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PHYTOCHEMICAL INVESTIGATION AND IN VITRO ANTIINFLAMMATORY ACTIVITY OF BLUMEA OXYODONTA ,DC LEAF EXTRACT

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Abstract

The study was conducted to investigate the phytochemical profile and evaluate the in vitro anti-inflammatory activity of Blumea oxyodonta leaf extract. The methanol and ethanol extracts of B. oxyodonta leaves were found to contain bioactive compounds such as alkaloids, catechins, coumarins, flavonoids, phenols, glycosides, saponins, steroids, terpenoids, sugars, and xanthoproteins. The ethanol extract demonstrated notable anti-inflammatory effects by inhibiting protein denaturation, proteinase, heat-induced hemolysis, hypotonicity-induced hemolysis, and lipoxygenase activities, with inhibition percentages of 87.63%, 84.83%, 82.72%, 88.46%, and 80.73%, respectively, at a concentration of 1000 µg/mL. These results indicate that the ethanol extract of Blumea oxyodonta leaves has significant potential as an anti-inflammatory agent.

Keywords: Blumea oxyodonta, phytochemical, flavonoid, anti-inflammatory, lipoxygenase inhibition.

Introduction

Plants have been vital in human health care since ancient times, producing various substances that have biological activities as part of their adaptation to combat pathogens and environmental stress. These small organic molecules, which are products of secondary metabolism, have a range of biological effects, with anti-inflammatory properties being particularly significant (Locatelli et al., 2016; Virshette et al., 2019).

Inflammation is the body's automatic protective response to tissue injury or foreign invaders. A regulated inflammatory response is crucial for maintaining tissue homeostasis (Jenssen and Henson, 2012). The duration and severity of inflammation are critical in determining its outcomes. Acute inflammation involves processes such as phagocytosis, apoptosis, and the activation of pro-inflammatory mediators, all aimed at clearing harmful stimuli and restoring normal function. However, chronic inflammation is harmful and contributes to various diseases, including Alzheimer's disease, cancer, rheumatoid arthritis, type 2 diabetes, obesity, and cardiovascular and pulmonary disorders (Varela et al., 2018; Tasneem et al., 2019).

Chronic inflammatory diseases are a leading cause of death worldwide, accounting for 60% of global deaths.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat inflammation and related pain, but their use is linked to numerous side effects. This has led to increased research focused on finding safe, natural alternatives to traditional anti-inflammatory drugs. In this context, the current study evaluates the in vitro anti-inflammatory activity of Blumea oxyodonta DC leaves, a plant from the Asteraceae family. Traditionally, the root decoction of this plant has been used to treat conditions such as impotence and spermatorrhea (Quattrocchi, 2012). Therefore, this study also includes a preliminary phytochemical analysis and investigation of the anti-inflammatory effects of ethanol extracts from Blumea oxyodonta leaves.

Materials and Methods

Collection of leaf sample

Fresh leaves of Blumea oxyodonta DC (BOL) were collected from Karumathra village, near Thrissur, Kerala. The plant specimens were taxonomically identified with the assistance of local floras, and the botanical identity was verified and authenticated by the Botanical Survey of India, Coimbatore, Southern Circle, Tamil Nadu, India. The collected leaves were then chopped into small pieces and shade-dried. Once dried, the samples were ground into a fine powder using a blender and sieved to ensure a uniform consistency.

Preliminary phytochemical screening

Qualitative tests were performed to identify various chemical components in the solvent extracts (petroleum ether, benzene, ethyl acetate, methanol, ethanol, and aqueous) of B. oxyodonta leaves, following the methods outlined by Brinda et al. (1981). The extracts were screened for the presence of alkaloids, anthraquinones, catechins, coumarins, flavonoids, phenols, quinones, saponins, steroids, tannins, terpenoids, glycosides, sugars, xanthoproteins, and fixed oils. The ethanol extract was then selected for the in vitro anti-inflammatory activity study.

In vitro anti-inflammatory activity

Inhibition of protein denaturation

The anti-inflammatory activity of the ethanol extract of B. oxyodonta leaves was assessed using the protein denaturation inhibition method, as described by Sakat et al. (2010) with modifications from Gunathilake et al. (2018). The reaction mixture (5 ml) included 0.2 ml of 1% bovine albumin, 4.78 ml of phosphate buffer saline (pH 6.4), and 0.02 ml of varying concentrations of the extract. The mixture was incubated in a water bath at 37°C for 15 minutes, then heated at 51°C for 5 minutes. After cooling, turbidity was measured at

660 nm using a UV/VIS spectrophotometer (Model SL 150, Elico India Ltd). Phosphate buffer solution served as the control, and aspirin was used as the standard drug for reference absorbance measurement. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition of denaturation} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Proteinase inhibitory activity

