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# Impact of Seasonal Changes on the Phytochemical Composition, Analgesic and Anti-Inflammatory Properties of Ethanol Extract from the Aerial Parts of *Waltheria indica* L. in Rodents

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## ABSTRACT

**Background and Objective:** Seasonal changes significantly influence the phytochemical compositions and therapeutic potentials of medicinal plants. This study seeks to examine the influence of seasonal changes on the phytochemical composition and the analgesic and anti-inflammatory effects of ethanol extracts from the aerial parts of *Waltheria indica* L. in rodents. **Materials and Methods:** Samples collected during the wet and dry seasons were subjected to standard extraction and spectrophotometric methods to quantify their phytochemical levels. The extract's analgesic and anti-inflammatory properties were evaluated using established experimental methods. **Results:** Phytochemical screening of the ethanol extract from the aerial parts confirmed the presence of alkaloids, flavonoids, tannins, saponins and terpenes. The winter extract demonstrated significant ($p<0.05$) analgesic and anti-inflammatory effects, as evidenced by the inhibition of acetic acid-induced writhing in mice and a reduction in carrageenan-induced paw edema in rats, compared to controls. At doses of 200 and 400 mg/kg, the summer extract significantly ($p<0.05$) reduced acetic acid-induced writhing by 48.05 and 57.41%, respectively, against control values. Both extracts reduced carrageenan-induced rat paw edema, with the summer extract showing a 64.52% reduction and the winter extract achieving a 79.60% reduction at a 400 mg/kg dose, 4 hrs post-carrageenan injection. Comparative analysis revealed that the winter extract produced a higher inhibition of carrageenan-induced paw edema than the summer extract (79.60 vs 64.52%). **Conclusion:** The ethanol extract of *W. indica* aerial parts collected in winter exhibited superior analgesic and anti-inflammatory activity, suggesting that winter may be the optimal season for harvesting *W. indica* for medicinal purposes.

## KEYWORDS

Analgesic activity, anti-inflammatory properties, ethanol extract, Northern Nigeria, paw edema, dry season extract, wet season extract



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## INTRODUCTION

*Waltheria indica* L. (Malvaceae) is a Tropical West African plant commonly called monkey bush, velvet leaf, sleeping morning and marshmallow. In Northern Nigeria, this plant is frequently utilized, particularly by the Fulani tribe to treat inflammatory and pain disorders[1-3]. Its extracts are used to relieve pain and inflammation in other regions of the world, particularly by practitioners of Hawaiian traditional medicine[4].

Due to its widespread distribution in Nigeria and its ability to grow throughout the year, this plant may be a cheap source of raw materials for analgesic and anti-inflammatory medications. It may also offer an alternative to the ever-rising costs of medications like Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and the negative side effects that come with them[4]. Generally, traditional medicine practitioners in Nigeria believe that seasonal variation affects the Phyto active components of medicinal plants. Botanicals collected during dry seasons have a higher concentration of bioactive compounds (personal communication). This implies that the pharmacological potentials of plant material are influenced by the period of collecting for therapeutic purposes.

The anti-inflammatory properties and bioactivities of *W. indica* extracts were attributed to its phenolic components, specifically flavonoids, alkaloids and saponins[5-7]. Epicatechin, quercetin, alkaloids, waltherione, kaempferol and tiliroside, among others, are additional secondary metabolites necessary for the plant's pharmacological actions[6]. Adouetin X, Y, Y1 and Z are the first known cyclopeptide alkaloids found in the leaves and roots of the plant[7]. Recent investigations have demonstrated a wide range of pharmacological effects, including antioxidant, analgesic, anti-inflammatory, sedative, anti-fungal and anti-parasitic properties for both crude and refined substances derived from the whole plant[7-9]. The hydro-alcoholic extract of the plant contained alkaloids, flavonoids, hormones, terpenoids, phenols, tannins and biochemical carbohydrates. Phytochemical analysis confirmed the presence of flavonoids, terpenoids, phenols and carbohydrates; however, flavonoids and resin phytoconstituents were present in a chloroform extract[10].

When tested in lipopolysaccharide and interferon-activated peritoneal macrophages, flavonoid derivatives like tiliroside, (-)- epicatechin and quercetin derived from sequentially fractionated ethanol whole plant extract demonstrated dose-dependent inhibition of inflammatory mediating cytokines, Tumor Necrosis Factor (TNF)-α, nitric oxide (NO) and interleukin (IL-12) without causing any cytotoxicity[7]. In carrageenan-induced edema, methanol leaf extract and an aqueous extract of the stem and leaves also demonstrated dose-dependent reduction of both acute and chronic inflammations[11].

The body uses inflammation as a defense mechanism against pathogens including bacteria, viruses, parasites, or other stimuli. It is a self-regulating process intended to prevent tissue damage or infection[12]. Pro-inflammatory cytokines, prostaglandins and reactive oxygen species (ROS) are all released during the inflammatory process. Overproduction of these inflammatory mediators causes chronic inflammation and maintains inflammation[8]. Enzymes like cyclooxygenase (COX) and lipoxygenase (LOX) can catalyze the metabolism of arachidonic acid, which is a component of inflammation. Leukotrienes and prostaglandins are inflammatory mediators generated from arachidonic acids, respectively by lipoxygenase and cyclooxygenase[13].

