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**AN *IN-VIVO* STUDY: COMBINED THERAPY OF LEAVES AND
ROOTS OF *ALTERNANTHERA PHILOXEROIDES* IN RELIEVING
PAIN AND INFLAMMATION**

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ABSTRACT

Aim: *Alternanthera philoxeroides* (Amaranthaceae) weed has a great importance in Ethnobotany among different community in Assam and other regions of India. The main focus of present study was to evaluate synergistic *in-vivo* analgesic and anti-inflammatory activity of *Alternanthera philoxeroides* leaves and roots based on the traditional practice by the villages of lower Assam, India.

Methods: Equal portion of powdered plant materials were extracted successively using petroleum ether (40-60°C), Chloroform, Ethyl acetate, Acetone, Methanol and Hydro-alcohol (9:1) followed by their phytochemical screening. Synergistic anti-inflammatory activity was evaluated by carrageenan and egg albumin induced paw edema while synergistic analgesic activity was evaluated by acetic acid induced writhing and Eddy's hot plate method for different doses of methanolic extract.

Results: Results revealed that methanol extract contained most of the phyto-constituents. The 500 mg/kg bodyweight dose of extract showed very potent anti-inflammatory activity (% inhibition at 240

minute: standard 57.9%, extract 42.84%) as well as analgesic activity (% inhibition in acetic acid induced method: standard 72.43%, extract 53.86%; mean reaction time at 120 minute in Eddy's hot plate method: Standard 13.09 ± 0.08 , extract 3.67 ± 0.04).

Conclusions: This study revealed potentiality of the plant materials with considerable analgesic and anti-inflammatory activities which have a great scope of development of drugs and nutraceuticals.

Keywords: *Alternanthera philoxeroides*, Synergistic, Anti-inflammatory activity, Analgesic activity

INTRODUCTION

Traditional medication portrays a gathering of medical care practices and items with a long history of utilization. It often alludes to clinical information created by native societies that joins plant, creature and mineral-based drugs, profound treatments and manual procedures intended to treat ailment or look after prosperity. Traditional medication will in general be polished outside of allopathic medication (otherwise called biomedicine, traditional or western medication) which is the predominant arrangement of medication in the created world. In many cultures, traditional medicine functions as a comprehensive system of health care refined over hundreds or even thousands of years. The absolute most popular conventional medication frameworks incorporate customary Indian medication (Ayurveda), customary Chinese medication (TCM) and conventional Arabic medication (Unani). The World Health Organization (WHO) defines traditional

medicine as “the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether applicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses”. Indian Systems of Medicine include: Ayurveda, Siddha, Unani, Yoga, Naturapathy, etc. [1, 2].

The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence often for hundreds of years, before the development and spread of current medication are as yet being used today. Conventional medication is the union of remedial experience of ages of rehearsing doctors of native frameworks of medication. The traditional preparations comprise medicinal plants, minerals,

organic matter, etc. Natural medications establish just those customary prescriptions which basically utilize restorative plant arrangements for treatment. The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The traditional Indian writings incorporate Rigveda, Athurveda, Charak Samhita and Sushruta Samhita. Therefore, the herbal medicines or traditional medicaments have been derived from rich traditions of ancient civilizations and scientific heritage [3].

Alternanthera philoxeroides with the family Amaranthaceae commonly known as alligator weed is a non-woody summer perennial aquatic plant. *Alternanthera philoxeroides* is allegedly local to South America as well as in India. The plant has an extraordinary significance in Ethnobotany among various local areas in Assam just as in other parts of India. The people of north-east region of India blessed with rich biological diversity of plants and a high degree of traditional knowledge about medicinal plants. North-east is been situated in a floristically assorted region, jungle and such variety significantly increment the quantity of plants accessible,

it in this manner upgrades the probability that plants with great pharmacological responses upon administration. There is a need for documentation of research work carried out on traditional medicine, and it is become important to make an effort towards the standardization of the plant material to be used as medicine. The process of standardization can be achieved by step wise Pharmacognostic studies. These studies help to identification and authentication of plant material [4].

The plant is characterized by dark-green waxy leaves which are lanceolate shaped and opposite. The leaves of the plant are 12-14 cm length and 1.5-2.5cm width. The inflorescence is white, ball-molded, 1.5cm in width and papery. It does not always set viable seed under field conditions but reproduces vegetative from axillary buds at each node. One of the primary distinguishing highlights of alligator weed developing over water is that the stems grow up to 60 cm high and have bigger leaves and empty internodes. However on the land stems are shorter and internodes are smaller and much less hollow [5].

