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Study on the anti-inflammatory and in vivo antioxidant effects of crude leaf extract and fractions of *Ipomoea involucrata* in Albino Wistar Rats

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Abstract

Traditional African herbalist uses *Ipomoea involucrata* to treat fever, gonorrhea, and asthma among other diseases. An animal model has not been used to compare its crude leaf extract and fractions on anti-inflammatory and antioxidant effects. The study aims to assess the anti-inflammatory and antioxidant activities of crude leaf extracts and fractions of *Ipomoea involucrata* using albino Wistar rats. Phytochemical analysis was carried out using standard methods. Crude extract and fractions of were tested for anti-inflammatory activity against Carrageenan-induced edema in Wistar rats weighing 160-180 g. The rats were separated into 13 groups (n=5) that received low (250 mg/kg) and high (500 mg/kg) doses of crude and fractions of the extracts. Data was analyzed with one-way analyses of variance (ANOVA) followed by post hoc turkey's test. Results were presented as the mean ± standard deviation (SD) of sample replicates. $P<0.05$ was considered statistically significant. The crude extract and the fractions exhibited significant ($p<0.05$) potent anti-inflammatory activity, with the ethyl acetate fraction at 500 mg/kg producing the highest percentage (90.94%) reduction in paw edema induced by carrageenan. In the antioxidant biochemical assay, ethyl acetate, n-hexane, and ethanol extracts elicited significant ($P<0.05$) increases in superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) levels. However, there was a significant decrease in malondialdehyde (MDA) better than the other extracts, as well as the control group. Our findings provided additional evidence to support the traditional use of *Ipomoea involucrata* for the treatment of inflammation. Moreover, the study unveils its antioxidant properties and healing effects.

Keywords: Anti-Oxidant; Inflammation; *Ipomoea involucrata*; Fractions

1. Introduction

Medicinal plants are the most primordial form of medication, having been utilized in traditional medicine in numerous countries worldwide for thousands of years [1]. For millennia, medicinal plants have played an important role in human health and healthcare systems. Their significance stems from their potential to offer natural, effective, and long-term solutions for illness prevention and treatment. With a diverse array of bioactive substances such as alkaloids, flavonoids, terpenoids, and polyphenols, these plants play versatile roles in promoting well-being via antioxidant, anti-inflammatory, antibacterial, and anticancer[2] effects. Global studies have been conducted to assess the efficacy of some medicinal plants, with some findings resulting in the development of plant-based medications. The worldwide market value of medical plant products surpasses $100 billion annually[3].

Medicinal plants have always been acknowledged for their efficacy in treating inflammatory diseases. Infection or tissue injury induces the cardinal indications of inflammation: redness, swelling, heat, and pain, which are traditionally used

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to define the condition[4]. Inflammatory disease is an extensive phrase that includes several medical diseases impacting numerous organs and tissues. Inflammatory disorders encompass inflammatory bowel disease, atherosclerosis, rheumatoid arthritis, multiple sclerosis, asthma, and psoriasis[5].Certain plants, including Curcuma longa, *Zingiber officinale, Rosa canina, Cassia fistula, Salvia officinalis, Borago officinalis, Rosmarinus officinalis,* evening primrose*, Ribes nigrum, Olea europaea, Harpagophytum, and Elaeagnus angustifolia*[6], have demonstrated anti-inflammatory properties.

Moreover, medicinal plants are abundant in antioxidants owing to their varied phytochemical composition, which encompasses phenolic compounds, flavonoids, tannins[7], and other bioactive components. Plants having therapeutic properties are becoming increasingly important in the food and pharmaceutical industries due to their ability to prevent and treat diseases[8]. Oxidative stress has been established as a primary factor of the onset and progression of various illnesses. Exogenous antioxidant supplementation or enhancing the body's endogenous antioxidant defenses is a promising approach to treating the negative consequences of reactive oxygen species (ROS)-induced oxidative damage. Plants have a natural ability to produce a wide spectrum of non-enzymatic antioxidants that can reduce ROS-induced oxidative damage[9], [10].

