

# INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY AND BIO SCIENCES

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Pharmaceutical Sciences

RESEARCH ARTICLE .....!!!

## “ANALGESIC ACTIVITY OF HYDRO ALCOHOLIC EXTRACTS OF STEMS AND ROOTS OF *EUPATORIUM TRIPLINERVE VAHL.*”

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### ABSTRACT

#### KEYWORDS:

*Eupatorium triplinerve*,  
Ayapana, Stem and root  
extracts, Analgesic  
activity.

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*Eupatorium triplinerve* commonly known as ayapana is a most important plant used in traditional medicine. The plant is considered to be a therapeutic agent for the treatment of various diseases. The main aim of this research was to study the analgesic activity of the stem and root extracts. The hydroalcoholic extract of stem and roots of *E. triplinerve* was subjected to preliminary phytochemical screening. Acute toxicity studies were carried out in Swiss albino mice. Analgesic activity was evaluated by well established models like acetic acid induced writhing. Acute toxicity studies showed that the stem and root extracts was non-toxic upto a maximum dose of 2000 mg/kg body weight. The hydroalcoholic extract of the stem and root exhibited significant inhibition of acetic acid induced writhing. The present study indicates that the stem and root extracts of *Eupatorium triplinerve* has potential analgesic activity.

## **1. INTRODUCTION:**

*E. triplinerve* is a member of large Asteraceae plant family. Ayapana is an ornamental erect Perennial herb with aromatic leaves. The leaves of *Eupatorium triplinerve* are reported to be useful in pain and inflammatory disorder. The essential oil from the plant has been reported to possess a number of medicinal properties such as central nervous system (CNS) depressant, analgesic and sedative effects. The methanol extract of ayapana leaves shows antifungal activity and potent antibacterial activity. Ayapana is a rich source of naturally occurring coumarin chemicals. Pain is a complex unpleasant phenomenon composed of sensory and emotional experiences associated with actual and potential tissue damage. Analgesics is defined as the agents which selectively relieve pain by acting in CNS or by peripheral pain mechanisms. Here the acetic acid induces the writhing. Writhing means the twisting body movements as in pain or struggle or abdominal constrictions. The present study has been designed to investigate the hydro alcoholic extracts of the stem and root, exhibits significant inhibition of acetic acid induced writhing, therefore it has analgesic activity.

## **2. MATERIALS AND METHODS:**

### **2.1 collection of plant material:**

The whole plant of *Eupatorium triplinerve* were collected from Thrissur district in Kerala in month of January 2016.

### **2.2 Animals**

Swiss albino mice (20-25gm) were taken from the institutional animal house. The animals are fed with food and water. All the experiments were conducted according to the CPCSEA.

### **2.3 Preparation of extract**

#### **Maceration**

The stem and roots of ayapana were collected, shade dried for 7 days. Stem and roots are crushed in grinder then were extracted with 80% aqueous ethanol by double maceration process at room temperature. The extract was filtered and then filtrate was concentrated.

### **2.4 Preliminary chemical tests**

The hydro alcoholic extract of stem and roots of ayapana was subjected to phytochemical screening and plant extract contain alkaloids, carbohydrates, saponins, flavonoids, tannin, and glycoside using appropriate reagents.

### **2.5 Acute toxicity study**

Acute oral toxicity study for the test was carried out according to Organization for Economic

Co operation and Development OECD 423. The test procedure is to minimize the no. of animal required to estimate the oral toxicity. Food was withheld for the study with a dose of 2000mg/kg of body weight. The animals are weighed and the extract was administered in a single dose as 1% suspension in Carboxy methyl cellulose by oral intubation. After dosing periodically during the first 24 hours daily after for a total of the 14days. The LD 50 of the compound was estimated to be more than 2000mg/kg, so that doses of 100,200,400,1000mg/kg orally were safe for the dose.

### **2.6 Acetic acid induced abdominal constriction**

Acetic acid induced writhing method was adopted for evaluation of analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen, any writhing is considered as a positive response.

Mice (20-25gm) of either sex were divided to 3 different groups each containing six animals and marked (food, but not water should be withheld) .Mice's were orally treated with hydro alcoholic extract of eupatorium triplinervis, after 60 minutes later acetic acid (0.6% v/v in saline) volume 0.1ml/10g was injected intra peritoneally. The numbers of abdominal constrictions are counted for 15 minutes following acetic acid injection. Any significant reduction in number of abdominal constriction by treatment compared to the vehicle treatment and standard treatment.

Percentage of inhibition was evaluated using following formula:  $(C-T/C) \times 100$

where C is the number of abdominal constrictions recorded in vehicle treated animals and T is the number of abdominal constrictions in the treatment group

### **Statistical analysis**

All the values of in vivo analgesic studies of hydro alcoholic extract of ayapana were expressed as mean and standard error of mean (S.E.M) and were examined for significance by ANOVA (Analysis of variance) and groups were compared by Dunnet's test for individual comparison of groups with control. P value were measured moderate significant at  $P < 0.01$ ,  $< 0.001$  level

## **3. RESULTS:**

### **Phytochemical screening**

The percentage yield of hydro alcoholic extract of the stems and roots of ayapana was found to be 9.8%w/w. The chemical tests indicate the presence of alkaloids, carbohydrates, saponins, flavonoids, tannin, and glycoside.

### Acute toxicity studies

In acute oral toxicity studies no mortality was recorded in these animals up to 14 days. Thus the extract was non toxic up to 2000mg/kg.

### Analgesic activity

The writhing was counted .There is a significant reduction in number of writhing between the vehicle treated and standard treated animals.

