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Original Research Article

Analgesic and Anti-inflammatory Activities of Leaf, Stem and Root Essential Oils of *Borassus aethiopum* (Arecaceae)

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Abstract

Purpose: Pain and inflammation have received man's attention from time immemorial. *Borassus aethiopum*, a large fan shaped tree is found in Africa, with reported nutritional and biological activities. This study was designed to investigate the analgesic and anti-inflammatory activities of the leaf, stem and root essential oils of *Borassus aethiopum*.
Methods: Plant sample was obtained from Gboko, Nigeria. Essential oils from leaf, stem and roots of this plant were extracted by hydro distillation using all-glass Clevenger apparatus. Acute toxicity test on essential oil samples of *Borassus aethiopum* was carried out using various dosages. Analgesic activity of these essential oils was investigated by measuring the number of acetic acid induced writhing in rats while oedema volume of rat's paw was used to determine the anti-inflammatory activity of the essential oil samples.
Results: Essential oil samples were safe at 25 ml/kg bodyweight. Analgesic activity of essential oil was best on leaf (71.4%) at 15 ml/kg; stem (94.64%) at 10 ml/kg; root (78.2%) at 10 ml/kg and root (77.6%) at 15 ml/kg bodyweight. During investigation of anti-inflammatory activity, all three essential oil samples: leaf, stem and roots showed better activity than the standard drug, indomethacin. However, leaf essential oil showed early phase inhibitory activity (0 minute) while late phase inhibitory effect (120 minutes) was seen in root essential oils of *Borassus aethiopum*.
Conclusion: Results show that leaf, stem and root essential oils of *Borassus aethiopum* exhibited analgesic and anti-inflammatory activities and suggests their promise as drugs.

Keywords *Borassus aethiopum*, Essential oil, Anti-inflammatory, Analgesic, Diclofenac, Indomethacin

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INTRODUCTION

Chemical compounds isolated from plants, fungi, microbial, animal, or other natural sources are referred to as Natural products [1,2]. Generally, natural products are produced by living organisms. These products include lipids, carbohydrates, proteins and nucleic acids, all of which are important in metabolic reactions. Natural products, over the years, have played a key role in drug

discovery, including those for cancer, infectious diseases and other therapeutic uses [3]. The use of natural products of plant origin including essential oils has been widespread.

An essential oil is a product of plant raw material obtained using distillation [4]. Essential oils (EOs), are also known as volatile oils, etheric oils, essences or aetheroleum [4]. They are mostly found in aromatic plants and are complex natural mixtures of volatile, lipophilic, and odoriferous substances [4].

Essential oils are mostly composed of about 20–60 components at various concentrations, whereas others may consist of 300 or more different substances [5]. While most essential oil constituents are usually present in small concentrations, about two or three components may be in large proportions of between 20 – 70 % [5]. Some examples include, eucalyptol (70–90%) as major component of *Eucalyptus globulus* Labill essential oil [5], rotundifolone (50–65%) of *Mentha villosa* Hudson leaf essential oil [4], and cinnamaldehyde (60–90%) of *Cinnamomum zeylanicum* Blume leaf and bark essential oil [6]. Often, major components of essential oils dictate their biological properties. Minor compounds may also ensure bioactivity by enhancing the action of major components or through antagonistic action [7]. These minor compounds may effect activity by singular or synergistic action [7].

Essential oil components are divided into mainly two groups: terpenoids (major group) and non-terpenoids (mainly phenylpropanoids). All of these are hydrocarbons and their oxygenated derivatives [4]. They can be found in the form of several chemical classes, such as aldehydes, ketones, alcohols, oxides, esters, amines, amides, phenols, nitrogen and sulfur compounds, and heterocycles [4]. Essential oils (EOs) have been studied widely for their pharmacologic potentials in various pathologies [4,8,9]. Promising results in several areas of science have been reported through the use of EOs and their chemical constituents [10,11]. Essential oils have been reported to exhibit antimicrobial, anti-inflammatory, antitumor, antioxidant and analgesic activities, among others [4].

Pain is stimulated by naked nerve endings and attends almost all ailments. The desperate quest to manage pain is directly linked to various researches on analgesic and anti-inflammatory activities of various plants and plant products including *Corchorus olitorius* [1], Propolis [12], *Jatropha curcas* [13] among several others.