MATERIALS AND METHODS

Selection, collection and authentication of plant materials: Fresh and healthy

plants were selected and collected from Fulgasa, Chirakuti, Dhubri, Assam, India. Finally, leaves and roots from the plants were collected by hand picking method. Herbarium specimen of the plant was sent to Gauhati University for authentication and identified the plants as *Alternanthera phyloxeroides* (Mart.) Griseb, (Accession number: GUBH19918).

Pharmacognostic and physicochemical analysis: Microscopy, moisture content, extractive values, ash values were determined as per the standard methods. Plant materials were extracted successively in soxhlet apparatus using various solvents like petroleum ether (40-60°C), Chloroform, Ethyl acetate, Acetone, Methanol, Hydro-alcohol (9:1) in the increasing polarity order followed by their phytochemical screening [6, 7, 8].

Ethical approval: This research is approved by the Institutional Animal Ethics Committee (IAEC) of our university (Approval number: AdtU/IAEC/2022/10).

Animal grouping: Albino rat were divided into 5 groups (6 animals each group) and evaluated the *in-vivo* analgesic and anti-inflammatory activities as follows-

Group I: Control (Normal food and water).

Group II: Standard (Indomethacine 10

mg/kg body weight for anti-inflammatory activity and diclofenac sodium 10 mg/kg body weight for analgesic activity).

Group III: Test 1 (Methanol extract 500 mg/kg body weight).

Group IV: Test 2 (Methanol extract 300 mg/kg body weight).

Group V: Test 3 (Methanol extract 200 mg/kg body weight).

Anti-inflammatory activity: Synergistic anti-inflammatory activity was carried out by the carrageenan induced paw edema and egg albumin induced paw edema methods using indomethacine (10 mg/kg bodyweight) as standard for various doses (500 mg/kg, 300 mg/kg and 200 mg/kg bodyweight) of methanol extract of the plant material [9, 10, 11, 12]. The percentage of inhibition was calculated according to the following formula:

$$\% \text{ Inhibition} = 1 - (A - X / B - Y) \times 100$$

Where,

A= Mean paw volume of treated rats after administration of carrageenan at time 't'.

X= Mean paw volume of treated rats before administration of carrageenan.

C= Mean paw volume of treated

control rats after administration of carrageenan at time 't'.

Y= Mean paw volume of treated control rats before administration of carrageenan.

Analgesic activity: A synergistic Analgesic evaluation was carried out by acetic acid (writhing and abdominal contraction) using diclofenac sodium (10 mg/kg bodyweight) as standard for several doses (500 mg/kg, 300 mg/kg and 200 mg/kg bodyweight) of methanol extract of the plant material [13, 14] and the % protection was calculated using the formula:

$$\% \text{ Inhibition} = (W_c - W_t) / W_c \times 100$$

Where,

W_c = Mean values of number of writhing in control group.

W_t = Mean values of number of writhing in the test group.

Also the same synergistic activity was evaluated by Eddy's hot plate method using diclofenac sodium (10 mg/kg bodyweight) as standard for several doses (500 mg/kg, 300 mg/kg and 200 mg/kg bodyweight) of methanol extract of the plant material [15] and the % protection was calculated using the formula:

$$\% \text{ Inhibition} = (W_t - W_c) / W_t \times 100$$

Where,

W_c = Mean values of number of writhing in control group.

W_t = Mean values of number of writhing in the test group.

RESULTS

Pharmacognostic and physicochemical analysis: Transverse section of leaf and root of the plant were prepared, washed with 70% methanol, stained using safranin and examined under digital microscope. Where, leaf showed the presence of cuticle, upper epidermis, xylem, phloem, palisade cells and lower epidermis (**Figure 1**) while root contained the cortex, epidermis, vascular cylinder, etc. (**Figure 2**).

Physicochemical parameters such as moisture content, ash values, extractive values etc. were performed as per the standard procedures and found the optimum results (**Table 1**).

The phytochemical screening of the plant extract using various solvents shows a number of phytoconstituents present, which are responsible for the biological activities. From the preliminary phytochemical analysis it was found that the methanolic extract of leaves and roots of *Alternanthera philoxeroides* contained the most phyto-constituents such as carbohydrates, tannins, flavonoids, phytosterols, steroids,

etc. (Table 2).

Acute toxicity study: Acute toxicity study was performed as per the OECD guideline 423 and animals were found safe both at 2000mg/kg and 5000mg/kg bodyweight.

