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ANTIPYRETIC POTENTIAL OF AQUEOUS LEAF EXTRACT OF *ANNONA MURICATA*.L AND *SPERMACOCE ARTICULARIS.* L.F. ON YEAST-INDUCED PYREXIA IN RATS

MARUVOOR ARASI K[1]*, KRISHNAVENI C[2]

[1]PhD scholar, Department of Botany, PSGR Krishnammal College for Women (Autonomous), Coimbatore-641004, Tamil Nadu, India. [2]Assistant Professor, Department of Botany, PSGR Krishnammal College for Women (Autonomous), Coimbatore-641004, Tamil Nadu, India. Email: arasibiology@gmail.com

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ABSTRACT

Objective: The objective of this study is to evaluate the potential of *in vivo* antipyretic activity of the aqueous leaf extracts of *Annona muricata* L. and *Spermacoce articularis*. L.f.

Methods: The acute oral toxicity was determined by the Organization of Economic and Cooperation Development-423 class methods, and the *in vivo* antipyretic activity was determined by brewer's yeast induced pyrexia method.

Results: The results showed that the aqueous leaf extract of *A. muricata.* L and *S. articularis* L.f plants is non-toxic and possessed significant antipyretic effect.

Conclusion: This study provides evidence for the antipyretic activity of *A. muricata.* L and *S. articularis* L.f. The aqueous leaf extract of *S. articularis* L.f at a dose of 400 mg/kg showed a more significant effect ($p<0.01$) in lowering the hypothermia than the extract of *A. muricata* L but found to have a similar effect as the standard drug aspirin (100 mg/kg).

Keywords: *Annona muricata L,* Antipyretic activity, Brewer's yeast, *Spermacoce articularis.* L.f., Pyrexia.

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INTRODUCTION

Fever or pyrexia [1] is a condition characterized by the elevation in the body's thermoregulatory set point. "Carl Wunderlich" discovered that "fever is not a disease, but a symptom of disease." It is one of the most adaptive immune responses to inhibit the growth of microorganisms. Body thermostat is normally maintained by the periphery of the brain. The slightest disturbance in the thermostat set point due to microbial infection results in triggering of pyrogens. Lipopolysaccharide produced by Gram-negative bacteria stimulates exogenous pyrogen such as interleukin-1 (IL-1), IL-6, interferon-γ (IFN-γ), and tumor necrosis factor. These molecules act directly on the hypothalamic thermoregulatory centre and induce fever. Likewise, endogenous pyrogen and inflammation enhance the innate immune defenses by stimulating leukocytes to kill pathogens. Many evidence suggest that iron sequestering compounds from the liver also stimulate fever to starve out the iron-dependent microbes [2].

The hypothalamus increases heat production by vasodilation of blood vessels in the skin and shivering and stimulates hormones like epinephrine (adrenaline), and the negative feedback includes prevention of heat loss by vasoconstriction. Increased heart rate and vasoconstriction contribute to increased blood pressure in fever.

Reliving fever and discomfort is achieved by antipyretics such as paracetamol and ibuprofen, but many clinical evidence show that it has been complicated and may increase the risk of overdose and medication errors. The presence of phytochemical constituents such as flavonoids, saponins, glycosides, and tannins [3] has a major contribution toward antipyretic activity without any side effects. The inhibitory activity on prostaglandins (PGs) may be the characteristics of antipyresis [4]. The yeast-induced hyperpyrexia in rats was employed to bring out the potential of antipyretic activity.

According to the World Health Organization, it is estimated that 80% of people worldwide depending on herbs for their primary health care and thus it gains the attention of many researchers [5].

Annona muricata L., commonly known as sour soup, graviola, guanabana, pawpaw, and sirsak, is a member of the Annonaceae family comprising approximately 130 genera and 2300 species [6,7].