Borassus aethiopum is a palm tree with large fan shaped leaves. It is known as African palm or Borassus palm. In Nigeria, it is known as *Giginya* by the Hausas of northern Nigeria while the Yorubas of South-Western Nigeria call it *Agbonolodu* [14]. In South Eastern Nigeria, among the Ibos, it is known as *Ubiri* [14]. *Borassus aethiopum* popularly known among the Tiv people of North-Central Nigeria as *Kuuv* [15] is a dioecious plant and can reach up to 20m high on average and 1m in diameter [16] and grows widely in Africa. Leaves of this tropical plant specie, have been used for making baskets and mats while the trunk has been found useful as timber for roofing, door frames, poles, and tool handles and for construction of bridges, due to its toughness and resistance to termites [17]. Roots, leaves, flowers and fruits of this plant are used for many purposes such as nutrition agents, treatment for sexually transmitted diseases (e.g., herpes), cutaneous fungal infections, and viral infections especially measles [18]. The antipyretic and anti-inflammatory activities [19], antimicrobial activity [18,20] and antioxidant activity [21] of this crude plant extract have also been reported.

This study seeks to evaluate the analgesic and anti-inflammatory activities of leaf, stem and root essential oils of *Borassus aethiopum* obtained from Gboko, Benue State, Nigeria with the intention of addressing the challenges pain has posed. This may also address the attendant side effects of the already existing remedies for pain.

MATERIALS AND METHODS

Collection of Plant Materials

Borassus aethiopum plant was collected from Mkar in June, 2023. Mkar, a settlement in Gboko, Benue State, Nigeria, lies between longitude (7º 47’ 0” and 10º 0’ 0” E) and latitude (6º 25’ 0” and 8º 8’ 0” N). Plant sample was identified and authenticated at the Department of Forestry and Environmental Technology, Akperan Orshi Polytechnic, Yandev, Benue State and assigned Voucher number FHY 014. The collected plant was separated into leaf, stem and root parts.

Preparation of Plant Material

The freshly obtained plant material was washed with water to exclude contaminants. Leaf, stem and root parts were separated and weighed.

Drugs and Chemicals

Diclofenac sodium was purchased from Normed Pharmacy Ltd, a registered pharmacy in Gboko, Nigeria. All other chemicals were purchased from Sigma-Aldrich (Germany).

Experimental Animals

Rats of both sexes weighing between 15 to 32 g obtained from the animal house of Benue State University, Makurdi were used for the study. Animals were acclimatized at room temperature (29ºC) with a relative humidity of 70% in standard cages for 4 to 5 days prior to commencement of the experiment. Throughout the period of study, animals were kept under hygienic conditions by constant cleaning and removal of faeces and spilled feeds from cages daily. The study was approved by the Animal Welfare and Ethics Committee of University of Mkar, Mkar, Benue State, Nigeria and issued an Approval number: UMM/DOR/ECSBS/21/01. All conditions of animals used were as approved by United States National Institute of Health (NIH) guide for Care and Use of Laboratory Animals.

Extraction of the Essential Oil Components

Essential oil extractions were carried out by the use of an all-glass Clevenger-type apparatus designed to British Pharmacopeia specifications. 250 g each of leaf, stem and roots of *Borassus aethiopum* were crushed and hydro-distilled for 2 hours. 2.0 ml each of distilled hexane were used to remove the essential oils. The oils were then stored in vials and refrigerated[1,22].

Acute Toxicity Studies

The acute toxicity (median mean dose, LD_{50}) was determined using albino rats. Twenty five rats were grouped into five groups labeled A, B, C, D and E, with five rats per group for each essential oil sample; leaf, stem and root. For each essential oil sample, rats in each group were administered doses of 5, 10, 15, 20 and 25 ml/kg bodyweight respectively. The experimental animals were observed for 72 hours for signs and symptoms of toxicity after treatment [23,24].

Drug

Diclofenac was used as a standard drug for the analgesic activity at a dose of 25 mg/kg bodyweight.