Anti-inflammatory activity: The evaluation of anti-inflammatory activity was carried out using different doses of methanolic extract (200, 300 and 500 mg/kg body weight). The 500 mg/kg bodyweight methanol extract of plant materials inhibited the carrageenan induced paw edema formation, at both early phase (0.85 ± 0.04) and later phase (0.73 ± 0.03), tend to a significant inhibitory action 47.37% as compared to the standard (67.9%) while the %inhibition at 200mg/kg and 300mg/kg bodyweight was found

10.52% and 10.53% respectively. Similarly, 200mg/kg, 300mg/kg and 500mg/kg body weight doses of the extract showed the %inhibition 9.62%, 12.44% and 44.57% respectively which is 65.39% for standard drug (Figure 3).

Analgesic activity: The analgesic activity was evaluated using acetic acid induced method and Eddy's hot plate method using different doses of methanolic extract (200, 300 and 500 mg/kg body weight). The highest dose of the extract showed significant analgesic activity, %inhibition 53.86% in acetic acid induced method (Table 3 and Table 4) and mean reaction time 3.67 ± 0.04 seconds at 120 minute in Eddy's hot plate method (Table 5).

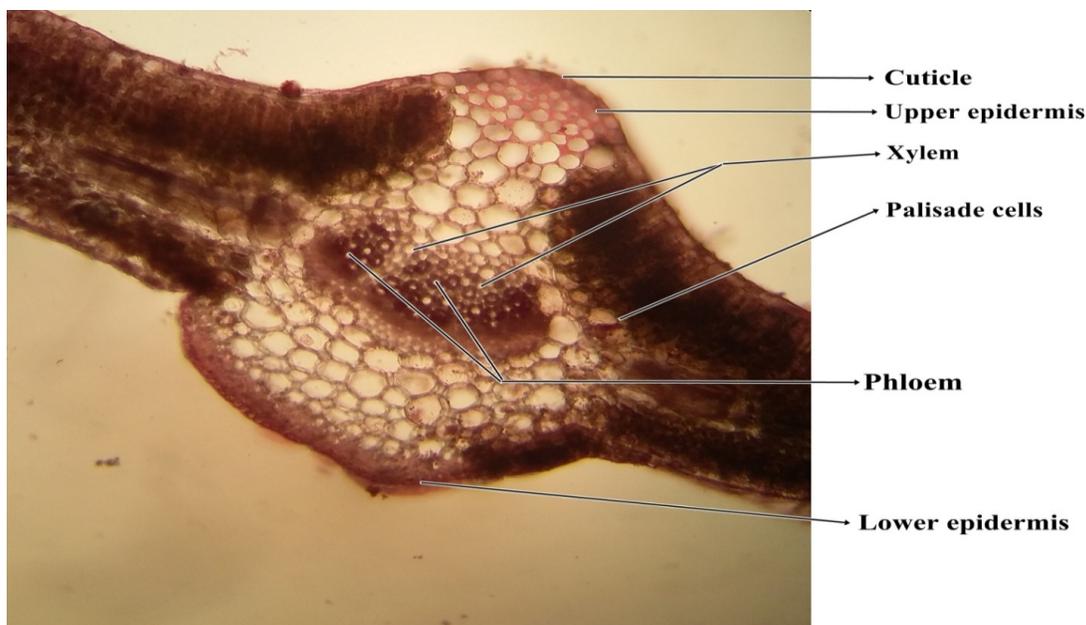
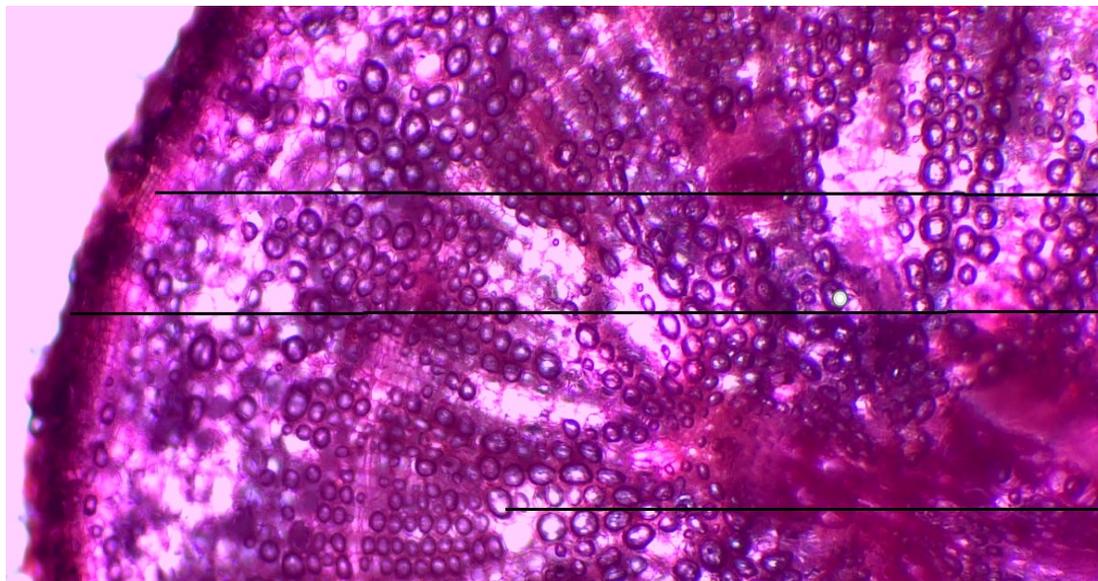


