Antioxidant capacity and phytochemical content of *Cyphostemma glaucophilla* Aqueous Leaf Extract

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ABSTRACT

Cyphostemma glaucophilla is used in the treatment of several degenerative diseases. Phytochemical analyses was carried out on aqueous leaves extract and the anti oxidant activity were investigated using albino rats, which were divided into five groups of five animals each. Group A received (0.85% NaCl; 5ml/kg) control while single daily oral doses of 10, 15, 20, 25mg/kg body weight of extract were administered to groups B, C, D and E for 21 days respectively. Animals were fasted overnight and sacrificed with ether anaesthesia and the liver homogenates were used for the assessment of protein, malondialdehyde and assay of glutathione peroxidase (GPx), superoxide dismutase (SOD), anti lipid peroxidation and 2, 2- diphenyl-1-piccrylhydrazylhydrate (DPPH) radical scavenging activities by standard methods. Results confirmed the presence of flavonoids, vitamin C, proteins, carbohydrates, steroids, O and C glycosides, traces of vitamin E and A. The extract induced significant ($p<0.05$) dose dependent increase in the concentration of proteins and inhibited significant ($p<0.05$) dose related decrease in the concentration of malondialdehyde. It produced significant ($p<0.05$) dose dependent increase in the concentration of glutathione peroxidase and SOD peaks at 25mg/kg (55 and 35%) relative to control, there was also a significant ($p<0.05$) inhibition of lipid peroxidation by 18.80% in group B and 25.42% in E, the DPPH radical scavenging activity increased with increased concentration of extract by 14.31% and 37.23% in groups B and E respectively. Study has shown that extract contains phytochemicals of biological and pharmacological importance and has antioxidant capacity which can be utilized to alleviate the symptoms of chronic and degenerative diseases.

Key words: Anti-oxidant, phytochemicals, *Cyphostemma glaucophilla*, degenerative disease.

1.0 Introduction

Cyphostemma glaucophilla is a flowering plant which belongs to the family of vitaceae. These species are cruciform and used to belong to the genus cissus. It is used in herbal medicine in Kogi and Kwara states of Nigeria for the treatment and management of diverse ailments ranging from malnutrition disorder, infertility and systemic disease in different locations. The Igalas, macerate the leaves in water and the aqueous extract is administered daily to malnourished children and hypertensive patients, Ebiras used the stem prepare in form of decoction as internal cleanser for new born babies while the Yorubas use the leaves for the treatment of infertility in women and stomach discomfort in children .

Omale et al., (2006) had attributed its medicinal properties to the presence of bioactive compound in its leaves, root and stem. Result of acute toxicity recorded by Ojogbane et al., (2010) indicated that extract has no adverse effect at the limit per oral dose of 5000mg/kg body weight administration to rats. The efficacy of Cyphostemma glaucophilla aqueous extracts in boosting protein synthesis and minerals has been investigated *(*Ojogbane and Nwodo 2010*;* Omale et al*.,* 2009) also, the potential of extract as an anti inflammatory agent has been examined and confirmed *(*Ojogbane et al*.,* 2011).

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction. The potential of the antioxidant constituent of plant material in the maintenance of health and protection from disease is also of raising interest among scientists and food manufacturers as consumer move toward functional foods with specific health effects (Al-Duais et al*.,* 2009). The antioxidants compounds present in edible plant have recently been promoted as food additives because they display little or not oxic effects (Han et al*.,* 2004). In this communication, the photochemical screening and antioxidants activity of the aqueous extract of the leaves of *C. glaucophilla* was investigated.

2.0 Materials and method

2.1 Plants Material

The leaves of *C. glaucophilla* was collected from Egah in Idah local government of Kogi State Nigeria. They were washed to remove dirt, dried in the laboratory at room temperature and pulverized with a milling machine into a coarse powder.

2.2 Preparation of Ethanol and Chloroform Extraction

A 400g quantity of pulverized dried leaves of *C. glaucophilla* was macerated twice in five volumes (w/v) of chloroform–ethanol mixture (2:1) for 18 hours with 2 changes of solvent the Whatman number 4 filtrate of the macerate was shaken with 0.2 volume water in a separating funnels. The two emerging layers: viz upper ethanol layer and lower chloroform layer were separated and dried in *vacuo*.

