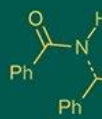


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Anti-inflammatory and antioxidant potential of ethanolic leaf extract of *Artabotrys hexapetalus*: *In vitro* and *in vivo* approach

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Abstract

Artabotrys hexapetalus, a medicinal plant of the Annonaceae family, is traditionally used for inflammatory and oxidative stress-related conditions. The present study evaluated the antioxidant and anti-inflammatory activities of the ethanolic leaf extract of *A. hexapetalus* using *in vitro* and *in vivo* models. Phytochemical screening revealed alkaloids, flavonoids, phenols, tannins, saponins, terpenoids and carbohydrates. The extract exhibited concentration-dependent DPPH radical scavenging activity with an IC_{50} of 161.31 $\mu\text{g/mL}$. It also stabilized red blood cell membranes against heat and hypotonicity-induced hemolysis and inhibited albumin denaturation, with maximum effects at 500 $\mu\text{g/mL}$ and 400 $\mu\text{g/mL}$, respectively. In the carrageenan-induced paw edema model in rats, the extract significantly reduced paw swelling, with efficacy comparable to phenylbutazone. These findings indicate that *A. hexapetalus* leaf extract possesses appreciable antioxidant and anti-inflammatory properties, likely mediated by phenolics, flavonoids and alkaloids. The plant may serve as a potential source of natural anti-inflammatory agents warranting further pharmacological and mechanistic studies.

Keywords: *Artabotrys hexapetalus*, antioxidants, anti-inflammatory, rats

Introduction

Inflammation is a conserved protective response that eliminates the initial cause of cellular injury and facilitates removal of damaged tissue and subsequent repair. It is elicited by diverse stimuli, including physical injury, ischemia, autoimmune reactions and infectious agents. Clinical manifestations may be transient or persist for days to weeks, as observed in bronchitis, pharyngitis, appendicitis and dermatitis (Laveti *et al.*, 2013) [1]. When inflammatory responses are sustained, however, they become maladaptive and contribute to chronic pathology. Persistent inflammation is implicated in the pathogenesis of multiple chronic diseases, including cancer, diabetes, asthma, myocardial infarction and arthritis (Allegra *et al.*, 2019) [2]. Experimental and clinical studies further demonstrate a close interplay between oxidative stress mediated by free radicals and other reactive species and inflammatory signalling pathways. Accumulation of reactive oxygen and nitrogen species has been associated with disease processes such as tumorigenesis, hepatitis, hepatic injury and immune dysfunction (Sinmisola *et al.*, 2019) [3].

Artabotrys hexapetalus, commonly known as climbing ylang-ylang or harichampa, is a woody evergreen climber of the family Annonaceae. Native to India and other regions of South and Southeast Asia, it is widely cultivated as an ornamental species because of its intensely fragrant yellow-green flowers. The plant has simple opposite leaves and bears bisexual, actinomorphic flowers that occur singly or in small clusters on hooked peduncles. Various parts of the plant have long been used in traditional medicine and several biological activities of its leaves and other parts have been reported (Bailey *et al.*, 2022) [4]. However, to the best of our knowledge, no previous study has examined the antioxidant and anti-inflammatory potential of the ethanol leaf extract of *A. hexapetalus*. Accordingly, the present study aimed to investigate the antioxidant and anti-inflammatory activities of the ethanol extract using *in vitro* and *in vivo* approaches.

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Materials and Methods

Collection and authentication of *A. hexapetalus* leaves

The leaves of *A. hexapetalus* were collected in and around Namakkal District, Tamil Nadu and it was identified, duly authenticated by Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai. The collected leaves were shade dried, powdered and stored for further preparation of ethanolic extract.

Preparation of extract

The powdered leaves of *A. hexapetalus* were extracted with ethanol by maceration method (Pandey and Tripathi, 2014) [5].

Phytochemical analysis

Phytochemical screening was carried out by the method described by Odebedy and Sofowara, (1978) [6].

DPPH radical scavenging activity

The radical scavenging activity of the extract was determined as described by Aouachria *et al.* (2017) [7]. DPPH radical scavenging activity was estimated using the following equation:

$$\text{Scavenging activity (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

The half-maximal inhibitory concentration (IC₅₀) was then obtained from a linear regression plot of percentage inhibition against the extract concentration.

Determination of *in vitro* anti-inflammatory studies

The *in vitro* anti-inflammatory activity of the extract was evaluated by membrane-stabilizing activity and inhibition of albumin denaturation.

