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# Anti-inflammatory Activity of *Azima tetracantha* Root Bark Extract in Rats

R. Nagulsamy[1], L. Kalaiselvi[1], S.P. Preetha[1], R.P. Aravindh Babu[1], S. Ramesh[1]

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## ABSTRACT

Inflammation is associated with many pathological conditions such as arthritis, inflammatory bowel syndrome, cancer, etc. The side effects associated with the long term use of current anti-inflammatory drugs necessitates the need for the development of new drug with fewer side effects. The present study was aimed at evaluating anti-oxidant and anti-inflammatory activity of *Azima tetracantha* root bark, a plant widely used by tribal people and in Siddha medicine for the treatment of arthritis and rheumatism. Aqueous and methanolic extracts of *Azima tetracantha* root bark were prepared. Total phenol, flavoid, alkaloid, terpenoid and saponin content were analyzed. The anti-oxidant activity was determined by DPPH assay and total antioxidant capacity. The anti-inflammatory activity was evaluated by carrageenan induced paw edema model in rats. The total phenol and flavonoid contents were higher in methanolic extract compared to aqueous extract. The extracts were found to have anti-oxidant activity and dose-dependent anti-inflammatory activity in both male and female rats with highest average inhibition at 400 mg/kg. The results confirm the traditional use of this plant against inflammatory disorders and could be source for the development of novel drugs.

**Key words:** Anti-inflammatory, Antioxidant, *Azima tetracantha,* Caraageenan.

Inflammation plays a very important role in tissue healing but uncontrolled chronic inflammation is associated with many disease conditions such as arthritis, asthma, atherosclerosis, cancer, diabetes, Alzheimer's disease, Parkinsons disease, *etc* (Borquaye *et al*., 2020). Chronic inflammatory diseases continues to be one of the major health problem and it is usually treated using non-steroidal anti-inflammatory drugs (NSAIDs), steroids and immunosuppressants (Hussain *et al*., 2020). NSAIDs are most commonly used to control inflammation and pain in those conditions but their long term treatment can cause serious adverse effects such as gastric ulceration and bleeding, liver damage, kidney damage, stroke, cardiovascular complications and so on. Hence, there is an absolute need for alternate drugs with maximal efficacy and minimal side effect, which necessitates the pursuit of novel drugs from alternate systems of medicine.

*Azima tetracantha* is widely used in the alternative systems of medicine for the treatment various disease conditions including rheumatoid arthritis, cough, cold, fever, body pain, bronchitis, asthma, dropsy, *etc.* (Kekuda and Raghavendra, 2017). The roots of the plant are used in the herbal formulation called Pilavaikkalimbu, which is used topically in the treatment of tumors. The root of this plant is one of the components of *Parangichakkai Choornam*, polyherbal Siddha formulation which is indicated in the treatment of various diseases of pitha and kabha origin (Kumarasamy and Kumarswamy, 2014). There are studies that have corroborated pharmacological actions of leaf extract of this plant. However, there is little information about phytochemical composition and pharmacological actions of root extracts. Since the root bark is used by traditional healers and in Siddha medicine, this study was attempted to elucidate phytochemical composition and to validate anti-oxidant and anti-inflammatory potential of aqueous and methanolic extract of root bark of *A. tetracantha.*

[1]Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Chennai-600 007, Tamil Nadu, India.

**Corresponding Author:** L. Kalaiselvi, Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Chennai-600 007, Tamil Nadu, India. Email: kalaiselvivet@gmail.com

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The present study was conducted at Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Chennai from March 2022 to May 2022.

*Azima tetracantha* whole plant was collected and authenticated by Botanist at Department of Medicinal Botony, Government Siddha Medical College, Arumbakkam, Chennai. Aqeuous and methanolic extracts of root bark were prepared by maceration technique, lyophilized and stored at 4°C. The total yield of aqueous and methanolic extracts was 12% and 4%, respectively.

