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LIPOXYGENASE (LOX) INHIBITORY ACTIVITY OF LEAVES OF *CEIBA PENTANDRA* (L.) GAERTN: A NEGLECTED VEGETABLE FROM NIGERIA

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ABSTRACT
In the search for inhibitorsof lipoxygenase (LOX) for therapeutic significance, this present study was aimed at evaluating the anti-lipoxygenase (anti-LOX) of the leaves of *Ceiba pentandra*-a Nigeria's underutilized vegetable. This study tends to assess its antioxidant activity beside the anti-inflammatory of the leaves through 2, 2′-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging and anti-lypoxygenase (anti-LOX) activity tests. The positive control employed are indomethacin and ascorbic acid. The IC_{50} value for the anti-inflammatory activity of the extract is significant (102.5µg/mL) when compared with that of the positive control 90.4µg/mL (Indomethacin), the extract showed a good antioxidant effect with an IC_{50} of 0.99µg/mL, ascorbic acid was used as a positive control (0.65µg/mL). Hence, the results support the fact that many edible but neglected vegetables (the leaves of *C. pentandra*) in West Africa possess huge medicinal importance beside culinary uses.

Keywords: *C. pentandra*; ABTS; anti-LOX; neglected vegetable

INTRODUCTION

Medicinal plants' role in maintaining and preserving human life and health is well recognized and accepted (Bello *et al.*, 2018; Moerman, 1996). In developing countries like Nigeria and many parts of the World, many of these medicinal plants are employed as condiments, food ornaments, spices and for managing array of diseases (Bello *et al.*, 2018). Nigeria has one of the major economy in Africa with a projected per capital income of >$300, as most of her population living in abject poverty (WHO, 2007). This means more than half of her population may not be able to afford orthodox medicine for managing their health coupled with serious side effects accompanying synthetic drugs (Chindo *et al*., 2002: Adegboye *et al*., 2008)

Ceiba pentandra (L.) Gaertn. belongs to the genus *Ceiba* which is in the family Malvaceae of the order Malvales (formerly of the family Bombacacea), it is a tropical tree (ThePlantList, 2013). ThePlantList, 2013 includes ninety-seven (97) scientific plants names that belongs to the genus *Ceiba* and also gave twelve others names synonymous to *Ceiba pentandra* (The Plant List, 2013). *Ceiba pentandra* is native to South America with tropical West Africa, and it is easily found all over southern Asia and East Indies though in a lesser measure (Anigo *et al.*, 2012). The tree is mostly known as the Ceiba, Java cotton, Hara kapok, Silk cotton and Samauma (Anigo *et al*., 2012; Enechi *et al*., 2013). *C. pentandra* is commonly referred to as Kapok and the fibre gotten from its seed pods is often called Kapok It is one of the largest forest tree in West Africa, Kapok is also known as Rimi (Hausa), Bamtami (Fulani), Arabaogungun (Yoruba) and Akpi (Igbo) (Aloke *et al.*, 2010; Anosike *et al.*, 2014).

In Nigeria, the leaves of *C. pentandra* are cooked as vegetables in form of slurry sauce comparable to Okra and used as livestock fodder (Anosike *et al.*, 2014). Different parts of *C. pentandra* have been described to be beneficial as effective remedies against constipation, diabetes, dizziness, fever, headache, hypertension, leprosy, mental diseases and peptic ulcer (Aloke *et al.*, 2010). Traditional medicine practitioners in Nigeria use the bark for the management of infections, it is employed as diuretics and to banish evil spirits. Its use in the treatment of diarrhea is very common in West African countries (Cowan, 1999; Kiritikar and Basu, 1987; Ueda *et al.*, 2002). The plant is mostly use in India and Malaya against bowel problems. In West Africa, it is generally

used in the treatment of diarrhea (Bello *et al.,* 2017; Bello *et al.,* 2017b).