It is a slender evergreen tree, 5–10 m in height and 15 cm in diameter, trunk straight, and bark smooth, rough, and fissured with age. It is widely distributed in tropical regions of the world. Young branches are hairy. Leaves are alternate, simple, 7.6–15.2 cm long, leathery, obviate to elliptic, glossy on top, and glabrous on underside. The leaves stalk is 4–13 mm long and without hairs [8]. The leaves of *A. muricata*. L have astringent, antiplasmodic, and gastric properties [9]. It is a traditional medicinal plant in Indonesia to treat breast cancer. They are rich in annonaceous acetogenins. The increasingly popular use of *A. muricata*. L as an anticancer treatment reported ethanol botanically may be related to reports of its selective cytotoxic activity. In ancient time, it had been used as herbal remedies in treating diabetes, hypertension, fever, vomiting, and against worm [10]. Other than that, it also has been used in treating headaches, cough, and asthma and as a sedative [11,12]. Many researchers demonstrated the potential of sour soup against blood pressure [13] in addition to that the bark as well as the leaves have antihypertensive, vasodilator, antispasmodic, and cardiac depressant activities in animals.

Spermacoce articularis. L.f. (Rubiaceae) was popularly known as "Nattaiccuri" in Tamil and "Shaggy button weed" in English. It is widely distributed in the Western Ghats of Kerala [14] and Maruthamalai forest, in Tamil Nadu. *S. articularis.* L.f. removes old age signs, improves vitality, and it was used by the tribes in Western Ghats of Kerala since ancient times [15]. *S. articularis.* L.f. is one of the crude materials used for the treatment of various ailments in the form of various preparations. The plant seed was used as a remedy to treat nerve and kidney injuries [16]. Its pharmacological properties include antioxidant [17] and anti-inflammatory [18]. Bioactive molecules isolated from plants served as the starting materials for isolation and laboratory synthesis of drugs as

well as a model for the production of biologically active compounds [19]. A study [20] reported that the extract from *S. articularis.* L.f. showed strong α,α-diphenyl-β-picrylhydrazyl free radical scavenging activity comparable with quercetin, butylated hydroxytoluene, and Vitamin C, whereas *Spermacoce exilis* showed only moderate activity.

The present study has been undertaken to investigate the antipyretic activity of the aqueous extracts of *A. muricata. L and S. articularis.* L.f. leaf by Brewer's yeast-induced pyrexia method.

METHODS

Collection of plant material

The leaves of *A. muricata* L. and *S. articularis.* L. were collected from Aanaikatti, Coimbatore district, Tamil Nadu, India, in November 2015. The plant was identified and authenticated by the Botanical Survey of India, Coimbatore. The leaves of *A. muricata.* L and *S. articularis.* L.f. were washed and cleaned to remove foreign organic matter, cut into small pieces, and then kept for drying in the shade. The dried plant parts were made into coarse powder. These powders were stored in airtight container and used for further extraction.

Preparation of extracts

The extract from the leaf powder was taken using Soxhlet extractor with water as a solvent. The collected extract was evaporated to dryness and stored in 4°C for experimental study. The extracts were stored in airtight container and used for further experimental purposes.

Experimental animals

Albino Wistar rats of either sex weighing between 180 and 200 g were used in this study. The animals were obtained from the animal house, Nandha College of Pharmacy, Erode, Tamil Nadu, India. They were maintained in polypropylene cages with paddy husk as bedding at a temperature of 24±2°C and relative humidity of 30–70% with 12:12 light and dark cycle. Animals were allowed free access to water and fed with standard commercial pelleted rat chow (M/s. Hindustan Lever Ltd., Mumbai). Animals were acclimatized to the laboratory conditions 1 week before the experiment and fasted overnight before the experiment. Experimental protocol was approved by the Institutional Animal Ethics Committee (No.688/PO/Re/S/02/CPCSEA).

Acute toxicity study

Acute toxicity studies were performed according to the Organization of Economic and Cooperation Development-423 guidelines [18]. The aqueous extract of *A. muricata.* L and *S. articularis.* L.f. was administered orally at a dose of 5 mg/kg initially and mortality if any was observed for first 24 h and after 72 h. If mortality was observed in two of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal of three animals, then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher (50, 300, 1000, and 2000 mg/kg) doses of the plant extracts were employed for further toxicity studies [22].

Brewer's yeast-induced pyrexia in rats

This antipyretic activity animal model was the slightly modified method described by Adams *et al*. [21].

Six groups of 5 rats each were injected subcutaneously with 10 ml/kg of yeast suspension (15% in 0.5% w/v methylcellulose) to induce pyrexia, after measuring the basal rectal temperature of each animal. 19 h after yeast injection, the rectal temperature was recorded again and animals showing a rise in temperature of <0.6°C were discarded.