Analgesic Activity Assay

Acetic Acid Induced Writhing Test

The abdominal constriction was induced in rats (weighing 15-32 g) by intraperitoneal injection of 1% (v/v) acetic acid (2.3 ml/kg), as described by Santos *et al*. [23] Animals were pretreated with leaf, stem and root essential oils of *B. aethiopum* at doses of 5, 10 and 15 ml/kg, intraperitoneally, 45 minutes before acetic acid administration. Control animals received 2 ml volume of hexane and the positive control animals were treated with the reference analgesic drug; Diclofenac sodium (40 mg/kg bodyweight). Animals were treated one at a time. The number of abdominal constrictions was cumulatively counted over a period of 20 minutes after acetic acid administration. The percentage inhibition of analgesic activity was calculated using equation (1) [25].

$$\%\ \text{Analgesic Activity} = \frac{\text{Mean writhing count (Control group} - \text{treated group)}}{\text{Mean writhing count of control group}} \times 100 \ldots\ldots\ldots\ldots (1)$$

Anti-inflammatory Activity Assay

Egg Albumin Induced Oedema

The test was conducted using a modified method of Winter *et al*. (1963) as described by [26]. The rats were divided into five groups of five rats of either sex per group and treated as follows: Group A which served as the negative control received hexane (2 mL), while group B which was the positive control received 25mg/kg bodyweight of indomethacin. Groups C, D and E were administered 5, 10 and 15 mL/kg bodyweight of the leaf stem and root essential oils of *Borassus aethiopum* respectively, orally. After 45 minutes of essential oil administration, oedema was induced by sub-plantar injection of 0.1mL of fresh raw egg albumin in the left hindpaw. This was carried out by wrapping a piece of cotton thread round the paw and measuring the circumference with a metre rule. The readings were taken at 20 minutes intervals, that is; 0, 20, 40, 60, 80, 100 and 120 minutes after albumin administration. The average increase in paw size of each group was calculated and compared with the controls; groups A and B.

Statistical Analysis

Data obtained was expressed as Mean± Standard Error of Mean and analyzed using the Analysis of Variance 'ANOVA' [25] and SPSS (version 20) where applicable. Values at $P<0.05$ were regarded as significant in comparison with appropriate controls.

RESULT AND DISCUSSION

Percentage yields of leaf, stem and root essential oils of *Borassus aethiopum* were 0.75, 0.36 and 1.07 % respectively (Table 1). Acute toxicity results of essential oils of these plant parts showed they were all safe at 25 mL/kg bodyweight (Table 2).

Chemical constituents commonly found in essential oils are monoterpenes, sesquiterpenes,

and phenylpropanoids [28]. Monoterpenes present in certain essential oils have been reported to include menthol, linalool, limonene, myrcene and 1,8-cineole [29]. Essential oils are reported to exhibit various pharmacological properties, such as antimicrobial, anticonvulsant, hypnotic, anxiolytic, or anticancer [28,29], antioxidant, analgesic [1,30,31] and anti-inflammatory [30].

Table 1: Yield of Leaf, Stem and Root essential oils of *Borassus aethiopum*

Plant part	Weight of sample (g)	Weight of essential oil procured (g)	Yield of essential oil (%)
Leaf	80.0	0.6	0.75
Stem	83.3	0.3	0.36
Root	65.0	0.7	1.07

Table 2: Acute toxicity determination for Leaf, Stem and Root Essential oils of *Borassus aethiopum*

Dose (ml/kg)	Plant Part		
	Leaf	Stem	Root
5	0/5	0/5	0/5
10	0/5	0/5	0/5
15	0/5	0/5	0/5
20	0/5	0/5	0/5
25	0/5	0/5	0/5

Key: 0 = number of death, 5 = number of rats

Analgesic Activity

Writhing test is a chemical method used to induce pain of peripheral origin by administration of irritant principles like phenylquinone or acetic acid in mice. Analgesic activity of the test compound is inferred from decrease in the frequency of writhing. This method was employed to evaluate the possible peripheral analgesic effects of leaf, stem and roots essential oils of *B. aethiopum*. Leaf essential oils of *Borassus aethiopum* at three different dose levels of 5, 10 and 15 mL/kg exhibited a significant analgesic activity in the acetic acid-induced abdominal writhes in mice in a non-dose dependent manner. This is similar to the analgesic report on *Artemisia sieberi* essential oil in which the activity was not dose-related [30]. The highest percentage inhibition of abdominal constriction for the leaf oil (71.4%) was observed at 15 mL/kg ($p<0.05$) compared to diclofenac (71.4%) at 25 mg/kg (Figure 8).

