis. There are about five classes of diuretics which act at different segments of the renal tubular system [13]. Diuretics are particularly important in the treatment of edema in heart failure, nephrotic syndrome and other conditions leading to edema. They are also important in the treatment of hypertension [24]. Diabetes mellitus is a metabolic disorder with multiple causes characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs [8]. Cataract, microangiopathy, epitheliopathy, nephropathy, neuropathy, and retinopathy are some of the complications associated with diabetes mellitus [6]. There are type 1 (insulin dependent) and type 2 (non-insulin dependent) diabetes mellitus. The use of oral hypoglycemic agents in the management of diabetes mellitus has stimulated great research interest over the years.

Chrysophyllum albium G. is a medicinal plant from the Sapotaceae family. Its natural occurrence has been reported in Nigeria, Uganda, Niger Republic, Cameroon and Cote d’Ivoire by Adewusi and Bada [29]. In Nigeria, C. albium (white star...
apple), is found in the southwestern and southeastern parts. It is often called ‘Agbalumo’ in the southwest and ‘Udara’ in the southeast. The fruit is seasonal (December to March). It is generally regarded as a plant with many ethno-medicinal uses. It is used in traditional medicine, for the treatment of yellow fever, malaria, diarrhea, vaginal and dermatological infections among others [2]. The incidence of hypertension, diabetes mellitus, and various diseases caused by free radicals is increasing by the day. Appropriate medication management targeting glycemic control, hypertension, and lipid management is important for reducing morbidity and mortality, and improving long-term quality of life for patients diagnosed with type 2 diabetes mellitus (T2DM) [21]. Therefore, the present study was designed to investigate the antioxidant, hypoglycemic and diuretic activity of *C. albidum*.

**MATERIALS AND METHODS**

**Sample collection**

Fresh leaves of *C. albidum* plucked from the tree were collected from LohumImenyi, a village in Bende Local Government of Abia State in March 2016. The leaves were identified by Dr. GarubaOmosun of the plant science and biotechnology department of Michael Okpara University of Agriculture Umudike.

**Preparation of extract**

The method described by Adebayo et al. [3] was adopted with slight modifications. The leaves of *C. albidum* were obtained and air-dried for 14 d. They were subsequently mashed into fine powder. 100g of the ground leaves was weighed using a weighing balance and soaked in 50 ml of 95% ethanol. The solution was left for 7 h. After 72 h, it was filtered using Whatman no. 1 filter paper. The filtrate obtained was left to evaporate. A concentration of 200 mg/ml was obtained after evaporation and was stored at room temperature for further use.

**Experimental animals**

Twenty one (21) male Wistar albino rats weighing between 150g and 200g obtained from University of Nigeria Nsukka, Enugu State, Nigeria were used for the experiment. The animals were housed in the animal house of the department of biochemistry, Michael Okpara university of Agriculture Umudike, Abia State and they were maintained in 12-h light and 12-h dark at controlled temperature and humidity. The animals were allowed to acclimatize for six weeks. Feed (Vital growers mash by grand cereals and oil mills, Nigeria) and water were given ad libitum. All animals were treated in accordance with the recommendations of National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals [19].

**Evaluation of in vitro antioxidant activity**

**DPPH radical scavenging activity**

DPPH radical scavenging activity was measured by following standard method [27].

**Table 1: DPPH radical scavenging activity of ethanol leaf extract of *Chrysosyllum albidum***

| Concentration (mg/ml) | DPPH radical scavenging activity (%) | IC$_{50}$ (101.74 mg/ml) |
|-----------------------|--------------------------------------|--------------------------|
| 12.5                  | 0.00±0.00                            |                          |
| 25                    | 20.78±8.61                           |                          |
| 50                    | 34.55±5.40                           |                          |
| 100                   | 59.13±5.22                           |                          |
| 200                   | 84.99±7.97                           |                          |
| Vit C (100 mg/ml)     | 97.68±0.84                           |                          |

*Mean values±standard error of the mean of three replicates.*

**Anti-lipid, peroxidation activity**

The inhibition of lipid peroxidation was determined using deoxyribose assay [10] and the reactions of peroxides with thiobarbituric acid using egg yolk homogenate as lipid-rich media [23].

**Nitric oxide inhibition activity**

The nitric oxide scavenging activity was conducted based on the standard methodology [11].

