Original Research Article

In vitro Anti-inflammatory activity of endemic *Artocarpus nobilis* Thw found in Sri Lanka.

ABSTRACT

Aims: To investigate *in vitro* anti-inflammatory activity of aqueous, methanol, dichloromethane, and hexane extracts of *Artocarpus nobilis* Thw. leaves and stem bark using heat-induced protein denaturation test (egg albumin denaturation).
Methodology: About 500 g of each matured, fully expanded leaves and stem bark of *Artocarpus nobilis* Thw were collected. Washed and air-dried. Leaves and stem bark parts were grounded to obtain a fine powder material. The extractions were obtained using the decoction extraction method. Anti-inflammatory activity was evaluated using the heat-induced egg albumin denaturation method. Diclofenac sodium was used as the positive control.

Results: Results showed that Diclofenac sodium exhibited an IC_{50} value of 243.4 µg/mL and methanolic stem bark extract had an IC_{50} Value of 249.8 µg/mL) for heat-induced egg albumin protein denaturation test. R^2 and P values for aqueous, methanol, dichloromethane, and hexane extracts indicated that there was a strong, statistically significant correlation ($P<0.01$) between concentration and percentage inhibition for all extracts of *A. nobilis* Thw. However, methanol stem bark extract demonstrated the highest efficacy and potency with similar activity observed for the positive control Diclofenac sodium.

Conclusion: Methanol stem bark extract of *Artocarpus nobilis* Thw. have marked *in vitro* anti-inflammatory activity. Further studies are necessary to determine the mechanism and the active constituents responsible for the anti-inflammatory activity of the plant parts of *Artocarpus nobilis* Thw.

Keywords: Artocarpus nobilis Thw., Anti-inflammatory activity, ethanomedicine, protein denaturation, ~~heat induced egg albumin protein denaturation test~~, Sri Lanka, extract, leaves, stem.

1. INTRODUCTION

Inflammation is a nonspecific response from the body's immune system [1-3]. This reaction occurs when bacteria, trauma, chemicals, heat, or some other cause damages to the tissues, and chemical mediators are released by damaged cells. Blood vessels are stimulated by these chemicals to leak fluid into the tissues, causing swelling. This allows the foreign material to be removed from further contact with the body [2]. When inflammation is

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not regulated it can become rapid and aggressive causing more damage [3]. Anti-inflammatory agents control inflammation and prevent excessive tissue damage [4]. One of the most widely prescribed anti-inflammatory drugs is Non-steroidal anti-inflammatory medications (NSAIDs) [5]. Even though NSAIDs are effective for anti-inflammation they have demonstrated many side effects, such as gastrointestinal mucosal damage, increased blood pressure, congestive heart failure, and hormonal imbalances [6]. Therefore, it is important to identify new drug molecules with minimum side effects. Plants have many phytoconstituents that are effective for inflammation such as flavonoids, terpenoids, polyphenols, etc., and screening of medicinal plants for anti-inflammatory activity~~ion~~ is therefore important [7]. Traditional medicinal plants are a great alternative to find novel treatments and for the development of novel antimicrobials to combat many diseases/infections. Hence, screening of medicinal plants for anti-~~inflammation~~ inflammatory and anti-microbial activity is in all time high demand [7,26,27, 28,29, 30].

Artocarpus nobilis Thw. belongs to the family *Moraceae* with 40 genera and 60 species [10,11,17]. It is an economically and medicinally important endemic tree in Sri Lanka, which is listed under the "vulnerable" category by the World Conservation Monitoring Centre [8]. *Artocarpus nobilis* Thw. commonly found in the wet zone, the mid-country homesteads, and the wet zone forests in Sri Lanka [9]. It is known as "Wal Del", "Badi Del", "Sinhala Del" or as "Hingala Del" in Sinhala [10], and Aresini-pilaka, Asiri-pillakai in Tamil, Ceylon wild breadfruit in English [11]. Presently the *Artocarpus nobilis* Thw. is used in folk and ayurvedic medicine for its well-known anti-helminthic, and anti-microbial properties. Bark and latex are used for abscesses and blisters~~,~~. Edible fruits and seeds possess good nutritional value and are mainly used for worm infections (e.g. *Ascariasis*) and dysentery [10-12]. As anti-inflammatory activity can be mediated through anti-microbial activity, the presence of such properties may be taken as evidence of possible anti-inflammatory activity [13]. Various parts of this plant consisted of phytoconstituents which are responsible for anti-inflammatory activity and other pharmacological properties. In the *Artocarpus* genus as phytoconstituents flavonoids, terpenoids, triterpenes, polyphenols, geranyl chalcone derivatives, geranylated phenolic constituents, stilbene derivatives, xanthones, and cycloartane-type triterpenoids are contained [14]. Apart from these phytochemical constituents, pharmacological properties such as radical scavenging, anti-oxidant, anti-fungal [15-18], and acetylcholinesterase inhibitory activities had already been investigated [19]. Upon the review of previous studies, we identified that, there is a lack of research regarding anti-inflammatory activity on *Artocarpus nobilis* Thw. Therefore, we initiated the first set of in-vitro experiments to evaluate the anti-inflammatory activity of aqueous and solvent (methanol, hexane, and dichloromethane) extracts of leaves and stem bark of *Artocarpus nobilis* Thw. by using the *in- vitro* egg albumin denaturation method ~~[20].~~