Figure 1: TS of *Alternanthera philoxeroides* leaf

Figure 2: TS of *Alternanthera philoxeroides* rootTable 1: Physicochemical analysis of *Alternanthera philoxeroides*

S. No.	Physicochemical parameters	Results (%)
1	Moisture content	9.60
2	Water soluble extractive	1.03
3	Alcohol soluble extractive	1.00
4	Total ash	10.38
5	Acid insoluble ash	8.33
6	Water soluble ash	5.16

Table 2: Phytochemical screening of *Alternanthera philoxeroides*

Phytoconstituents	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Hydroalcohol
Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve
Glycosides	-ve	-ve	-ve	-ve	+ve	-ve
Carbohydrates	-ve	+ve	-ve	+ve	+ve	+ve
Fixed oils and Fats	+ve	+ve	-ve	-ve	-ve	-ve
Tannins & phenolic compounds	-ve	-ve	+ve	+ve	+ve	-ve
Proteins	-ve	-ve	-ve	-ve	-ve	-ve
Flavonoides	-ve	-ve	+ve	+ve	+ve	-ve

'+ve' indicates the presence of phytoconstituents; '-ve' indicates absence of phytoconstituents

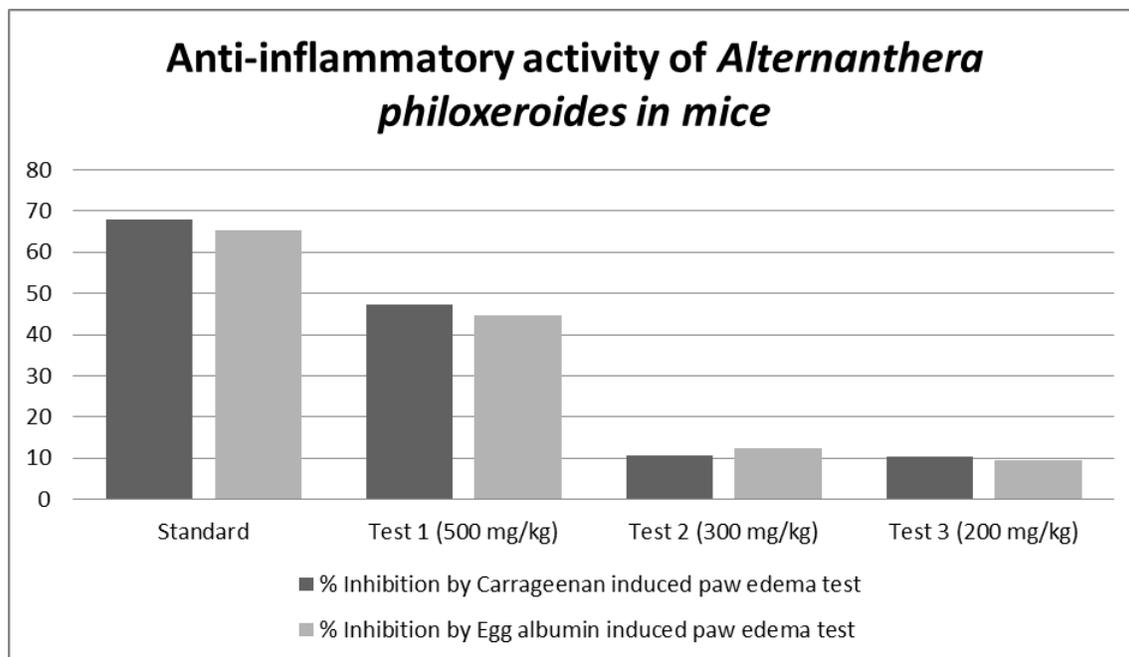


Figure 3: Synergistic anti-inflammatory effect of methanol extract of *Alternanthera philoxeroides* leaves and roots in rat