The isozymes Cox-1 and Cox-2 are found in normal human skin through the epidermis, but Cox-2 mostly localizes in suprabasal keratinocytes[13]. Pro-inflammatory cytokines such as Interleukin-16 (IL-16) are produced in greater quantities when PGE-2 levels are raised and neutrophils and macrophage immune cells react to this. The isozymes COX-1 and 2 are the targets of Non-Steroidal Anti-Inflammatory Medications (NSAIDs), which are used to treat inflammation[14]. Long-term NSAIDS use is known to cause a number of adverse consequences such as peptic ulcers, nephrotoxicity and gastrointestinal erosion. The negative effects and rising costs of conventional medications prompted the search for alternative plant derivatives.

In Nigeria, traditional medicine practitioners utilize the aerial parts decoction to heal wounds, abscesses, asthma, toothaches, swellings and rheumatism[5,15,16]. The time and season in which plant samples were collected for medicinal uses have a great quantitative impact on the phytochemicals in plant extracts[17,18]. Research findings on the impact of seasonal variations on the phytochemical composition of certain medicinal plants stressed that environmental conditions influence the phytochemical composition of medicinal plants[19]. By studying the analgesic and anti-inflammatory effects of the plant, this study sought to determine the pharmacological potentials of ethanol extract of *W. indica* aerial parts (stem, leaves and flowers) collected in August, (wet season) and March, (dry season). This will serve as baseline information about the optimal time of year to collect *W. indica* parts with the highest concentration of phytochemicals for effective bioactivity.

## MATERIALS AND METHODS

**Study duration:** The research began in March, 2022 (summer) and August, 2023 (winter) with the collection of plant material while laboratory investigation commenced by September, 2023 and ended in July, 2024.

**Animals:** Research animals were handled and treated in according with the Principle of Laboratory Animal Care (National Institute of Health Publication No. 85-23) and ethical guidelines for the examination of experimental pain in conscious animals[20]. All experimental procedures were carried out in compliance with the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals. The (36) male Swiss mice (21.5-29.5 g) were used for analgesic studies and 36 Wistar rats (150-200 g) for the inflammatory studies. They were acclimatized under standard laboratory conditions, (temperature: 21±2°C; 12 hrs/12 hrs light-dark cycle) for 2 weeks and were allowed free access to water and feed (ECWA FEEDS, Jos, Nigeria).

**Plant materials:** Fresh *W. indica* aerial parts (stem, leaves and flowers) were collected from open fields around Samaru. The Campus of Ahmadu Bello University, Zaria (11.1247°N, 7.7254N°E), Kaduna State, Nigeria. The fresh plant materials were collected in March, 2022 (dry season,) and August, 2023 (wet season), respectively. Taxonomic identification was carried out with a herbarium voucher specimen (NPR 2006) in the herbarium of the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria.

**Extraction from plant materials:** The plant materials were cleaned, allowed to air dry and then oven-dried for two hours before it was sieved and ground into powder with a pestle and mortar[21]. Using ethanol as the solvent, 500 g of the powdered aerial portion of each collection was put in a Soxhlet apparatus, with 60% v/v aqueous ethanol for thorough extraction. After concentration, the aqueous ethanol extract produced by the dry season collection gave a residue known as extract (14.85%). Following that, the solvent was extracted in rotavapor at 52°C and lower pressure. This process was repeated for the samples collected during the wet season (August) and an extract (15.10 %) was produced.

The yield was calculated using the formula:

$$\text{Extract yield (\%)} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of aerial part (g)}} \times 100$$

**Phytochemical screening:** Thin layer chromatography (TLC) was used to screen the ethanol extract of both extracts for phytochemicals using chromatoplates (60 F254, 10×5 cm, 10×20 cm glass support, Merck)[22]. Following their solubilization in their respective extraction solvents, 5 µL of dry extract from each collection was placed on the plate to observe the chromatogram's progression.

Path chromatography, which uses thin-layer chromatography (TLC) to screen for chemical groups including steroids, terpenes and phenolics, was developed using polar and nonpolar solvent systems. TLC was used to identify the extracts through phytochemical screening and physicochemical analysis.

Various spraying reagents were used for specific compounds, including Dragendorff's for alkaloids, AlCl3 for flavonoids, $FeCl_3$ for tannin and phenolic, Lieberman Burchard for steroids and sulphuric acid for saponin/triterpenoid[23]. Water content, total ash content, acid-insoluble ash content and soluble extractive materials[24].

**Flavonoids (qualitative and quantitative):** The alkaline-reagent test was used to identify flavonoids: 2 µL of 5% Sodium Hydroxide (NaOH) was added to test tubes that contained 2 µL of the dry season extract (DSE) and wet season extract (WSE) crudes, respectively. The presence of flavonoids is confirmed by a yellow solution that is decolorized when 1 mL of 50% $H_2SO_4$ is added. Employing aluminum chloride and the colorimetric method[25], the extract's total flavonoids were ascertained. The 100 µL of a 2% methanol solution of Aluminum Trichloride ($AlCl_3$) was combined with 100 µL of plant extract (1 mg/mL). After 10 min, a spectrophotometer (Spectrophotometer UV, Epoch Biotech Instruments and U.S.A.) was used to measure the absorbance at 415 nm against a blank, which was 100 µL of methanol and 100 µL of extract without $AlCl_3$. The standard curve was created using quercetin as a reference chemical ($y = 0.01x+0.0128$, $R^2 = 0.9998$). The total flavonoid concentration was measured in milligrams of quercetin equivalent (QE)/g of extract and the assays were run twice.