Thereafter, treatment as follows:

The rats were divided into six groups of 5 each

1. The Group I - Normal control (distilled water, 1 ml/kg).
2. Group II - Reference control (Aspirin-100 mg/kg).
3. Group III - Aqueous leaf extract of *A. muricata. L* (200 mg/kg).
4. Group IV - Aqueous leaf extract of *A. muricata. L* (400 mg/kg).
5. Group V - Aqueous leaf extract of *S. articularis.* L.f. (200 mg/kg).
6. Group VI - Aqueous leaf extract of *S. articularis.* L.f. (400 mg/kg).

The test drugs were administered orally using gastric gavages by dissolving in distilled water. The rectal temperature was then recorded in 20, 21, 22, 23, and 24 h after yeast injection. Percentage reduction in rectal temperature was calculated by the following formula.

$$\% \text{ Reduction } = \frac{B - Cn}{B - A} \times 100$$

a. Normal temperature.
b. Rectal temperature at 18 h after yeast administration.
c. Rectal temperature after drug administration.

Statistical analysis

All the results were expressed as mean ± standard error mean. The data were analyzed using one-way analysis of variance followed by Dunnett's *t*-test using Graph Pad software of version 3 $p<0.05$ was considered as statistically significant.

RESULTS

Acute toxicity study

The results of acute toxicity study of aqueous extracts of *A. muricata*. L and *S. articularis.* L.f. were summarized in Table 1. Mortality was not produced by both aqueous extracts up to 2000 mg/kg on oral administration after 24 and 72 h. On general behavior, the aqueous extract of *A. muricata. L* showed moderate analgesic and mild muscle relaxant activity, and all other general behavior observed were normal, whereas the aqueous extract of *S. articularis.* L.f. *showed* mild analgesic activity and other general behavior remains normal. No lethality or toxic reactions were found with both the extracts, during and after the study period

Brewer's y*e*ast-induced pyrexia in rats

The effects of aqueous leaf extracts of *A. muricata.* L and *S. articularis.* L.f. were studied in albino rats by observing its antipyretic activity induced by Brewer's yeast. The experiment showed (Table 2) that the leaf extract of *S. articularis.* L.f exhibited was statistically more significant effect ($p<0.01$) at a dose of 400 mg/kg (Group-VI) after 24 h of administration of the extract and found to have a similar effect as the standard drug aspirin administration.

The aqueous leaf extract of *S. articularis.* L.f at a dose of 200 mg/kg (Group-V) has moderately significant effect ($p<0.01$) compared to standard drug aspirin. In addition to this, the effects of an aqueous leaf extract of *A. muricata. L at* a dose of 400 mg/kg (Group-IV) exhibit very less antipyretic activity.

Table 1: Oral acute toxicity study of aqueous extract of *A. muricata. L* and *S. articularis.* L.f. (2000 mg/kg) in mice

General behavior	*A. muricata. L*	*S. articularis* L.f
Sedation	–	–
Hypnosis	–	–
Convulsion	–	–
Ptosis	–	–
Analgesia	++	+
Stupor Reaction	–	–
Motor activity	–	–
Muscle Relaxant	+	–
CNS stimulant	–	–
CNS depressant	–	–
Piloerection	–	–
Skin color	–	–
Lachrymation	–	–
Stool consistency	–	–

+: Present and –: Absent. CNS: Central nervous system, *A. muricata. L: Annona muricata, S. articularis*. L.f.: *Spermacoce articularis* L.f.

Table 2: The antipyretic effect of aqueous extract of *A. muricata. L* and *S. articularis.* L.f. on yeast-induced pyrexia in rats

Drug Treatment	Rectal temperature (°C)						
	Normal	After yeast	After drug administration				
		19 h	20 h	21 h	22 h	23 h	24 h
Control distilled water (10 ml/kg)	37.42±1.27	38.72±0.93	39.05±1.03	39.15±0.95	39.47±0.83	39.58±0.82	39.83±1.73
Reference control aspirin (100 mg/kg)	37.51±0.85	38.94±1.05	38.67±1.23	38.17±1.27*	37.73±0.87**	37.54±1.32**	37.50±0.92**
A. muricata (200 mg/kg)	37.62±1.20	38.76±1.22	39.02±1.04	39.07±0.96	38.94±0.76	38.67±0.74	38.32±0.62*
A. muricata (400 mg/kg)	37.60±1.18	38.85±1.42	39.02±1.12	38.86±0.84	38.46±1.62*	38.12±1.22*	37.93±0.78**
S. articularis (200 mg/kg)	37.50±1.38	38.83±0.98	38.95±1.17	38.63±1.47	38.16±1.25*	37.84±1.62*	37.66±1.52**
S. articularis (400 mg/kg)	37.48±1.56	38.97±0.96	38.69±1.26	38.22±1.35*	37.85±1.62**	37.66±0.87**	37.52±0.73**

