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Screening of Analgesic Activity of Methanolic Extract and Its Fractions of *Alternanthera Pungens*

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Abstract: *The purpose of the present study was to prepare the methanolic extract and various fractions of whole plant of Alternanthera pungens and to perform preliminary phytochemical analysis and screening of analgesic activity using Tail Immersion method on Swiss albino mice. The whole plant of Alternanthera pungens was collected, powdered and extracted with methanol by maceration method. The obtained methanolic extract was fractioned into various fractions (P_2 and C_2) using different solvents of increasing order of polarity viz., Petroleum ether and chloroform successively and the residue left over is Hydro alcoholic fraction (H_2). Preliminary phytochemical analysis of the obtained extract and fractions revealed the presence of steroids, triterpenoids, phenols and flavonoids rich in methanolic and chloroform fractions (M_2 and C_2). The extract and its fractions were significantly ($p<0.001$) reduced the pain as compare to the control group. Among all extract and fractions chloroform fraction (C_2) was found to possess good analgesic activity.*

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I. Introduction

Pain has been defined by the International Association for the Study of Pain (1979) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage." The pain generated by physical stimuli may be treated with analgesic and anti-inflammatory drugs [1]. Analgesics are those drugs or agents that reduce or inhibit the pain sensation temporarily. Several synthetic and plant origin analgesics are being tested for their efficacy and potency on different animal models which include hot plate, tail flick, tail clip, cold pain, filament pain, tail immersion technique, acetic acid induced writhing test, formalin induced writhing test [2]. Most of the pain-relieving drugs produced noticeable side effects on the physiology of the body. In the indigenous system of medicine, several plants possess an analgesic property and many investigators screened the plant crude extracts for their analgesic property [3]. In light of above, in the present study, we aimed to explore the beneficial effects of *Alternanthera pungens* as possible analgesic agents.

Plants acts as a natural repository of a wide spectrum of bioactive compounds which are responsible for their biological properties. *Alternanthera pungens* (Khaki weed) is a kunth creeping, variable, perennial pioneer plant of the family Amaranthaceae L. synonyms are *Alternantherarepens (L.)* and*Telantherapungens* (Kunth) [4]. The vernacular name of the plant in Telugu is Mulluponnaganti. It is a branched and prostrate herb. The leaves are widely used as vegetable. The plant can grow upto a length of 50cms. The species forms dense mats of stems and leaves during the rainy season. The plant is reported to be effective in the treatment of naso-pharyngeal infections, diarrhoea, HIV, dysentery, vermifuges, dropsy, swellings, oedema, gout, venereal diseases and used as lactation stimulants, abortifacients, fabrifuges [5]. From the literature review, *Alternanthera pungens* was found to contain saponins, alkaloids, steroids, triterpenoids, leucoanthocyanidins, choline and exhibited spasmogenic properties, antimicrobial, diuretic, antidiarrhoeal, antidiabetic, antioxidant and anti-HIV properties [6]. The presence of C-glycoflavones and betalaineshave proven potent antioxidant properties for the ethanolic extract of *Alternanthera pungens* [7]. The plant is diuretic. A decoction is used internally to treat gonorrhoea [8]. In traditional and ayurvedic medicine it was used as painkiller, for stomachache, swelling and nasopharyngeal infections and also reported for lactation stimulus in veterinary [9]. Hence the present work is undertaken to validate its traditional claim scientifically, keeping in view of the above observations.


***Fig.1 Alternanthera pungens* grown on road side Fig.2 Leaves with bracteoles spine-tipped**


Fig.3 & 4 Dried *Alternanthera pungens* whole plant material

II. Material And Methods

Drugs and Chemicals

Diclofenac sodium, Saline solution, Methanol (95% v/v), Petroleum ether (60-80^{o} C), Chloroform, Distilled water.

