



ANTI-INFLAMMATORY ACTIVITIES OF POWDERED EXTRACTS OF *VERNONIA AMYGDALINA* AND *LAURUS NOBILIS* ON ALBINO RATS

FATIMAH ABDUSSALAM OLUWAKEMI

Department of Science Laboratory Technology, Federal Polytechnic, Ilaro, Ogun State

Email: fatimah.abdussalam@federalpolyilaro.edu.ng

Abstract

Several African countries and its dwellers depend on traditional medicine for their health needs and medicinal plants are the main source of remedies for this therapy. These medicinal plants are used for the treatment of various disease conditions such as microbial infections, skin diseases, hypertension, pains, and inflammation. This study aims at investigating the anti-inflammatory activities of powdered extracts of *Vernonia amygdalina* (Va) and *Laurus nobilis* (Ln) using albino rats. The anti-inflammatory activities of these plants were determined using twenty albino rats divided into four groups of five albino rats each. Oedema was produced by injecting each albino rat with 0.2 ml of a solution of 1% egg albumin (EA) in the hind paw. Group I, the negative control was administered with egg albumin. Group II were treated with 10 mg/kg of ketoprofen (standard drug). Group III and IV were treated with 100 mg/kg of powdered extracts of Va and Ln respectively. Animals were observed for oedema inhibition by Ketoprofen, Va and Ln at 1, 2 and 3 hours post injection of EA. Findings from this study showed that Va and Ln contain phytochemicals such as alkaloids, tannins, flavonoids, and phenols among others. Also, the result from the study showed that the powdered extracts of Va and Ln has high anti-inflammatory activities reducing oedema to 67% and 89.2% at 3 hrs post injection of EA compared to ketoprofen (33.9%) used in this study. Ln extract showed the most promising effect; as oedema was drastically reduced by 89.2% at 3 hrs post injection of EA. Hence, it was concluded that the powdered extracts of *Vernonia amygdalina* and *Laurus nobilis* can be effectively used as an alternative drug to treat inflammation.

Key words: *Vernonia amygdalina*, *Laurus nobilis*, phytochemicals, ketoprofen

Introduction

Inflammation is a complex biological phenomenon of body tissues responding to harmful and exogenous irritants such as pathogens or damaged cells (Medzhitov, 2010; Chandrasekhar & Rao, 2013). It is an important aspect of the body defense mechanism. The process is characterized by various occurrence such as itching, redness, warmth, swollen and pains. Most times, inflammation is an auto signal of the onset of various infections such as staphylococcal, syphilis, warts, leprosy, celiac diseases, auto-immune diseases etc.

Inflammation can be short-lived (acute). Example of such acute inflammation are redness, heat or swollen or can persist for months or years (chronic) if not given proper or immediate attention. Such examples of prolonged or chronic inflammation are warts, diabetes, rheumatoid arthritis etc. Sometimes inflammation may be silent without any physical symptoms. Individual may only feel tired, generally unwell or fever (Adam, 2023).

In most cases of inflammation, Non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, hydroxychloroquine and immune system suppressing drug are used to suppress the effect of pains and other associated difficulties in an individual, but these relieving agents also carry some potential risk. The use of NSAIDs sometimes cause gastro-intestinal disorder, cardiovascular and various toxicities effect when used for a prolonged period (Eswaraiyah et al., 2012). Immunosuppressive drugs such as cyclosporine, tacrolimus and mycophenolate mofetil can also pose severe adverse effect that prevents the immune system's competence to fight infection (Tian et al., 2018). Due to their lack of specificity and potential risk, there is always demand for better therapeutic alternative which can combat inflammation without deleterious effect on the user. The search for alternatives birthed the use of natural drugs (botanicals). Many have tested various botanicals which some have proven to be a remedial on inflammation but there is lack of scientific evidence to substantiate the claim of their effectiveness. Therefore, the background and objectives of this study aimed to examine the potential anti-inflammatory effect of powdered extracts of *Vernonia amygdalina* and *Laurus nobilis* by solution of egg albumin induced paw oedema in Albino rats.



Materials and methods

Sample Collection

Egg albumin was purchased from biotech store, Idumota, Lagos State. Ketoprofen was purchased from Sango, Ogun state.