Stem essential oil of *Borassus aethiopum* at three dose levels of 5, 10 and 15 ml/kg exhibited significantly, analgesic activity in acetic acid induced abdominal writhes in rats in a dose dependent manner. The highest percentage inhibition of abdominal constrictions for the essential oil extract (94.64%) was observed at 10 ml/kg ($p<0.05$) compared with diclofenac sodium (71.4%) at 25 mg/kg (Figure 8). [30] also reported better analgesic activity by *Artemisia sieberi* oil than diclofenac sodium.

Roots of *Borassus aethiopum* essential oil at the three dose levels of 5, 10 and 15 ml/kg exhibited significantly, analgesic activity in acetic acid induced abdominal writhes in rats in a non-dose dependent manner similar to report by[30] on essential oil of *Artemisia sieberi* in which the analgesic activity was non-dose dependent. The highest activity was exhibited by root essential oil at dose levels of 10 ml/kg bodyweight (78.2%) and 15 ml/kg bodyweight (77.6%) when compared to diclofenac (71.4%) (Figure 8).

Stem essential oils were more active at 5ml dosage than leaf and root essential oil samples. Best analgesic activity was shown at 10 ml/kg bodyweight of stem essential oil administration. Analgesic activity at 10 ml/kg bodyweight of stem and root essential oils and 15 ml/kg bodyweight of leaf, stem and root essential oils gave better activity than the standard drug, diclofenac, at 25 mg/kg bodyweight. [30] also reported better essential oil activity than diclofenac.

Anti-inflammatory Activity

Inflammatory paw edema in rats

Cardinal signs of inflammation, hyperalgesia, and erythema develop immediately after subcutaneous injection of carrageenan, resulting from the activity of pro-inflammatory agents such as bradykinin, histamine, tachykinins, complement and reactive oxygen and nitrogen species [30]. The result of the anti-inflammatory screening shows that the leaf essential oil of *Borassus aethiopum* exhibited marked inhibition of albumin-induced hind paw oedema in rats. The anti-inflammatory activity shown by the extract over a period of 2 hours reveals that at 20 minutes, the extract at 15 ml/kg, significantly inhibited the inflammation at the early phase compared to indomethacin (25 mg/kg) (Figure 2). At 120 minutes, indomethacin (25 mg/kg) significantly reduced the paw size, suggesting its late rapid inhibitory effect (Figure 7). The mechanism of action could possibly involve inhibition of serotonin, histamine and prostaglandins.

The result of anti-inflammatory screening shows that the stem essential oil of *Borassus aethiopum* exhibited marked inhibition of albumin induced hind paw oedema in rats. Anti-inflammatory activity exhibited by the sample over a period of 2

hours revealed that at 40 minutes, the extract at 10 ml/kg significantly inhibited inflammation at the early phase compared to indomethacin (25 mg/kg) (Figure 3). At 100 minutes, indomethacin (25 mg/kg) significantly reduced the paw size suggesting that it has late rapid inhibitory effect (Figure 6). The essential oil acts on both early and late phases of inflammation. The mechanism of action could possibly involve inhibition of histamine and prostaglandins.

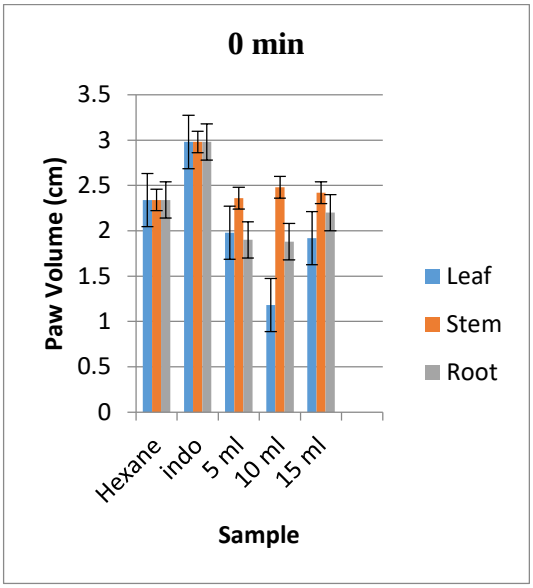

Figure 1: Comparative Anti-inflammatory Activities of Leaf, Stem and Root Essential oils of *Borassus aethiopum* 0 minutes after Administration

