ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF THE FLAVONOID FRACTION FROM THE LEAVES OF DICHROSTACHYS GLOMERATA (FORSSKAL)

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Abstract

Oxidative stress has been implicated in a number of diseases such as atherosclerosis, chronic inflammatory disease, rheumatoid arthritis, cancer and aging. Flavonoids are phytochemicals characterised by a wide range of biological activities such as antioxidant activity, the ability to modulate enzyme or cell receptor activity patterns, and interference with essential biochemical pathways. Flavonoids, have also been suggested to limit oxidative damage in humans, thereby lowering the risk of certain chronic diseases. The present work showed antioxidant and anti-inflammatory activities of flavonoid fraction of *D. glomerata* leaves using standard protocols. The flavonoid fraction (FF) of *D. glomerata* leaves at moderate concentrations (50, 100, 200, 400 µg/ml) significantly decreased dipheny-picryl-hydraxyl (DPPH) – absorbance in a concentration dependent manner comparable to vitamin C – a potent antioxidant agent. In the carrageenan-induced paw oedema test in rats, the FF (50, 100, 200 and 400 mg/kg *p.o*) caused significant ($P < 0.05$) decrease in paw oedema 2 – 4 hours after Carrageenan administration, comparable to indomethacin a potent anti-inflaming agent. The FF (100, 200, 400 mg/kg *i.p*), also caused significant ($P < 0.05$) inhibition of the acetic acid-induced writhing in mice in a dose dependent manner comparable to the effect of Acetyl salicylic acid (ASA), a potent analgesic drug. These findings suggest that the leaves of *Dichrostachys glomerata* have potent anti-inflammatory, analgesic and antiradical effects.

Keywords: *Dichrostachys glomerata*, antioxidant and anti-inflammatory properties

Introduction

Herbal medicinal products are assuming roles in the lives of the people across the world in the face of global upsurge of resistance, toxicity, adverse effects and escalating cost of synthetic medicinal products (Okpara *et al*., 2007; Das *et al*., 2011; Imafidon *et al*., 2012). The search for antioxidant based drugs/formulations for the prevention and treatment of complex diseases like stroke, diabetes; cancer and chronic diarrhea have appeared during the last three decades (Aquil *et al*., 2006). Subsequently a worldwide trend towards the use of natural phytochemicals present in plants has increased (Das *et al*., 2011). *Dichrostachys glomerata* (Forsskal) commonly called sickle bush (*Dundu* (Hausa), *Burli* (Fulani), *Kora* (Yoruba) and *amiogwu* (Igbo) is a gregarious, very invasive shrub. All Nigerian *Mimosaecae* have bipinate alternate leaves with one to twenty-five pairs of pinnae. The stipules of some genera are modified into spines. The bark and leaves are used to treat dysentery and diarrhea in many parts of Nigeria (Daziel, 1963). This work was aimed at evaluating the possible antioxidant and anti-inflammatory properties of flavonoid fraction from the leaves of *Dichrostachys glomerata* using standard protocols.

MATERIALS AND METHODS

Animals: Wistar rats (*Rattus norvegicus*) weighing between 160 – 210g and Swiss albino mice (*Mus musculus* 25 – 30g) of male sex used for this studies were bred and housed in the Teaching Laboratory of the Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University (ABU), Zaria. The animals were kept in a photo period controlled environment (12 hours light – dark cycle). All the rats were kept in cages with solid floors covered with wood shavings, and they were given growers mash (Vital Feed Plc, Bukuru, Nigeria) and water *ad libitum*.

Collection and Preparation of the Plant Materials: Leaves of *Dichrostachys glomerata* tree situated at the premises of the National Veterinary Research Institute, Vom, Nigeria were used throughout the study. The plant material was collected in the month of October and was authenticated by Mallan Shehu Gallah (a taxonomist) in the Department of Biological Sciences, Ahmadu Bello University, Zaria. Voucher specimen OJO2011 were preserved for reference purpose in Biological Sciences Herbarium of the ABU, Zaria. The fresh leaves 2.4 kg were air-dried for 4 weeks in the Teaching Laboratory of the Department of Pharmacology and Toxicology, ABU, Zaria. The dried leaves 950 g were grounded to powder using pestle and mortar, sieved, weighed and carefully packed in clean polythene bag and stored under room temperature until use.