**Tannins (qualitative and quantitative):** The ferric chloride test was used to ascertain this[26]. Dry and wet season powdered samples weighing 0.5 g each were gently boiled and chilled. The 10% Iron (III) Chloride ($FeCl_3$) solution was added to 1 mL of the resultant solution in a test tube and the mixture was stirred. The presence of tannin is confirmed by a blue-black or brownish-green hue. Hydrolysable tannins: The method[27] was used to determine which tannins were hydrolyzable. 3.5 mL of the reagent (Ferric Chloride $FeCl_3$ 10-2 M in hydrochloric acid HCl 10-3 M) was mixed with 1 mL of each extract (5 mg/mL). After 15 sec, the mixture's absorbance at 660 nm was measured using a Shimadzu UV spectrophotometer. The following formula was used to calculate the hydrolyzable tannin content T (%):

$$T\ (\%) = \frac{A \times PM \times V \times FD}{\varepsilon mole} \times P$$

Where:

A = Absorbance
εmole = 2169 (for gallic acid)
PM = Gallic acid weight (170.12 g/mol)
V = Extract volume
P = Sample weight
FD = Dilution factor

**Condensed tannins:** The method of Jedrejek *et al.*[26] was modified to determine condensed tannins. About 2 mL of vanillin 1% (1 g of vanillin dissolved in 100 mL of 70% sulphuric acid) and 1 mL of extract (5 mg/mL) were combined. After the combination was incubated for 15 mins at 20°C in a water bath, its absorbance was measured at 500 nm using a Shimadzu spectrophotometer UV. The following formula was used to calculate the condensed tannins content:

$$T\ (\%)\text{: } 5.2.\ 10\text{-}2 \times (A \times V/P) = T\ (\%)\ 5.2$$

Where:

A = Absorbance
V = Extract volume
P = Sample
10-2 = Constant in cyanidin equivalency

**Alkaloids:** To identify alkaloids, Mayer's technique (potassium mercuric iodide) was modified. Two drops of Wagner's reagent (aqueous iodine in potassium iodide solution) are placed along the side of a test tube that contains 1 mL of crude extract. The presence of alkaloids is confirmed by the production of a reddish-brown or yellow precipitate.

**Steroids:** Two mL of acetic anhydride were added to 0.5 g of powdered SDS and SWS, respectively. About 2 mL of $H_2SO_4$ was then added and the color shift was utilized as an indicator.

**Terpenes:** Using the Salkowski test procedure, 5 g of powdered extracts were combined with 2 mL of chloroform each. After carefully adding 3 mL of concentrated $H_2SO_4$ to each to create a layer, conclusions were drawn using color shifts.

**Saponins:** According to Auwal *et al.*[27], the frothing test was employed to identify saponins. About 20 mL of distilled water is added to test tubes that contain 5 mL of each extract. The mixture is then violently agitated for fifteen minutes. The presence of saponins was shown by the formation of froth layers. If no froth forms, the observed findings are reported negative; when present, they were recorded as positive (1.2 cm high), strongly positive (>2cm in height) and weakly positive (<1 cm in height).

**Anti-inflammatory**

**Properties rat paw edema test:** The anti-inflammatory effects of the dry-season extract were evaluated on rat paw edema caused by carrageenan[28]. Different inflammatory mediators have been implicated in the developing stages of the carrageenan-induced paw edema model. As a result, it is frequently employed to access plant extract's anti-inflammatory properties. The different groups of rats were administered with ethanol extracts (100, 200 and 400 mg/kg p.o.) and indomethacin (10 mg/kg p.o.). The control group received vehicle (distilled water, 10 mL/kg p.o.). One hour after treatment, paw edema was induced by the injection of carrageenan (an edematogenic agent). The paw volume was measured using a Plethysmometer (Ugo Basile model: 37141). The measurements were determined at 0 hr (Vo: before carrageenan injection) and 1, 2, 3, 4 and 5 hrs intervals later (Vt). The difference between Vt and Vo was taken as the change in paw volume (edema).

**Treatment regimen:** The 30 male and female rats were divided into 5 groups (n = 6 rats):

- **Group 1:** Received vehicle (NaCl 0.9%, i.p.) [negative]
- **Group 2:** Positive control, received the NSAID, indomethacin (10 mg/kg)
- **Group 3:** Received 100 mg/kg
- **Group 4:** Received 200 mg/kg
- **Group 5:** Received the extract at the 400 mg/kg

The extract was administered intraperitoneally.

Then the paw volume of each rat was measured at 1, 3 and 5 hrs (Vt) after carrageenan injection using a plethysmometer (Model Ugo Basil, N°7141, Italy). Edema was expressed as an increase in the volume of paw (ΔV) and Percentage Inhibition (I %) for each treatment was derived by the formula[28]:

$$V = Vt - Vo$$

$$I(\%) = \frac{\Delta Vc - \Delta Vtr}{\Delta Vc} \times 100$$

Where:

Vtr = Right hind paw average increased volume in treated group

Vc = Right hind paw average increase in control group

**Test of lipid peroxidation inhibition:** The old procedures[29,30] was used to assess the extracts' inhibitory effect on lipid peroxidation in an *in vitro* setting. About 50 µL of $FeCl_2$ (0.5 mM), 50 µL of $H_2O_2$ (0.5 mM), 1 mL of rat liver homogenate 1% (100 mL, comprising 1 g of liver) and 0.2 mL of standard or extract at a concentration of 1.5 mg/kg were added to create a combination.