The proteinase inhibitory activity of the ethanol extract of B. oxyodonta was evaluated using the method outlined by Sakat et al. (2010) and modified by Gunathilake et al. (2018). Briefly, the reaction mixture (2 ml) contained 0.06 mg of trypsin, 1 ml of 20 mM Tris-HCl buffer (pH 7.4), and 1 ml of the extract at varying concentrations (100–1000 µg/ml). The mixture was incubated at 37°C for 5 minutes, after which 1 ml of 0.8% (w/v) casein was added, and the incubation continued for another 20 minutes. After incubation, 2 ml of 70% perchloric acid was added to terminate the reaction. The mixture was centrifuged, and the absorbance of the supernatant was measured at 210 nm, with the buffer used as a blank. Phosphate buffer solution served as the control, and aspirin was used as the standard drug. The experiment was conducted in triplicate, and the percentage inhibition of proteinase activity was calculated using the following formula:

$$\% \text{ inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Membrane Stabilization

Preparation of Red Blood Cell (RBC) Suspension (Sadique et al., 1989; Sakat et al., 2010)

Blood was collected from a healthy human volunteer who had not taken any NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) for at least two weeks prior to the experiment. The blood was placed in centrifuge tubes and centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded, and the cells were washed three times with an equal volume of normal saline (0.9% NaCl). Following the washes, the blood volume was measured and then reconstituted into a 10% (v/v) red blood cell (RBC) suspension using an isotonic buffer solution (10 mM sodium phosphate buffer, pH 7.4).

Heat-Induced Hemolysis (Shinde et al., 1999; Sakat et al., 2010)

The reaction mixture (2 ml) comprised 1 ml of the test extract at varying concentrations (100–1000 µg/ml) and 1 ml of the 10% RBC suspension. For the control group, saline was used in place of the test extract, and diclofenac sodium was used as the standard drug. All reaction mixtures were incubated in a water bath at 56°C for 30 minutes. After incubation,

the tubes were cooled under running tap water. The mixture was then centrifuged at 2500 rpm for 5 minutes, and the absorbance of the supernatant was measured at 560 nm. The experiment was performed in triplicate. The percentage inhibition of hemolysis was calculated using the following formula:

$$\% \text{ inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Hypotonicity-Induced Hemolysis (Azeem et al., 2010)

Various concentrations of the ethanol extract (ranging from 100 to 1000 µg/ml), along with the reference sample and control, were separately combined with 1 ml of phosphate buffer, 2 ml of hyposaline, and 0.5 ml of HRBC suspension. Diclofenac sodium (100–1000 µg/ml) was used as the standard drug. The mixtures were incubated at 37°C for 30 minutes and then centrifuged at 3000 rpm. The supernatant was discarded, and the hemoglobin content was measured spectrophotometrically at 560 nm. The inhibition of hemolysis was calculated using the same formula as in the heat-induced hemolysis assay.

Lipoxygenase Inhibition Activity (Shinde et al., 1999)

Lipoxygenase inhibition was evaluated using linoleic acid as the substrate and lipoxygenase as the enzyme. The test solution was dissolved in 0.25 ml of 2 M borate buffer (pH 9.0) and mixed with 0.25 ml of lipoxygenase enzyme solution (final concentration 20,000 U/ml), then incubated for 5 minutes at 25°C. After incubation, 1 ml of linoleic acid solution (0.6 mM) was added and thoroughly mixed, and the absorbance was measured at 234 nm using a UV/Vis spectrophotometer. Indomethacin was used as the reference standard, and phosphate buffer solution served as the control. The percentage inhibition of lipoxygenase was calculated using the following formula:

$$\% \text{ inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Statistical Analysis

The results are expressed as Mean ± SD. Differences between experimental groups were compared using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison tests (Control vs. Test) using GraphPad Instat software. Experimental groups were compared with control **P< 0.01 considered extremely significant; *P < 0.05 considered significant.

Results and Discussion

Qualitative phytochemical analysis

The distribution of various phytochemical constituents in the petroleum ether, benzene, ethyl acetate, methanol, ethanol, and aqueous extracts of B. oxyodonta leaves (BOL) was assessed qualitatively, and the results are presented in Table 1. The ethyl acetate, methanol, and ethanol extracts of B. oxyodonta leaves contained alkaloids, catechins, coumarins, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenoids, sugars, and xanthoproteins. This preliminary phytochemical analysis aids in identifying the chemical classes present in the extract, which can contribute to their quantitative evaluation and help identify sources of pharmacologically active compounds. These detected phytocompounds in B. oxyodonta leaves suggest the plant's potential for addressing various ailments, as they may possess significant therapeutic properties.