The leaves of *Vernonia amygdalina* (Bitter leaf) used were obtained from Iyana sawmill, Ilaro, Ogun State while leaves sample of *Laurus nobilis* (Bay leaf) were obtained from Sayedero market in Ilaro, Ogun state. Leaves samples were confirmed by Mr Adeniji, a Botanist in the department of Science Laboratory Technology. The two leaves samples were transported via hand down to the laboratory of the Department of Science Laboratory Technology, Federal polytechnic, Ilaro.

Twenty-five healthy albino rats (150–200g), bred in the experimental animal house in Crystal animal farm, Abiola way, Abeokuta, Ogun state, Nigeria were used for the study. The animals were kept in cages within the animal house and allowed free access to water and feed during acclimatization. The animals were examined and found to be free of wounds, swellings and infections before the commencement of the experiment.

Sample Preparation

The leaves sample were washed separately under clean running tap water to remove dirt and air-dried at room temperature. The samples were then pulverized separately with Binatone blender (model BLG401). The powder obtained from the samples were stored separately in an aseptic air tight container for further use.

Two (2) sets of two (2) 120 ml conical flasks each containing 10 g of powdered *Vernonia amygdalina* leaves and other conical flask containing 10 g of *Laurus nobilis* with 100 ml of water were used. The mixtures were stirred for 5 minutes and then allowed to stand for 12 hours. The mixtures were then filtered using Whatman filter paper. The filtrates were then transferred into 100 ml airtight containers and stored in cool temperature for subsequent use. The separate extracts had a concentration of 10g/100ml each.

Phytochemical Screening

Phytochemical screening was done to determine presence or absence of secondary metabolites such as tannins, alkaloids, flavonoids, saponins, sterols, anthraquinones, terpenoids and glycosides. This was done according to established procedures in literature.

Anti-inflammatory Evaluation

Experimental rats were divided into four groups of five animals per group. 0.2 ml of a solution of 1% Egg albumin was injected in the hind paw of each of the rats to produce inflammation (Oedema). The groups are in the following order;

- I. Group I received 0.1ml of the 1% solution (Toxicant/negative control group);
- II. Group II received 10 mg/kg of ketoprofen (Standard/positive control group)
- III. Group III were treated with 100 mg/kg solid extract of *Vernonia amygdalina* by oral route
- IV. Group IV received 100 mg/kg of solid extract of *Laurus nobilis* by oral route.

Following the injection of egg albumin into the rats, they were left for 48 hours to allow the even circulation of the egg albumin in the rats causing the inflammation to spread rapidly. One-hour post drug administration, 0.1 ml of 1% egg albumin was dissolved in distilled water and injected into the sub plantar region of right hind paws of all groups. The volume of paw oedema was measured with the help of plethysmometer at zero hour (immediately after injecting Egg albumin). This procedure was repeated at 1, 2, and 3 hours interval post egg albumin injection. Actual oedema volume was obtained by the difference between the initial and subsequent reading of oedema. Anti-inflammatory response was determined by the reduction in paw volume compared to the control animals. The percentage inhibition was determined using the formula below

$$\text{Percentage Inhibition} = \left[\frac{V_c - V_t}{V_c} \right] \times 100$$



Where,

V_t = Paw volume of test group;

V_c = Paw volume of control group.

Control Test (Standard);

The standard anti-inflammatory drug used in this study is ketoprofen.

Results

Table 1 shows the phytochemical constituents of *Vernonia amygdalina* and *Laurus nobilis* leaves. *Vernonia amygdalina* contains tannins, flavonoids, alkaloids, steroids, phenols, oxalate, phylate, cyanogenic glycoside and anthraquinone. *Laurus nobilis* contains tannins, flavonoids, alkaloids, steroids, phenols, eugenols, citric acid and triterpenoids.

Table 2: Phytochemical Constituents of *Vernonia amygdalina* and *Laurus nobilis*.