**Dose preparation**

2g of the ethanol leaf extract of *C. albidum* was dissolved in 1 ml of distilled water and a small quantity of ethanol to yield a concentration of 2000 mg/ml, double dilutions were made to obtain various concentrations which includes: 1000 mg/ml, 500 mg/ml, 250 mg/ml, and 125 mg/ml.

**Evaluation of diuretic activity**

The method of Lipschitz et al. [33] was employed for the assessment of diuretic activity.

**Evaluation of hypoglycemic activity**

Experimental diabetes was induced by a single intraperitoneal injection of alloxan at 140 mg/kg. This was administered after overnight fast of about 12 h. After 3 d, the fasting blood glucose level of the rats was checked to confirm that they were diabetic. The diabetic rats were grouped into 7 groups with 3 rats per group. Group 1 (positive control) received the standard drug, glibenclamide (5 mg), group 2 (negative control) received normal saline, group 3 (non-diabetic control) received normal saline, groups 4, 5, 6, and 7 received 250, 500, 1000, and 2000 mg/kg of the extract respectively for 10 d and the fasting blood sugar level was checked at an interval of three days afterwards, using Accu-Chek active glucometer and strips.

**Statistical analysis**

Data obtained were analyzed by one way analysis of variance (ANOVA) and the means were analyzed by Duncan’s multiple range tests (SPSS version 22). Differences were considered significant at p<0.05. Values were expressed as mean±standard error of the mean.

**RESULTS**

**Results of the in vitro antioxidant activity**

The result shows that there was a significant (p<0.05) increase in DPPH radical scavenging activity (%) in a concentration-dependent manner. The highest activity was seen at 200 mg/ml (84.99±7.97) and is significant when compared with that of Vit C (100 mg/ml) (97.68±0.84) which was used as the control.
Table 2: Anti-lipid peroxidation activity of ethanol leaf extract of *Chrysophyllum albidum*

| Concentration (mg/ml) | Anti-lipid peroxidation activity (%) | IC$_{50}$ (118.39 mg/ml) |
|-----------------------|-------------------------------------|--------------------------|
| 12.5                  | 2.34±2.04                           |                          |
| 25                    | 18.71±4.52                          |                          |
| 50                    | 28.09±4.57                          |                          |
| 100                   | 43.26±9.08                          |                          |
| 200                   | 79.82±6.10                          |                          |
| Vit C (100 mg/ml)     | 99.05±0.24                          |                          |

Mean values±standard error of the mean of three replicates.

Table 3: Nitric oxide inhibition activity of ethanol leaf extract of *Chrysophyllum albidum*

| Concentration (mg/ml) | Nitric oxide inhibition activity (%) | IC$_{50}$ (115.41 mg/ml) |
|-----------------------|-------------------------------------|--------------------------|
| 12.5                  | 19.90±7.12                          |                          |
| 25                    | 21.51±2.19                          |                          |
| 50                    | 37.69±0.45                          |                          |
| 100                   | 50.04±2.23                          |                          |
| 200                   | 69.99±2.22                          |                          |
| Vit C (100 mg/ml)     | 98.15±0.29                          |                          |

Mean values±standard error of the mean of three replicates.

The result shows that there was a significant (p<0.05) increase in anti-lipid peroxidation activity (%) in a concentration-dependent manner. The highest activity was seen at 200 mg/ml (79.82±6.10) and is significant when compared with that of Vit C (100 mg/ml) (99.05±0.24).

The result shows that there was a significant (p<0.05) increase in nitric oxide inhibition activity (%) in a concentration-dependent manner. The highest activity was seen at 200 mg/ml (69.99±2.22) and is significant when compared with that of Vit C (100 mg/ml) (98.15±0.29).

The result shows a dose-dependent increase in urinary sodium. The increase in urinary sodium at 2000 mg/kg (109.60±0.57) of the extract is significant (p<0.05) when compared with that of the standard drug which is furosemide (40 mg).

Like urinary sodium, the result shows a dose-dependent increase in urinary chloride. Urinary chloride at 2000 mg/kg (106.00±1.41) of the extract is significant (p<0.05) when compared with that of the standard drug which is furosemide (40 mg).

The result shows that urinary potassium levels were low when compared to those of sodium and chloride ions. However, the urinary potassium levels were shown to increase with increasing dose of the extract.