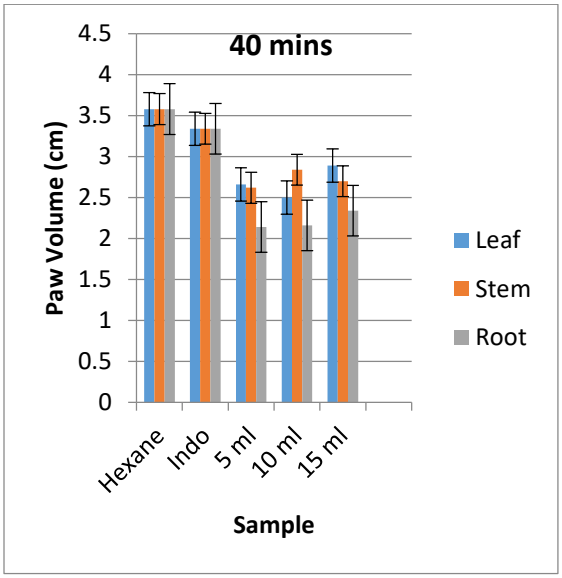

Figure 3: Comparative Anti-Inflammatory Activities of Leaf, Stem and Root essential oils of *Borassus aethiopum* 40 minutes after administration

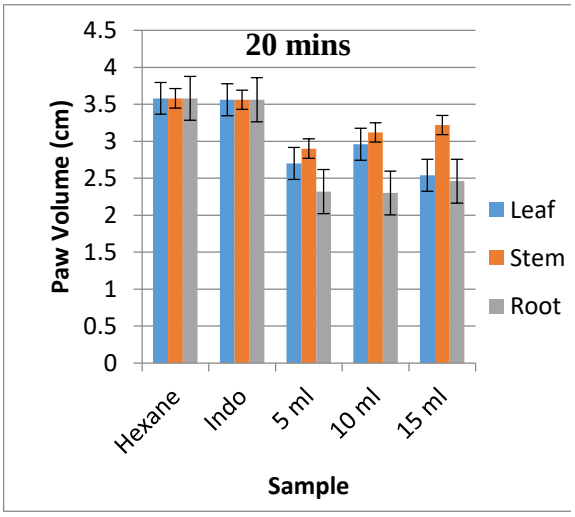

Figure 2: Comparative Anti-Inflammatory Activities of Leaf, Stem and Root essential oils of *Borassus aethiopum* 20 minutes after administration

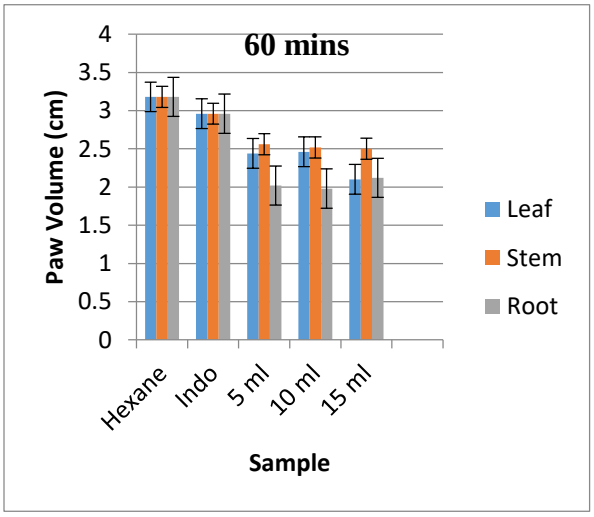

Figure 4: Comparative Anti-Inflammatory Activities of Leaf, Stem and Root essential oils of *Borassus aethiopum* 60 minutes after administration