Membrane-stabilizing activity was determined as described by Oyedapo *et al.* (2010) [8] using 2% (v/v) bovine erythrocyte suspension, with phenylbutazone used as the standard drug. The percentage of membrane stabilization was calculated using the following formula:

$$\text{Percentage of membrane stability} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

The ability of the extract to inhibit albumin denaturation was evaluated according to the method described by Sakat *et al.* (2010) [9]. The percentage inhibition of albumin denaturation was calculated using the following equation:

$$\text{Percentage inhibition of albumin denaturation} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Determination of *in vivo* anti-inflammatory studies

The *in vivo* anti-inflammatory activity of the extract was evaluated using the carrageenan-induced paw edema model in rats (Kalitha *et al.*, 2018) [10]. Male Wistar rats were divided into three groups: control, test (treated with the extract), and standard (treated with phenylbutazone). Edema was induced by injecting 0.1 mL of 1% carrageenan solution into the right hind paw of each rat. The test extract and standard drug were administered 30 minutes before carrageenan injection. Paw volume was measured at 0, 0.5, 1.0, 2.0, 3.0, and 4.0 h post-injection using a digital plethysmometer. The percentage inhibition of edema was

calculated to assess the efficacy of the extract in comparison with the standard drug.

Statistical analysis

All measurements were performed in triplicate and the results are expressed as mean $\pm$ Standard error of the mean (SEM) using GraphPad Prism software. Statistical analysis was carried out using one-way analysis of variance (ANOVA), and a *p* value of less than 0.05 was considered statistically significant.

Results

Qualitative phytochemical analysis

The preliminary qualitative phytochemical analysis of *A. hexapetalus* leaf ethanolic extract revealed the presence of alkaloids, carbohydrates, flavonoids, phenols, saponins, tannins and terpenoids, whereas glycosides was not detected. The results are presented in Table 1.

DPPH radical scavenging activity

As shown in Table 2, the IC₅₀ values for *A. hexapetalus* leaf extract and ascorbic acid (standard) were 161.31 and 106.15 $\mu\text{g/mL}$, respectively. The plant extract exhibited concentration-dependent DPPH radical scavenging activity, with the highest activity observed at 500 $\mu\text{g/mL}$.

Membrane stability

For *A. hexapetalus* leaf extract, the ability to stabilize red blood cell membranes against heat and hypotonicity-induced hemolysis was evaluated to investigate its underlying anti-inflammatory mechanism. As shown in Table 3, the extract protected RBCs against hemolysis in a concentration-dependent manner. The highest inhibition was observed at 500 $\mu\text{g/mL}$. Phenylbutazone was used as the standard drug.

Inhibition of albumin denaturation

The anti-inflammatory activity of *A. hexapetalus* leaf extract was evaluated by its ability to inhibit albumin denaturation, with phenylbutazone as the standard. As shown in Table 4, the extract exhibited its highest inhibitory activity at 400 $\mu\text{g/mL}$.

In vivo anti-inflammatory activity

Table 5 illustrates the anti-inflammatory effect of *A. hexapetalus* leaf extract on carrageenan-induced paw edema in rats. The negative control group (carrageenan only) showed the highest paw volume, confirming successful induction of inflammation. The standard drug, phenylbutazone, produced a significant reduction in paw edema, validating its known anti-inflammatory effect. *A. hexapetalus* leaf extract produced a comparable reduction in paw volume to phenylbutazone, indicating similar efficacy.

Discussion

In the present study, we evaluated the antioxidant and anti-inflammatory effects of *A. hexapetalus* leaf extract. Phytochemical analysis indicated that the extract contained phenolic compounds. Kousalya and Doss (2020) [11] demonstrated that *A. hexapetalus* aqueous leaves extract showed the presence of various phytochemicals like alkaloids, flavonoids, tannins, phenols, glycosides, diterpenes, saponins, carbohydrates, proteins and amino acids. These compounds demonstrated radical-scavenging activity, protected RBC membranes against heat and hypotonicity-induced hemolysis and inhibited albumin

denaturation. As primary natural antioxidants, phenolic compounds likely account for the extract's total phenolic content, its free-radical scavenging and antioxidant activities and its observed anti-inflammatory effects. DPPH is a stable nitrogen-centered free radical that is violet and turns yellow upon reduction by hydrogen or electron-donating antioxidants. Substances with this activity are termed antioxidants or free-radical scavengers (Pavithra and Vadivukkarasi, 2015) [12]. In the present study, *A. hexapetalus* leaf extract scavenged DPPH radicals in a dose-dependent manner, with scavenging activity increasing as extract concentration increased. Thus, *A. hexapetalus* extract may be an important source of free-radical scavengers and could support treatment of oxidative stress-related ailments. These results agree with Krishna Satya *et al.* (2017) [13], who reported potent DPPH activity and high total phenolic and flavonoid contents in *A. hexapetalus* leaf extract. The extract's flavonoids and phenols likely contribute to the observed radical-scavenging activity and overall antioxidant potential. RBC membrane stabilization is used to assess *in vitro* anti-inflammatory activity because the RBC membrane resembles the lysosomal membrane. *A. hexapetalus* leaf extract inhibited heat and hypotonicity-induced hemolysis in a dose-dependent manner, indicating RBC membrane stabilization. This activity may result from phytoconstituents interacting with membrane components and preventing release of phospholipase A2, thereby reducing phospholipid hydrolysis and formation of inflammatory mediators. These findings support membrane stabilization as one mechanism underlying the extract's anti-inflammatory effect and suggest the extract helps preserve lysosomal and RBC membrane integrity during inflammation. Lysosomal enzymes released into the cell can exacerbate inflammation and tissue damage by preventing their release, the extract may limit such damage. The extract's maximum percentage inhibition of hemolysis was comparable to that of phenylbutazone, corroborating the observations of Bajaj and Wakode (2017) [14]. Protein denaturation is associated with the inflammatory process. In the present study, *A. hexapetalus* leaf extract effectively prevented albumin denaturation in a concentration-dependent manner, indicating the presence of secondary metabolites, such as phenolic compounds, that preserve protein structure and protect against denaturation (Kousalya and Doss, 2020) [11]. The carrageenan-induced paw edema model is a standard laboratory test used to study acute inflammation and screen the anti-inflammatory properties of drugs and plant extracts. In the present study, the ethanolic leaf extract of *A. hexapetalus* showed significant anti-inflammatory activity in carrageenan-induced acute paw edema in albino rats (Suman *et al.*, 2015) [15]. Alkaloids and polyphenols present in *A. hexapetalus* likely contribute to