Fig. 1: the effect of *Chrysophyllum albidum* on urinary sodium levels over a period of 24 h
## Results of the diuretic activity

### Table 4: Effect of ethanol leaf extract of *Chrysophyllum albidum* on urine volume and concentration of electrolytes

| Groups                  | Total urine volume (ml/24 h) | Na⁺ (mEq/24 h) | K⁺ (mEq/24 h) | Cl⁻ (mEq/24 h) | HCO₃⁻ (mEq/24 h) |
|-------------------------|------------------------------|----------------|---------------|----------------|------------------|
| Control                 | 0.15±0.07                    | 42.90±1.13     | 5.00±0.28     | 41.95±1.34     | 18.20±1.27       |
| Std drug                | 1.85±0.07                    | 115.20±0.57    | 15.15±0.35    | 112.85±0.49    | 21.15±0.35       |
| 125 mg/kg Extract       | 0.35±0.07                    | 57.15±1.48     | 6.45±0.35     | 55.80±0.57     | 20.20±1.13       |
| 250 mg/kg Extract       | 0.70±0.14                    | 75.50±0.99     | 8.30±0.14     | 71.30±0.99     | 22.55±0.78       |
| 500 mg/kg Extract       | 1.15±0.07                    | 87.95±0.07     | 9.80±0.28     | 81.45±1.34     | 22.35±0.64       |
| 1000 mg/kg Extract      | 1.35±0.07                    | 97.40±1.27     | 11.60±0.28    | 95.30±0.99     | 20.95±0.07       |
| 2000 mg/kg Extract      | 1.55±0.07                    | 109.60±0.57    | 13.70±0.28    | 106.00±1.41    | 19.85±0.64       |

Values shown are mean±standard error of the mean (n=2) and the values are compared with the control group and standard drug and considered significant at p<0.05.

![Fig. 2: The effect of *Chrysophyllum albidum* on urinary chloride levels over a period of 24 h](image1)

![Fig. 3: The effect of *Chrysophyllum albidum* on urinary potassium levels over a period of 24 h](image2)
The result shows that urinary bicarbonate levels were low when compared to those of sodium and chloride ions. There was no significant (p<0.05) difference between the control (18.20±1.27), the standard drug (21.15±0.35), and the test groups 20.20±1.13, 22.55±0.7, 22.35±0.64, 20.95±0.07, 9.85±0.64.

The result shows that the extract caused a significant (p<0.05) increase in the volume of urine produced over a period of 24 h in a concentration-dependent manner. The maximum effect was seen at 2000 mg/kg (1.55±0.07) and is comparable to that of the standard drug (1.85±0.07).

Results of the hypoglycemic activity

| Groups                  | Day 0     | Day 3      | Day 6       | Day 10     |
|-------------------------|-----------|------------|-------------|------------|
| Positive control (Glibenclamide) | 152.07±11.15 | 90.33±3.51 | 86.00±5.57 | 70.67±2.08 |
| Negative control (normal saline) | 145.00±6.25 | 143.00±16.09 | 120.00±13.23 | 108.33±8.50 |
| Non-diabetic control    | 70.00±4.00 | 78.33±12.58 | 67.33±4.62 | 78.00±7.00 |
| 250 mg/kg Extract       | 126.00±7.94 | 133.00±3.60 | 128.67±1.53 | 125.67±0.58 |
| 500 mg/kg Extract       | 147.00±20.66 | 116.33±14.57 | 115.00±12.00 | 107.33±24.00 |
| 1000 mg/kg Extract      | 184.33±53.15 | 135.67±24.54 | 128.33±26.54 | 126.67±29.01 |
| 2000 mg/kg Extract      | 169.33±35.56 | 131.00±10.82 | 122.33±11.24 | 109.00±7.81 |

Values shown are mean±standard error of the mean (n=3). The mean difference is significant at p<0.05.
DISCUSSION

Free radicals are implicated in a number of diseases including cancer, cardiovascular diseases, aging, etc [27]. Antioxidants can stop the reactive oxygen radical formation and also in detoxification [32]. The phytochemical screening of *C. albidum* leaves, have shown the presence of flavonoids, steroids, terpenes, cardiac glycosides and saponins [30]. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals and inhibitors of lipid peroxidation. The mechanisms of action of flavonoids are through chelating or scavenging [15].

According to Adebayo et al. [2], a glycoside flavonoid, myricetin-3-rhamnoside was isolated from *C. albidum* leaves and was found to rapidly scavenge DPPH radicals. Its effect was comparable to that of Vitamin C. The result showed that the extract was capable of neutralizing the DPPH free radicals via hydrogen donating activity by 20.78, 34.55, 59.13, and 84.99% at concentrations of 25, 50, 100, and 200 mg/ml respectively. DPPH scavenging activity showed an enhancement and directly proportional to the concentration when compared with ascorbic acid (Vit C) which was used as the positive control.