The qualitative phytochemical screening tests were carried out as per the procedure described by Deyab *et al.* (2016). The total phenol content in the extracts was determined by Folin- Ciocalteau method using gallic acid as a standard (Singh and Maurya, 2009). The total flavonoid

content of the extracts was determined by aluminium chloride method (Kamtekar *et al*., 2014) using Catechin as a standard. The alkaloid content of the extract was determined by titrimetric method as described by Debnath *et al.* (2015). The terpenoid and saponin contents of the extract were determined as per the method described by Malik *et al*. (2017) and Ezeonu and Ejikeme (2016). respectively

The free radical scavenging activity of plant extracts were evaluated using DPPH (2, 2-diphenyl-1-picryl hydrazyl) (Zahin *et al*., 2009). The total antioxidant capacity of the extract was measured by phosphomolybdenum method as described by Wan *et al.* (2011) and expressed as ascorbic acid equivalent (AAE) per g of extract.

The anti-inflammatory activity was evaluated by carrageenan induced paw edema model in Wistar rats. The rats were maintained under standard conditions of relative humidity and temperature and provided with rodent pellet feed and water *ad libitum.* The protocol was approved by

**Table 1:** Experimental design.

| Group | Details | Dose | No. of animals |
|---|---|---|---|
| I | Carrageenan control | 0.1 ml of 1% w/v | 6 Male + 6 Female |
| II | Carrageenan+*A. tetracantha* aqueous extract @ 200 mg/kg | 200 mg/kg | 6 Male + 6 Female |
| III | Carrageenan+*A. tetracantha* aqueous extract @ 400 mg/kg | 400 mg/kg | 6 Male + 6 Female |
| V | Carrageenan+*A. tetracantha* methanolic extract @ 200 mg/kg | 200 mg/kg | 6 Male + 6 Female |
| V | Carrageenan+*A. tetracantha* methanolic extract @ 200 mg/kg | 400 mg/kg | 6 Male + 6 Female |
| VI | Carrageenan+Diclofenac sodium | 10 mg/kg | 6 Male + 6 Female |