Table 3: Synergistic analgesic effect of methanol extract of *Alternanthera philoxeroides* leaves and roots in acetic acid induced writhing method (writhing movements) in rat

Groups / treatment	Dose (mg/kg body weight)	No. of writhing movements (per 10 min.)	% Inhibition
control	-	59.33±0.04	-
Standard	10	13.33±0.02	77.53
Test 1 (500 mg/kg)	500	20.83±0.03	64.89
Test 2 (300 mg/kg)	300	25.67±0.04	56.73
Test 3 (200 mg/kg)	200	52.00±1.05	12.36

Values are expressed as mean ± SEM (n = 6)

Table 4: Synergistic analgesic effect of methanol extract of *Alternanthera philoxeroides* leaves and roots in acetic acid induced writhing method (abdominal contraction) in rat

Groups / treatment	Dose (mg/kg body weight)	No. of abdominal contraction (per 10 min.)	% Inhibition
control	-	8.17±0.01	-
Standard	10	2.67±0.04	67.32
Test 1 (500 mg/kg)	500	4.67±0.03	42.84
Test 2 (300 mg/kg)	300	5.83±0.04	28.64
Test 3 (200 mg/kg)	200	6.83±0.04	16.40

Values are expressed as mean ± SEM (n = 6)

Table 5: Synergistic analgesic effect of methanol extract of *Alternanthera philoxeroides* leaves and roots in eddy's hot plate method in rat

Groups / treatment	Mean reaction time (seconds)					% Inhibition
	0 min	30 min	60 min	90 min	120 min	
control	1.72±0.09	1.40±0.07	0.95±0.06	1.57±0.10	1.40±0.07	-
Standard	1.88±0.04	3.87±0.13	7.75±0.11	11.12±0.16	13.09±0.08	89.30
Test 1 (500 mg/kg)	1.45±0.06	1.98±0.003	4.33±0.09	6.87±0.07	3.67±0.04	61.85
Test 2 (300 mg/kg)	1.23±0.09	1.67±0.06	3.33 ±0.07	4.80±0.09	2.33±0.13	39.91
Test 3 (200 mg/kg)	1.22±0.03	1.45±0.09	2.89±0.10	3.33±0.06	1.57±0.05	10.83

Values are expressed as mean ± SEM (n = 6)

DISCUSSION

People believe that the use of natural products is much more active and safer than the use of other synthetic medicines. Therefore, people use various natural products or plant products in the form of juices, pastes, solution and or even in the raw form for the treatment of various diseases. *Alternanthera philoxeroides* is also found to be used by various tribes of

Assam and North-East India. Basically the young stems and leaves Alligator weed is widely used as vegetable in various regions of Assam. The stems and leaves of alligator weed are used to treat dysentery, asthma as well as to reduce headache by the villagers in the various regions of Assam. The plant is widely distributed throughout the world (Figure 4) [16].