2.3 Preparation of Aqueous Extract

An 80g quantity of pulverized leaves was macerated in distilled water (1:5 w/v) for 18 hours and then filter the filtrate was evaporated in a water baths to get the dried residue.

2.4 Animals

Experimental animals used in this study were albino rats of either sex aged between seven and nine weeks weighing 100-150g. They were purchased from the Animal House of Faculty of Biological Sciences University of Nigeria Nsukka.

2.5 Phytochemical Screening

Aqueous and chloroform extract were screened for the presence of bioactive components following the method of WHO (1998)

2.6 Experimental Design

Five groups of five rats each weighing (110-120g) were fed with rat feed obtained from Top feeds and allowed drinkable water throughout the period of experiment. Group A received daily oral doses of saline (0.85% NaCl 5ml/kg) while groups B, C, D and E were administered 10, 15, 20, 25mg/kg body weight of extract using intubator for 21 days respectively.

24 hours after the last administration, animal were sacrificed. The livers were excised and homogenized and the liver homogenate were used for the assessment of total proteins by the method of Lowry et al., (1951), malondialdehyde concentrations by the method of Wallen et al. *(*1993), activities of glutathione peroxidase (GPx) the method of Plagia and Valentine (1967), superoxide dismutase (SOD) activity by Wolliams et al. (1983) and lipid peroxidation by the modified method of Nabasree and Bratati (2002).

2.7 Anti-Lipid Peroxidation Assay

The anti–lipid peroxidation assay was determined using a modified thiobarbituric acid reactive species (TBARS) assay of (Nabasree and Bratati, 2002). Briefly, to 0.5ml of a 0.1g/ml liver homogenate was added 0.1ml of varying concentration of the extract (5.0, 2.5, 1.25, 0.625 and 0.3125mg/ml) in a test tube followed by the addition of 1.0ml of distilled water. Then 50µl of 0.07M iron II tetraoxosulpahte (v) was added to the reaction mixture, vortexed and allowed to stand at room temperature for 30 minutes. 1.5ml of 20% (w/v) acetic acid and 1.5ml of 0.8% (w/v) thiobarbituric acid in 1.1% (w/v) sodium dodecyl sulphate were added. The resulting mixture was then incubated in a water bath at 37^0C for 1 hour. After cooling, 4.0ml of butan-1-ol was added to each tube, shaken vigorously and centrifuged at 3000rpm for 10 minutes. The absorbance of the organic upper layer was measured at 532nm.

2.8 Determination of 2, 2 Diphenyl-1-Piccrlhydrazyl Hydrate (DPPH) Radical Scavenging Activity The radical scavenging property of the extract was determined by the modified method of Brace (2001). To 1.0ml of different concentrations (0.5, 0.25, 0.125, 0.0625, 0.03125mg/ml) of the extract was added 1.0ml of 0.3mm DPPH in methanol. The mixture was vortexed and incubated in a dark chamber for 30minute. The absorbance was measured at 517nm against DPPH control containing only 1.0ml of methanol in place of extract.

2.9 Data Analysis

The result were expressed as mean±SEM and analysis of variance (ANOVA) was used to test for the difference among the group at $p<0.05$ regarded as significant

3.0 Results

3.1 Phytochemical screening

The bioactive compound are concentrated more in the aqueous extract than in the chloroform extract. The chloroform extract has only the fractions that are soluble in non-polar solution.

Table 1: Phytochemical screening

Compounds	***Aqueous extract***	***Chloroform extract***
Alkaloid	-	-
Anthraquinones	-	-
Carbohydrates	++	-
Flavonoids	+	-
Cardiac glycoside	++	-
Proteins	++	-
Lipids	+	++
Tannins	-	-
Saponins	++	-
Steroids	+	+
Vitamin E	-	++
Vitamin C	++	++
Vitamin A	-	+

- *: Absence of bioactive compound*
++ *: Presence of bioactive compound in high concentration*
+ *: Presence of bioactive compound in low concentration*

3.2 Extract effects on % inhibition of lipid peroxidation, MDA and liver proteins (mg/dL).

Table 2: shows that the extract produced a significant (P<0.05) dose dependent increase in the concentration of total proteins in rat plasma. It caused the total protein concentration to increase from a control value of 4.08 ± 0.02 by 1.94mg/dl in group B of rats which received the lowest dose of the extract and by 3.72mg/dL in group E that received four times group B dose . This treatment also affected the concentration of album in in a scale manner. It induced an increase of 0.89mg/dL from a control value of 3.02 ± 0.04 in group B and 1.84mg/dL in group E.