Essential oil hydrodistilled from roots of *Borassus aethiopum* showed marked inhibition of albumin induced hind-paw oedema in rats compared to indomethacin (25 mg/kg). Anti-inflammatory activity of this essential oil over a period of 2 hours showed that from 0-120 minutes, there is significant ($p<0.05$) increase in activity compared to indomethacin (25 mg/kg) (Figures 1-7). Marked essential oil activity at 0 minutes (Figure 1) showed early phase activity while activity at 100 (Figure 6) and 120 minutes (Figure 7) showed late phase reaction. This, then suggests both early and late phase rapid inhibitory effect for root essential oil of *Borassus aethiopum*. [30] reported that inhibitory activity shown by *A. sieberi* essential oil (1 mg/kg) during a 4 h period in the carrageenan-induced paw inflammation test was somewhat better than that exhibited by the group treated with standard drug, especially after the first hour. Similar study on aqueous extract of *Euphorbia heterophylla* also reported good anti-inflammatory activity comparable to indomethacin [32] These confirms good anti-inflammatory activity of some reported essential oils compared to indomethacin.

This anti-inflammatory activity of *Borassus aethiopum* oil may be related to the inhibition of Nitric Oxide (NO) formation/release since NO contributes to oedema formation [33]. Nitric oxide (NO) is one of those chemical mediators highly involved in the process of pain transmission [34]. It is implicated in the mechanisms of pain generation and transmission throughout the peripheral and central nervous systems and locally released pain mediators (including formation of inflammation and vascular oedema) [34].

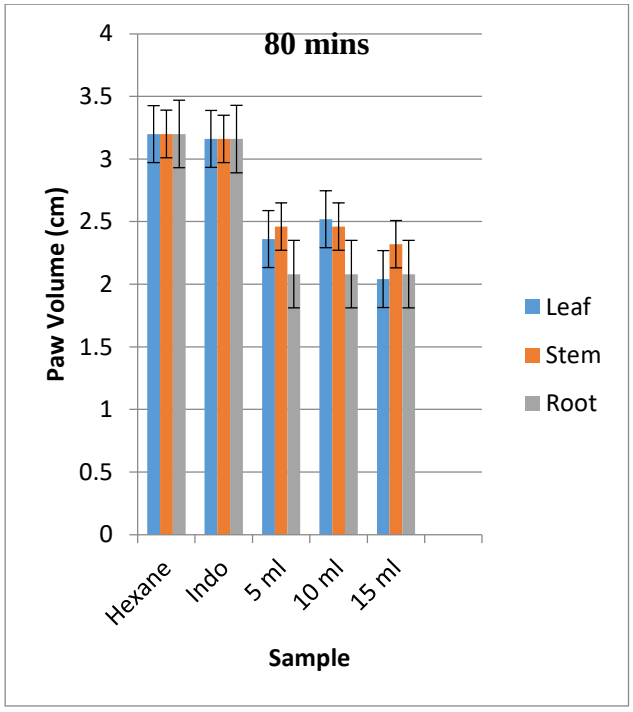

Figure 5: Comparative Anti-Inflammatory Activities of Leaf, Stem and Root essential oils of *Borassus aethiopum* 80 minutes after administration