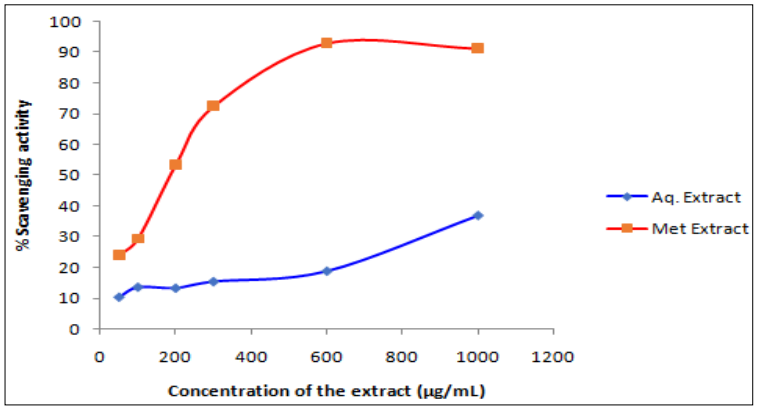


**Fig 1:** DPPH free radical Scavenging activity.

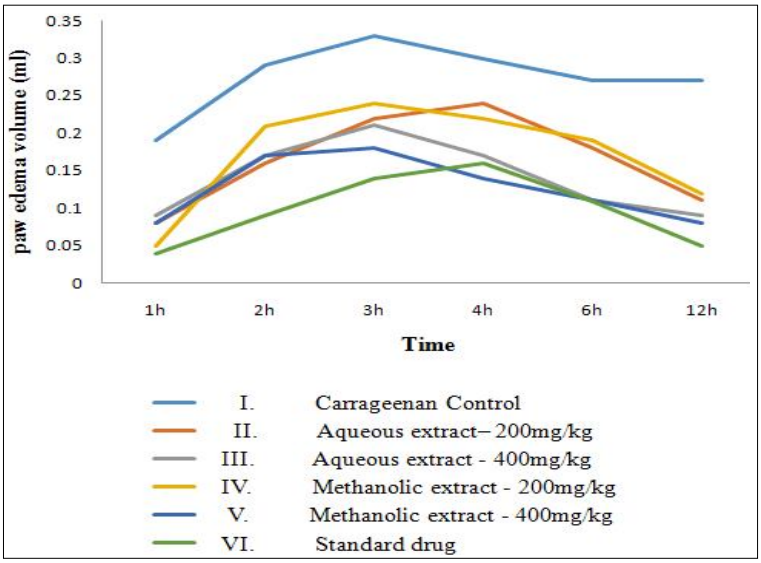


**Fig 2:** Changes in Paw edema volume in male rats.

the Institutional Animal Ethical Committee, Madras Veterinary College (12/SA/IAEC/2022). The animals were acclimatized and randomly divided into 6 groups as given in the experimental design below (Table 1).

**Table 2:** Phytochemical screening of *Azima tetracantha* root bark extract.

| Compound | Aqueous extract | Methanolic extract |
|---|---|---|
| Cyanin (beta cyanin) | + | + |
| Coumarin | + | + |
| Amino acids | - | - |
| Free sugar | + | + |
| Terpenoid | + | + |
| Phenol | + | + |
| Alkaloid | + | + |
| Flavonoid | + | + |
| Saponin | + | + |
| Tannin | + | + |
| Glycosides | - | - |
| Protein | - | - |
| Steroid | - | - |
| Quinine | + | + |
| Carbohydrates | + | + |

+ indicates presence and - indicates absence.

Carageenan was injected into sub-plantar region of hind paw to induce acute inflammation. Paw edema was measured before and at various time intervals after carrageenan injection. The anti-inflammatory activity was calculated as percentage inhibition of oedema in comparison to the carageenan control using the formula,

$$\frac{Vc - Vt}{Vc} \times 100$$

Where,

Vc = Oedema volume of carrageenan control group.

Vt = Oedema volume of treatment group.

The results were expressed as mean±standard error of the mean (SEM) and statistically analysed by one-way ANOVA followed by Duncan's Posthoc analysis. Values were considered significantly different at $P<0.05$.

The alternative systems of medicine have gained attention in the recent years. With the increased use of herbal drugs, validation of efficacy of the herbal plants by scientific studies becomes essential. *A. tetracantha* root is widely used in the siddha medicine and also by tribal people and traditional healers in the treatment of various disease conditions including rheumatoid arthritis. In the present study, the preliminary phytochemical analysis of *A. tetracantha* root bark extracts revealed the presence of various phytochemicals

