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ANTIFUNGAL, ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF *AILANTHUS EXCELSA* BARK

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ABSTRACT

Various extracts of *Ailanthus excelsa* bark evaluated for antifungal at a dose 1 mg/ml, anti-inflammatory and analgesic activity at a dose 200 mg/kg and 50 mg/kg of body weight. The experimental methods were used cup plate method for antifungal and formalin induced rat hind paw oedema measured by plethysmograph (mercury displacement method) for anti-inflammatory and Tail flick method for analgesic activity. Flucanazole (1 mg/ml), diclofenac sodium (200 mg/kg, orally) and Novelgin (50 mg/kg, orally) clinically used drugs were used as standards. The ethyl acetate extract showed good antifungal activity against fungal strains *A. terrus, A. niger* and *A. flavus* at 1 mg/ml and the remaining extracts showed moderate activity compared to standard flucanzole. Extracts of *Ailanthus excelsa* bark showed significant anti-inflammatory and analgesic activity in the above study.

INTRODUCTION: *Ailanthus* found generally around villages and old forts and in forests. The plant is identified by light grey bark with large conspicuous leaf scars and long peri-pinnate leaves crowded at the end of the branches. The bark of the plant was bitter, refrigerant, astringent and appetizer. The juice of the bark is used for local applications like diarrhea, dysentery and it is used for the treatment of the skin diseases and troubles of the rectum. The bark is aromatic and used for dyspeptic complaints and it is also regarded as a tonic and febrifuge in cases of debility, expectorant and antispasmodic, given in chronic bronchitis and asthma. Also used as an astringent in diarrhea and dysentery [1]. A novel triterpenoid isolated from the root bark of *Ailanthus excelsa* Roxb (Tree of Heaven), AECHL-1 as a potential anti-cancer agent [2]. The stem of *Ailanthus excelsa* Roxb. (Simaroubaceae) may develop vascular occlusions and gum-resin cavities in the xylem as a response to injury and infection [3]. In continuation of our research work on medicinal plants, we report here antifungal, anti-inflammatory and analgesic activities of *Ailanthus excelsa* bark.

MATERIALS AND METHODS: The plant *Ailanthus excelsa* was collected from the area around Gulbarga, Karnataka, India during the period around November - December 2006 and authenticated at the Herbarium, Department of Botany, Gulbarga University, Gulbarga. The shoot system of the plant includes stem, leaves and flower. Only bark of the plant was dried and chopped into small pieces and powdered (200 g) and was extracted with petroleum ether, ethyl acetate and methanol in a soxhlet extractor exhaustively for 20-24 h. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50^{0}C). The petroleum ether extract yielded dark brown gummy solid (2 g), ethyl acetate extract gave dark brown solid (3.8 g) and methanol extract yields light brown solid (4.5 g). The crude extracts were kept in desiccator and then stored in refrigerator. Phytochemical tests of these extracts showed the presence of alkaloids, steroids and glycosides.

RESULTS AND DISCUSSION:

- **Antifungal activity:** The various extracts of Ailanthus excelsa bark were also screened for antifungal activity [4-6] against the fungal strains *A. terrus, A. niger* and *A. flavus*. The petroleum ether extract showed less activity against the *A. terrus, A. niger* and *A. flavus* organisms when compared with standard drug flucanzole. The methanol extract exhibited moderate activity against the A. terrus, *A. niger* and *A. flavus* and the ethyl acetate extract showed good activity against *A. terrus, A. niger* and *A. flavus* organisms at 1 mg/ml compared to standard flucanzole. Results are tabulated in **Table 1**.

TABLE 1: ANTIFUNGAL ACTIVITY OF VARIOUS EXTRACTS OF *AILANTHUS EXCELSA* BARK

Extract	Dose (µg/ml)	Antifungal Activity		
		Zone of Inhibition (mm)		
		A. terrus	A. niger	A. flavus
Pet. ether extract	1000	16	15	16
Ethyl acetate extarct	1000	20	19	20
Methanol extract	1000	15	14	15
Control (DMF)	-	Nil	Nil	Nil
Flucanazole	1000	19	18	19

* Zone of inhibition excluding well size 6 mm

- **Anti-inflammatory activity:** The anti-inflammatory activity was studied by formalin induced rat hind paw oedema measured by plethysmograph (mercury displacement method) [7-11]. Wistar strain rats of either sex weighing between 150-200 g were divided into five groups of six animals each. The first group served as the control and received the vehicle i.e., Tween 80, second group of animals was administered with standard drug diclofinac sodium, 200 mg/kg body weight, (subcutaneous). The third, fourth and fifth groups of animals were treated with crude extracts of *Ailanthus excelsa* bark at a dose of 200 mg/kg body weight, orally. The volume of paw oedema was measured in control and standard and treated groups accordingly 1, 2, 3, and 4 h after formalin injection. The percent inhibitions of oedema were calculated and are tabulated in **Table 2**.