Ipomoea involucrata, a species in the Morning Glory family, has been traditionally used for various medicinal purposes in African medicine, including wound treatment, antimicrobial properties, malaria treatment, and digestive health[11]. It promotes tissue regeneration and reduces infection risk. The plant's leaves are crushed for healing and used topically for skin infections. Herbal preparations for malaria symptoms reportedly use its antimalarial properties[12]. Although few of the medicinal applications of *Ipomoea involucrata* have been scientifically evaluated, many of the medicinal local claims have not. The study aims to assess the anti-inflammatory and antioxidant activities of crude leaf extracts and fractions of *Ipomoea involucrata* using albino Wistar rats.

2. Materials and Methods

2.1. Apparatus and Equipment

The following materials were used in the course of this study: spectrophotometer (B. Bran Scientific and Instrument Company, England), analytical weighing balance (Mettler H30, Switzerland), glass column, flasks, beakers, test tubes, measuring cylinders, rotary evaporator, electric oven (Gallenkamp, England), water bath (Techmel and Techmel, Texas, USA), national blender (Japan), micropipette (Finn pipette® Lab systems, Finland), syringes and needles, medical hand gloves, and dissection kits.

2.2. Reagents and Drugs

The following chemicals and drugs were used in the course of the research: ethanol, butanol, n-hexane, ethyl acetate (JHD, Guangdong Guanghua Schi-Tech. Ltd China), formaldehyde 40% w/v, carrageenan, diclofenac, and povidone (Sigma LTD USA). All reagents and chemicals were supplied and purchased from Sigma-Aldrich, USA.

2.3. Plant Collection and Authentication

Fresh *Ipomoea involucrata* leaves were picked from the botanical garden of the Faculty of Pharmaceutical Sciences at Nnamdi Azikiwe University in Awka between 6:30 and 7:35 a.m. A taxonomist at the same university's Department of Botany authenticated the plant, and voucher number PCG/474/C/017 was issued by the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu campus.

Figure 1 *Ipomoea involucrata* photograph taken from the Department of Pharmacognosy and Traditional Medicine's herbarium at Nnamdi Azikiwe University's Faculty of Pharmaceutical Sciences in Awka

2.4. Animals

Healthy albino Wistar rats of either sex, with an average weight of 160–180 g, were obtained from the Animal Facility of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka. The animals were confined in clean metabolic cages, provided with clean drinking water ad libitum, and fed commercial pelleted feed (Guinea Feed®, Nigeria) in the animals' house at the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. The National Research Council's Guide for the Care and Use of Laboratory Animals [13] was followed in the handling of the animals. They were permitted to acclimate for a period of ten days prior to their utilization.

2.5. Extraction

Freshly collected leaves of *Ipomoea involucrata* were washed under a running tap and dried at room temperature for two weeks. Crisply dried leaves were pulverized using a mechanical grinder and cold-macerated using 16.6 liters of 80 % aqueous ethanol (ratio 1:3) in batches for a period of 48 h, with occasional agitation. The filtrate was recovered with the aid of a muslin cloth. The leaves were cold macerated in fresh 80% ethanol for another 48 h on two occasions. The final filtrate recovered was concentrated using a water bath set at 40°C to form a greenish paste. Percentage yield was calculated similar to [14]

$$\text{Percentage yield (\% yield)} = \frac{\text{Weight of extract}}{\text{Weight of plant material used}} \times \frac{100}{1}$$

2.6. Fractionation

The extract underwent liquid-liquid partitioning with n-hexane, ethyl acetate, and butanol, following the methodology outlined by Omoirri *et al.* [15]. The extract (80 g) was dispersed in 250 mL of distilled water. The solution was placed into a 1000 ml separating funnel, mixed with n-hexane (500 ml) by inversion, and allowed to stand until two-phase separation occurred. The upper layer (n-hexane partition) was separated, and the lower section was exposed to new n-hexane solvent until the upper layer became transparent. Following the n-hexane phase, the remaining fraction was treated with ethyl acetate, followed by butanol. The leftovers were used as the water fraction. The method was repeated with different extract sets to get appropriate amounts of the individual fractions. All of the fractions were filtered and concentrated to dryness in a water bath set at 40°C.

2.7. Phytochemical Analysis

The ethanol leaf extract of *Ipomoea involucrata* was screened for the presence of proteins, carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids using the methods of Yadav and Agarwala [16]. A 1 ml volume of each sample was used for each test. Moreover, water (1 ml) served as a control in each test carried out.