The extract produced a significant (P<0.05) dose depended decrease in the concentration of MDA. There was a change of 0.25mg/dl from the control value of 0.38 ± 0.10mg/dl in group B and 0.34mg/ml in group E (table 2). The doses of extract at 5, 10 , 15 and 20mg/kg bw caused inhibition of lipid peroxidation by 41.49±1.31, 43.03±0.18, 48.82±0.51 and 51.50±1.00 respectively.

Table 2: Extract effects on % inhibition of lipid peroxidation, MDA and liver proteins (mg/dL).

Group	**Treatment (mg/kg)**	**Total Protein**	**Albumin**	**MDA**	**(%) Iinhibition**
A	Normal saline 5ml/kg	4.08 ± 0.02^{a}	3.02 ± 0.04^{a}	0.38 ± 0.10^{c}	38.99 ± 2.31^{a}
B	05.0 mg/kg	6.02 ± 0.03^{b}	3.89 ± 0.02^{b}	0.13 ± 0.01^{b}	41.49 ± 1.31^{b}
C	10.0 mg/kg	6.59 ± 0.01^{c}	4.43 ± 0.02^{c}	0.10 ± 0.01^{b}	43.03 ± 0.18^{b}
D	15.0 mg/kg	7.17 ± 0.02^{c}	4.71 ± 0.03^{c}	0.08 ± 0.01^{a}	48.82 ± 0.51^{c}
E	20.0 mg/kg	7.80 ± 0.01^{d}	4.86 ± 0.02^{d}	0.04 ± 0.01^{a}	51.50 ± 1.00^{d}

Values with different superscripts in a column are statistically significant (P<0.05)

3.3 Effect of the extract treatment on SOD, GPx and DPPH radical scavenging activity.

Table 3 shows that extract treatment induced dose dependent significant (p<0.05) increase in the antioxidant activity of DPPH radical from a value of 14.31% obtain for the control by 11.13% at the extract concentration of 0.5mg/ml, increasing doses of the extract at 0.75, 1.0 and 1.25mg/ml induced increases in antioxidant activity by 16.20, 19.56 and 23.92% respectively. There was a s ignificant (p<0.05) dose dependent increase in the activities of SOD and GPx. Extract at the lowest dose of 10mg/kg effected a change of 1.11 ± 0.06 µ/ml in SOD and 0.60±0.10µ/ml in GPx. Scalar doses in group B and C cause a higher change in SOD and GPx. The effect of extract was at peak in group E with a change of 2.15±0.07u/ml in SOD and 6.74±0.03u/ml in GPx. However the effect of extract on GPx was more compared to SOD (table 3).

Table 3: Effect of the extract treatment on SOD, GPx and DPPH radical scavenging activity.

Concentration of the extract (mg/ml)	Antioxidant activity (%)	Change in antioxidant activity (%)	SOD (U/ml)	GPx (U/ml)
Normal saline (5ml/kg)	14.31		1.01±0.06[a]	11.80±0.41[a]
0.5	25.44	11.13	1.11±0.06[a]	12.40±0.51[b]
0.75	30.51	16.20	1.53±_0.04[b]	14.78±0.55[c]
1.0	33.87	19.56	1.95±0.06[bc]	15.35± 0.50[cd]
1.25	38.23	23.92	2.15±0.07[c]	18.54 ±0.44[d]

Values are mean of triplicate determinations

4.0 Discussion

In Table 1 the presence of proteins, vitamins, lipids and carbohydrates support its uses in providing energy: building up of worn out tissues and regulation of internal body temperature. flavonoids, vitamin C,E,A are antioxidant which are useful in free radical scavenging in living system. Vitamin C is also necessary for connective tissues and promote the healing of fracture and wounds These justified the use of extract in malnutrition and healing of bone fracture. However the aqueous extract will be more effective than chloroform extract. This result is in agreement with the report of (Omale *et al.,* 2006).

