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HPTLC FINGERPRINTING AND PHARMACOLOGICAL EVALUATION OF EXTRACT OF *PHYLLANTHUS FRATERNUS WEBSTER* LEAVES

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ABSTRACT

The objective of present investigation was to develop HPTLC fingerprinting and evaluate analgesic activity of pet ether and methanolic extract of *Phyllanthus fraternus webster* leaves in rats. Extraction method used was maceration. Three solvents were used for extraction of phytoconstituents (pet ether, methanol and hydromethanolic). Various mobile phase were tried and the optimized mobile phase was n-Hexane : ethylacetate (6:4) v/v. used for fingerprinting. Analgesic activity of both extract of *Phyllanthus fraternus Webster* leaves was tested at a dose of 500 mg/kg & 1000 mg/kg. Activity was evaluated by Digital randall selitto and tail immersion method using Wistar rats of either sex (n = 6 in each group). The results indicated that the methanolic extract exhibited significant analgesic activity. It may be due to presence of saponin, steroids & alkaloids in it.

KEY WORDS: *Phyllanthus fraternus webster*, HPTLC fingerprinting, Digital randall selitto, tail immersion method, analgesic activity.

INTRODUCTION

BHUIAMLA (*Phyllanthus fraternus Webster*) is an annual herb commonly occurring in gardens, waste places and roadside. This plant belongs to Euphorbiaceae family.[1] The family has about 750 -1200 genera. The majority of genera are pan tropical weeds and they are distributed in all tropical and subtropical regions on the earth. The common uses of whole

plant reported were in treatment of dyspepsia, vertilago, malaria, diabetes, menorrhagia, sores, chronic dysentry, tubercular ulcers, wound, bruises, scabies, ringworm, dropsical infection, gonorrhoea, genito-urinary disorders, jaundice, indigestion, intermittent fever, anemia, cough, gout, urinary disease, dermatosis, miscarriage, abdomen tumour, vaginitis and skin eruption. Leaves are used in scabies, bruises, wound, poultice lesions, swelling, ulcer, spleen and liver disorders and problem of joints. Bark is purgative. Stem is used in ophthalmia.[2] [3] [4]

Pain is an ill-defined, unpleasant, sensation usually evoked by an external or internal noxious stimulus. It is a warning signal primarily protective in nature, but causes discomfort. Analgesics are the drugs that selectively relieve pain by acting on the central nervous system or on peripheral pain mechanism without significantly altering consciousness. Therapy with plant extract can provide an alternative to other selective and traditional NSAIDs in treating patients with arthritis and other painful conditions. The other species of phyllanthus (P*hyllanthus lawii*) has been reported to possess analgesic activity[5]. Thus the objective of the research work was to carry fingerprint analysis of the extracts and pharmacological evaluation of analgesic activity of the extracts.

MATERIAL AND METHODS

Plant collection and preparation

Fresh leaves of phyllanthus fraternus were collected from Botanical garden of Indira College of Pharmacy, Wakad, Pune. Their botanical identifications and authentication were confirmed at the Agharkar research institute, Pune. Then the leaves were separated and washed with water and allowed to dry. Later fine ground powder was prepared.

Animals used

Wistar rats, weighing 200-250 g were procured from the animal house of the AISSMS College of Pharmacy, Pune, Maharashtra, India. All the animals were kept under standard laboratory conditions of temperature (25 ± 2ºC) and humidity (55 ± 5 %) under 12:12 light dark cycle. The animals were fed with standard pellet diet purchased from Nutrivet, Pune, India and drinking water ad libitum. The experiment were designed and conducted in accordance with ethical norms approved by committee for the purpose of control and supervision on experiments on animals (CPCEA) and institutional animal ethical committee (IAEC) Approval no CPCSEA/IAEC/QA/03/12-2K 13

Crude Extraction

100 g of finely powdered drug was weighed and defatted with 100 ml of pet. ether. The solution was kept overnight with continuous stirring at 350 rpm with the help of magnetic stirrer. Later this solution was filtered through whatmann filter paper and filtrate was concentrated on rotary evaporator to get pet ether extract. The marc obtained was treated with 100 ml of methanol and processed in similar manner as that of pet ether extract to get methanolic extract. Further hydromethanolic extract was obtained by treating marc of methanolic extract with hydromethanolic solution (Methanol: water; 60:40) in similar manner.

HPTLC fingerprinting of *Phyllanthus fraternus*

HPTLC of all three extracts was carried out using solvent system Hexane: ethyl acetate (6:4 v/v) on precoated silica gel aluminium plate 60 F_{254} (5 cm ×10 cm) with 250 µm thickness (E. MERCK, Darmstadt, Germany). The samples were applied onto plates as a band with 5 mm width using a CAMAG Linomat 5 sample applicator (Switzerland).

