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IDENTIFICATION OF PHYTOCHEMICALS AND ANTI-PYRETIC ACTIVITY OF *Enicostema axillare* Lam.

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ABSTRACT

In the present study to investigate the phytochemicals and anti-pyretic activity of *Enicostema axillare* Lam Antipyretic effect of alcoholic extract of *Enicostema axillar* (Lam.) A. Raynal., (Gentianaceae) leaf against brewer yeast induced pyrexia model in albino rats was investigated. Pyrexia was induced by subcutaneously injecting 20% w/v Brewer's yeast suspension (20 ml/kg). Twenty four hours after the injection, the rectal temperature of each rat was measured. The temperature was measured at every 30mints upto 4hrs after *Enicostema axillar* extract administration of different concentrations (100, 250 and 500mg/kg b.wt.). Paracetamol (150 mg/kg p. o.) was used as standard drug. The group received alcoholic extract 500mg/kg showed significant decrease in rectal temperature as compared with the group received standard drug. The statistically processed results support the conclusion, that the alcoholic extract of *Enicostema axillar* (500mg/kg) possesses dose dependent, significant antipyretic activity due to the phytochemicals Flavonoids and phenolic compounds present in the plant. From the study it was concluded that plant of *Enicostema axillar* possesses significant antipyretic activity.

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INTRODUCTION

The World Health Organization (WHO) estimates that 80% of the people of developing countries rely on traditional medicines, mostly plant-derived drugs, for their primary health needs. Medicinal plants are commonly used in treating and preventing specific ailments and are considered to play a significant role in health care. Use of plants in Traditional medicinal systems is an indispensable source of medicinal preparations. Hundreds of species are recognized as having medicinally value. Indeed, 'Phytomedicines' are beginning to link traditional and modern medicines. (WHO, 1993).

Fever or pyretic is defined as the elevation of core body temperature above normal; in normal adults, the average oral temperature is 36.98°C (98.58°F). In oncology practice, a single temperature of more than 38.3°C (101°F) or three readings (at least 1 hour apart) of more than 38°C (100.4° F) are considered significant. Lower temperature elevations in the very young or old and in patients receiving steroids or other immunosuppressants are considered abnormal. Fever of an Unknown Origin (FUO) is defined as a febrile illness lasting more than 3 weeks, with temperatures exceeding 38.3°C on several occasions, and lacking a definitive diagnosis after 1 week of evaluation in the hospital (Aronoff and Neilson, 2001). In the present study to investigate the phytochemicals of *Enicostema axillare* extract by qualitative analysis and evaluate the antipyretic effects in brewer's yeast-induced pyrexia in rats.

MATERIALS AND METHODS

Plant materials:

The fully mature *Enicostema axillare* leaves were collected in December 2015 from Vannarapettai, Thanjavur district, Tamil Nadu, India.

Preparation of alcoholic extract:

The leaf of *Enicostema axillare* was first washed well and dust was removed from the leaves. Leaf was washed several times with distilled water to remove the traces of impurities from the leaf. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% methanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Animals

Male albino rats of Wistar strain approximately weighing 160-180g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The animal feed composition is crude protein (22.3%), crude oil (4.01%), crude fibre (4.02%), Ash (8.02%) and sand silical (1.02%).

Phytochemical analysis

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

Antipyretic activity

Yeast-induced hyperpyrexia in rats

Antipyretic activity was measured by slightly modifying the method described by Adams *et al*. (1968). Rats were fasted overnight with water *ad libitum* before the experiments. Animals were divided into five groups containing six animals each. The first group was given saline, the second, third and fourth group was given 100, 200 and 300 mg/kg of the extract, respectively, and the fifth group was given as standard Paracetamol (150mg/kg b.w.). Pyrexia was induced by subcutaneously injecting 20% w/v brewer's yeast suspension (10ml/kg) into the animal's dorsum region. 19hrs after the injection, the rectal temperature of each rat was measured using a digital thermometer. Only rats that showed an increase in temperature of at least 0.7℃ were used for experiments. *Enicostema axillare* (100, 250mg/kg and 500mg/kg b.w.), Paracetamol (150mg/kg b.w.) was administered orally and the temperature was measured at 1, 2, 3 and 4 hrs after treatment.

Experimental Design

Group I:Pyretic rats ; Group II: Pyretic rats treated with 100mg of *Enicostema axillare* leaf extract; Group III; Pyretic rats treated with 250mg of *Enicostema axillare* leaf extract Group IV: Pyretic rats treated with 500mg of *Enicostema axillare* leaf extract. Group V: Pyretic rats treated with 150mg of Paracetamol (Standard)

RESULTS AND DISCUSSION

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Attractions of pollinators, natural defense system against predators and diseases, etc., are examples of the roles of secondary metabolites (Sofowara, 1993).

In the present study was carried out on the *Enicostema axillare* leaves revealed the presence of medicinally active constituents. The phytochemical characters of the *Enicostema axillare* leaves investigated and summarized in Table-1. The phytochemical screening *Enicostema axillare* leaves showed that the presence of flavonoids, polyphenolics, tannin, saponins, glycosides, alkaloids, carbohydrate, terpenoids, triterpenoids and anthroquinones, steroids, phlobatannins while protein was absent.