Table 1: Preliminary phytochemical screening of different solvent extracts of B. oxyodonta leaf

	Nature of extract					
Bioactive components	**Petroleum ether**	**Benzene**	**Ethyl acetate**	**Methanol**	**Ethanol**	**Aqueous**
Alkaloids	-	-	+	+	+	-
Anthraquinones	-	+	-	-	-	-
Catechins	+	+	+	+	+	-
Coumarins	-	-	+	+	+	-
Flavonoids	-	+	+	+	+	+
Glycosides	-	+	+	+	+	-
Phenols	+	+	+	+	+	+
Quinones	-	-	-	-	+	-
Saponins	-	-	+	+	+	+
Steroids	+	+	+	+	+	-
Tannins	-	+	+	+	+	+
Terpenoids	+	+	+	+	+	-
Sugars	+	+	+	+	+	+
Xanthoproteins	+	-	-	-	-	-
Fixed oil	+	-	+	+	+	+

In vitro antiinflammatory activity

Inhibition of protein denaturation

In the current study, the ethanol extract of B. oxyodonta leaves demonstrated a concentration-dependent inhibition of protein denaturation. The highest inhibition, 87.63%, was observed at

a concentration of 1000 µg/mL, which is similar to the 91.75% inhibition observed with aspirin at the same concentration (Fig. 1). A previous study on cold and hot water extracts of Ficus racemosa bark reported protein denaturation inhibitions of 27.71% and 27.65%, respectively (Dharmadeva et al., 2018). Additionally, another study found that leaf and root extracts of Euphorbia hirta exhibited 87.51% and 64.20% inhibition of protein denaturation, respectively (Das et al., 2022). Protein denaturation is known to produce auto-antigens in conditions such as rheumatoid arthritis, cancer, and diabetes—diseases linked to inflammation. Therefore, by inhibiting protein denaturation, the inflammatory response can also be suppressed (Dharmadeva et al., 2018).

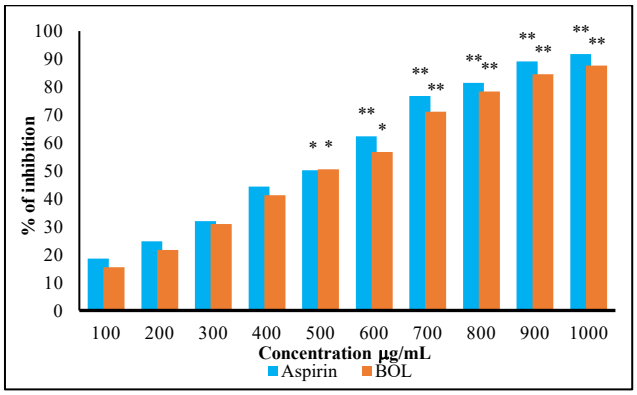

Figure 1: Inhibition of protein denaturation

Proteinase inhibitory activity

In this study, the ethanol extract of B. oxyodonta leaves demonstrated notable antiproteinase activity at various concentrations, as shown in Figure 2. The maximum inhibition of proteinase, 84.83%, was observed at a concentration of 1000 µg/mL. In comparison, aspirin exhibited the highest antiprotease activity of 92.13% at the same concentration. A previous study on methanol extracts of Punica granatum fruit epicarp, Solanum xanthocarpum aerial parts, Adansonia digitata leaves, and Vitex negundo leaves reported antiproteinase activities of 81.07%, 60.65%, 84.10%, and 78.55%, respectively (Modi et al., 2019). Proteinase inhibition is linked to a reduction in inflammation, either by indirectly inhibiting neutral proteinases or by preventing their infiltration into inflammatory sites (Shigatomi et al., 2010).

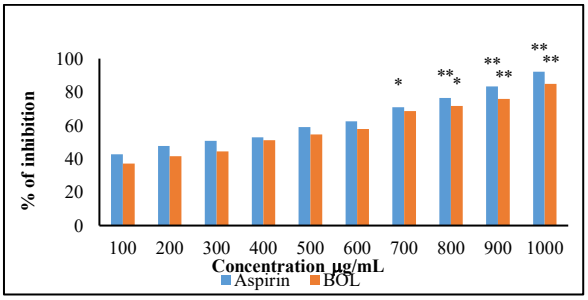

Figure 2: Inhibition of proteinase inhibitory action

Membrane stabilization

Head induce hemolysis

The ethanol extract of B. oxyodonta leaves demonstrated a concentration-dependent effect in inhibiting hemolysis (Fig. 3). At a concentration of 1000 µg/mL, the extract provided significant protection, preventing 82.72% of erythrocyte membrane lysis induced by heat. In comparison, diclofenac sodium offered 85.19% protection against heat-induced hemolysis. A similar study by Govindappa et al. (2011) also reported effective inhibition of heat-induced hemolysis by the ethanolic extract of Wedelia trilobata.