Recently, free radicals-induced lipid peroxidation was found to be involved in many pathological conditions [4]. The results of this investigation showed that the extract inhibited lipid peroxidation in a concentration-dependent manner.

The ethanol leaf extract of *C. albidum* significantly reduced (or inhibited) the generation of NO, by 19.90, 21.51, 37.69, 50.04, and 69.99% at concentrations of 12.5, 25, 50, 100, and 200 mg/ml respectively. NO plays multiple roles in a variety of biological processes. It serves as an effector molecule, neuronal messenger, vasodilator, antimicrobial agent, etc [7]. It has been reported that NO reacts with oxygen radicals to form peroxynitrite radicals (ONOO⁻) that are toxic to biomolecules such as lipids, proteins and nucleic acids [7]. *C. albidum*, was found to inhibit the generation of NO⁻ and HO⁻ radicals and is directly proportional to the concentration.

Diuretic use in clinical medicine spans conditions like edema, hypertension, metabolic acidosis and hyperkalemia [12]. Diuretics have been used effectively to treat millions of hypertensive patients during the past four decades. In majority of hypertensive patients, they reduce both systolic and diastolic blood pressure [24]. In heart failure, diuretics are primarily used to reduce pulmonary and/or systemic congestion, edema, and associated symptoms [16]. Studies have shown that there are numerous compounds which could be accountable for a plant’s diuretic effects. These include flavonoids, saponins, and organic acids. The diuretic activity of flavonoids may be due to their binding with adenosine receptors. Some studies have also shown that the diuretic activity might be a consequence of alkaloids [5]. Therefore, the precise site, molecule and cellular mechanisms responsible for diuretic activity remain to be elucidated.

The result of the present study showed that the ethanol leaf extract of *C. albidum* caused a dose-dependent increase in urine volume, urinary sodium, and urinary chloride, excreted over a period of 24 h. The results also showed that urinary potassium increased in a dose-dependent manner. However, the values were low when compared with those of Na⁺ and Cl⁻. It is likely that the mechanism of action of the extract may be the same as that of potassium-sparing diuretics. Therefore, the diuretic activity of *C. albidum* may be attributed to the inhibition of aldosterone action or inhibition of epithelial sodium channels. Further studies are required to verify this. The result also showed that like potassium, the urinary excretion of bicarbonate was not so significant and that there was no significant difference between the control group, the group treated with the standard drug and the test groups. Again, this may point to the fact that the mechanism of action of the extract may be the same as that of the potassium-sparing diuretics which decrease K⁺ and H+ secretion in the distal convoluted tubule.
Diabetes mellitus is probably the fastest growing metabolic disorder. Currently, its management is a global problem [14]. Medicinal plants have been used for so many years in the treatment of diabetes however, only a few have been scientifically evaluated. Alloxan is known for its selective cytotoxicity to the β-cells of the pancreatic islet and has been extensively used to induce diabetes in experimental animals [8]. The cytotoxic action of alloxan is mediated by reactive oxygen species (ROS). The source of generation of ROS is diuretic acid, a reduction product of alloxan [31]. Increased thirst, very significant weight loss and loss of appetite were some of the symptoms observed in the animals following alloxan administration. The first two are symptoms associated with diabetes mellitus (Stang and Story, 2005). Plants with anti-diabetic (hypoglycemic) properties have been said to stimulate the β-cells of the pancreas by activating the regeneration of the pancreatic cells [31]. The result of the study shows that there was only a slight decrease in the blood glucose level of the groups treated with different concentrations of the extract. The highest effect was seen in the group that received 2000 mg/kg of the extract. Free radicals (ROS) are implicated in the pathogenesis of diabetes mellitus therefore antioxidants (which scavenge these free radicals) may be useful in the management of the disorder. The present study has shown that C. albium has antioxidant properties possibly because of its flavonoid content. The antioxidant property of the flavonoids may have played a role in the slight reduction in blood glucose level that was observed. It is suggested that a longer duration of treatment and also a higher concentration of the extract (>2000 mg/kg) may significantly reduce blood glucose level.

CONCLUSION
The result of the study showed that the ethanol leaf extract of C. albium has a dose-dependent antioxidant and diuretic activities. The result also showed that the extract exhibited a non-significant hypoglycemic activity.

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