2.8. Acute toxicity test

This was carried out using a modified Lorke's [17] method. A total of 13 animals were used. In phase one, nine animals were divided into three groups of three animals each. Each group of animals was administered different doses (10, 100, and 1000 mg/kg) of the crude extract. The animals were observed for 24 hours to monitor their behavior as well as if mortality will occur. In the absence of mortality, the animals were introduced to higher doses of the extract in phase two: 2000, 3000, 4000, and 5000 mg/kg of plant extract. The animals were carefully monitored for signs of toxicity and mortality. The acute toxicity (LD50) was determined by the formula LD50 = √(Da x Db), where Da = the highest dose of extract that caused no mortality, while Db = the lowest dose that produced mortality.

2.9. Study Design

2.9.1. Carrageenan-induced arthritis

The effect of the crude extract and fractions of *Ipomoea involucrata* on arthritis was studied using the carrageenan-induced arthritis model in rats as described by Udegbunam *et al.* [18]. A total of 65 healthy Wistar rats were used. They were grouped into 13 groups of 5 rats per group (n=5) as follows:

- Group 1 received 10ml/kg of distilled water
- Group 2 received carrageenan only.
- Group 3 received 50 mg/kg of diclofenac.
- Group 4 received 250 mg/kg of crude extract.
- Group 5 received 500 mg/kg of crude extract.
- Group 6 received 250 mg/kg of ethyl acetate fraction.
- Group 7 received 500 mg/kg of ethyl acetate fraction.
- Group 8 received a 250 mg/kg n-hexane fraction.
- Group 9 received 500 mg/kg n-hexane fraction.
- Group 10 received a 250 mg/kg butanol fraction.
- Group 11 received 500 mg/kg butanol fraction.
- Group 12 received 250 mg/kg of water fraction.
- Group 13 received 500 mg/kg of water fraction.

After one hour of treatment, the animals were injected with 0.1 mL of 1% w/v suspension of carrageenan into the sub-plantar region of the right hind paw. The paw volumes were measured as follows: 0, 1, 2, 3, 4, and 5 after carrageenan injection using a plethysmometer, and the mean increase in paw volume was noted. The edema volumes in the control (Vc) and in the groups treated with test compounds (Vt) were calculated. The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition } = \frac{(VC - VT)}{VC} \times 100$$

Where

- Vc: mean edema volume in the control group (untreated).
- Vt: mean edema volume in the treated group. This formula quantifies the anti-inflammatory efficacy of test fractions by comparing edema reduction to untreated controls.

2.9.2. Antioxidant assay

Blood samples collected from the studied animals were used to evaluate the following biochemical assays: superoxide dismutase (SOD), catalase level (CAT), malondialdehyde (MDA), and reduced glutathione (GSH) level. The SOD was carried out similar to Pal *et al.*, [19] while catalase was carried out using the spectrophotometric assay procedure for assessments of catalase activity in biological samples as described by Hadwan and Ali [20]. Also, the MDA levels was tested similar to Qurnianingsih *et al.*, [21] while serum reduced glutathione (GSH) level was determined similar to Ojuederie *et al.*, [22] and Ojetola *et al.*, [23] .

2.9.3. Statistical Analysis

The data obtained from the study were analyzed utilizing the Statistical Package for the Social Sciences (SPSS-27). The data were subjected to one-way analyses of variance (ANOVA), followed by a post hoc Tukey's test. P-values within the

range ($p<0.05$) were deemed statistically significant. Results were expressed as the mean ± standard deviation (SD) of sample replicates.

3. Results

3.1. Phytochemical content of *Ipomoea involucrata*

The ethanol extract and fractions of *Ipomoea involucrata* showed the presence of various phytochemical constitutions such as saponins, alkaloids, flavonoids, tannins, cardiac glycosides, reducing sugar, phenols, etc., as shown in Table 1 below.