Collection and authentication of plant material

The whole plant material of *Alternanthera pungens* was collected in rainy season from road side and surrounding areas of Kakatiya University, Warangal. The plant was authenticated by Prof. AjmeeraRaagan, Plants systemic Laboratory, Department of Botany, Kakatiya University, Warangal, Telangana.

Preparation of herbal extract and fractions:

The shade dried plant materials were powdered, sieved and macerated with methanol for 7 days with occasional stirring, then filtered and extract was collected. Again the marc was re macerated with fresh methanol for more 7 days and re maceration process repeated 2-3 times until solvent turns to colorless. All the macerated solutions were filtered, pooled together and solvent recovered in a Rotary evaporator. Finally the dried methanolic extract of *Alternanthera pungens* was collected and labelled as M_2 and preserved in a desiccator for further evaluation.

The dried methanolic extract was prepared into methanol solution in separating funnel and various fractions were prepared as shown in Fig.5.

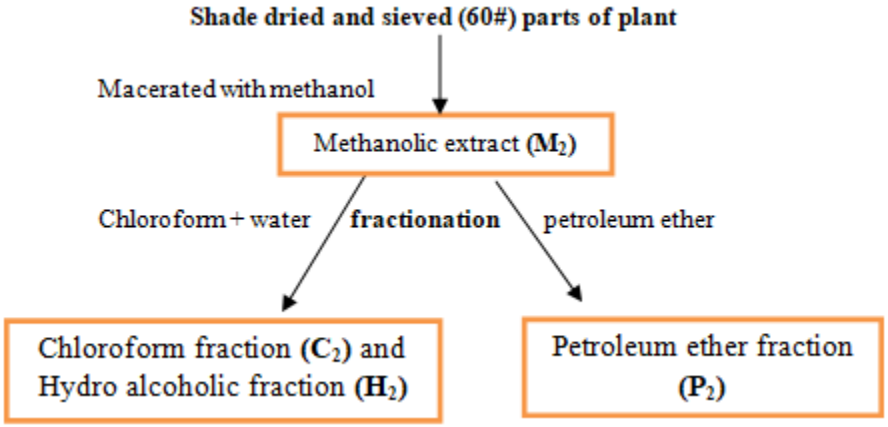

Fig.5 Preparation of methanolic extract and its fractions of Alt*ernanthera pungens*

Preliminary phytochemical screening

The preliminary phytochemical screening was done by following standard qualitative chemical methods [9]. The methanolic extract and its fractions of *A. pungens*were screened for the presence of carbohydrates, alkaloids, triterpenoids, saponins, phenols, lipids, sterols, volatile oils, proteins and flavonoids.

Evaluation of analgesic activity by Tail Immersion Method:

Selection of Experimental Animals:

The animal care and handling will be done according to the guidelines set by the CPCSEA. All animals will be housed in a polypropylene cage containing sterile paddy husk as bedding throughout the experiment. Six to eight week old Male adult Swiss Albino mice weighing 21-25g will be selected from an inbred colony maintained under the controlled conditions of temperature (23 ± 2ºC) and 12 hr light/dark cycle with food and water provided *ad libitum.* The experiment was performed in pharmacology department of the institute in the time period between 10.00 to 1.00pm.

Preparation and Mode of Administration of Drugs:

All drug solutions are freshly prepared and suspended in saline solution

Standard drug: Diclofenac sodium (20mg/kg/10ml, b.w,orally)

Table 1.Group Classification:

Sl. No	Groups	No. of Mice	Inducing PD
I	**Normal Control**	06	Distilled water + Feed
II	**Standard**	06	Diclofenac sodium (20mg/kg,b.w, orally)
III	**Test (C_2)**	06	C_2 (100mg/kg,b.w, orally)
IV	**Test (H_2)**	06	H_2 (100mg/kg,b.w, orally)
V	**Test (M_2)**	06	M_2 (100mg/kg,b.w, orally)
VI	**Test (P_2)**	06	P_2 (100mg/kg,b.w, orally)

Experimental Procedure:

Healthy Swiss albino mice weighing between 21-25g were selected and divided into five groups, of 6 mice each. Group I received the drinking water and feed, served as normal control. Group II received Diclofenac sodium 20mg/kg, b.w, orally served as standard. Group III, IV and V received test drugs extracts (C_2, H_2, M_2, and P_2) at doses of 100mg/kg, b.w, orally respectively for 1 day. After 30 minutes of standard drug administration and 1 hour after extract administration 3 to 4 cm area of the tail was marked and immersed in the water bath thermostatically maintained at 55^{O}C. The time taken by mice to withdraw the tail from hot water (in seconds) and body jerk was noted as the reaction time or tail flick latency. The maximum cut off time for immersion was 15 s to avoid the injury of the tissues of tail. Responses were recorded using stop clock starting from the instant dipping to instant tail flick. The latency was recorded at 5, 15, 30 and 60 minutes for vehicle, standard and test drug administration [1].

Statistical analysis

Statistical analysis was determined using statistical package PRISM 7.0 version and one way analysis of variance (ANOVA) in Dunnett's multiple comparisons test. Results values were expressed as Mean ± SEM

for six mice in each group. The significant difference between and within various groups were determined. The values of $P < 0.001$ were considered very statistically significant.

In Tail immersion method all the test and standard drugs significantly ($p<0.001$) reduce the pain as compare to the control group. By applying Dunnett's multiple comparisons test, it was shown that there is significant ($p<0.001$) effect of standard, C_2, M_2, H_2 and P_2 as compared to the control group.

III. Results

Preliminary phytochemical analysis

Preliminary phytochemical analysis showed the presence of phytoconstituents such as carbohydrates, alkaloids, triterpenoids, lipids, saponins, phenols, steroids, glycosides and flavonoids whereas volatile oils are absent.

Table 2. Preliminary phytochemical screening of extract and fractions of *A.pungens:*

S.No	Phytochemicals	Crude plant methanolic extract and its various fractions			
		Methanolic extract (M2)	Pet. ether fraction	Chloroform fraction	Hydro alcoholic fraction
1	Carbohydrates and glycosides	+	-	-	++
2.	Alkaloids	+	-	+	+
3.	Saponins	+	-	-	+
4.	Phenolic compounds	++	+	+	++
5.	Lipids	++	+++	+	-
6.	Triterpenoids and Steroids	+++	+	++	+
7.	Flavonoids	++	-	++	+

Analgesic activity of extract and its fractions of *Alternanthera pungens* by using Tail Immersion Method:

Table 3. Analgesic activity of extract and its fractions of *Alternanthera pungens.*

S.No	Groups	Treatment	Dose	Basal reaction time (s) (mean ± SEM)				
				5 Min	15 Min	30 Min	60 Min	90 Min
1.	Group I	Normal Control	Distilled water	2.017±0.017	2.93± 0.024	2.967±0.021	3.00±0.020	2.993±0.003
2.	Group II	Standard	20mg/kg	22.34±0.082	23.02±0.048	24.42±0.187	25.15±0.310	24.05±0.034
3.	Group III	Test (C_2)	100mg/kg	21.34±0.082	22.02±0.048	23.08±0.040	23.85±0.043	23.46±0.021
4.	Group IV	Test (H_2)	100mg/kg	9.9 ± 0.052	9.288±0.007	9.362±0.010	10.77±0.016	10.03±0.042
5.	Group V	Test (M_2)	100mg/kg	19.79±0.05	20.77±0.09	21.87±0.05	22.1±0.10	21.8 ±0.08
6.	Group VI	Test (P_2)	100mg/kg	17.67±0.120	18.83±0.10	15.90± 0.05	12.18± 0.08	11.02 ± 0.03

Values were expressed as **Mean ± SEM values (N = 6).**$P < 0.001$ extremely significant on comparing group II vs. group I.$P < 0.001$ extremely significant on comparing group III, IV, V,VI vs. group I