### 4. DISCUSSION:

*E.triplinerve Vahl* (synonyms: *Eupatorium ayapana*, *Ayapana triplinervis*) belongs to the Asteraceae family and is commonly known as ayapana in Hindi. The present study was conducted to scientifically validate the traditional claims of *Eupatorium triplinerve* with particular reference to its analgesic activity. In acute toxicity testing no mortality was observed in mice even in a dose of 2g/kg of petroleum-ether extract of *E.triplinerve* which indicates the safe nature of the extract. Acetic acid induced abdominal constriction is regarded as a very sensitive method which induces minimal noxious stimulus. The advantage of this method is that even compounds with weaker analgesic property can be detected from the results of this test .the acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins, bradykinins and substance P. Here we conclude that the hydro alcoholic extracts of stems and roots of ayapana shows analgesic activity.

Table no.1 shows there is an decrease in writhing by administration of ayapana extract with reference to the vehicle treated mice and plant extracts shows analgesic activity.

Group	Treatment	Dose	No. of wriths in 15 mins (mean±SEM)	Inhibition %
Control	Vehicle(carboxy methyl cellulose)	1.3ml	20.6±0.33**	-
Test	Plant extract	4.8mg in cmc orally	13.16±0.30**	36%
Standard	Diclofenac potassium	1mg orally	5.1±0.30**	75%

Table no.1 Effect of plant extract on acetic acid induced writhing in mice

n = 6. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  show a comparison of group # 2,3 vs. group # 1 (One-way ANOVA followed by Dunnett's test).

## 5. Acknowledgement:

The authors are very thankful to all teaching and non teaching staffs of Pushpagiri College of pharmacy, tiruvalla, kerala for providing all facilities to carry out this work.

## 6. Conclusions

The traditional claims of the usefulness of *Eupatorium triplinerve* in the treatment pain has been scientifically validated by the results of the present study .The stem and root extracts of ayapana plant has analgesic activity. The study indicates that the data obtained will be basis or further studies and applications of this plant.

## 7. REFERENCES:

1. Chaurasia,sc: kher,a: (1978) Activity of essential oils of three medicinal plants against various pathogenic and nonpathogenic fungi indian journal hospital pharmacy 15 pp. 139-141.
2. Sukanlaya Leejae\*, Teeratad Sudsai, and Chat Krobthong (2015) *Eupatorium ayapana*, a natural source of anti-biofilm, anti-inflammatory, and anti-oxidant agents Rangsit Journal of Arts and Sciences, Vol. 5 No. 2, pp. 141-149.
3. Sugumar, N., Karthikeyan, S., & Gowdhami, T. (2015). Chemical composition and antimicrobial activity of essential oil from *Eupatorium triplinerve* Vahl aerial parts. International Letters of Natural Sciences, 4, 14-21.
4. Sharath, R., Harish, B. G., Channarayappa, S., Preetham, J., & Sushma, S. (2014). Evaluation of antioxidant activity of coumarin isolated from *Eupatorium triplinerve*. Research and Reviews: a Journal of Pharmacognosy, 1(1), 18-22.
5. Unnikrishnan, P. K., Varughese, T., Sreedhar, S., Balan, N., Balachandran, I., & Shree, A. B. (2014). Study on *Eupatorium triplinerve* Vahl from South India, a rich source for thymohydroquinone dimethylether and its antimicrobial activity. Journal of Essential Oil-Bearing Plants, 17, 652-657.
6. Arung, E. T., Kuspradini, H., Kusuma, I. W., Shimizu, K., & Kondo, R. (2012). Validation of *Eupatorium triplinerve* Vahl leaves, a skin care herb from East Kalimantan, using a melanin biosynthesis assay. Journal of Acupuncture & Meridian Studies, 5(2), 87-92.
7. Kokate C and Verma KC Pharmacological studies on the essential oil of *Eupatorium triplinerve*. Effects on the central nervous system and antimicrobial activity. Flavor Industries 2 (3)1971; 177-180.

8. Garg, S. C., et al. "Studies on the essential oil from the flowers of *Eupatorium triplinerve*." *Indian Perfum* 1993; 37 (4): 318-323.
9. Sharma, S. K., et al. "The antifungal activity of some essential oils." *Indian Drugs Pharm Ind.*1979; (14) 1: 3-6.
10. Sewell RDE and Spencers PS. Antinociceptive activity of narcotic agonists and partial agonists, analgesics and other agents on the tail immersion test in mice and rats. *Journal of Neuropharmacology*. 1976; 15: 683-685.
11. Koster R, Anderson M and Dee Beer AJ. Acetic acid for analgesic screening. *Fed Proc.*1959; 18: 412-416.
12. Facknath, S., et al. (1999)"Response of three important insect pests of horticultural crops in Mauritius to extracts of *Ayapana triplinervis*." *Journal of Applied Entomology*.
13. Bose, P. K., et al. "Haemostatic agents. Part I. Experiments with ayapanin and ayapin." *Nature*, 1937; 139: 515.
14. Shafiqur Rahman and Mohammad Junaid "Antimicrobial activity of leaf extracts of *Eupatorium triplinerve*" Against some human Pathogenic bacteria and phytopathogenic fungi" *Bangladesh J. Bot.* 37(1): 89-92, 2008 (June).
15. Parimala K., Cheriyan, B. V., & Viswanathan, S. (2012). Antinociceptive and anti-inflammatory activity of petroleum ether extract of *Eupatorium triplinerve* Vahl. *International Journal of Life science & Phama Research*, 2(3), 12-16.