The linear ascending development was carried out in 10 cm×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland). The optimized chamber saturation time for mobile phase was 20 min. The length of chromatogram run was 8 cm and development time was approximately 15 min. TLC plates were dried in a current of air with the help of a hair drier. Plates were then observed under UV lamp at 254 nm, 364nm and in day light. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 350 nm with slit dimensions 4 mm × 0.45 mm and scanning speed of 20 mm/sec was employed for all developments operated by WINCATS software version 1.4.2.

ANALGESIC ACTIVITIY

Digital randall selitto method [6]: - Rats were divided into six groups each consisting of six animals per group. They were starved for 12 h. The treatment regimen was as follows:

1. Group 1(Control): Vehicle.
2. Group 2 (Test 1): Methanolic extract of *PF Webster* (500 mg/kg)
3. Group3 (Test2): Methanolic extract of *PF webster* (1000 mg/kg).
4. Group 4(Test 3): Pet ether extract of leaves of *PF Webster* (500 mg/kg)
5. Group 5(Test 4): Pet ether extract of leaves of *PF webster* (1000 mg/kg).
6. Group 6(Standard): Diclofenac (4 mg/kg)

Pet ether extract was reconstituted in 2 % DMSO and methanolic extract was reconstituted in water. Dosing was done for 7 days and pressure threshold was measured on 7th day. Mechanical hyperalgesia of left hind paw was evaluated by Randall and Selitto test using analgesiometer (UGO Basile ,ITALY). The left hind paw was placed between flat surface and blunt pointer applying, steadily increasing pressure. The threshold was determined when rat exhibited a stereotype flinch response and attempted to remove the foot from apparatus. The cut-off pressure was 450 gm to avoid injury.

% increase in pain threshold = (Applied force of vehicle - Applied force of test drug)/Applied vehicle force of control

Tail-immersion method [7]

Rats were divided into six groups each consisting of six animals per group. The treatment regimen was as follows:

1. Group 1(Control): Vehicle.
2. Group 2 (Test 1): Methanolic extract of leaves of *PF* Webster (500 mg/kg)
3. Group3 (Test2): Methanolic extract of leaves of *PF Webster* (1000 mg/kg).
4. Group 4 (Test 3): Pet ether extract of leaves of *PF webster* (500 mg/kg).
5. Group 5 (Test 4): Pet ether extract of leaves of *PF Webster* (1000 mg/kg)
6. Group 6 (Standard): Aspirin (100 mg/kg)

The distal part of the tail of the animals was immersed in hot water maintained at 55 ± 1.0 ^{0}C. The time taken to withdraw the tail was noted as the reaction time. A cut off time of 10 Sec. was maintained at 55 ^{0}C to prevent tissue damage. The reaction time was checked at 0, 30, 60, 120, 180, 240 min, respectively after treatment.

RESULT AND DISCUSSION

Extraction of the Phyllanthus *fraternus* leaves was carried out by cold maceration process. The fingerprint for these extracts was obtained with optimized mobile phase n-hexane: ethylacetate (6:4 v/v). Fingerprint analysis of extracts of Phyllanthus *fraternus* leaves showed major components in methanolic extracts. The peaks are well separated as shown in Figure 1 and Figure 2.

Tail immersion test is useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level. In *Randall–Selitto* method Methanol extract and the standard drugs significantly ($p<0.001$) reduced the pain as compare to the control by applying ANOVA followed by Dunnett's test.

In Tail immersion method all the test and standard drugs significantly ($p<0.001$) reduced the pain as compare to the control. By applying ANOVA followed by Dunnett's test, it was shown that there is significant ($p<0.01$) effect of Test-1 & Test-2 as compare to the standard at 60 minutes and there is significant ($p<0.05$) effect of Test-2 and standard group at 60 and 120 minutes. This indicate onset of action is fast with methanolic extract, which also prolonged at higher dose.