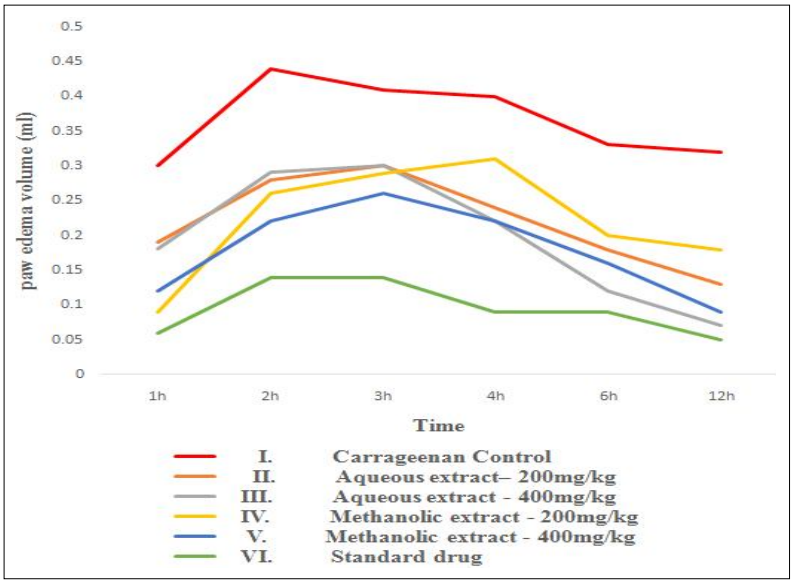


**Fig 3:** Changes in paw edema volume in female rats.

**Table 3:** Quantitative phytochemical analysis of *Azima tetracantha* root bark extract.

| | Aqueous extract | Methanolic extract |
|---|---|---|
| Phenol (mg Gallic acid Equivalent/g extract) | 89.14±0.56 | 191.6±0.05 |
| Flavonoid (mg Catechin equivalent/g extract) | 55.61±2.84 | 105.88±10.46 |
| Alkaloid (mg/g extract) | 137.70±1.79 | 143.00±1.33 |
| Terpenoid (%) | 36.10±1.1 | 73.80±3.2 |
| Saponin (%) | 48.60±0.26 | 24.20±0.79 |

Values are expressed as Mean±S.E.M (n=3).

**Table 4:** Per cent inhibition of Inflammation in Male rats.

| Group | % inhibition of Inflammation | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 h | 2 h | 3 h | 4 h | 6 h | 12 h | Average inhibition |
| II - *A. tetracantha* aqueous extract 200 mg/kg | $59.20^{abc}$±8.44 | $45.78^{ab}$±7.88 | $32.42^{ab}$±3.47 | $21.14^{a}$±3.65 | $34.07^{a}$±8.25 | $58.96^{a}$±9.97 | $41.93^{a}$±0.67 |
| III - *A. tetracantha* aqueous extract 400 mg/kg | $51.39^{a}$±10.47 | $42.92^{ab}$±10.78 | $37.50^{ab}$±9.31 | $41.83^{a}$±12.90 | $60.32^{a}$±7.10 | $65.18^{a}$±9.76 | $49.86^{ab}$±7.77 |
| IV - *A. tetracantha* methanolic extract 200 mg/kg | $75.70^{bc}$ ±4.59 | $29.80^{a}$ ± 9.84 | $27.34^{a}$±10.69 | $26.18^{a}$±8.22 | $30.40^{a}$±10.27 | $56.47^{a}$±13.34 | $40.98^{a}$±8.03 |
| V - *A. tetracantha* methanolic extract 400 mg/kg | $56.60^{ab}$±8.68 | $42.35^{ab}$±10.06 | $46.65^{ab}$±10.10 | $51.90^{a}$±13.83 | $61.54^{a}$±16.32 | $69.53^{a}$±14.21 | $54.76^{ab}$±9.91 |
| VI - Diclofenac 10 mg/kg | $81.77^{c}$±4.41 | $68.04^{b}$±5.64 | $57.83^{b}$±6.32 | $47.43^{a}$±9.07 | $59.10^{a}$±6.85 | $82.59^{a}$ ±5.91 | $66.12^{b}$±4.47 |

Values are expressed as Mean±S.E.M (n= 6), Means bearing different superscripts in a column differ significantly ($p<0.05$).

**Table 5:** Per cent inhibition of inflammation in female rats.

| Group | % inhibition of Inflammation | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 h | 2 h | 3 h | 4 h | 6 h | 12 h | Average inhibition |
| II - *A. tetracantha* aqueous extract 200 mg/kg | $38.33^{a}$±4.85 | $36.36^{a}$±5.31 | $26.56^{a}$±4.41 | $39.88^{a}$±5.03 | $44.11^{a}$±9.50 | $60.94^{ab}$±12.12 | $41.03^{a}$±4.64 |
| III - *A. tetracantha* aqueous extract 400 mg/kg | $40.00^{a}$±8.39 | $35.23^{a}$±2.61 | $28.17^{a}$±7.49 | $44.44^{a}$±7.34 | $63.92^{ab}$±8.78 | $79.69^{bc}$±6.69 | $48.58^{a}$±2.89 |
| IV - *A. tetracantha* methanolic extract 200 mg/kg | $69.44^{b}$±6.17 | $40.15^{a}$±8.15 | $28.98^{a}$±9.58 | $22.89^{a}$±8.25 | $38.01^{a}$±9.47 | $44.80^{a}$±6.64 | $40.71^{a}$±6.20 |
| V - *A. tetracantha* methanolic extract 400 mg/kg | $60.56^{ab}$±10.38 | $50.76^{ab}$±10.18 | $36.64^{a}$±11.16 | $45.69^{a}$±7.82 | $52.74^{ab}$±6.48 | $71.88^{bc}$±5.17 | $53.04^{a}$±6.82 |
| VI - Standard drug (Diclofenac sodium 10 mg/kg) | $81.67^{b}$±6.93 | $68.56^{b}$±7.29 | $65.70^{b}$±7.84 | $77.20^{b}$±7.31 | $73.58^{b}$±7.90 | $58.94^{c}$±5.02 | $75.44^{b}$±6.68 |