Table 1 Phytochemical screening of *Ipomoea involucrata*

Phytochemicals	**Crude**	**Ethyl Acetate fraction**	**Butanol fraction**	**n-hexane fraction**	**Water fraction**
Alkaloids Wagner's test	++	 +++	++	+	+
Flavonoids Shinoda test Alkaline reagent test	 +++	 +++	 ++	 +++	 ++
Steroids Liebermann-Burchard test	 +++	 ++	 ++	 +++	 +
Terpenoids Salkowski test	 ++	++	 ++	 ++	 ++
Anthroquinone Borntrager s test	++	 +	++	+++	++
Phenols Ferric chloride test Lead acetate test	 + ++	 +++ ++	 ++ +	 ++ ++	 +
Saponin Frothing test	+++	+++	++	++	+
Tannin Gelatin test	++	++	+++	++	++
Carbohydrates Iodine test	 ++	- 	 +	 +	 +
Proteins and Amino acids Ninhydrin test Millon's test	-	 + +	-	-	-
Resins	++	++	+	++	+
Glycosides Keller- Killani test Liebermann's test	+++ ++	+ ++	+++ +	++ +	+

(-) => Not Present, (+) => Faintly Present, (++) => moderately present, (+++) => Highly present

3.2. Acute toxicity test

The extract as well as fractions never recorded any signs of toxicity. This implies that the LD50 of *Ipomoea involucrata* ≥ 5000mg/kg.

Table 2 LD50 *Ipomoea involucrata*

Phases	Dose (mg/kg)	Mortality
Phase 1	10	0/3
	100	0/3
	1000	0/3
Phase 2	2000	0/1
	3000	0/1
	4000	0/1
	5000	0/1

3.3. Effects of *Ipomoea involucrata* on Carrageenan-induced edema in rats

The effect of ethanol extract of *Ipomoea involucrata* on carrageenan-induced edema in rats is as shown below. The extracts (250 and 500 mg/kg) exerted a significant ($P<0.05$) anti-inflammatory effect in a dose-dependent manner, which was comparable to the standard drug, diclofenac, 15 mg/kg. It was seen from results that ethyl acetate fraction (500 mg/kg) gave the highest reduction rate (90.94%) after 5 hours.

Table 3 Anti-inflammatory activities of *Ipomoea involucrata* in Wistar rats

Treatment	**Changes in paw diameter (mm) after dose administration**					
	0hr	**1hr**	**2hr**	**3hr**	**4hr**	**5hrs**
Normal control	100.00±0.00 (0.00)	100.00±0.00 (0.00)	100.00±0.00 (0.00)	100.00±0.00 (0.00)	100.00±0.00 (0.00)	100.00±0.00 (0.00)
Carrageenan	100.00±0.00 (0.00)	104.32±0.16 (-4.32)	104.90±0.38 (-4.9)	105.09±0.11 (-5.09)	105.22±0.32 (-5.22)	106.31±0.23 (-6.31)
Carrageenan + Diclofenac (15 mg/kg bw)	100.00±0.00 (0.00)	69.43±0.07 (30.57)	60.56±1.33 (39.44)	53.22±0.10 (46.78)	34.77±0.56 (65.23)	18.05±0.13 (81.95) ***
Carrageenan + (Crude extract 250 mg/kg bw)	100.00±0.00 (0.00)	87.66±0.13 (9.88)	73.44±0.67 (20.59)	70.09±0.79 (24.61)	63.76±0.17 (28.42)	50.05±0.23 (32.84) *
Carrageenan + (Crude extract 500 mg/kg bw)	100.00±0.00 (0.00)	71.43±0.21 (28.57)	60.09±0.14 (35.04)	48.77±0.12 (39.91)	42.12±0.36 (57.88) *	30.52±0.18 (69.48) *
Carrageenan + (Ethyl acetate fraction 250 mg/kg bw)	100.00±0.00 (0.00)	75.15±0.15 (24.85)	64.37±0.21 (35.63)	45.56±0.26 (54.44)	41.07±0.74 (58.93) *	19.12±1.38 (80.88) *
Carrageenan + (Ethyl acetate fraction 500 mg/kg bw)	100.00±0.00 (0.00)	60.65±0.76 (39.35)	50.12±0.13 (49.88)	40.27±0.39 (59.73)	32.31±0.54 (67.69) *	9.06±0.20 (90.94) *
Carrageenan + (n-hexane fraction 250 mg/kg bw)	100.00±0.00 (0.00)	89.28±0.18 (10.72)	76.54±0.76 (23.46)	63.55±1.22 (36.45)	58.32±0.77 (41.68)	45.37±1.43 (54.63) *
Carrageenan + (n-hexane fraction 500 mg/kg bw)	100.00±0.00 (0.00)	77.86±0.55 (22.14)	71.24±1.42 (28.76)	60.11±1.54 (39.89)	51.15±0.49 (48.85)	40.03±1.31 (9.97) *
Carrageenan + (butanol fraction 250 mg/kg bw)	100.00±0.00 (0.00)	75.43±0.11 (24.57)	64.42±1.65 (35.58)	52.11±1.46(47.89)	43.12±0.33 (56.88) *	26.11± (73.89) *
Carrageenan + (butanol fraction 500 mg/kg bw)	100.00±0.00 (0.00)	65.43±0.61 (28.57)	54.21±1.32 (35.04)	41.17±0.64(58.83) *	33.43±0.60 (66.57) *	19.11±0.18 (80.89) *
Carrageenan + (water fraction 250 mg/kg bw)	100.00±0.00 (0.00)	85.23±0.27 (10.72)	79.04±0.43 (23.46)	60.28±0.65 (36.45)	54.21±1.42 (41.68)	47.07±0.31 (54.63) *
Carrageenan + (water fraction 500 mg/kg bw)	100.00±0.00 (0.00)	77.09±1.06 (22.91)	68.03±0.32 (31.97)	55.15±0.54 (44.85) *	43.21±1.09(56.79) *	34.54±0.28 (65.46) *