In table 2, the extract produced a significant ($p<0.05$) increase in the concentration of liver total proteins and a graded increase in albumin concentration. This result further confirms the result of earlier studies by Ojogbane and Nwodo (2010). This extract induced concentration of plasma proteins can alleviate the protein deficiency in kwashiorkor(r, also the hallmark of kwashiorkor, oedema is caused by albumin deficiency (Cohen and Lehman, 2002) because albumin is used for the maintenance of colloid osmotic pressure at the capillary membrane to prevent plasma fluid from leaving into the interstitial cell. A decrease in albumin (less than 5g/dl) results in the lowering of plasma colloid osmotic pressure in a way that it can no longer counteract the effect of the hydrostatic pressure of blood. This results in an increased outward movement of fluid from the capillary wall and decreased inward movement of fluid from the interstitial space causing oedema. The impaired immune response and high risk of infections which are consequent on reduced synthesis of protein can be tampered with an increase in plasma protein concentrations (Heird, 2008).

The extract induced a significant ($p<0.05$) dose dependent inhibition of malondialdehyde in table 2. This observation is an indication of the inhibition of lipid peroxidation by the extract. This finding is a reflection on enhancement of antioxidant enzymes caused by the extract and is in accordance with earlier studies (Al-duais et al.,2009)

The increase in the activity of SOD and GPx in table 3 is of interest. SOD is an enzyme that repairs cells and reduce the damage done to them by superoxide, by catalyzing the dismutation of the superoxide radical (O_2) into hydrogen peroxide (H_2O_2) and elemental (O_2). Studies carried out by Muth (2004) have shown that SOD act as both antioxidant and anti inflammatory agent in the body neutralizing the free radicals that can lead to wrinkles and pre cancerous cell changes. It also helps the body to use Zinc, copper and manganese and a likely key to the production of healthy fibroblast; one of the deficiencies in protein energy malnutrition. The extract induce increase in SOD indicates the antioxidant and anti inflammatory potentials of *C. glaucophilla* leaves.

Glutathione peroxidase (GPx) is the most abundant antioxidant enzyme that protects vision and boosts the immune system (Farmer, 2009). It also help in the detoxification of harmful compounds via the bile (Marnett, 1999). GPx keeps the red and white blood cell healthy to maximize the efficiency of the immune system. It has been shown by (Farmer, 2009) that low level of glutathione peroxidase makes the body more vulnerable to damage by free radicals. From the result in table 3, it is obvious that extract was able to enhance the activity of GPx which could explain the potency in treating various ailments which was also reported by (Hans *et al.,* 2004).

There was a dose dependent percentage inhibition of peroxidation in table 2. Peroxidation of lipids as recorded by Languerre (2000), can disturb the assembly of the membrane, causing alteration of ion transport and inhibition of some metabolic processes. The observation in this study reveals that *C. glaucophilla* leaf extract could be used to alleviate this challenges (Spector et al*.,* 2005) had also observed that inhibition of peroxidation is enhanced by phenolic anti oxidants, *C. glaucophilla* could possess some natural antioxidant property which makes it exhibit this effect. The DPPH scavenging activity in table 3 increased with increase in the concentration of extract. DPPH is a phenolic compound which inhibits lipooxygenase and scavenges free radicals (Hu et al., 2005). DPPH scavenging activity as describe by Aruoma (2008) plays a role to inhibit reactive oxygen species that are responsible for various diseases like neurodegenerative disorder, heart disease

and inflammation hence *C. glaucophilla* leave extract could be used to tamper these ailments. This result further confirms the studies of [4] which justify its use in herbal medicine.

The results of these studies provide scientific evidence for the use of *C. glaucophilla* aqueous leaf extract in traditional medicine. In conclusion, extract contain phytochemicals of biological and pharmacological importance and exhibits antioxidant activity which can be utilized to alleviate the symptom of chronic and degenerative disease. Consequently, the leaf extract of *C. glaucophilla* contains some bioactive agent that might account for this activity.

Acknowledgement

We appreciate the technical assistance of Mr. F. Emmanuel, Mr . S. Olupinyo and Mr. G. Adah of the Department of Biochemistry Kogi State University, Anyigba, Nigeria.

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