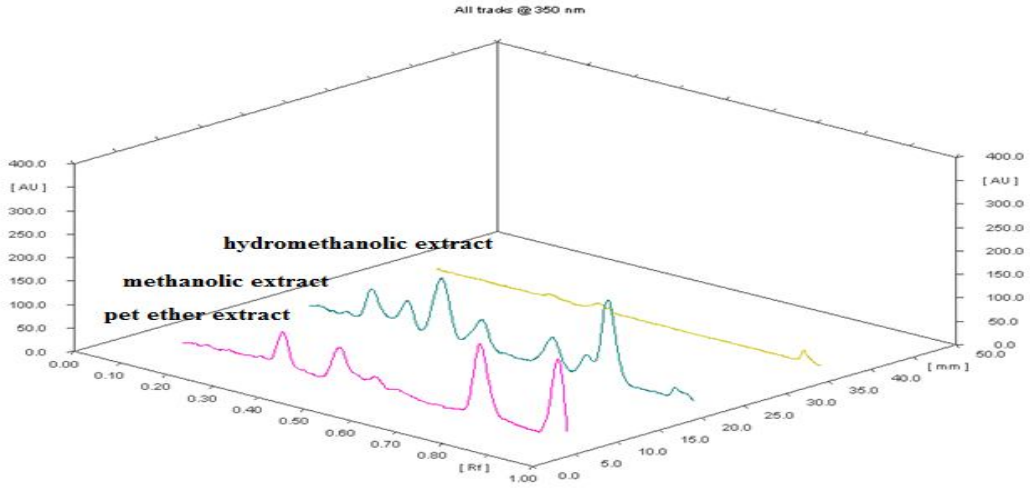

Fig 1: 3 D densitogram of extracts at 350 nm

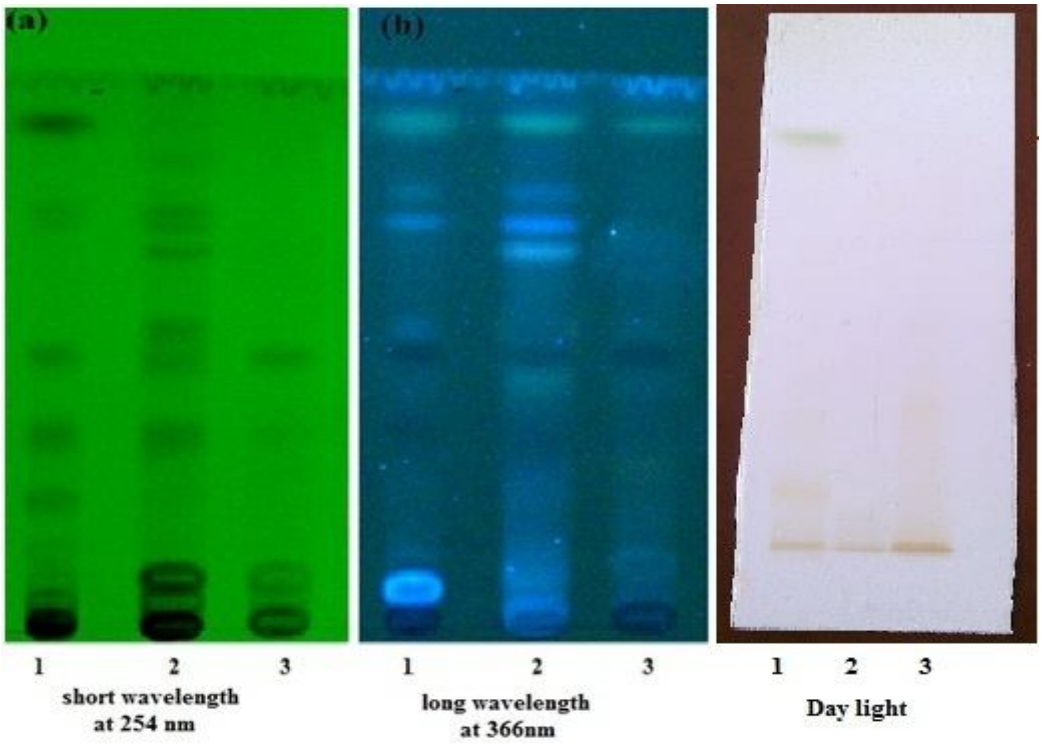

Fig 2: TLC plate at 254 nm, 364 nm and in day light (Track 1: Pet ether extract; Track 2: Methanolic extract; Track 3: Hydromethanolic extract)