Values are expressed as Mean±S.E.M (n= 6), Means bearing different superscripts in a column differ significantly ($p<0.05$).

which includes terpenoid, phenol, alkaloid, flavonoid, saponin, tannin, quinine and carbohydrates (Table 2).

The total phenolic and total flavanoid content was found to be higher in methanolic extract compared to aqueous extract (Table 3). Our findings is consistent with several reports of higher total phenol content in methanol extract than the other solvents which could be due to more effective extraction of polyphenolic chemicals in methanol (Jung *et al*., 2004; Choi *et al*., 2007). The terpenoid content in the methanolic extract was higher than aqueous extract whereas aqueous extract had higher saponin content. Phytochemical data on *A. tetracantha* root bark are very limited and to the best of our knowledge, this is the first report of the phytoconstituent in aqueous root extracts of *A. tetracantha*.

In DPPH assay, methanolic extract showed higher antioxidant activity with an $IC_{50}$ of 0.226 mg/mL compared to aqueous extract ($IC_{50}$-1.19 mg/mL) (Fig 1). The total antioxidant capacity of aqueous and methanolic extracts of *A. tetracantha* root were 117.81±13.91 and 126.35±126.35 mg AAE per g extract.

Phytochemicals such as phenols and flavonoids contributes to the antioxidant activity of the plant extracts (Ravipati *et al*., 2012). In the present study, we found higher amount of total phenol and flavonoids in methanolic extract compared to aqueous extracts and this was reflected by higher antioxidant activity in methanolic extract compared to aqueous extract.

The anti-inflammatory effect of the extracts was evaluated in carrageenan-induced paw edema model. In the present study, carrageenan control rats showed increased paw edema which increases in time with maximum edema at 3-4 h after carrageenan injection. Both the extracts of *A. tetracantha* caused reduction in paw edema compared to carrageenan control which was significant at all time points in both male and female rats (Fig 2 and 3). The % anti-inflammatory activity are given in Table 4 and Table 5. Both the extracts showed dose-dependent inhibition of paw edema from 1 h and it continued up to 12 h in both male and female rats with maximum activity at 400 mg/kg. In male rats, inhibition of inflammation in rats treated with high dose of extracts was higher and did not differ significantly from the diclofenac standard.

The carragenan induced paw edema is a well established animal model to evaluate anti-inflammatory property of the herbal products. The development of carrageenan induced paw edema occurs in two phases. The first phase is observed within 1-2 h of induction and is mediated by histamine, serotonin and bradykinin. The second phase (3-6 h) is mediated by prostaglandins, nitric oxide and various cytokines including IL-6, IL-1β, TNF-α (Karim *et al*., 2019). In present study, we observed inhibition of edema formation by the extracts of *A. tetracantha* in both first phase and second phase and maximum inhibition was noticed with methanolic extract at 400 mg/kg.

## CONCLUSION

The results of the study demonstrated antioxidant and anti-inflammatory activity of *A. tetracantha* and provides scientific validation and justification for the traditional use of this plant in inflammatory conditions. The activity could be due to phytochemicals such as to phenols, flavonoids and terpenoids present in the extract. Further studies are warranted to isolate the bioactive compound and to elucidate the mechanism of action.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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