Following a 60 min incubation period, the liquid was cooled after 0.1 mL of trichloroacetic acid and 1 mL were added. At 532 nm, the absorbances were measured.

The expression below was used to calculate the inhibitory capacity of the lipid peroxidation standards and products:

$$\text{Inhibitory (\%)} = \frac{1 - (Ab - As)}{Ao} \times 100$$

Where:
As = Absorbance substance (extract or standard)

**Analgesic assessment:** This study used chemical models (abdominal writhing caused by acetic acid). Every test was carried out by the International Association for the Study of Pain's ethical criteria[29].

**Acetic acid-induced writhing test in mice:** With a few minor adjustments, the acetic acid-induced abdominal writhing test was performed as described by Wateman and Mole[29]. The 60 min after the oral administration of extract dosages of 100, 200 and 400 mg/kg, respectively, mice were given an intraperitoneal injection of 0.6% acetic acid (10 mg/kg) to cause pain in the peritoneal cavity. Between 5 and 15 min following the delivery of acetic acid, the total number of abdominal constrictions was tallied. The decrease in the average number of abdominal constrictions in the test groups relative to the control group demonstrated the analgesic action of the extracts. Indomethacin (cyclooxygenase inhibitor) at a dose of 10 mg/kg served as the reference medication, while the control mice group received 10 mL/kg of pure water:

$$\text{Inhibition (\%)} = \frac{NC_{neg} - NC_{pos}}{NC_{pos}} \times 100$$

Where:
$Nc_{neg}$ = Number of contortions of the negative control and
$Nc_{pos}$ = Number of contortion batch test or the positive control

**Experimental design:**

- **Group 1:** Control group given (saline solution)
- **Group 2:** Indomethacin treated group 10 mg/kg (standard group)
- **Group 3:** 100 mg/kg of ethanol extract aerial part of *W. indica* (DSE)
- **Group 4:** 200 mg/kg of DSE
- **Group 5:** Received 400 mg/kg of DSE
- **Group 6:** Administered 100 mg/kg of WSE
- **Group 7:** Received 200 mg/kg WSE
- **Group 8:** Received 400 mg/kg WSE

**Statistical analysis:** Statistical analysis was carried out by One-way Analysis of Variance (ANOVA) test using a statistical package program (SPSS 10.0). The significance of the difference between means was determined by Duncan's multiple range test at ($p<0.05$) significant level. Analysis was carried out in duplicate and Mean±SD of the two measurements.

## RESULTS

The extract yields, the residual moisture content for WSE and DSE are, respectively 6.16±1.33% and 6.90±0.87% and the results are presented in Table 1. Also, the results of the preliminary phytochemical analysis of *W. indica* aerial collected in different seasons were summarized below in Table 1; while some physicochemical parameters also evaluated were presented in the follow-up Table 2. The physicochemical analysis of *W. indica* aerial parts showed significant seasonal variation. Wet season samples had lower water-soluble extractive (19.6%) and ethanol-soluble extractive (32.9%) compared to dry season samples (36.3 and 50.1%, respectively). Water content was slightly higher in the dry season (7.9%) than in the wet season (6.4%). Total ash content remained similar across seasons (3.1-3.3%), while acid-insoluble ash showed minimal variation (1.7 vs 1.5%).

**Anti-inflammatory effect:** Inflammatory edema was brought on by the sub-plantar administration of carrageenan; it began to form gradually within the 1 hr and peaked in both extracts at the 5 hrs mark (Table 3 and 4). The 5 hrs after carrageenan injection, both aerial component extracts (DSE and WSE) significantly ($p<0.05$) decreased the paw edema caused by the injection by 74.76 and 71.56%, respectively, at a higher dose of 400 mg/kg. When compared to the control, the 400 mg/kg dose of DSE demonstrated a significant ($p<0.05$) anti-inflammatory effect. This effect was gradual and peaked 5 hrs after the carrageenan injection, with a maximum inhibition of edema of 74.76% compared to the standard drug, indomethacin (79.19%).

**Lipid peroxidation test:** Lipid peroxide formation in experimental animals was reduced by the extract. The WSE inhibited the formation of lipid peroxide by 71.38% at a high dose (400 mg/kg), but the DSE inhibited the production by 78.43% at a similar level. The administration of both extracts (DSE and WSE) at doses of 200 and 400 mg/kg, respectively, significantly ($p<0.05$) decreased the acetic acid-induced abdominal writhing in mice as compared to the normal control animals. The WSE provided a protective effect of 48.08 and 57.31% at dosages of 200 and 400 mg/kg, respectively, whereas the DSE produced a protective effect of 53.85 and 62.54% at doses of 200 and 400 mg/kg. The analgesic activity of the reference medication, indomethacin (10 mg/kg), was 60.05%, which is comparable to the dry season extract (62.54%) at a dose of 400 mg/kg (Table 5-7).