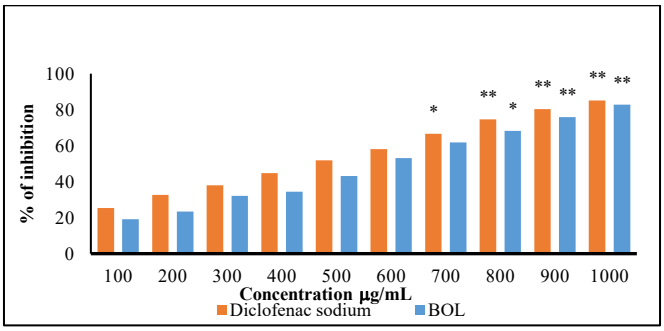

Figure 3: Inhibition of hemolysis of erythocyte by heat

Hypotonicity induced hemolysis

In this study, the results showed that the ethanol extract of B. oxyodonta leaves, at a concentration of 1000 µg/mL, provided significant protection by preventing 88.46% of erythrocyte membrane lysis induced by a hypotonic solution. Similarly, diclofenac sodium at the same concentration demonstrated a significant protective effect, preventing 92.95% of the damage caused by the hypotonic solution (Fig. 4). A recent study by Chirumamilla and Taduri (2023) reported that Solanum khasianum plant extracts exhibited notable anti-inflammatory activity by stabilizing the RBC membrane, thus preventing the release of lytic enzymes and other inflammatory mediators.

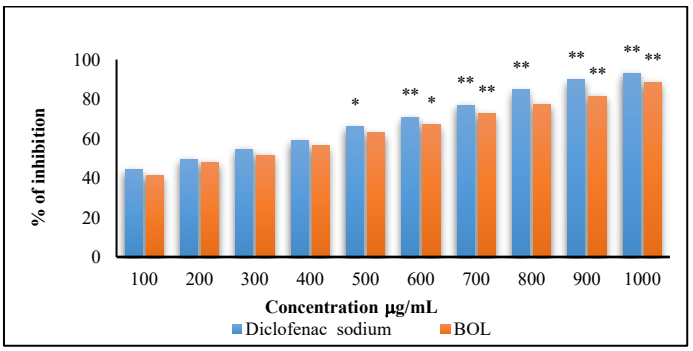

Figure 4: Inhibition of haemolysis of erythrocyte induced by hypotonicity

Lipoxygenase inhibitory activity

The results for the lipoxygenase inhibitory activity of B. oxyodonta leaves are presented in Figure 5. The inhibition levels ranged from 38.53% to 80.73% across concentrations of 100–1000 µg/mL. The drug indomethacin showed 82.11% inhibition at a concentration of 1000 µg/mL. A previous study on ethanol extracts of Euphorbia hirta leaves and roots reported inhibition activities of 94.43% and 48.21%, respectively, at a concentration of 100 µg/mL (Das et al., 2022). Lipoxygenases are crucial enzymes in the biosynthesis of leukotrienes, which play a key role in various inflammatory conditions such as arthritis, asthma, cancer, and allergic diseases. Research has shown that polyphenols can inhibit lipoxygenase activity, potentially interfering with the arachidonic acid metabolism pathway.

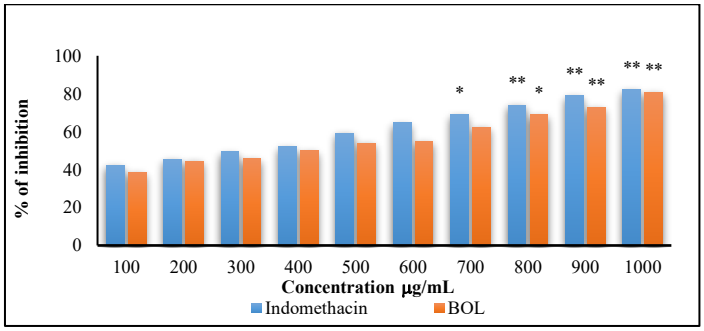

Figure 5: Inhibition of lipoxygenase activity

The present study shows that the ethanol extract of Blumea oxyodonta leaves possesses significant anti-inflammatory properties. These effects are likely due to the high concentration of bioactive compounds such as alkaloids, phenols, flavonoids, tannins, steroids, and glycosides. These compounds may be beneficial in treating various illnesses and combating oxidative stress. The findings could therefore support the development of herbal medicines aimed at treating diseases associated with inflammation caused by oxidative stress.

Conflicts of interest

All authors declare that there is no conflicts of interest.

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