Values are expressed as Mean ±SD for five animals per group. Values with the same superscript letter are not significantly different (one way ANOVA followed by Tukey‟s test) ($p<0.05$) is statistically significant. Percentage reduction in the size of the edema is given within parenthesis.

3.3.1. Effects of crude extract and fractions of Ipomoea involucrata in vivo antioxidant parameters

In table 4, there is significant reduction in SOD, CAT, and GSH levels, along with an increase in MDA levels, was seen in Carrageenan-treated rats. The oral administration of the crude extract and fraction at doses of 250 and 500 mg/kg, as well as diclofenac at 15 mg/kg, significantly ($P<0.05$) reversed the effect. The reversal of the effects was dependent upon the dose. Ethyl acetate, n-hexane fraction, and ethanol extract significantly ($P<0.05$) enhanced SOD, CAT, and GSH levels while reducing MDA more significantly than the other extracts when compared to the control group, as illustrated in table 4 below.

Table 4 Effects of *Ipomoea involucrata* fractions on activities of SOD, CAT, MDA and GSH in Wistar rats

Group	Treatment	SOD (U/mg protien)	CAT (µg/mg)	MDA (µg/mg)	GSH (ug/mg protein)
1	Normal Control	8.24±0.05	84.43±0.60	45.52±0.32	1.89 ± 0.66
2	Carrageenan	3.34±0.08#	38.54±0.44#	98.83±0.54#	1.12 ± 0.49#
3	Carrageenan + Diclofenac (15 mg/kg bw)	8.65±0.07*	82.14±0.40*	46.14±1.21*	1.83 ± 1.04*
4	Carrageenan + (Crude extract 250 mg/kg bw)	6.85±0.07	77.66±0.50	56.14±1.21	1.55 ± 0.51
5	Carrageenan + (Crude extract 500 mg/kg bw)	8.87±0.06*	83.55±0.43*	46.55±0.42*	1.80 ± 0.27*
6	Carrageenan + (Ethyl acetate fraction 250 mg/kg bw)	6.40±0.07	78.65±0.30	54.45±1.11	1.50 ± 0.71
7	Carrageenan + (Ethyl acetate fraction 500 mg/kg bw)	8.03±0.05*	81.46±0.87*	45.56±0.60*	1.77 ± 0.66*
8	Carrageenan + (n-hexane fraction 250 mg/kg bw)	7.95±0.08	68.99±0.80	65.34±0.28	1.45 ± 0.32
9	Carrageenan + (n-hexane fraction 500 mg/kg bw)	8.58±0.07*	85.86±0.35*	46.21±0.44*	1.87 ± 1.33*
10	Carrageenan + (butanol fraction 250 mg/kg bw)	6.17±0.09	78.11±0.60	61.15±0.65	1.40 ± 0.14
11	Carrageenan + (butanol fraction 500 mg/kg bw)	8.66±0.08*	80.15±0.27*	46.20±1.44*	1.68 ± 0.25*
12	Carrageenan + (water fraction 250 mg/kg bw)	6.11±0.07	78.09±0.49	54.89±0.77	1.48 ± 0.42
13	Carrageenan + (water fraction 500 mg/kg bw)	8.60±0.08*	82.63±0.27*	46.36±0.29*	1.85±0.86*

All the values are expressed as mean ± SD , n=5 in each group. Data analyzed with ONE WAY ANOVA followed by Tukey‟s test. #P<0.001: significant difference from Carrageenan control. *P<0.05 compared with normal control.