TABLE 2: ANTI-INFLAMMATORY ACTIVITY OF EXTRACTS OF *AILANTHUS EXCELSA* BARK

Group	Dose (mg/kg)	Paw volume (ml)			
		1 h	2 h	3 h	4 h
Standard	200	48.69*(+ 0.010)	70.96**(+ 0.021)	76.80** (+ 0.022)	70.76** (+ 0.015)
Control	-	0.421 (+ 0.021)	0.458 (+ 0.0514)	0.4 59 (+ 0.0159)	0.47 (+ 0.043)
Pet ether extract	200	8.00 (+ 0.010)	13.00 (+ 0.011)	13.00 (+ 0.12)	8.00 (+ 0.119)
Ethyl acetate extract	200	24.22 (+ 0.017)	39.95*(+ 0.018)	57.44**(+ 0.029)	52.28**(+ 0.022)
Methanol extract	200	18.5 (+ 0.012)	23.58*(+ 0.014)	36.17*(+ 0.139)	29.19** (+ 0.154)

Standard: diclofenac sodium, Control 1% Tween 80, *indicates significance difference at $p<0.05$ compared to control, ** indicates significance difference at $p<0.01$ compared to control

The data were analyzed by one-way ANOVA. According to this test, there was a significant difference between the drug treated groups and control at the level of $P < 0.05$. To analyze the spectrum of anti-inflammatory activity of various fractions was used. At 1 h, diclofenac sodium exhibited good anti-inflammatory activity compared to crude extracts (Table 2). At 2 h, diclofenac sodium and ethyl acetate fraction showed good anti-inflammatory compared to other groups. Similarly at 3 h, diclofenac sodium and ethyl acetate fraction, methanol fraction showed good anti-inflammatory activity. Where as at 4 h, anti-inflammatory activity was statistically different in all the test groups except petroleum ether fraction. It means, diclofenac sodium showed highest anti-inflammatory activity followed by crude extracts. Hence, the results of the present investigation conclude that the ethyl acetate and methanol extract of *Ailanthus excelsa* bark is accountable for the anti-inflammatory activity.

- **Analgesic activity:** Analgesic activity was carried by tail flick method by using analgesiometer [12]. Weigh and number the mice 20-25 g of an either sex and take basal reaction time to radiant heat by placing the last 1-2 cm of the tail on the radiant heat source. The mouse withdrawing its tail within 3-5 seconds. A cut off period of 10 s is rejected from the study. Inject Novalgin 50 mg/kg body weight and note the reaction time at 0, 30, 60 and 90 min after drug. As the reaction time reaches 10 sec, it is considered maximum analgesia and the tail is removed from the source of heat to avoid tissue damage. In the same way the test sample pet ether, ethyl acetate and methanol extracts at the dose of 200 mg/kg body weight and note the reaction time at 0, 30, 60 and 90 min. Percent

protection against tail flicking was calculated using the formula,

$$\% \text{ protection} = (1\text{-Wt/Wc}) \text{ X } 100$$

Where, Wt and Wc are the mean value of the tail flicking in the test and control groups respectively. The results are given in **Table 3**. The data were analysed by student's t test and the level of significance was set at $p<0.001$. From Table 3, it is evident that the petroleum ether extract and ethyl acetate extract of *Ailanthus excelsa* bark showed good activity after 60 min at dose 200 mg/kg body weight compared with standard novelgin and the methanol extract showed less activity when compared with standard.

TABLE 3: ANALGESIC ACTIVITY OF VARIOUS EXTRACTS OF *AILANTHUS EXCELSA* BARK

Group	Dose (mg/kg)	Time taken to remove the tail at different intervals of time (Mean + SEM)			
		0 min	30 min	60 min	90 min
Standard	50	8.021**(+ 0.362)	8.075**(+ 0.257)	8.33**(+ 0.276)	8.625** (+ 0.297)
Control	-	4.290 (+ 0.162)	4.231 (+ 0.178)	4.412 (+ 0.174)	4.775 (+ 0.159)
Pet ether extract	200	4.850 (+ 0.269)	5.57*(+ 0.217)	6.00**(+ 0.102)	6.275**(+ 0.15)
Ethyl acetate extract	200	6.36 (+ 0.371)	7.025*(+ 0.292)	7.031**(+ 0.219)	7.687**(+ 0.187)
Methanol extract	200	4.96 (+ 0.33)	5.325 (+ 0.349)	5.65(+ 0.328)	5.937 (+ 0.328)

Standard: Novelgin, Control 1% Tween 80,* indicates significance different at $p < 0.05$ compared to control, ** indicates significance different at $p < 0.01$ compared to control

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