The anti-inflammatory activity was studied using the anti-lypoxygenase (anti-LOX) activity of the plant extract. Lipoxygenases (LOX) are members of a family of non-heme iron-containing dioxygenases that catalyze the addition of molecular oxygen to polyunsaturated fatty acids in lipids containing a cis, cis-1,4-pentadiene system to give an unsaturated fatty acid hydroperoxides. It has been found that the lipoxygenase (LOX) products play a key role in many inflammatory diseases (Steinhilber, 1999).Therefore, based on paucity of literature on anti-inflammatory activities of the leaves of this plantas shown in Table **5**, hence the study aimed to provide anti-lypoxygenase (anti-LOX) and antioxidant activities of leaves extract of *C. pentandra*, an underutilized wild vegetable in Nigeria for the first time.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Fresh leaves of *C. pentandra* were collected in the month of February, 2017 at different market in Ilorin metropolis. The plant was identified and authenticated at Plant Biology Department, University of Ilorin with voucher number. The leaves were harvested from the authenticated plant, air dried for three weeks to completely remove the moisture content. Subsequently the dried leaves were crushed using pre-washed pestle and mortar and was stored in an air tight polythene container.

Preparation of Plant Material

The powdered *C. pentandra* (400g) was macerated in 3 L of n-hexane in extraction jar such that the level of the solvent was above that of the plant materials. The macerated mixtures were then left for 72 hours at ambient temperature. The extracts were filtered out from the macerated mixture using Whatman 185 µm filter paper. The n-Hexane extracts were concentrated in a vacuum rotary evaporator under reduced pressure and suitable temperature, transferred to appropriately labelled 250 mL beaker and allowed to stand at ambient temperature to permit evaporation of residual solvents. The procedure was repeated using methanol after the residue of the n-hexane extract had been air-dried

Determination of 2, 2′-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity.

The 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonate, ABTS radical cation decolourization assay based on the scavenging of ABTS+ radicals by antioxidants component of the extracts was used. The study was evaluated *in vitro* at various concentrations (100, 200, 300, 400,500) of the extract. The assay follows the procedure of Atolani *et al.* (2013), with slight modifications (Bello *et al.,* 2018; Atolani *et al*., 2013). All analyses were determined in duplicate.

Anti-inflammatory Activity of the Extract of *C. pentandra*

This activity was studied using the anti-lypoxygenase (anti-LOX) activity of the plant extract. The anti-Lipoxygenase (anti-LOX) activity was studied using linoleic acid as substrate and lipoxidase as enzyme according to Steinhilber, (1999). Test samples were dissolved in 0.25 ml of 2 M borate buffer at pH 9.0 and added 0.25 ml of lipoxidase enzyme solution (20,000 U/ml) then incubated for 5 min at 25 ^{0}C. After which, 1.0 ml of lenoleic acid solution (0.6 mM) was added, and thoroughly mixed.

$$\%\ inhibition = \frac{(A_{control} - A_{sample})X100}{A_{control}} \text{ ----------(1)}$$

where; A = absorbance

The absorbance was measured at 234 nm. Indomethacin was used as reference standard and the percent inhibition was also calculated using equation 1 above (Bello *et al*., 2018).

Analysis of Data

GraphPad Prism 3 software (San Diego, USA) was used to determine the IC_{50} on through a non-regression analysis. The IC_{50} was taken as the concentration of sample that scavenged 50 % of the radicals. Results are presented as mean ± standard deviation (±SD) of the mean.

Table 1: ABTS Activity of Methanol Extract of *Ceiba pentandra*

Concentration (µg/mL)	Methanol Extract of *C. pentandra* (% Mean ± SD)	Ascorbic acid (% Mean ± SD)
100	27.94±6.54	18.55 ± 0.61
200	29.19±5.90	21.42 ± 0.38
300	29.63±5.79	25.94 ± 6.03
400	28.70±4.81	26.46 ± 4.96
500	31.59±7.28	22.35 ± 0.15

Table 2: IC_{50} ABTS Activity of Methanol Extract of *C. pentandra*

Test materials	IC_{50} (µg/mL)
Methanol extract of *C. pentandra*	0.99 ±0.11
Ascorbic acid	0.65 ±0.02