Percentage reduction was given in parentheses. Values are in mean±SEM (n=5) *significantly different from the control at the corresponding time *p<0.05, SEM: Standard error of the mean, **p<0.01 **moderately significant compared with the control and ***p<0.001 highly significant compared with the control.
A. muricata: Annona muricata, S. articularis: Spermacoce articularis

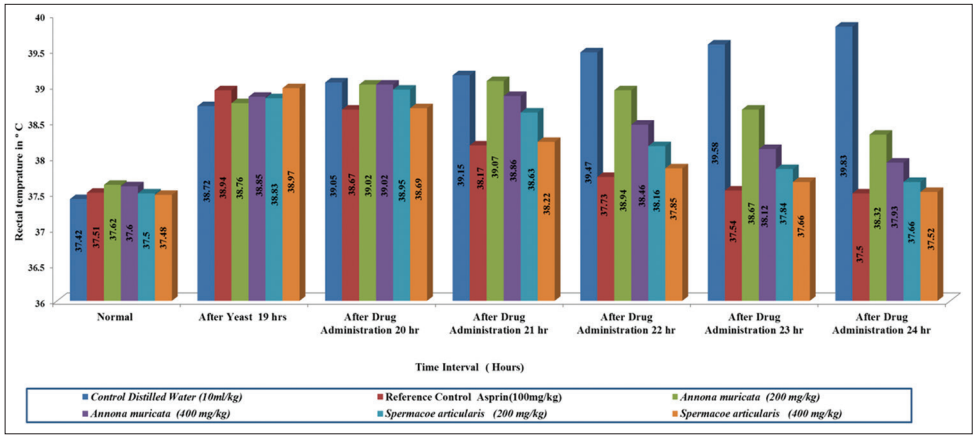

Fig 1: Antipyretic effect of aqueous extract of *Annona muricata. L* and *Spermacoce articularis* L.f. on yeast-induced pyrexia in rats

However, the *S. articularis.* L.f (Fig 1) fraction showed better rectal temperature reduction when compared to other plant.

DISCUSSION

The results of the above experiment suggested that the effects of aqueous leaf extracts of *A. muricata.* L and *S. articularis.* L.f. possess antipyretic activity at the tested doses.

Phytochemical studies on *S. articularis.* L.f. originated from India indicated the presence of flavonoids, triterpenoids, and ursolic acid [23,24] *A. muricata* L. leaves also have phytochemicals such as β-sitosterol, triterpenes, flavonoids, saponins, glycosides, tannins, alkaloids, proteins, lipids, and carbohydrates. Innate immunity can be boosted by these phytocomponents mediated by adaptogen [25]. The presence of steroids, tannins, and flavonoids in turn inhibits synthetase, carboxylase, or lipoxygenase enzymes. This inhibition helps in antipyretic activity [26]. In addition to that *A. muricata.* L has also been extensively used in targeting cancer cells rather than normal cells, which is a new challenging therapy along with the potential antipyretic effect [27].

The present results show that the aqueous leaf extract of *S. articularis.* L.f has more significant antipyretic activity than aqueous leaf extracts of *A. muricata* L. in yeast-induced pyrexia in rats, and this effect is comparable to standard drug aspirin.

To conclude, there are many immune factors which contribute to antipyretic action, but inhibition of certain enzymes and PGs like aspirin [28] may be one of the factors for significant antipyresis. The several multiprocessor or mediators by pathogenesis can be screened, and inhibition of these mediators can have a potential antipyretic activity [29].

CONCLUSION

The outcome of this study indicates that the aqueous leaf extract of *A. muricata*. L and *S. articularis.* L.f. possesses antipyretic property at the tested doses. However, *S. articularis.* L.f leaf showed better action when compared to others. This could provide a rationale for the use of these plants in fever as an herbal medicine without any side effects.

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