4. Discussion

Acute toxicity of *Ipomoea involucrata recorded* in our study indicates that the plant is relatively safe since the lethal dose at 50% exceeds 5000 mg/kg, having observed no visible signs of toxicity and mortality. Previous study by Gbadamosi *et al.* [24] has shown no mortality in tested animals following oral treatment with 2000 mg/kg body weight, indicating a high margin of safety. Moreover, another study demonstrated that aqueous extracts of the leaves and stem of *Ipomoea involucrata* are considerably safe, suggesting potential for therapeutic use without significant toxicity[25]. Therefore, our study aligns with several studies that involved acute toxicity testing, biochemical and hematological analyses, and in vivo pharmacological assessments, all of which have confirmed that *Ipomoea involucrata* is safe at the doses tested in animal models. This supports its traditional medicinal use and potential for drug development [26] ,[27] ,[28].

Plants are a prolific source of secondary metabolites, referred to as phytochemicals, which are categorized as alkaloids, polyphenols, flavonoids, saponins, carotenoids, and terpenes[29]. In our study, as seen in table 1, *Ipomoea involucrata* extract contains abundant phytochemicals such as steroids, flavonoids, alkaloids, and saponins. These phytochemicals interact with several molecular targets and signaling pathways, including NF-κB, MAPKs, STAT, and Nrf2, to diminish the expression of inflammatory mediators, oxidative stress, and pathological inflammation[30], [31]. This multi-target modulation enhances their potential as natural anti-inflammatory medicines with reduced adverse effects relative to conventional medications[32]. Our findings therefore align with a study analyzing the leaves, stems, and petals of *Ipomoea involucrata* that detected saponins, tannins, flavonoids, alkaloids, and anthraquinones in all parts, suggesting multiple therapeutic potentials[33].

In table 3, the ethanol extracts of *Ipomoea involucrata* and fractions were evaluated for anti-inflammatory activities using low (250 mg/kg) and high (500 mg/kg) respectively. In the carrageenan-induced edema, the extract (250 and 500 mg/kg) was observed to have exerted a significant dose-dependent effect at the early stage of inflammation, indicating an effect probably on histamine, serotonin, and kinins that are involved in the early stage of carrageenan-induced edema [11]. The extract's further reduction of the later stage of the edema may be due to its ability to inhibit prostaglandin, which is known to mediate the second phase of carrageenan-induced inflammation. Similarly, diclofenac (15 mg/kg), whose mechanism of action involves inhibition of prostaglandin, produced a reasonable inhibition of the paw swelling induced by carrageenan injection. The extract also demonstrated that it can inhibit inflammation by blocking the release of histamine and 5-HT, two mediators that are released by carrageenan [34].

Although low (carrageenan + butanol fraction 500 mg/kg bw) and high (carrageenan + butanol fraction 500 mg/kg bw) doses of butanol fractions exhibited statistically significant ($p < 0.05$) reduction in paw edema, low (250 mg/kg) and high (500 mg/kg) doses of ethyl acetate fractions produced the highest percentage (80.88% and 90.94%) reduction in paw edema induced by carrageenan. Other fractions could not exhibit better reduction after 5 hours. Based on results of our study, we established that the extraction process using various solvents has a substantial impact on the yield and activity of bioactive compounds, which in turn leads to variations in biological activities. Ethyl acetate extraction is favored due to its ability to dissolve a diverse array of bioactive phytochemicals, including flavonoids, phenolic compounds, and some alkaloids, which are frequently associated with antioxidant, antibacterial, and anti-inflammatory properties. According to Pochapski *et al.* [35] and Islam *et al.*[36] ,these phytochemicals have reduced solubility in non-polar solvents such as hexane in table 3 and increased solubility compared to water, rendering ethyl acetate an ideal solvent for their extraction.