Vikas Kumar *et al*. (2009) examined leaves of *Paederia foefida* for the pharmacognostial and phytochemical studies. Aiyelaagbe and Osamundiemen (2009)were screened *Mangifera indica* for the chemically active compounds. Qualitative analysis was made for the active compounds present in the four important medicinal plants *Acalypha indica, Cassia auriculata, Eclipta alba* and *Phyllanthus niruri* (Chitravadivu *et al*., 2009).

Table: 1 Phytochemical screening of *Enicostema axillare* leaf

S.No	Phytochemical analysis	Observation	Results
1	Tannin	Blue black	+
2	Phlobatannins	Red precipitated	+
3	Saponin	Emulsion	+
4	Flavonoids	yellow	++
5	Steroids	Blue	+
6	Terpenoids	Redish brown	++
7	Triterpenoids	Violet	+
8	Alkaloids	White precipitated	++
9	Carbohydrate	Red precipitated	+
10	Protein	No characteristic colour	-
11	Anthroquinone	Pink	+
12	Polyphenol	Bule green	+
13	Glycoside	Brown ring	+

(+) Presence (-) Absence

Antipyretic activity

The effect of the *Enicostema axillare* on normal body temperature in rats is presented in Table 4. It was found that the *Enicostema axillare* at doseof 500 mg/kg body wt. caused significant lowering of body temperature up to 4 h following its administration. This effect was maximal at 4 hrs and doses of 100 and 250 mg/kg body wt. in a dose-dependent manner and caused significant lowering of body temperature up to 4 h after its administration. The administration of a yeast suspension elevated the rectal temperature markedly after 19 h of administration. Treatment with the Enicostema axillare at doses of 100, 250 and 500 mg/kg body wt. decreased the rectal temperature of the rats in a dose-dependent manner. The antipyretic effect started as early as 1 h, and the effect was maintained for 4 h, after its administration. The standard drug paracetamol at 150 mg/kg body wt. reduced the yeast-provoked elevation of body temperature significantly. The results obtained for standard, drug treated and *Enicostema axillare* -treated rats were compared with the control group and we observed a significant reduction in the yeast-elevated rectal temperature.

Body temperature rises due to derangement of heat regulating mechanism in the brain. The rise in body temperature above 37°C is called fever. Fever generally occurs due to the infections by virus, bacteria, protozoa and other microorganisms that produce pyrogens. These pyrogens act on WBC, which in turn produce endogenous toxins. They act on the anterior hypothalamus and the body temperature is elevated causing fever. Fever leads to the disturbance of metabolism and it increases blood pressure, pulse rate, cardiac output, respiration rate etc. Most Traditional Medicine Systems believe that fever is not a disease in itself but the symptom of some other diseases. The antipyretic agents treat these symptoms and completely eliminate fever. Herbal antipyretic agents are favored over the chemical ones for their compatibility to the human physiological system, easy availability and the rich knowledge about the traditional healing systems. The body's ability to maintain a natural balance of Cycloxygenase 1 and 2 that regulate inflammatory response play a crucial role in supporting cardiovascular, immune, neurological role and joint and connective tissue system (Cheng, 2005).

Table 2 represents the effect of *Enicostema axillare* on yeast induced pyrexia in rats.

Groups	Doses (mg/kg b. wt)	Mean Total temperature Mean ± SD (^{o}C)				
		After 18hrs of Yeast Injection				
		0 hrs	1 hrs	2 hrs	3 hrs	4hrs
I	--	37.14±0.52	37.10±0.51	37.08±0.53	37.02±0.55	37.02±0.50
II	100	40.24±0.25	39.24±0.25	38.24±0.23	37.24±0.25	36.84±0.23
III	250	40.34±0.28	39.09±0.25	38.60±023	37.83±0.29	36.68±0.29
IV	500	40.54±0.32	39.94±.023	38.73±0.22	37.14±0.20	36.27±0.17
V	Paracetamol (150mg/kg)	40.74±0.46	39.78±.0.22	38.02±0.21	37.37±0.21	36.35±0.12

Values are expressed as Mean ± SD for six rats

The results showed that *Enicostema axillare* possesses a significant antipyretic effect in maintaining normal body temperature and reducing yeast- induced elevated body temperature in rats and its effect is comparable to that of the standard antipyretic drug paracetamol. The potential activity of the plant may be due to the phytochemical constituents present in it. In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through inhibition of prostaglandin synthetase within hypothalamus. Although, there is no direct evidence of *Enicostema axillare* to interfere with prostaglandin synthesis in hypothalamus by it can be supported by a related study in which *D. odorifera* extract was found to inhibit prostaglandin biosynthesis (Fadeyi *et al*., 2004). Therefore, it appears that antipyretic inhibition of prostaglandin synthesis in hypothalamus.

Based on the results of the present study it can be concluded that the ethanolic extract of *Enicostema axillare* leaf contain rich source of phytochemicals and has potential dose dependent antipyretic activity, it may due to active compounds present in the extract.

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