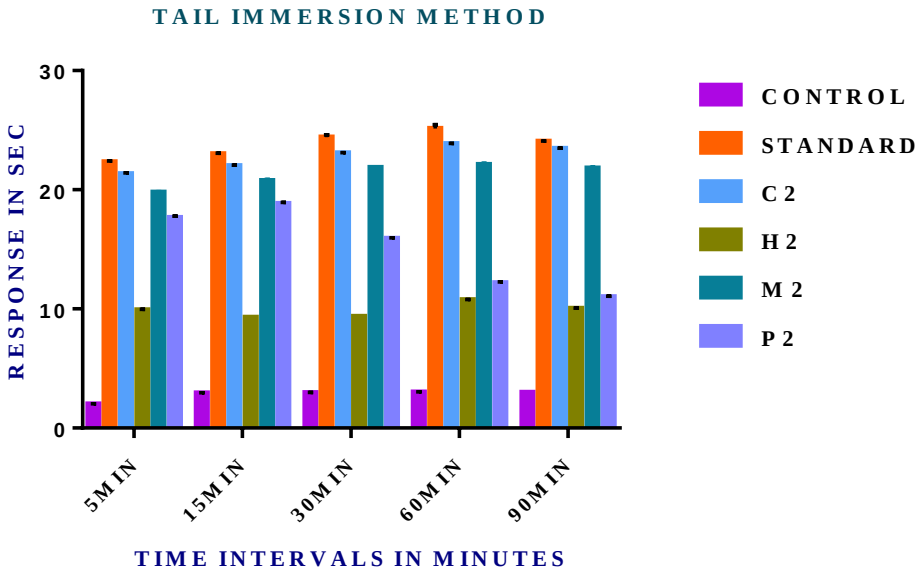

Fig.6. Analgesic effect of different extracts of *Alternanthera pungens*

Evaluation of analgesic effect with graphical representation of different extracts of *Alternanthera pungens* by using tail immersion method are shown in Fig.6.

IV. Discussion

From preliminary phytochemical analysis of methanolic extract and its fractions revealed that, both methanolic extract (M_2) and chloroform fraction (C_2) are rich in terpenoids, steroids, flavonoids and phenolic compounds, whereas Hydro alcoholic fraction (H_2) is rich in carbohydrates, glycosides, alkaloids, saponins and phenolic compounds. Most of the lipid compounds and few of nonpolar phenolic, terpenoidal compounds were separated into Petroleum ether (P_2) fraction. In tail immersion model, the extract and fractions (M_2, C_2, H_2 and P_2)from *Alternanthera pungens* exhibited significant analgesic activity by increasing the reaction time of the mice compared to control group at all-time intervals. Diclofenac sodium is used as standard drug, which is considered mild and moderate to severe analgesic. Among the tested plant extracts the basal reaction time of chloroform extract (C_2) was found be significant at 60 minutes. All the remaining extracts also reported their significant analgesic activity at the same duration (60 minutes). The order of analgesic activity was found to be Diclofenac sodium >chloroform fraction (C_2) > methanolic extract (M_2) > Petroleum ether fraction (P_2)>hydro alcoholic (H_2) > distilled water.

V. Conclusion

- From the preliminary phytochemical analysis of extract and fractions of *Alternanthera pungens*, it is concluded that flavonoids, steroids, phenols and terpenoids are more present in both methanolic extract (M_2) and chloroform fraction (C_2) whereas Hydro alcoholic fraction shows the presence of carbohydrates, glycosides, phenols in high quantity and flavonoids and terpenoids in less quantity.
- All the extracts demonstrated significant analgesic activity amongst them chloroform extract of *Alternanthera pungens* exhibited maximal analgesic profile.
- This analgesic activity may be due to the presence of flavonoids, steroids and terpenoids in chloroform fraction (C_2) of *Alternanthera pungens*.
- Further isolation of bioactive constituents responsible for the analgesic activity is needed to develop as a promising analgesic drug.

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