Table 1: Phytochemical analysis of *W. indica* aerial parts extracts

| | Dry season aerial part | Wet season aerial part |
|---|---|---|
| Phytochemical compound | (WSE) | (DSE) |
| Alkaloids | + | ++ |
| Flavonoids | + | ++ |
| Steroids | + | ++ |
| Saponins | + | + |
| Tannins | - | + |
| Terpenes | + | + |
| Residual moisture content (%) | 6.16±1.33 | 6.90±0.87 |
| Extract yields (%) | 14.85 | 15.10 |

Table 2: Physicochemical analysis of *W. indica* aerial part

| | Values obtained w/w on dry weight extract | |
|---|---|---|
| Parameter | Wet season sample (%) | Dry season sample (%) |
| Water soluble extractive matters | 19.6±0.9[a] | 36.3±0.7[b] |
| Ethanol soluble extractive matter | 32.9±0.4[a] | 50.1±1.9[b] |
| Water content | 6.4±0.8[a] | 7.9±1.5[b] |
| Total ash content | 3.1±0.9 | 3.3±0.8 |
| Acid insoluble ash content | 1.7±0.7 | 1.5±0.9 |

All values are Mean±SD and Mean values followed by different superscripts in a column were significantly different ($p<0.05$)

Table 3: Anti-inflammatory effect of DSE varying doses on rat hind paw edema induced by carrageenan

| | | Increase in paw volume (ΔV mL) | | | Edema inhibition (%) | | |
|---|---|---|---|---|---|---|---|
| Sample | Doses (mg/kg b.wt.) | 1 hr | 3 hrs | 5 hrs | 1 hr | 3 hrs | 5 hrs |
| Control | - | 0.25±0.08 | 0.39±0.11 | 0.43±0.05 | - | - | - |
| Indometh. | 10 | 0.17±0.32* | 0.18±0.13* | 0.17±0.09* | 47.69 | 58.80 | 79.19 |
| DSE | 50 | 0.24±0.17 | 0.30±0.11* | 0.25±0.12* | 03.59 | 24.62 | 47.05 |
| DSE | 100 | 0.23±0.17* | 0.31±0.02* | 0.22±0.08* | 11.01 | 22.07 | 55.00 |
| DSE | 200 | 0.24±0.21 | 0.22±0.06* | 0.1±0.13* | 14.61 | 40.27 | 67.48 |
| DSE | 400 | 0.11±0.25* | 0.16±0.07* | 0.12±0.11* | 58.65 | 62.54 | 74.76 |

All data represent Mean±SD of six mice, comparisons were made using one-way ANOVA, followed by Dunnett's *post-hoc* test and*p<0.05 compared to control is considered statistically significant

Table 4: Anti-inflammatory effect of WSE varying doses on rat hind paw edema

| | | Increase in paw volume (ΔV mL) | | | Edema inhibition (%) | | |
|---|---|---|---|---|---|---|---|
| Sample | Doses (mg/kg b.wt.) | 1 hr | 3 hrs | 5 hrs | 1 hr | 3 hrs | 5 hrs |
| Control | - | 0.25±0.08 | 0.40±0.15 | 0.42±0.09 | - | - | - |
| Indometh | 10 | 0.17±0.12* | 0.21±0.19* | 0.18±0.19* | 49.76 | 58.88 | 79.06 |
| DSE | 50 | 0.28±0.07 | 0.31±0.24* | 0.23±0.07* | 05.55 | 26.72 | 50.09 |
| DSE | 100 | 0.23±0.13* | 0.29±0.02* | 0.22±0.18* | 13.04 | 29.08 | 57.05 |
| DSE | 200 | 0.25±0.01 | 0.22±0.06* | 0.11±0.17* | 17.63 | 48.27 | 69.48 |
| DSE | 400 | 0.12±0.05* | 0.17±0.11* | 0.12±0.11* | 58.95 | 62.54 | 71.56 |

All data represent the Mean±SD of six mice, comparisons were made using one-way ANOVA, followed by Dunnett's *post-hoc* test and *p<0.05 compared to control is considered statistically significant

Table 5: Effect of DSE varying doses on acetic acid-induced writhing in mice

| Sample | Doses (mg/kg b.wt.) | Number of writhes | Inhibition (%) |
|---|---|---|---|
| Control | - | 63.27±2.36 | - |
| Indomethacin | 10 | 32.30±3.64* | 60.05 |
| DSE | 100 | 33.32±3.54* | 44.58 |
| DSE | 200 | 32.51±5.01* | 53.85 |
| DSE | 400 | 33.53±2.42* | 62.54 |

Values are Mean±SD, n = 6, comparisons were made using ANOVA followed by Dunnett's *post-hoc* test and*p<0.05 was considered significant compared to the control

Table 6: Effect of WSE varying doses on acetic acid-induced writhing in mice

| Sample | Doses (mg/kg b.wt.) | Number of writhes | Inhibition (%) |
|---|---|---|---|
| Control | - | 63.27±2.36 | - |
| Indomethacin | 10 | 32.30±3.64* | 60.05 |
| DSE | 100 | 34.61±2.79* | 42.38 |
| DSE | 200 | 33.76±4.08* | 48.08 |
| DSE | 400 | 31.98±2.08* | 57.41 |

Values are Mean±SD, n = 6, comparisons were made using ANOVA followed by Dunnett's *post-hoc* test and *p<0.05 was considered significant compared to the control