Preparation of Crude Methanolic Extract from the Powdered Leaves of *D. glomerata*

The extraction procedure was as described by Harborne (1998). Four hundred gram (400 g) of the powdered leaves material of *D. glomerata* was packed into a thimble and transferred into Soxhlet Extractor. The extraction was carried out in 95%, methanol at 60^{o}C until there was no more colour change in the

methanol, indicating that the extraction was complete. This took about 72 hours, the methanolic extract harvested was then concentrated *in vacuo* using rotary evaporator, coupled to a thermoregulator. The extract was subsequently placed in the oven at 80^{o}C to remove the residual methanol until a constant weight was obtained. The extract was then collected in a plastic container, closed tightly (air and water tight) and stored in a refrigerator at 4^{o}C until required.

Isolation of the Flavonoid Fraction: 1 g of the extract was subjected to column Chromatography (column 40 cm long) on silica gel (70 – 230 mash) using hexane and EtOAc (1:1) 10 ml fractions were collected and the elution of the flavonoids monitored by TLC using EtOAc (1:1) as the developing solvent. The plates were spread with $AICI_3$ in 95% EtOH (Olaleye *et al*., 2004). The flavonoids were revealed by UV light at 254 and 365 nm).

Free Radical Scavenging Activity: This was determined using the Diphenyl-picryl-hydraxyl (DPPH) method (Olaleye et al., 2004). Briefly, 0.1mM ethanol DPPH solution was added to different concentrations of the flavonoid fraction (FF50, 100, 200, 400 µg/ml) and 25 µg/ml of Vitamin C (VC), respectively, with gentle shaking. Triplicate measurement of the optical density change was obtained 10 minutes later with Spectrophotometer – Spectronic – 20 (Model UV 160, Kyoto, Japan), at the wavelength of 517 nm. The free radical scavenging activity was measured as the decrease in absorbance of samples versus DPPH standard solution. The results were expressed as the percentage activity (Oke and Hamburger, 2002; Olaleye *et al*., 2004).

Evaluation of the Anti-inflammatory Activity of the of the Flavonoid Fraction of *D. glomerata* Leaves

Carrageenan-induced Paw Oedema in Rats: Acute inflammation in albino rats (10 – 12 weeks old) weighing 160 – 210 g was produced according to the method described by Olaleye *et al*. (2004). An injection was made of 0.1 ml 1% carrageenan on the sub-planter surface of the right hind-paw of the rats which had been fasted for 12 hours. Oedema was assessed for 3 hours at 30 minutes interval after administration of the flavonoid fraction in terms of an increase in circumference of the carrageenan injected paw compared to the non-injected one. Animals were given agents under test-FF *per os* at doses of 50, 100, 200, 400 mg/kg one hour before carrageenan injection. At the same time, the control received 5 ml/kg of distilled water and the reference group received 10 mg/kg of Indomethacin *per os*. Measurement of paw size was carried out by wrapping a piece of cotton thread around the paw and measuring the circumference with a meter rule. The inhibition activity was calculated according to the following formula.

Percentage Inhibition = (Ct – Co) control – (Ct – Co) treated x 100 (Ct – Co) control

Where Ct = Linear circumference of paw after carrageenan injection Co = Linear circumference of paw before carrageenan injection

The inhibitory values at 3 hours, representing peak Oedema were adopted as a measure of effect.

Acetic Acid-Induced Writhing Test

This method was based on that described by Santos *et al*. (1994). Male albino mice weighing 25 – 30 g were divided into groups. The various doses of FF (100, 200, 400 mg/kg) and acetyl salicylic acid (ASA) (50 mg/kg) were administered through the intraperitoneal route 45 minutes before intraperitoneal injection of acetic acid (0.6%) solution in distilled water at a dose of 10 ml/kg. Control animals received 5 ml/kg of 0.9% NaCl solution. Immediately after administering the acetic acid, the number of writhings and stretchings (a syndrome characterized by a wave of contraction of the abdominal muscle together with a stretching of the hind – limbs) occurring between 5 – 20 minutes were counted. A reduction in writhing number as compared to passive control was considered as evidence for the presence of analgesia, which was expressed as percentage inhibition of writhing. The acetylsalicylic acid treated group served as a positive control. Data were calculated according to the formula:

% inhibition = mean number of writhings (control) - mean number of writhings (treated) X100 Mean number of writhings (control)

