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Total Phenolic Contents and Anti-lipooxygenase Activity of the Methanolic Extracts from *Durio zibethinus* Murr

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Abstract

Durio zibethinus parts (leaves, stem bark, and root) used to treat malaria fever, vermifuge, diabetes, jaundice, inflammation, and oxidative stress management prompted the investigation of their total phenolic contents and anti-lipooxygenase activities. The total phenolic concentration of D. zibethinus parts was determined spectrophotometrically after collection and extraction. The anti-inflammatory activity was estimated using a lipooxygenase assay. Total phenolic content measured in gallic acid equivalent (GAE) gave the highest levels of 6.88 ± 1.54, 23.32 ± 1.73, and 29.00 ± 3.43 mg GAE/g sample weight leaves stem bark, and root extracts, respectively. The plant extracts showed lower anti-lipooxygenase activity in leaves (IC50 µg/mL- 1.464) and stem bark (IC50 µg/mL- 1.203), but had significant activity in root (IC50 µg/mL- 1.400) compared to standard indomethacin (IC50 µg/mL- 1.660). The plant extract's declining order of anti- lipooxygenase activity follows the same trend with the phenolic contents, indicating that the extract's magnitude of anti- lipooxygenase activity is comparable with the quantity of phenolic compounds present in the extracts. Hence, the *D. zibethinus* extracts are a potential candidate for a plant-derived anti-inflammatory agent.

Keywords: *Durio zibethinus;* Phenolic contents; Anti-lipooxygenase; Inflammation; Indomethacin.

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1. Introduction

Phenolics (phenolic compounds) are defined by Youngsu *et al*. [1] as compounds that possess aromatic ring(s) added to hydroxyl groups and functional derivatives. Phenolics in plants consist of flavonoids, phenolic acids, tannins, lignans, and stilbenes, which protect against ultraviolet radiation or aggression by pathogens, predators, and parasites adding to plants' colors [2]. There are two classifications of phenolic acids, particularly benzoic acid derivatives such as gallic acid and cinnamic acid derivatives, for example, ferulic,

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coumaric, and caffeic acids. Caffeic acid is a sufficient phenolic acid in numerous fruits and vegetables, while ferulic acid exists in cereals [3].

Phenolics are the prevalent constituents of plant-based foods and beverages, adding to the bitterness and astringency of fruit and fruit juices due to the relation between phenolics (procyanidin) and the glycoprotein in saliva [4]. Meanwhile, some authors have earlier reported that some phenolics can serve as antibacterial [5], antiviral [6], and anti-inflammatory [7] agents. Moreover, some phenolics show antioxidant properties [8] due to their chemical structures that decrease the hazard of oxidative diseases in humans [9].

Lipoxygenases are important enzymes that are liable for transforming arachidonic and linoleic into bioactive substances involved in immune and inflammatory responses. These are vital enzymes useful in leukotrienes' biosynthesis that play crucial roles in some inflammation-related conditions such as allergic reactions, cancer, asthma, colitis ulcerosa, psoriasis, and rheumatoid arthritis [10-13].

Durio zibethinus (Durian), belonging to the Bombacaceae family, was cultivated in the tropical region of Malaysia and the Southeast Asian countries [14]. Durian is well known as "King of Tropical Fruit" due to its high nutritional status and physical appearance, resembling the thorny thrones of Asian kings [15].

Durian fruit was earlier investigated to possess potential medicinal and therapeutic properties, including its ability to raise the immune system and wound healing [16]. It is reported by Ang *et al*. [17] that durian fruit has antioxidant, anti-cardiovascular, anticancer, anti-diabetic [18,19], and anti-obesity properties [20] and can cure insomnia, enhance digestion, reduce blood pressure and lessen the symptoms of depression, anxiety, and stress disorders [21,22]. With this background, it was disclosed that the anti-lipooxygenase activity of *D. zibethinus* parts (leaves, stem-bark, and root) had not been reported. Hence, this study focused on estimating the magnitude of phenolic compounds and evaluated the in vitro anti-lipooxygenase activity of methanol extracts of *D. zibethinus* parts.

2. Materials and Methods

2.1. *Study area*

The project was carried out at the Chemistry Department of the University of Ilorin, Kwara State, Nigeria from February-November, 2019.