Table 7: Effects of varying doses of WSE on acetic acid-induced writhing in mice

| Samples | Doses (mg/kg b.wt.) | Number of writhes | Inhibition (%) |
|---|---|---|---|
| Control | - | 63.27±2.36 | - |
| Indomethacin | 10 | 32.30±3.64* | 60.05 |
| WSE | 100 | 34.61±2.79* | 44.58 |
| DSE | 100 | 33.51±3.54* | 53.85 |
| WSE | 200 | 33.76±4.08* | 62.54 |
| DSE | 200 | 32.51±5.01* | 53.91 |
| WSE | 400 | 31.98±2.08* | 57.41 |
| DSE | 400 | 33.53±2.42** | 62.54 |

Values are Mean±SD, n = 6, comparisons were made using ANOVA followed by Dunnett's *post-hoc* test, *p<0.05 was considered significant compared to control and while **p<0.05 significant when DSE compared to WSE

**Lipid peroxidation test:** Lipid peroxide formation in experimental animals was reduced by the extract. The WSE inhibited the formation of lipid peroxide by 71.38% at a high dose (400 mg/kg), but the DSE inhibited the production by 78.43% at a similar level. The administration of both extracts (DSE and WSE) at doses of 200 and 400 mg/kg, respectively, significantly ($p<0.05$) decreased the acetic acid-induced abdominal writhing in mice as compared to the normal control animals. The WSE provided a protective effect of 48.08 and 57.31% at dosages of 200 and 400 mg/kg, respectively, whereas the DSE produced a protective effect of 53.85 and 62.54% at doses of 200 and 400 mg/kg. The analgesic activity of the reference medication, indomethacin (10 mg/kg), was 60.05%, which is comparable to the dry season extract (62.54%) at a dose of 400 mg/kg (Table 5-7).

## DISCUSSION

The use of *W. indica* in the treatment of inflammations, rheumatism and discomfort is a popular practice in traditional medicine. It is believed that the aerial parts have more bioactive components during the dry (winter) season, traditional medicine practitioners typically collect the aerial parts during the dry season (December to March). The wet season (winter) in Northern Nigeria spans from April to September, while the dry season (summer) spreads from December to March, marked by high/extreme weather conditions with respect to temperature (Climate Research Unit, CRU).

The study assessed the phytochemical composition of aerial portions of *W. indica* collected during the wet and dry seasons, respectively. The anti-inflammatory and analgesic qualities of the ethanol extract of these plant components were evaluated *in vivo*. Aerial parts collected in the dry season confirmed the presence of alkaloids, flavonoids, steroids, saponins, terpenes and tannins, while extracts from the wet season had flavonoids, steroids, saponins, terpenes and tannins, albeit with less intensity in composition. This study also established that extracts of aerial parts collected in dry season had majority of the phytochemicals and a higher percentage of extract yield when compared to those collected in wet season.

Despite being in distinct plant species, these results are consistent with previous study on *Carissa*, it was observed that *C. macrocarpa* leaf extract collected during the dry season had a much higher pharmacological activity and a significantly higher amount of bioactive chemicals than the same material gathered during the wet season[31-35]. According to Mattoli *et al.*[33], plants under stress create more phytochemicals, such as flavonoids and anthocyanins, to help them survive adverse conditions. In a different study[35], plant samples taken during the dry season had higher amounts of phytochemicals than those taken during the rainy season. This is probably because the harsher environmental circumstances cause the production and storage of secondary defensive metabolites. It was previously documented that seasonal variation in the biological activities of medicinal plants is caused by variations in the seasonal phytochemical concentration of their leaves[16].

This study demonstrated that administering ethanol extracts from the aerial parts of *W. indica* significantly decreased carrageenan-induced paw edema in rats, with effects peaking five hours post-injection in a dose-dependent manner. Oedema was progressively reduced at the tested doses for the WSE, while similar inhibitory effects were observed for the DSE at the same dosages.

Lipid peroxidation has been associated with inflammatory responses[36,37] and this study demonstrated that the extract prevented rat liver lipid peroxidation *in vitro*, indicating the extract's potential anti-inflammatory properties[38]. Mice's acetic acid-induced abdomen writhing was dose-dependently reduced by the dry and wet season extracts. Significant analgesia was produced by the extracts at doses of 100, 200 and 400 mg/kg while at 400 mg/kg, the DSE significantly demonstrated a higher analgesic effect compared to the WSE inhibition of acetic acid-induced writhing in mice, respectively.

## CONCLUSION

The study concluded that the dry season (DSE) is the ideal time to collect Waltheria indica aerial parts for the highest concentration of secondary metabolites responsible for its therapeutic bioactivities. Extracts from plants gathered during this period showed stronger anti-inflammatory and analgesic effects than those from the wet season (WSE). These findings highlight the importance of understanding seasonal phytochemical dynamics for optimizing harvest strategies, enhancing the plant's medicinal potential and aiding conservation efforts. The research underscores the significance of seasonal variations in bioactive compound levels for improving the quality and consistency of medicinal preparations.

## SIGNIFICANCE STATEMENT

Studies on *Waltheria indica* L. gathered in various seasons have demonstrated that seasonal changes have a substantial impact on the phytochemical composition and therapeutic efficacy of plant extracts. These variations may affect the concentration of bioactive substances that have anti-inflammatory and analgesic properties, changing their pharmacological efficacy and potency. The medicinal qualities of *Waltheria indica* may change with seasonal variations, as evidenced by studies evaluating the ethanol extract of the plant's aerial portions in rodents. This emphasizes the significance of harvesting time in maximizing therapeutic advantages. This realization emphasizes the necessity of more research to standardize extraction procedures, guaranteeing constant phytochemical profiles and therapeutic effects throughout the year.