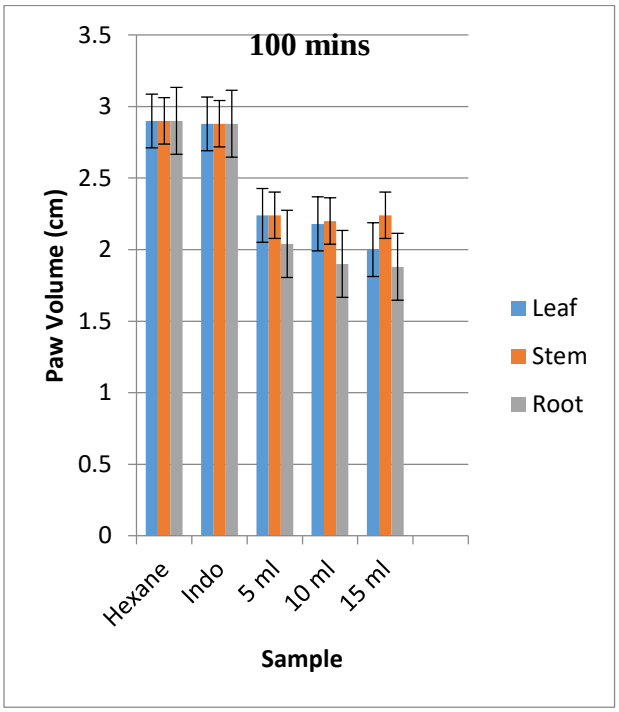

Figure 6: Comparative Anti-Inflammatory Activities of Leaf, Stem and Root essential oils of *Borassus aethiopum* 100 minutes after administration

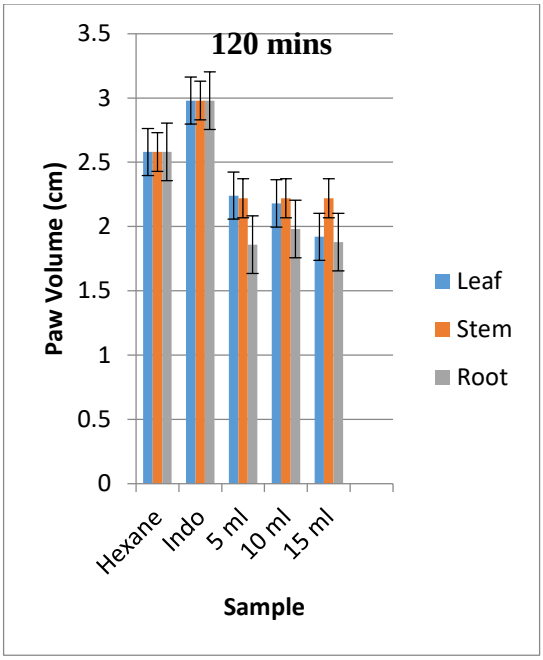

Figure 7: Comparative Anti-Inflammatory Activities of Leaf, Stem and Root essential oils of *Borassus aethiopum* 120 minutes after administration

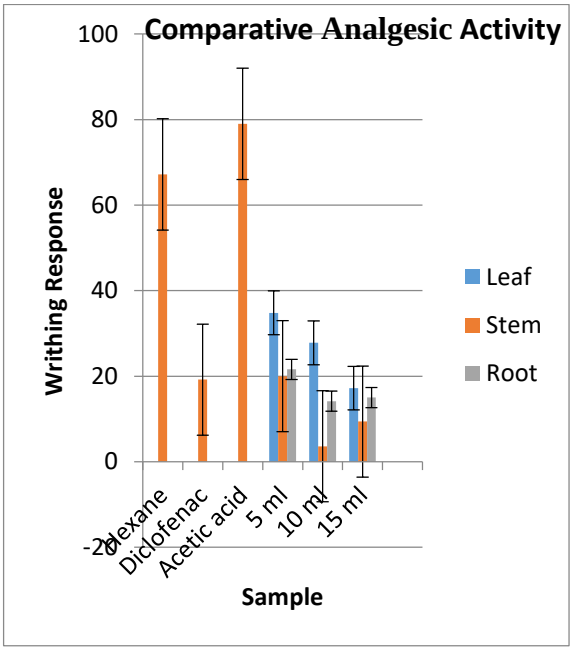

Figure 8: Comparative Analgesic Activities of 5, 10 and 15ml of Leaf, Stem and Root essential oils of *Borassus aethiopum.*

CONCLUSION

Various pharmacological activities of essential oils have been reported. This report highlights that analgesic activity of root essential oils of *Borassus aethiopum* at dose levels of 10 ml/kg bodyweight (78.2%) and 15 ml/kg bodyweight (77.6%) showed better activity than diclofenac (71.4%). Anti-inflammatory screening shows that stem and root essential oils of *Borassus aethiopum* exhibit activity at both early (40 minutes) and late phases (100 minutes). In conclusion, leaf, stem and root essential oils of *Borassus aethiopum* have both analgesic and anti-inflammatory activities at various dosages. This suggests it to be a drug of promise. A further study to determine the compounds present in these essential oils and their various activities is recommended.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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