these anti-inflammatory effects.

Table 1: Phytochemical screening of ethanol extract of *Artabotrys hexapetalus*

S. No.	Phytochemicals	Ethanol extract of <i>A. hexapetalus</i>
1.	Tannins	Present
2.	Flavonoids	Present
3.	Glycosides	Absent
4.	Terpenoids	Present
5.	Alkaloids	Present
6.	Phenols	Present
7.	Carbohydrates	Present
8.	Saponins	Present

Table 2: DPPH radical scavenging activity of ethanol extract of *Artabotrys hexapetalus*

Concentration (µg/mL)	Ethanol extract of <i>A. hexapetalus</i>	Ascorbic acid
100	40.49±1.40	48.64±0.68
200	55.87±0.68	64.92±0.37
300	62.86±1.89	85.66±0.63
400	67.22±0.76	85.44±0.24
500	72.88±1.10	85.50±0.35
IC50	161.31 µg/mL	106.15 µg/mL

Each value represents the mean ± SEM of three replicates.

IC50: Inhibitory concentration

Table 3: Effect of various concentrations of ethanol extract of *Artabotrys hexapetalus* on RBC membrane-stabilizing in response to heat-induced hemolysis

Concentration	% Hemolysis	
	<i>Artabotrys hexapetalus</i>	Phenyl butazone
100	21.08±0.97	10.35±0.24
200	44.27±1.45	16.35±0.53
300	42.81±0.73	28.26±1.09
400	48.57±0.82	36.67±1.26
500	51.08±1.82	53.11±1.24
IC50	423.45 µg/mL	489.23 µg/mL

Each value represents the mean ± SEM of three replicates.

IC50: Inhibitory concentration

Table 4: Inhibition of thermally-induced protein denaturation by ethanol extract of *Artabotrys hexapetalus*

Concentration	% Inhibition of Denaturation	
	<i>Artabotrys hexapetalus</i>	Phenyl butazone
100	67.32±1.00	12.40±0.81
200	71.32±0.14	44.98±1.74
300	82.02±1.63	60.43±0.25
400	86.55±0.31	72.71±1.42
500	86.54±0.47	84.33±1.19
IC50	41.51 µg/mL	241.58 µg/mL

Each value represents the mean ± SEM of three replicates.

IC50: Inhibitory concentration

Table 5: Effect of ethanol extract of *Artabotrys hexapetalus* treatment on carrageenan-induced paw edema volume in rats

	0 hr	0.5 hr	% inhibition	1hr	% inhibition	2 hr	% inhibition	3 hr	% inhibition	4 hr	% inhibition
Control	0.186±0.005	0.336 ^a ±0.016	-	0.484 ^a ±0.022	-	0.580 ^a ±0.018	-	0.645 ^a ±0.038	-	0.698 ^a ±0.024	-
<i>A. hexapetalus</i>	0.195±0.007	0.222 ^b ±0.005	33.92	0.319 ^b ±0.011	35.41	0.345 ^b ±0.015	41.37	0.296 ^b ±0.014	54.68	0.215 ^b ±0.006	69.56
Phenyl butazone	0.166±0.007	0.219 ^b ±0.011	36.36	0.234 ^c ±0.009	52.03	0.271 ^c ±0.008	53.44	0.210 ^c ±0.006	67.18	0.153 ^c ±0.006	78.26

Means bearing different superscripts within a column (between treatment groups) differ significantly

Conclusion

The ethanolic leaf extract of *A. hexapetalus* demonstrated significant antioxidant and anti-inflammatory activities in

both *in vitro* and *in vivo* models. Its effects on DPPH scavenging, membrane stabilization, albumin denaturation and carrageenan-induced paw edema support its traditional

use and suggest potential for development as a natural anti-inflammatory therapeutic.

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