2.2. *Sample collection*

Fresh plant materials (leaves, stem bark, and root) of *D. zibethinus* were obtained from Okada in Edo State, Nigeria. The plant samples were identified and authenticated at the Herbarium of the Faculty of Life Sciences, University of Ilorin, and voucher number UILH/001/1371 was assigned. The samples air-drying was crushed and subjected to extraction at ambient temperature.

2.3. *Chemicals and reagents*

Reagents and chemicals, including Folin-Ciocalteu, Gallic acid, linoleic acid, borate buffer, and indomethacin, were Sigma-Aldrich, USA. Sodium carbonate (Na_2CO_3) and other reagents used were analytical grades expected otherwise indicated. However, when required, solvents were redistilled before use.

2.4. *Evaluation of total phenolic contents*

The Folin-Ciocalteu (FC) method of Dewanto *et al*. [23] with slight modifications was followed to estimate the total phenolic contents (TPC) of D. zibethinus extracts. An aliquot part of the diluted extract was added to the mixture of 0.5 mL of distilled water and 0.125 mL of Folin–Ciocalteu reagent. The mixture was then shaken and allowed to settle down for 6 min before adding 1.25 mL of 7 % Sodium carbonate (Na_2CO_3). The solution was diluted to a final volume of 3 mL with distilled water, mixed thoroughly, and incubated in the dark. Sample blank for all the dilution of standard gallic acid was prepared similarly by replacing Folin-Ciocalteu solution with distilled water, filtered and absorbance was spectrophotometrically (UV-VIS spectrophotometer V-550 model, Japan) at 760 nm, measured against the suitable blank [24]. TPC was expressed as mg GAE/g DW (i.e., milligram gallic acid equivalent per gram of dried extract). The sample was analyzed in triplicate.

2.5. *Anti-inflammatory activity: lipoxygenase assay*

The method of Eshwarappa *et al*. [13] was adopted to determine the lipoxygenase activity of the sample using lipoxidase enzyme and linoleic acid as a substrate. Precisely, the assay was implemented by dissolving the test samples in 0.25 mL of 2 M borate buffer (pH 9.0), added to 0.25 mL of lipoxidase enzyme solution at 20,000 U/mL, and incubated for 5 min at 25 °C. Thereafter, 1.0 mL of linoleic acid solution (0.6 mM) was added and thoroughly mixed. The extent of reaction was compared to the standard indomethacin by measuring the formation of 13-hydroperoxyl linoleic acid from the linoleic acid (forming a new conjugated diene) at 234 nm on a multiscan absorbance reader. The percentage inhibition was calculated utilizing the expression: % Inhibition = 100 x (1 – Vt/Vc).

Where Vt = absorbance of test sample; Vc = absorbance of control A dose-response curve was plotted to obtain the IC50 values. IC50 is the inhibitory concentration at 50% of a maximum scavenging capacity. Triplicate tests and analyses were performed, and the average was calculated.

2.6. *Statistical analysis*

Results obtained were evaluated on GraphPad Prism 5 (San Diego, CA) using a one-way ANOVA and outcome triplicate values given as mean ± standard deviation (± SD). The

concentrations of samples showing a 50 % inhibition (IC50) were estimated on the GraphPad Prism 5 via a non-linear regression fit.

3. Results and Discussion

3.1. *Total phenolic contents*

The results of total phenolic contents of the three crude extracts (leaves, stem bark, and roots) of *D. zibethinus* are given in Table 1. Total phenolic content in gallic acid equivalent (GAE) gave the highest levels of 6.88 ± 1.54, 23.32 ± 1.73, and 29.00 ± 3.43 mg GAE/g, sample weights for the leaves, stem bark, and root extracts, respectively.

The results attained for the total phenolic content of the three crude extracts (leaves, stem bark, and roots) of *D. zibethinus* revealed that all the extracts have some amount of total phenolics. Reports have indicated that phenols have broad biological activity, including antithrombotic, cardio-protective, vasodilator, and antimicrobial activities [25]. Recently, phenolics were investigated as strong antioxidants in vitro and demonstrated to be more effective than Vitamin C and E and carotenoids [26].

Also, a lot of *in vitro* and *in vivo* systems have been employed to estimate the anti-carcinogenic and anticancer potential of these natural phenolic compounds or extracts [3]. Phenolic extracts or isolated polyphenols from different plant food are studied in many cancer cell lines revealing various evolutionary stages of cancer [27,28]. Furthermore, in vitro studies on cancer cell lines numerous in vivo experiments have also been carried out to verify the antitumor efficacy of plant food-derived phenolic extracts or compounds with tumor incidence and multiplicity (e.g., number of tumors per animal) as endpoints [29-32].