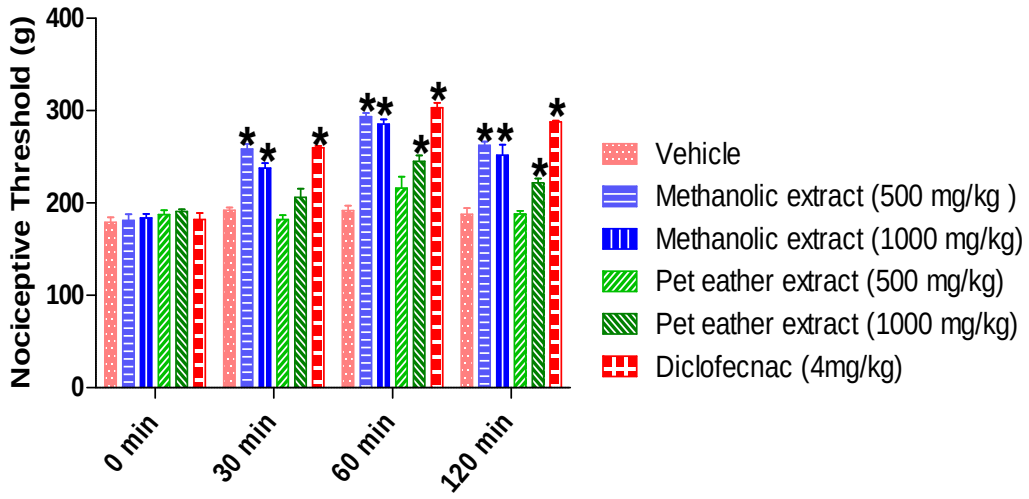

Fig 3: Analgesic activity of *Phyllanthus fraternus* using digital randall sellito method

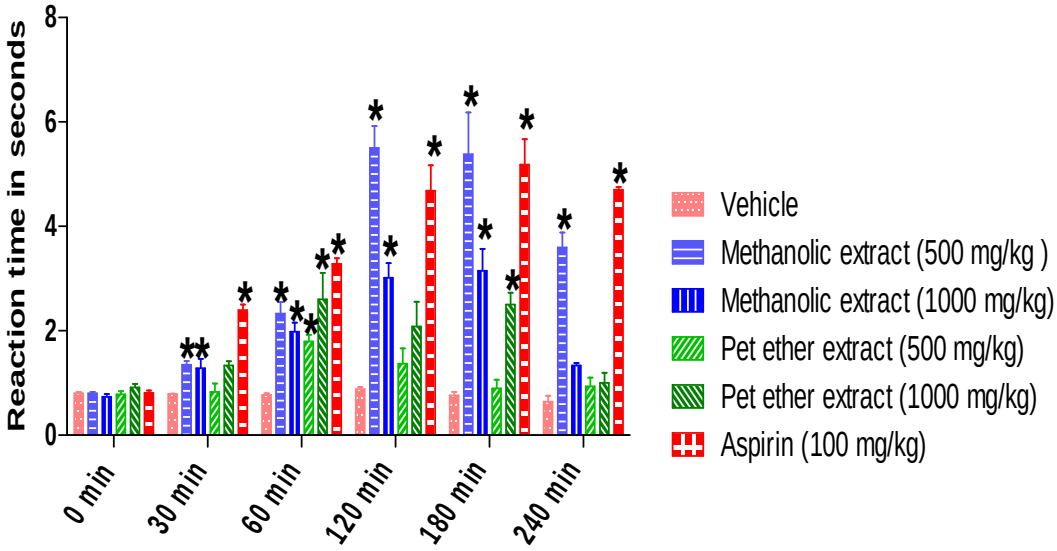

Fig 4: Analgesic activity of Phyllanthus fraternus by tail immersion method

CONCLUSION

It is concluded that the methanol and pet ether extract were more effective than the single traditional NSAIDs drug therapy, it may be due to different mechanism of actions of different drugs. The chances of side effects for methanol and pet ether extract are less as compare to the NSAIDs drug. More study on these natural drug therapies may overcome the problems of the conventional NSAIDs.

REFERENCES

1. Rajeshwar Y, Sreekanth T, Shyamsunder A, Tejaswi Divya B, Investigation of phytoconstituents, TLC profile and antimicrobial activity of methanol extract of *Asparagus racemosus* willd. roots. International Journal of Pharmacognosy and Phytochemical Research, 2014; 6(1):128-132
2. Christian M, Steroids – Chemical Constituents of Phyllanthus Fraternus Webster through TLC and HPTLC. International research journal of chemistry, 39-49
3. Sen B, Dubey SD., Tripathi k., Pharmacognostical study of Tamalaki(Phyllanthus Fraternus Webster) a herb used tamaka-svasa, An international Quaterly journal of research in ayurveda, 2011;32(3): 398-401
4. Mehta.k, Patel.B.N, Jain .B.K, Antimicrobial activity of root extract of phyllanthus fraternus webster: an ethanomedicinal plant, Research journal of recent science,2014; 3: 275-278.
5. Kodangala Subraya Chandrashekar*[1], Santanu Saha[2] and Prasanna Kodangala Subraya[3], Analgesic activity of *Phyllanthus lawii* Extract in Swiss Albino Mice, Pharmaceutical Crops, 2011; 2: 8-10.
6. Eva santos nogureia, Randall- sellito test: A new approach for the detection of neuropathic pain after spinal cord injury, Journal of neurotrauma, 2012; 29(5): 898–904.
7. C. Sumathy, Dr. S. Natarajan, Analgesic activity and phytochemical screening of ethanolic extract of musa sapientum on inflammation induced rats, World journal of pharmaceutical research, 2014; 3: 4404-4412.