## REFERENCES

1. Niang, S.A.A., M.S. Drame, A. Sarr, M.D. Toure, A. Gueye, S.O. Ndiaye and K. Talla, 2024. Seasonal performance of solar power plants in the Sahel Region: A study in Senegal, West Africa. Smart Grid Renewable Energy, 15: 79-97.
2. Owemidu, I.O., M.A. Olubori, O.S. Faborode, O.S. Oloyede and S.A. Onasanwo, 2018. Anti-nociceptive and anti-inflammatory activities of the methanol extract of *Waltheria americana* leaf in experimental animals. J. Complementary Med. Res., 9: 47-54.
3. Hamidu, L.J., J.O. Ayo, A.B. Adelaiye and M.S. Abubakar, 2008. Sedative and anticonvulsant effects of ethyl acetate fraction of *Waltheria indica* in mice. J. Pharmacol. Toxicol., 3: 261-266.
4. Hutchinson, J. and J.M. Dalziel, 1954. Flora of West Tropical Africa: The British West African Territories, Liberia, the French and Portuguese Territories South of Latitude L8 °N. to Lake Chad, and Fernando Po, Volume 1. 2nd Edn., Crown Agents for Oversea Governments and Administrations, Surrey, United Kingdom.
5. Zongo, F., C. Ribuot, A. Boumendjel and I. Guissou, 2013. Botany, traditional uses, phytochemistry and pharmacology of *Waltheria indica* L. (syn. *Waltheria americana*): A review. J. Ethnopharmacol., 148: 14-26.
6. Nirmala, C. and M. Sridevi, 2021. Ethnobotanical, phytochemistry, and pharmacological property of *Waltheria indica* Linn. Future J. Pharm. Sci., Vol. 7. 10.1186/s43094-020-00174-3.
7. Rao, Y.K., S.H. Fang and Y.M. Tzeng, 2005. Inhibitory effects of the flavonoids isolated from *Waltheria indica* on the production of NO, TNF-α and IL-12 in activated macrophages. Biol. Pharm. Bull., 28: 912-915.
8. Yabré, Z., W.L.M.E. Belem-Kabré, R. Boly, R. Ouédraogo and A.G.L. Boly *et al*., 2024. Evaluation of the antiasthmatic properties of stems and leaves of *Waltheria indica* L. (Malvaceae): Focus on antioxidant and anti-inflammatory activity, and quantification of phenolic compounds. Phytomed. Plus, Vol. 4. 10.1016/j.phyplu.2024.100600.
9. Yabré, Z., R. Boly, C. El Khattabi, M. Ouédraogo and R. Ouédraogo *et al*., 2024. Role of soluble guanylate cyclase and muscarinic receptors in the relaxant effect of *Waltheria indica* L. (Malvaceae) on tracheal smooth muscle. Afr. J. Pharm. Pharmacol., 18: 142-155.
10. Dubale, S., D. Kebebe, A. Zeynudin, N. Abdissa and S. Suleman, 2023. Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. J. Exp. Pharmacol., 15: 51-62.