Phytochemicals	<i>Vernonia amygdalina</i>	<i>Laurus Nobilis</i>
Oxalate	+	-
Eugenol	-	+
Phylate	+	-
Tannins	+	+
Flavonoids	+	+
Cyanogenic glycoside	+	-
Alkaloids	+	+
Anthraquinone	+	-
Citric acid	-	+
Steroids	+	+
Phenol	+	+
Triterpenoids	-	+

Key: '+' = Present; '-' = Absent

Presented in table 2 is the percentage inhibition of Odema by ketoprofen and powdered leaf extracts of *Vernonia amygdalina* and *Laurus nobilis*. In all the groups tested, group 1 (toxicant/negative control group) showed the least percentage inhibition while the powdered leaf extract of *Laurus nobilis* showed the highest level of inhibition. All the post hrs test showed significant anti-inflammatory effect.



Result shows that at 1hr post injection of egg albumin (EA), the positive control group (ketoprofen) showed the least percentage inhibition (13.8%) while group treated with *Laurus nobilis* showed the highest inhibition (55.5%). Toxicant/negative control group and group treated with extract of *V.amygdalina* had 18.0 and 38.4% inhibition respectively. At 2 hrs. post injection of EA, there is relatively reduced inflammation of the odema observed in Ketoprofen (30.7%), *V. amygdalina* (39.8%) and *L. nobilis* (67.0%) treated group with higher percentage inhibition observed in the *L. nobilis* treated group.

It was observed at 3 hrs. post injection of EA that there was drastic reduction of inflamed paw in *V. amygdalina* (67.0%) and *L. nobilis* (89.2%) treated group. All other groups also showed reduction at 3 hrs but the difference at Ketoprofen treated group was less pronounced compared to what was observed at 2 hrs.

Table 2: Percentage Inhibition of Odema Formation (%) by Ketoprofen, *V. amygdalina* and *L. nobilis*

Groups	Duration (hr.)		
	1	2	3
I (Negative control)	18.0	18.6	24
II (Positive control)	13.8	30.7	33.9
III (<i>V. amygdalina</i>)	38.4	39.8	67.0
IV (<i>L. nobilis</i>)	55.5	67.0	89.2

Discussion

For millenniums, botanicals have served humanity as potent remedy to combat many ailments; ranging from inflammation, pain, pyrexia and other infectious diseases. But the advent of medical technology paved way for the introduction and use of synthetic Non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and other anti-inflammatory agents sold over the counter. These synthetic drugs are known to suppress pain, reduce inflammation but many does more harm than good. They have the potential of causing adverse reaction which most times could be severe; leading to other ailments such as trivial nausea, vomiting, allergy, gastro-intestinal ulcer among others. Some of these undesirable adverse effects are irreversible while others may be expensive to cure.

Therefore, recently, there is an uptrend in the use of alternative medicines due to their medicinal values, benefits, potency and the fact that they are less toxic and harmful to the human health. In this study *Vernonia amygdalina* and *Laurus nobilis* proved effective as alternative medicines with their phytochemicals constituents serving as anti-inflammatory agent in albino rats.

The significance of phytochemicals in plants had been a topic of discussion amongst the researchers, traditional medicine practitioners and herbal trade fair across the globe (Maduibuke & Rosemary, 2017). Although, many of the claims by herbal practitioners lack scientific backings, therefore the current study evaluated the significance of the above-mentioned plants as anti-inflammatory in albino rats. The pharmaceutical quality and range of activity of some plant active principles have been reported to be due to the presence of various phytochemicals (Umar *et al.*, 2014). The presence of phytochemicals has demonstrated several pharmacological and biochemical actions which are beneficial to human health as well as possessing anti-inflammatory activities.

In the current study, oral administration of the leaf powdered extracts of *V. amygdalina* and *L. nobilis* have demonstrated potent effect in suppressing odema in albino rats at an impressive rate using a dose of 100 mg/kg. At 1hr. post injection of the EA, *V. amygdalins* and *L. nobilis* exhibit reduction in percentage odema (38.4% and 55.5% respectively) while the reduction of inflammation was progressive at each progressive time of 2 hours (39.8% and 67.0%) and 3 hours (67.0% and 89.2%). Their anti-inflammatory potency surpasses the conventional synthetic Ketoprofen popularly used as anti-inflammation which only reduces odema by 13.8, 30.7 and 33.9% at 1, 2 and 3



hours post injection of EA respectively. Ketoprofen was also observed to have a slow initial mode of action when observed at 1-hour post injection.