2. METHODOLOGY

2.1 Collection and authentication of plant parts

Well grown and fully expanded fresh leaves and stem bark parts of *Artocarpus nobilis* Thw. (about 500g of each) were collected during daytime from an estate in Gampaha district in Western Province, Sri Lanka (Latitude of 7^0 23' 59.99" N and Longitude of 79^0 98' 59.99"). Collected plant parts were identified and authenticated by a Botanist at National Herbarium, Peradeniya, Sri Lanka.

2.2 Preparation of extracts ~~aqueous, methanol, dichloromethane, and hexane extracts of *Artocarpus nobilis* Thw. (Bedi del / Wal del) leaves and stem bark~~

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The dried and well-grounded ~~plant~~ powders of leaves and stem bark parts of *Artocarpus nobilis* Thw. were used for the extraction process. Four ~~extractions~~ extracts such as aqueous, methanol, dichloromethane and hexane were obtained using the decoction extraction method. ~~Extraction method, in brief,~~For extraction, 50 g of the fine powder was weighed and added to 500 ml of solvent. This was added to the reflux apparatus and then it was boiled slowly for 4 hours. The prepared extract was left for cooling and then was concentrated from the rotary vacuum evaporator into sterile glass vials.

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2.3 Evaluation of *in-vitro* anti-inflammatory activity

Dimethyl sulfoxide was used to resuspend the extracts to prepare following dilution series (15.625, 31.25, 62.5, 125, 250, 500, 1000, 2000 µg/ml) of each extract. Diclofenac sodium was used as the positive control. Phosphate-buffered saline 2.8 ml (pH 6.4) and 0.2 ml of egg albumin (from fresh hen's egg) were used in the preparation of reaction mixtures. 2 ml of test extract was gently combined with reaction mixtures at concentrations of 15.625 to 2000 µg/ml. The mixtures were then incubated in a water bath for 15 minutes at 37± 2 ºC and then heated for 5 minutes at 70ºC. Then, the reaction mixture was allowed to cool down at room temperature. The absorbance of reaction mixtures was measured at 660 nm after cooling. Phosphate buffer was used as the blank. Diclofenac sodium (Positive control) was treated similarly for absorbance determination. The percentage inhibition of protein denaturation of each test sample was calculated by using the following formula:

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$$\% \, Inhibition = 100 \, X \, \frac{(1 - Vt)}{Vc}$$

where,
Vt = Absorbance of the test sample at 660nm, Vc = Absorbance of control at 660nm [21].

Statistics~~Statistics~~:

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3. RESULTS AND DISCUSSION

Plant extract dose-response data for aqueous, methanol, dichloromethane and hexane leaves of *A. nobilis* (both leaves and stem bark) along with positive control are shown in table 1. The dose-response curve for extracts and positive control are shown in figure 1. All the extracts show a strong positive statistically significant correlation (P = 0.05) between concentration and percentage inhibition.

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Table 1. Dose-response curve details for methanol, dichloromethane, and hexane leaves and stem bark extract samples of *A. nobilis* Thw plant parts and positive control

Tabular results	Positive control (Diclofenac Na)	Aqueous extracts	Methanol extracts	Dichloromethane extracts	Hexane extracts

		Leaves	Bark	Leaves	Bark	Leaves	Bark	Leaves	Bark
IC50 (µg/mL)	243.4	263.5	553.4	338.5	249.8	289.8	557.2	359.8	341.4
R-squared	0.9759	0.9676	0.9642	0.9738	0.9852	0.9696	0.9977	0.8607	0.9803
P-value	0.0057	0.0048	0.0011	0.0023	0.0035	0.0088	0.0002	0.0061	0.0016