11. Zongo, F., C. Ribuot, A. Boumendjel and I. Guissou, 2014. Bioguidage search of active compounds from *Waltheria indica* L. (Malvaceae) used for asthma and inflammation treatment in Burkina Faso. Fundam. Clin. Pharmacol., 28: 323-330.
12. Pasparakis, M., I. Haase and F.O. Nestle, 2014. Mechanisms regulating skin immunity and inflammation. Nat. Rev. Immunol., 14: 289-301.
13. An, K.P., M. Athar, X. Tang, S.K. Katiyar and J. Russo *et al*., 2002. Cyclooxygenase-2 expression in murine and human nonmelanoma skin cancers: Implications for therapeutic approaches. Photochem. Photobiol., 76: 73-80.
14. Termer, M., C. Carola, A. Salazar, C.M. Keck, J. Hemberger and J. von Hagen, 2021. Identification of plant metabolite classes from *Waltheria indica* L. extracts regulating inflammatory immune responses via COX-2 inhibition. J. Ethnopharmacol., Vol. 270. 10.1016/j.jep.2020.113741.
15. Flatie, T., T. Gedif, K. Asres and T. Gebre-Mariam, 2009. Ethnomedical survey of Berta ethnic group Assosa Zone, Benishangul-Gumuz Regional State, Mid-West Ethiopia. J. Ethnobiol. Ethnomed., Vol. 5. 10.1186/1746-4269-5-14.
16. Laczko, R., A. Chang, L. Watanabe, M. Petelo, K. Kahaleua, J.P. Bingham and K. Csiszar, 2020. Anti-inflammatory activities of *Waltheria indica* extracts by modulating expression of IL-1B, TNF-α, TNFRII and NF-κB in human macrophages. Inflammopharmacology, 28: 525-540.
17. Borokini, T.I. and F.O. Omotayo, 2012. Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria. J. Med. Plants Res., 6: 1106-1118.
18. Xia, C., Y. Liu, H. Qi, L. Niu and Y. Zhu *et al*., 2019. Characterization of the metabolic fate of *Datura metel* seed extract and its main constituents in rats. Front. Pharmacol., Vol. 10. 10.3389/fphar.2019.00571.
19. Gololo, S.S., L.J. Shai, N.M. Agyei and M.A. Mogale, 2016. Effect of seasonal changes on the quantity of phytochemicals in the leaves of three medicinal plants from Limpopo Province, South Africa. J. Pharmacogn. Phytother., 8: 168-172.
20. Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain, 16: 109-110.
21. Igbayilola, Y.D., O.S. Aina, O.O. Ogunkoya, O.D. Williams and F.A. Olaoye, 2022. Oxidative, hepatoprotective and ant-inflammatory responses to perinatal walnut (*Juglans regia* L.) supplemented diet in offspring of Sprague-Dawley rats. Int. J. Biochem. Physiol., Vol. 7. 10.23880/ijbp-16000205.
22. Harborne, J.B., 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd Edn., Springer Science & Business Media, Berlin/Heidelberg, Germany, ISBN-13: 9780412572708, Pages: 302.
23. Harborne, J.B., 1973. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 1st Edn., Chapman & Hall, Boundary Row, London, ISBN: 9780412105401, Pages: 278.
24. Scharenberg, F. and C. Zidorn, 2018. Genuine and sequestered natural products from the genus *Orobanche* (Orobanchaceae, Lamiales). Molecules, Vol. 23. 10.3390/molecules23112821.
25. Chang, C.C., M.H. Yang, H.M. Wen and J.C. Chern, 2002. Estimation of total flavonoid content in propolis by two complementary colometric methods. J. Food. Drug Anal., 10: 178-182.
26. Jedrejek, D., S. Pawelec, R. Piwowarczyk, Ł. Pecio and A. Stochmal, 2020. Identification and occurrence of phenylethanoid and iridoid glycosides in six polish broomrapes (*Orobanche* spp. and *Phelipanche* spp., Orobanchaceae). Phytochemistry, Vol. 170. 10.1016/j.phytochem.2019.112189.
27. Auwal, M.S., S. Saka, I.A. Mairiga, K.A. Sanda, A. Shuaibu and A. Ibrahim, 2014. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). Vet. Res. Forum, 5: 95-100.
28. Çilesizoğlu, N.B., E. Yalçin, K. Çavuşoğlu and S.S. Kuloğlu, 2022. Qualitative and quantitative phytochemical screening of *Nerium oleander* L. extracts associated with toxicity profile. Sci. Rep., Vol. 12. 10.1038/s41598-022-26087-0.
29. Waterman, P.G. and S. Mole, 1989. Extrinsic Factors Influencing Production of Secondary Metabolites in Plants. In: Insect-Plant Interactions, Bernays, E.A. (Ed.), CRC Press, Boca Raton, Florida, United States, ISBN: 9780429290916, pp: 107-134.

30. Iqbal, S. and M.I. Bhanger, 2006. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. J. Food Compos. Anal., 19: 544-551.
31. Su, X.Y., Z.Y. Wang and J.R. Liu, 2009. *In vitro* and *in vivo* antioxidant activity of *Pinus koraiensis* seed extract containing phenolic compounds. Food Chem., 117: 681-686.
32. Boly, R., Z. Yabre, M. Nitiema, B. Yaro and J. Yoda *et al*., 2021. Pharmacological evaluation of the bronchorelaxant effect of *Waltheria indica* L. (Malvaceae) extracts on rat trachea. Evidence-Based Complementary Altern. Med., Vol. 2021. 10.1155/2021/5535727.
33. Mattioli, R., A. Francioso, L. Mosca and P. Silva, 2020. Anthocyanins: A comprehensive review of their chemical properties and health effects on cardiovascular and neurodegenerative diseases. Molecules, Vol. 25. 10.3390/molecules25173809.
34. Uzkeser, H., E. Cadirci, Z. Halici, F. Odabasoglu and B. Polat *et al*., 2012. Anti-inflammatory and antinociceptive effects of salbutamol on acute and chronic models of inflammation in rats: Involvement of an antioxidant mechanism. Mediators Inflammation, Vol. 2012. 10.1155/2012/438912.
35. Ramasar, R., Y. Naidoo, Y.H. Dewir and A.N. El-Banna, 2022. Seasonal change in phytochemical composition and biological activities of *Carissa macrocarpa* (Eckl.) A. DC. leaf extract. Horticulturae, Vol. 8. 10.3390/horticulturae8090780.
36. Cordaro, M., R. Siracusa, R. Fusco, R. D'Amico and A.F. Peritore *et al*., 2020. Cashew (*Anacardium occidentale* L.) nuts counteract oxidative stress and inflammation in an acute experimental model of carrageenan-induced paw edema. Antioxidants, Vol. 9. 10.3390/antiox9080660.
37. Yougbare-Ziebrou, M.N., M. Lompo, N. Ouedraogo, B. Yaro and I.P. Guissoun, 2016. Antioxidant, analgesic and anti-inflammatory activities of the leafy stems of *Waltheria indica* L. (Sterculiaceae). J. Appl. Pharm. Sci., 6: 124-129.
38. Ncube, B., V.N.P. Ngunge, J.F. Finnie and J. van Staden, 2011. A comparative study of the antimicrobial and phytochemical properties between outdoor grown and micropropagated *Tulbaghia violacea* Harv. plants. J. Ethnopharmacol., 134: 775-780.