Table 3: Lipoxygenase Activity of Methanol Extract of *C. pentandra*

Concentration (µg/mL)	Methanol Extract of *C. pentandra* (% Mean ± SD)	Indomethacin (% Mean ± SD)
100	48.50 ± 0.00	29.21± 0.00
200	51.07 ± 0.00	39.73± 0.00
300	47.81±0.00	69.42± 0.00
400	37.69±0.00	82.42± 0.00
500	31.13±0.00	95.30 ± 0.00

Table 4: IC_{50} Anti-LOX Activity of Methanol Extract of *C.pentandra*

	Test materials	IC_{50} (µg/mL)
1	Methanol extract of *C. pentandra*	102.5±37.49
2	Ascorbic acid	90.4±18.05

RESULTS AND DISCUSSION

The antioxidant activity (ABTS) and lipoxygenase inhibitory effect of the methanol extract of the leaves of *C. pentandra* are shown in Tables **1** and **3,** respectively. The extract displays the highest antioxidant activity at 100 µg/mL concentration when compared with the positive control. From Table 1, it was observed that, as the concentration increases, there was a noticeable increase in antioxidant activity. The most significant anti-LOX activity was observed at a concentration of 500 µg/mL which is the highest in this study. Tables **2** and **4** showed the IC_{50} values for the activities (antioxidant and anti-inflammatory) evaluated in this study, these activities compared favourable at concentrations of 0.99 µg/mL and 102 µg/mL with the IC_{50} values of positive control of the extract at concentrations of 0.65 µg/mL and 90 µg/mL. The significance of the activity of the extract was determined by employing the positive control. Many authors have established the anti-inflammatory activity of some parts of *C. pentandra* (i.e. seeds) stem bark and oil from the seeds though no literature on the anti-inflammatory activity of the leaves was found. Alagawadi and Shah, (2011) reported the anti-inflammatory activity of the seed of *C. pentandra*, this extract wasadministered at various concentrations but doses of 200 mg/kg and 400 mg/kg reduced paw edema volume significantly. The result clearly shows anti-inflammatory effect of seed extracts. Elion Itou *et al*., (2015) investigated the anti-inflammatory and analgesic effects of aqueous extract of the stem of *C. pentandra* using mice, they reported thereafter that aqueous extract of the stem bark at doses 400 and 800 mg/kg showed a significant anti-inflammatory and analgesic activity. The anti-inflammatory activity of the oil from the seeds of *C. pentandra* have has been reported by Kiran and Rao, (2014) by *in vitro* and *in vivo*

(Kiran and Rao, 2014). These studies complement our work, which shows the anti-inflammatory activity of the leaves of *C. pentandra* (Table **5**). Many assays have been employed to assess the anti-inflammatory activity of isolated compounds and plants' extracts. One of the most commonly used one is the anti-LOX assay, this is mostly employed because anti-LOX agents play important role in preventing several inflammation-related diseases such as allergic diseases, arthritis, asthma, autoimmune diseases, cancer, leukemia and lymphoma (Dobrian *et al.,* 2011). Therefore, the search for anti-LOX is imperative since inhibitors of LOX antagonise the biological synthesis of leukotrienes which synonymous to these above mentioned diseases (Dobrian *et al.,* 2011; Rackova *et al.,* 2007). Pidgeon *et al.,* 2007, recommended that anti-LOX agents may help in finding new, biologically and pharmacologically active metabolites which may be useful in managing many diseases (Pidgeon et al., 2007). Since medicinal plants and their preparations have long history of use by humans for the treatment of many diseases, they can be sources of many anti-LOX agents. Hence the significant LOX inhibiting index that the leaves' extract of *Ceiba pentendra* show may have more important role than inflammation but related to preventing other diseases as shown above.