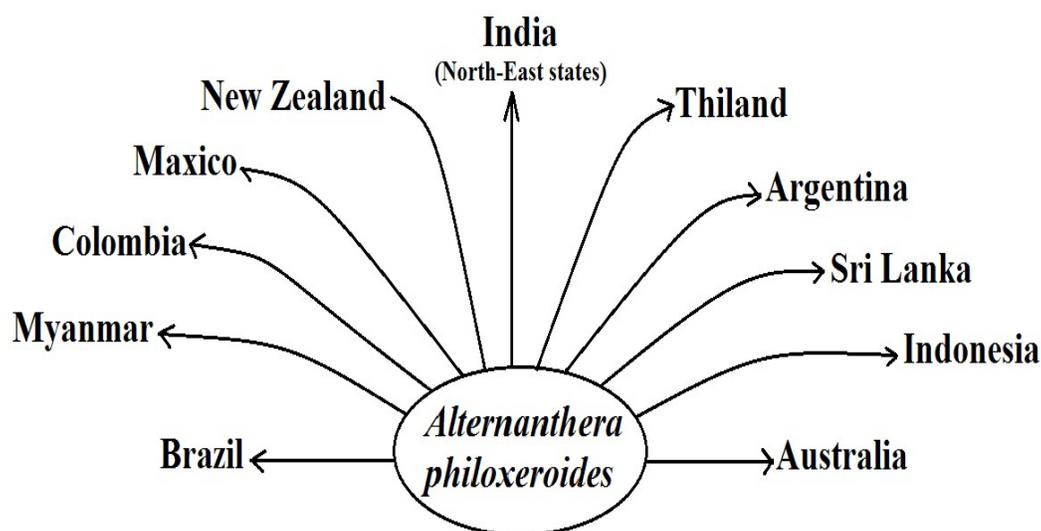


Figure 4: Worldwide distribution of *Alternanthera philoxeroides*

Youthful shoot and leaf juice (50-100 ml) with equivalent measure of water is given before sleep time for stomach pain routinely for seven days by Bodo, Santhal, and Goreswar tribes of North-East India [4, 17]. In Bangladesh, the plant is also used for pain and diabetes as a folk medicine [18].

Transverse section of leaf shown the presence of cuticle, upper epidermis, xylem, phloem, palisade cells and lower epidermis while root contained the cortex, epidermis, vascular cylinder, etc suggested the decotyledonous plant.

The various physicochemical parameters as mentioned in the **Table 1** reveal the quality of a plant material. The parameters such as moisture content, ash values, extractive values etc. were performed and found the optimum results at which the bacteria, fungi or yeast were not been grown during storage.

The phytochemical screening of the plant extract using various solvents shows a number of phytoconstituents present, which are responsible for the biological activities. From the preliminary phytochemical analysis it was found that the methanol extract of the plant *Alternanthera philoxeroides* contained more

phyto-constituents such as carbohydrates, tannins, flavonoids, phytosterols, steroids, etc. So the methanol extract was selected for the evaluation of the targeted activities.

It is believed that current anti-inflammatory and analgesic drugs are not useful in all cases because of their side effects and low potency. As a result, search for other alternatives became necessary and imperative. Novel anti-inflammatory and analgesic agents could be discovered from medicinal plants containing a wide variety of phyto-constituents. Traditional medicine for the treatment of various diseases is becoming more popular. Many medicinal plants provide relief of symptoms comparable to that of conventional medicinal agents. Therefore, the present study was aimed at evaluating the scientific basis for the traditional use of *Alternanthera philoxeroides* using *in vivo* anti-inflammatory and analgesic activity.

Carrageenan has been widely used as a harmful agent able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. This phlogistic agent, when injected locally into the paw, produced a severe inflammatory reaction, which was discernible within 30 min. Carrageenan

induced rat paw edema is a suitable *in vivo* model to predict the value of anti-inflammatory agents, which act by inhibiting the mediators of acute inflammation. Carrageenan-induced hind paw edema in rat is a biphasic event. The early phase (90 - 180 min) of the inflammation is due to the release of histamine, serotonin and similar substances; and the later phase (180-240 min) is associated with the activation of kinin-like substances, i.e., prostaglandins, proteases and lysosome. The 500 mg/kg bodyweight methanol extract of plant materials inhibited the carrageenan induced paw edema formation, at both early phase (0.85 ± 0.04) and later phase (0.73 ± 0.03). This result tends to suggest that the inhibitory effect of the extract (47.37%) on edema formation is probably due to the inhibition of the synthesis and/or release of the inflammatory mediators, especially the cyclooxygenase products, by the same way large dose of the methanol extracts reflect potent inhibitory effect. The carrageenan induced paw edema test is effectively controlled with the arachidonate cyclooxygenase (COX) inhibitors due to its COX-dependent mechanism, thus, it is suggested that the plant materials may

possess arachidonate COX inhibitory property.

Similarly the analgesic activity was evaluated using acetic acid induced method and Eddy's hot plate method. From these studies it was found that in the same way 500 mg/kg bodyweight methanol extract of the plant materials also inhibit the mediatory receptors responsible to produce the analgesia or pain, average percentage inhibition in acetic acid induced method is 53.86% and mean reaction time at 120 minute in Eddy's hot plate method 3.67 ± 0.04 seconds.

CONCLUSION

In the conclusion, the transverse sections of leaf and root of *Alternanthera philoxeroides* shows several histological characteristics of the plant. The methanol extract *Alternanthera philoxeroides* leaves and roots contains some potent ingredients and has a good and significant anti-inflammatory and analgesic activities, which can be considered as a strong literature for further researches for development of drugs and nutraceuticals.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest for publication of the article.

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