Statistical Analysis

Values were expressed as means ± SEM and subjected to one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Value of $P > 0.0\text{-}5$ was considered significant

Results

Table 1.0: Free Radical Scavenging Activity of the FF of *D. glomerata* Leaves Using the DPPH Method (n = 3, Mean ± SEM)

Concentration of FF and Vitamin C	Percentage (%) Decrease in DPPH Absorption
FF 400 µg/ml	78.20 ± 0.65^a
FF 200 µg/ml	75.10 ± 1.05^a
FF 100 µg/ml	73.05 ± 0.11^a
FF 50 µg/ml	60.30 ± 0.45^b
Vitamin 25 µg/ml	80.05 ± 0.20^a

Values with same superscripts are not statistically different ($P > 0.05$).

Radical Scavenging Effects

The flavonoid fraction of *D. glomerata* leaves at the highest concentration 400 µg/ml resulted in (78.20%) decrease in DPPH absorbance. It also produced a (60.39%) decrease in DPPH absorbance at the lowest concentration (Table 1.0). FF 400 µg/ml produced a significant ($P < 0.05$) percentage decrease in DPPH absorbance compared to the value obtained for FF 50 µg/ml. The percentage decrease in DPPH absorbance recorded for 100 µg/ml was non-significantly ($P > 0.05$) different when compared to the value obtained for 400 µg/ml and 200 µg/ml respectively. Also vitamin C at 25 µg/ml concentration resulted in 80.05% decrease in DPPH absorbance. The percentage decrease in DPPH absorbance recorded for 25 µg/ml vitamin C was non-significantly different ($P > 0.05$) when compared to the values obtained for (400, 200 and 100 µg/ml) of the FF respectively, but significantly higher ($P < 0.05$) when compared to the lowest concentration of FF (50 µg/ml). The radical scavenging activities of FF of *D. glomerata* leaves were concentration dependent (Table 1.0).

Effect of FF of *D. glomerata* on Carrageenan-Induced Paw Oedema in Rats

Carrageenan-Induced rat paw oedema was inhibited by various doses of FF (FF 100, 200, and 400 mg/kg) and Indomethacin (10 mg/kg) respectively. The FF of *D. glomerata* leaves inhibited the carrageenan-induced rat paw oedema in a dose related manner. There was no significant difference ($P > 0.05$) in between the groups, although FF (400 mg/kg) produced the highest inhibitory effect. The inhibitory effect of vitamin C, although higher, was non-significantly ($P > 0.05$) different compared to the values obtained for the various doses of Ff of *D. glomerata* leaves (Table 2.0).

Table 2.0: Effect of FF of *D. glomerata* Leaves on Carrageenan-Induced Paw Oedema in Rats

Group (n = 5)	Dose mg/kg Orally	Mean paw Size (cm)	Inhibition (%)
Control (DW)	**5 ml/kg**	**4.12** $\pm 0.20^b$	-
FF 400	400	2.15 ± 0.11^a	82
FF 200	200	2.30 ± 0.22^a	78
FF 100	100	2.45 ± 0.31^a	76
FF 50	50	2.51 ± 0.25^a	75
Indomethacin	10 mg	1.85 ± 0.01^a	87

Values with different superscripts are statistically significant ($P < 0.01$)

Effect of FF of *D. glomerata* Leaves on Acetic Acid-Induced Writhing in Mice

The results of the effect of Flavonoid Fraction (FF) of *D. glomerata* following acetic acid-induced writhing in mice are presented in Table 3.0. The various doses of FF significantly ($P < 0.05$) caused marked and dose – related inhibition of acetic acid-induced writhing in mice. The oral (intragastric administration) of various doses of FF (FF 400, 200, 100 mg/ml) or acetyl-salicylic acid (ASA) (100 mg/kg) respectively significantly reduced the number of writhing and stretching movements induced by intraperitonally injected acetic acid. The value obtained at various doses of FF is comparable to the antinociceptive effect of vitamin C.