In this study, it was perceived that the root extract has a substantial amount of phenolics compared to other extracts. But the minimum quantity of phenolics was obtained in the leaf extract. This indicates that phenolic compounds of the plant, *D. zibethinus*, can be well-chosen as lead compounds for designing potent antioxidant and anti-inflammatory drugs.

Table 1. Total phenolic content of methanolic extracts from *D. zibethinus* Murr.

Extract	Total Phenolics (mg GAE/g DW)
Leaf	6.88 ± 1.54
Stem bark	23.32 ± 1.73
Root	29.00 ± 3.43

The values are means ± SD of three replicates. GAE = Gallic acid equivalent, DW = Dried weight.

3.2. *Anti-lipooxygenase activity of Durio zibethinus*

The anti-lipooxygenase activity of the extracts of *D. zibethinus* was determined and compared with a standard drug, indomethacin, following the standard procedure already described. A dose-response activity comparable to the standard drug was obtained (Table

2). The activities recorded for the leaves, stem bark, and root extracts range from 29.15 to 62.97 %, 44.23 to 75.06 %, and 34.16 to 86.31 %, respectively, while the standard indomethacin ranges from 44.31 to 83.29 % with corresponding IC50 values of 1.464, 1.203 and 1.400 µg/mL for leaves, stem bark and root respectively compared to standard indomethacin 1.660 µg/mL at concentrations (10 to 150 µg/mL).

From Table 2, it was noted that lipoxygenase inhibition activity increased with an increase in concentration for the standard indomethacin and the methanol extracts of *D. zibethinus* parts. The plant lipoxygenase pathway is in many respects equivalent to the 'arachidonic acid cascades' in animals [33]. For this reason, the *in vitro* inhibition of lipoxygenase comprises a good model for screening plants with anti-inflammatory potential [34]. LOXs are susceptible to antioxidants, and their actions may consist of inhibition of lipid hydroperoxide formation owing to scavenging of lipidoxy or lipid peroxy- radical formed amid enzyme peroxidation. This can confine the availability of lipid hydroperoxide substrate required for the catalytic cycle of LOX.

In recent years, the search for phytochemicals possessing anti-inflammatory properties has been on the rise due to their potential use in the therapy of various chronic and some infectious diseases. Epidemiology and experimental studies have implicated oxidative cellular damage arising from an imbalance between free radical generating and scavenging systems as the primary cause of cardiovascular diseases, cancer, aging, etc. [35]. High total phenolic content values found in methanol extract *D. zibethinus* imply the function of phenolic compounds in contributing to these activities. Plant phenolic compounds were found to acquire effective anti-inflammatory activity [36,37]. The results of the studies on *D. zibethinus* showed a favorable anti-inflammatory activity as the plant extracts inhibited the lipoxygenase enzyme activity. This has proven that the *D. zibethinus* plant is more useful in inflammation studies and various related physiological studies, aging, and diseases such as cancer, neurological disorder, etc.

Table 2. Effect of methanolic extracts of *D. zibethinus* Murr. on lipoxygenase inhibitory action.

Sample Concentration (µg/mL)	Leaf % inhibition	Stem bark % inhibition	Root % inhibition	Indomethacin % inhibition
10	29.15 ± 0.03	44.23 ± 0.01	34.16 ± 0.02	44.31 ± 0.01
20	37.61 ± 0.01	55.17 ± 0.01	57.67 ± 0.02	52.09 ± 0.01
50	56.48 ± 0.05	55.40 ± 0.01	70.82 ± 0.01	62.65 ± 0.01
100	60.31 ± 0.01	63.40 ± 0.01	83.87 ± 0.01	80.05 ± 0.01
150	62.97 ± 0.01	75.06 ± 0.01	86.31 ± 0.01	83.29 ± 0.01
IC_{50} µg/mL	1.464	1.203	1.400	1.660

Indomethacin was used as the reference standard. Values were performed in triplicates and represented as mean ± SD.

4. Conclusion

The present results showed that the methanol extracts of *D. zibethinus* possess anti-inflammatory properties. These activities may be owing to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols. Isolation and purification of bioactive compounds in this plant are necessary, and this

purified form of the compounds may show increased activity. This study gives the idea that the *D. zibethinus* is conceivably used as lead compounds for designing effective anti-inflammatory drugs that can be employed to treat different diseases such as cancer, neurological disorder, aging, and inflammation.

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