CONCLUSION

From this study, it was observed that methanol extract of the leaves of *C. pentandra* has significant anti-inflammatory and antioxidant activity. Therefore, we suggest that *C. pentandra* constituents could provide a lead to the development of novel anti-inflammatory drugs. Hence, the traditional use of *C. pentandra* and its pharmacological importance, beside its basic nutritional values, is supported by this study. However, further isolation work is recommended to know its constituents and the structural activities relationship of the isolated compounds. This vegetable could play significant protective role against diseases after thorough clinical examination of the isolates from this medicinal plant. The plant can be planted more in household because of its many advantages and can be used as a health food.

Conflicts of Interest

The authors declare no conflict of interest.

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Table 5: Ethnomedicinal importance of *Ceibapentandra*.

	Parts Used	Country	Ethopharmacology	Biological Activity	Method	Isolated Compounds	Toxicity	References
1	Barks	Côte d'Ivoire	To manage symptoms as fever, abscess, paronychia, mental illness, conjunctivitis, dizziness, headache,	Antibacterial activity	*In vitro*	vavain 3′-*O*-*β*-*D*-glucoside, vavain, flavan-3-ol(+)-catechin		Julien *et al*., 2015; Arbonnier, 2000; Noreen *et al*., 1998
2	Stem Bark/ Heartwood	Nigeria, India, Malaysia, Nepal	*Ceiba pentandra* stembark decoction has been used as a diuretic, aphrodisiac, and to treat headache,	Antidiarrhoeal; hepaprotective effect; Antiulcerogenic Activity; Antibacterial and Antihelminthic activity;anti-sickle cell anemia; antiangiogenetic activity	*In vivo, In vitro*	Pentandrin, pentandrin glucoside, b-sitosterol, 3-O-b-D-glucopyranoside, 2,7-dihydroxy-8-formyl-5-isopropyl-3-methyl-1,4-naphthoquinone, 8-formyl-7-hydroxy-5-isopropyl-2-methoxy-3-methyl-1,4-naphthoquinone, L-rhamnose, D-xylose, L-arabinose, D-glucose, D-galactose, D-glucuronic acid, D-galacturonic acid		Sule *et al*., 2009; Burkill, 1985; Ngounou*et al*., 2000; Kishore *et al*., 2013; Bairwa *et al*., 2010; Ibara *et al*., 2007;Shah *et al*., 2017; Nam *et al*., 2003;
3	Seeds	India, Nepal, Nigeria	To treat asthma, dysentery, kidney problems and fever	Antioxidant and flavonoid content; Antibacterial, Anti-inflammatory activity	*In vitro; in vivo*			Pulok *et al*., 2008; Mohan *et al*., 2013; Parulekar, 2017;Alagawad and Shah, 2011
4	Root / Rootbark				Hypoglycemic and Antidiabetics effect	8-formyl-7-hydoxyl-5-isopropyl-2-methoxy-3-methyl- 1,4-naphthaquinone,		Rao *et al*., 1993; Kaimal *et al*., 1970; Dzeufiet *et al*., 2006a;

						7-hydroxycadalene, 2,7-dimethoxy-S-isopropyl-3-methyl-8, l-naphthalene carbolactone, 2-hydroxy-5-isopropyl-7-methoxy-3-methyl-8, l-naphthalene, carbolactone, cyclopropene fatty acids, dihydrosterculic, linoleic acids		Dzeufiet *et al.*, 2006b; Ladeji *et al*., 2003
5	Leaves	India, Nigeria, Indonesia, Nepal	infusion of the *C. pentandra*leaves is used totreatment of cough, intestinal and mucous membranes inflammation, and urethritis	Antipyretic activity, anti-fungal activity, Antibacterial and Antihelminthic activity; Against urinary tract infection, antioxidant activity, Ameliorative properties, anti-sickle cell anemia	*In vivo*, *In vitro*, disk diffusion and agar dilution techniques		$LD_{50}$was >5000 mg/kg b.w	Saptarini and Deswati, 2015; Nwachukwu *et al*., 2008; Shah *et al*., 2017; Padmalochana *et al*., 2018; Sarkiyayi *et al*., 2009; Muhammad *et al*., 2015; Mpianaa *et al*., 2007

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