Table 3: Effect of FF of *D. glomerata* on Acetic Acid-Induced Writhing in Mice (n = 5, mean ± SEM)

Group	Dose mg/kg	Mean Number of Writhing	Inhibition (%)
Control	5 ml/kg DW	52.00 ± 0.51	0.0[b]
FF 400	400 mg/kg	12.21 ± 0.22	81.4[a]
FF 200	200 mg/kg	13.02 ± 0.15	78.5[a]
FF 100	100 mg/kg	15.25 ± 0.20	75.2[a]
ASA	50 mg/kg	11.50 ± 0.54	82.5[a]

[a] = $P < 0.01$ compared to control DW

Discussion

Mammalian cells possess elaborate defence mechanisms for radical detoxification (Aquil *et al*., 2006). Free radicals are chemically highly reactive because the unpaired electron attempts to stabilize itself by pairing with another electron (Aquil and Ahmad, 2007), beginning a chain reaction cascade that leads to oxidative destruction of lipids and alteration of other macromolecules including proteins and DNA (Murray *et al*., 2000). If cellular constituents do not effectively scavenge free radicals, these lead to disease conditions due to oxidative stress (Das *et al*., 2011).

The results of this study showed that the flavonoid fraction of *D. glomerata* leaves has significant free radical scavenging activities comparable to that of vitamin C, although the effect is concentration dependent. The DPPH test provides information on the reactivity of test compounds with stable free radical. Usually, because of its odd electron 2, 2 – dephenylpicryl – hydroxyl radical (DPPH) gives a strong absorption band at 517 nm visible spectroscopy (deep violet colour). As the electron becomes paired off in the presence of a free radical scavenger, the absorption varnishes, thus resulting decolourization is stoichometric with respect to the number of electrons taken up (Olaleye *et al.*, 2004). Due to their polyphenol percentage, flavonoids are generally good scavengers of free radicals (Olayele *et al*., 2004; Aquil *et al*., 2006; Das *et al*., 2011). Free radicals from both endogenous and exogenous sources have been implicated in the etiologies of many disease conditions such as diabetes, cancer, chronic diarrhea, stroke, inflammation, aging and many poisoning cases (Halliwell and Gutteridge, 2007;
Ambali *et al*., 2011). Vitamin C is traditionally regarded as the most important water soluble antioxidant in human plasma Halliwell and Gutteridge, 2007), where it scavenges reactive oxygen and nitrogen species. Vitamin C is a chain breaking antioxidant vitamin involved in the prevention and restriction of free radical chain formation and propagation (Surai, 2002). It is conceivable that the FF of *D. glomrata* leaves mediated the observed antiradical effect through similar mechanism.

A large number of studies have emphasized the potential health promoting and disease preventing effects of fruits and vegetables in diet. The beneficial effects of fruits and vegetables have been frequently attributed to ascorbic acid and the carotenoids present in the foods. However, fruits and vegetables equally contain a multitude of flavonoids and related phenolic compounds that also act as natural antioxidants (Okpara *et al*., 2007; Iweala and Oludare, 2011; Adewale *et al*., 2014). This result strongly support the use of *D. glomerata* leaves, in folkloric medicine and further lend credence to the view that some traditionally used Nigerian medicinal plants are promising sources of antioxidant.
DiRosa *et al.* (2001) earlier reported that carrageenan-induced inflammation is useful in detecting orally active anti-inflammatory agents. Olaleye *et al.* (2004) also reported that oedema formation due to carrageenan in the rat paw is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The second phase of oedema is as result of liberation of prostaglandins, lysosomes, bradykinins, protease, and cyclooxygenase. DiRosa *et al.* (2001) had earlier stated that the second phase is sensitive to most clinically active anti-inflammatory drugs. The FF of *D. glomerata* shows high potency in inhibiting the carrageenan induced inflammation comparable to indomethacin – a known anti-inflammatory agent. Indomethacin mediates its anti-inflammatory activities by preventing the release of histamine and serotonin as well as inhibiting the release of pro-inflammatory enzymes. It is possible that FF of *D. glomerata* mediated the observed anti-inflammatory effects through similar mechanisms as indomethacin. These findings seem to, in part justify the folkloric uses of this plant. Further studies are in progress to isolate the particular flavonoid responsible for these actions by the leaves of *D. glomerata.*

The FF also showed strong anti-nociceptive action in mice by inhibiting the acetic acid-induced writhing. Acetic acid-induced writhing is a highly sensitive and useful test for analgesic screening (Olaleye *et al*., 2004). The effect is similar to ASA – a known

cyclooxygenase inhibitor, thus preventing the release of prostaglandin from arachidomic acid metabolic pathway. This finding seems to in part strengthen the folkloric use of this plant in arrays of condition including diarrhea and colic.

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