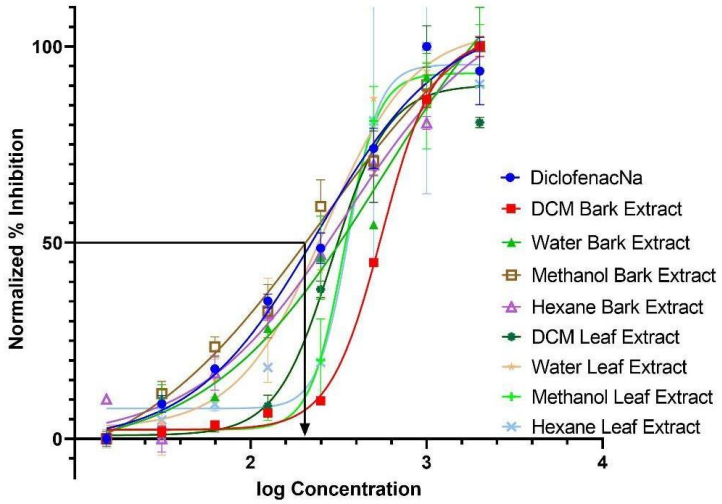

Fig. 1.: Dose-response curve for different extracts of *A. nobilis* Thw plant parts and positive control

This graph was prepared using Graphpad Prism 8 (version 8.2.1), using non-linear regression model according to the equation: Span = Top – Bottom, Y=Bottom + (Top-Bottom)/(1+10^((Log IC50-X)*HillSlope)), where X is the log of dose response or concentration, Y is response.

Diclofenac sodium exhibits an IC_{50} value of 243.4 µg/mL and a high R^2 value (R^2= 0.9759) conveying a strong positive relationship with the inhibitory percentage and log concentrations. Leaves extracts had following potencies aqueous (263.5 µg/mL) > dichloromethane (289.8 µg/mL) > methanol (338.5 µg/mL) > hexane (359.8 µg/mL). Stem bark extracts had following potencies: methanol (249.8 µg/mL) > hexane (341.4 µg/mL) > aqueous (553.4 µg/mL) > dichloromethane (557.2 µg/mL). In comparison of all leaves and stem bark extracts methanolic stem bark extract of *A. nobilis* Thw. exhibited the highest potency (249.8 µg/mL). This ~~was~~ activity was in par with the standard drug Diclofenac sodium (243.4 µg/mL). When compared the anti-inflammatory activity among extracts, methanolic stem bark extract shows almost 1.35 - fold higher activity compared to methanol leaves extract, 1.05 - fold higher activity compared to aqueous leaves extract, 2.22 - fold higher activity compared to aqueous stem bark extract, 1.16 - fold higher activity compared to dichloromethane leaves extract, 2.23 - fold higher activity compared to dichloromethane

stem bark extract, 1.44 - fold higher activity compared to hexane leaves extract and 1.37 - fold higher activity compared to hexane stem bark extract.

The differences in the results for each extract were due to the variations in hydrogen, hydrophobic, electrostatic, and disulphide bonding taking place due to the mechanisms of denaturation [22]. Anti-inflammatory activity is likely to be mediated via the synergistic effect of flavonoids, alkaloids, tannins, saponins, phenols, steroids, glycosides, and terpenoids [23]. According to the literature on phytochemical studies, the high presence of xanthone, xanthoangelol, terpenoids, stilbene, phenols, flavonoids, and flavone in different extracts might contribute to this positive anti-inflammatory activity. Flavonoids have analgesic and anti-inflammatory activity by inhibiting a number of inflammatory mediators [24]. Terpenoids have analgesic and anti-inflammatory properties. The ability to inhibit phospholipase A2 and thus block the metabolism of arachidonic acid has been attributed to these properties [24]. Polyphenols have anti-inflammatory activity, antioxidant activity, act as vasodilators, and also prevent endothelial dysfunction and thrombosis. The ability to inhibit the activity of Cyclooxygenase, Lipoxygenase, and inducible Nitric Oxide Synthase enzymes contribute to the anti-inflammatory property [25]. Environmental changes, the texture of the soil, the amount of rain, the average temperature of a particular area could also affect the formation of phytoconstituents and elemental composition of *A. nobilis* Thw.

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4. CONCLUSION

In conclusion, this study demonstrates, potent *in vitro* anti-inflammatory activity of methanol stem bark extract of *Artocarpus nobilis* Thw. compared to the positive control diclofenac sodium. Further elaborative studies are necessary to ascertain the mechanism and the active constituents responsible for the anti-inflammatory activities of the plant parts of *Artocarpus nobilis* Thw. Moreover, the results indicate a strong possibility of developing potent and cost-effective anti-inflammatory agents from the leaves and stem bark of *Artocarpus nobilis* Thw.

NOTE:

The study highlights the efficacy of " Panchagavya " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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Comment [a26]: Not in a propre order. Ref 30 in the Introduction part and not cited anywhere else? Please arrange the refernce properly.

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