In table 4, our findings demonstrated the effects of *Ipomoea involucrata* fractions on activities of SOD, CAT, MDA and GSH in Wistar rats. Carrageenan-induced inflammation is frequently used to study arthritis and the anti-inflammatory properties of certain compounds [37] .Inflammation and oxidative stress are two closely related events. Free radicals and reactive oxygen species (ROS) are continuously generated inside the body as a result of exposure to many exogenous drugs and xenobiotics. Under normal conditions, there is equilibrium between the ROS generated and the antioxidants present, as the ROS generated are neutralized by endogenous antioxidants[38]. ROS have detrimental consequences because of an imbalance between their generation and inactivation, which results in abnormalities in cellular physiology and many clinical conditions. In the study, the significant decrease ($P<0.05$) of GSH, CAT, and SOD levels in tissues observed might be one of the causes for the development of inflammation. Lower concentrations of GSH, CAT, and SOD have been implicated in inflammatory diseases by excessive accumulation of free radicals [39]. Oral administration of the extracts or diclofenac significantly ($P<0.05$) increased the level of GSH and SOD in the tissues. Observed significant increases in levels of GSH, CAT, and SOD in tissues of pretreated groups show that the extract tends to prevent the tissues' depletion of GSH and restore CAT and SOD enzymes.

Moreover, the significant increase ($P<0.05$) of MDA in the control group as compared to normal rats in our study may be attributed to the development of carrageenan-induced inflammation related to arthritis [37]. Higher concentrations of MDA have been implicated in inflammatory diseases. According to some studies by Raghavan *et al.*[40],Cordiano *et al.*[41] and Merino de Paz *et al.*[42], elevated MDA acts as both a marker and mediator of oxidative stress-induced inflammation, contributing to the pathogenesis and progression of multiple inflammatory diseases by activating inflammatory signaling pathways and cytokine networks. In our study, *Ipomoea involucrata* extract significantly ($P<0.05$) reduced the level of MDA compared to the control group in a dose-dependent manner.

Our study has demonstrated that *Ipomoea involucrata* extract fractions, especially ethyl acetate fractions, exhibit both anti-inflammatory effects and antioxidant properties**.** This is consistent with a study on *Annona senegalensis, Ipomoea batatas, Terminalia superba*, and *Psidium guajava*. Furthermore, a study of *Blighia sapida* leaves revealed substantial antioxidant and anti-inflammatory properties due to phenolic components such as tannins, flavonoids, alkaloids, and saponosides. The extract suppressed free radicals and protein denaturation, indicating its ability to counteract

oxidative and inflammatory processes associated with chronic diseases[43].Furthermore, the potential for strong antioxidant activities in certain Chinese traditional medicinal herbs, including *Salvia miltiorrhiza, Rabdosia rubescens*, and *Sanguisorba officinalis,* has been assessed. These herbs are believed to possess anti-inflammatory and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging properties[44] that are comparable to the results of our study with *Ipomoea involucrata*. Plants with anti-inflammatory and antioxidant properties offer potential for reducing oxidative stress, modulating inflammation pathways, and providing therapeutic agents for preventing or managing inflammation-related conditions.

5. Conclusion

Our study successfully evaluated the safety of crude extract of *Ipomoea involucrata* in African traditional medicinal therapeutic purposes, clarifying its safe use even in raw forms. It was established that *Ipomoea involucrata* is loaded with phytochemicals. *Ipomoea involucrata* demonstrated potent anti-inflammatory and antioxidant effects, which might explain some of its folkloric uses and pharmacological effects. This study unveils the bioactive nature of *Ipomoea involucrata, which* is more potent in the ethyl acetate fraction and exhibits significant activity. The study suggests that *Ipomoea involucrata* could be useful for managing cancer and conditions related to chronic arthritis or inflammation.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare that there is no conflict of interest.

Statement of ethical approval

The Nnamdi Azikiwe University-Animal Research Ethics Committee (NAU-AREC) granted ethical authorization for the study with approval number *NAU/AREC/ 2025/0007*. Throughout the study, representatives of the animal ethics committee supervised the animals to ensure that they were sacrificed humanely and that the bodies were disposed of correctly.

Authors Declaration

No competing interest is disclosed by any of the authors.

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