The presence of phytochemicals such as phenols, glycosides, tannins, triterpenoids, alkaloids and flavonoids could have given the plants used in this study their anti-inflammatory properties. Phenolic compounds have been reported to possess biochemical properties such as anti-apoptosis, antiaging, anticancer, anti-inflammatory, anti-atherosclerosis, improvement of endothelial function, inhibition of angiogenesis and cell proliferation activities. Phenols are also reported to have the ability to neutralize free radicals (Omale & Okafor, 2008).

Alkaloids are known to have metabolic functions and influence development in living system (Edeoga *et al.*, 2006). It also involves in cell division, hence their presence in *Vernonia amygdalina* and *Laurus nobilis* result in their use as anti-inflammatory agents. Flavonoids are expressed in plants in response to microbial infection suggesting their antimicrobial activity. Rabi and Bishayee (2009), reported terpenoid to possess antimicrobial, antifungal, anti-parasitic, antiviral, antispasmodic, and anti-inflammatory properties.

In this current study, powdered leaf extract of *L. nobilis* showed the most promising effect in inhibiting oedema in albino rat. This could be due to the presence of triterpenoids present which is absent in the extract of *V. amygdalina*. Several studies have shown that certain terpenes could reduce symptoms of inflammation through decreasing the release of pro-inflammatory cytokines such as the nuclear transcription factor-kappa B and the tumor necrosis factor-alpha (Maria *et al.*, 2021).

Conclusion

On the basis of this findings, the extracts of *Vernonia amygdalina* and *Laurus nobilis* can be used in the development of anti-inflammatory drugs since they both possess anti-inflammatory properties. Also, nutraceutical companies can make use of these plants for the production of nutraceuticals.

However, further studies could be conducted to evaluate the comprehensive analysis of *V. Amygdalina* and *L.nobilis* to ascertain their quantitative analysis, verify their structural features and assess their therapeutic activities with specific mechanism of action as an anti-inflammatory agent.

Reference

- Adam, F. (2023). Everything you need to know about inflammation. *Medical News Today*.
- Chandrashekar, R. & Rao, S. N. (2012). Acute anti-inflammatory activity of ethanolic extract of leaves of *Leucas indica* by carrageenan induced paw oedema in wistar albino rats. *International Journal of Basic & Clinical Pharmacology*, 2(3): 302-305.
- Edeoga, H. O., Omobuna, G., & Uche, L. C. (2006). Chemical Composition of Hyotissu aveoleus and *Ocimum gratissimum* Hybrids from Nigeria. *African Journal of Biotechnology*, 5(910): 892-895.
- Eswaraiah, M. C., Rahman, H., & Vakati, K. (2012). In-Vivo and In-Vitro Anti-Inflammatory Activity of *Aquilaria agallocha* Oil. *International Journal of Basic Medical Science and Pharmacology*, 2:7-10.
- Maduibuke, U. A., & Rosemary, C. A. (2017). Antimicrobial activity of Nigerian medicinal plants. *Journal of Intercultural Ethnopharmacology*. 6(2): 240–259.
- María- Luisa, D. P. A., Hernan, C., Isaac, H. C. F., Maykel, G. T., Lidia, E. G., Sergio, A. B. C., *et al.* (2021). Therapeutic Applications of Terpenes on Inflammatory Diseases. *Front Pharmacology*, 12: 704197.
- Medzhitov, R. (2010). New adventures of an old flame. *Cell*, 140:771-776
- Omale, J. & Okafor, P. (2008). Comparative Antioxidant Capacity, Membrane Stabilization, Polyphenol Composition and Cytotoxicity of the Leaf and Stem of *Cissus multistriata*. *African Journal of Biotechnology*, 7(17): 3129-3133
- Rabi, T., & Bishayee, A. (2009). Terpenoids and Breast Cancer Chemoprevention. *Breast Cancer Res Treat*, 115:223-239



- Tian J., Luo, Y., Wu, H., Long, H., Zhao M., & Lu, Q. (2018). Risk of adverse events from different drugs for SLE: A systematic review and network meta-analysis. *Lupus Science & Medicine*, 5:253.
- Umar, I. A., Ndidi, U.S., Muhammad, A., Anaedum, V. C *et al.* (2014). In vitro antitrypanosomal activity, antioxidant property and phytochemical constituents of aqueous extracts of nine Nigerian medicinal plants. *Asian Pacific Journal of Tropical Disease*,. 4(5): 348-355.