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Pharmacognostical and Analgesic Activity of Various Leaves Extracts of *Achyranthus Aspera* Linn

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ABSTRACT

Aim of present study was to investigate the analgesic activity of the leaves extracts of Achyranthus aspera linn. The leaves of drug is converted in uniform particle size and extracted by using Petroleum ether (60-800 c), Benzene, Chloroform, Ethyl acetate, n-Butanol, and Ethanol solvents. Then TLC and column chromatography is performing for isolation of active principle. This active principle is further carryout analgesic activity by Tail flick method and Writhing test method with the help of Aspirin (150 mg/kg) as standard drug. In Tail Flick Method the Ethanol extract showed good significant increase in reaction time as compared to pretreatment reaction time, where as n-Butanol, Chloroform, and Pet- ether extract, showed significant increase in reaction time, when compared to pretreatment reaction time However, Benzene and Ethyl acetate extract showed less significant increase in pretreatment reaction time. In Writhing Test Method the Ethanol extract showed good significant decrease in abdominal writhes as compared to standard group, where as n-Butanol extract, Ethyl acetate extract, Chloroform extract, Benzene extract, showed significant decrease in abdominal writhes, However, Pet-ether extract and Benzene extract failed to decrease in abdominal writhes when compared to standard group.

KEYWORDS

Achyranthus aspera, Analgesic activity, Column, Pretreatment, Aspirin

INTRODUCTION

Achyranthus aspera Linn. belonging to family Amarantaceae erect or procumbent annual or perennial herb, 1-2m. in height. It consist Achyrol, ecdysterone, ecdysterone, spooning A, saponin B, Hentricontane, ecdysterone[1], linolieic, Oleic palmitic[2]. Therapeutic it is uses eardrop to relieve pain, rheumatic pain, stomachic & bowel complaints, piles, boils, skin eruptions[3]. Poisonous insects, wasps, bee's[4]. digestant and dog-bites[5]. piles and snake bites. headache, leprosy & dyspepsia. inflammatory, arcdiatonic, diuretic, hydrophobia & itching[6].

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Fever is an elevation of body temperature above the normal circadian range as the result of a change in the thermoregulatory center located in the anterior hypothalamus[7]. Body temperature between 99^0F (37.22^0C) and 103^0F (40.57^0C) onward is called pyrexia while rise of body temperature above 107^0F (41.66^0C) is called hyper pyrexia[8]. A drug that selectively relieves pain by acting in CNS or on peripheral pain mechanisms, without significantly altering consciousness[9].

MATERIALS AND METHODS

Procurement, Drying & Size Reduction of Drugs

The leaves of *Achyranthus aspera* Linn were collected from local Area of Toranmar Dist. Nandurbar. Dried to coarse powder using mechanical grinder and passed through a sieve

No.40 to obtained powder of desired particle size[10].

Successive Extraction

About 250gm material was subjected to hot continues successive extraction with various solvents in increasing order of polarity from Petroleum ether (60-80^0 c), Benzene, Chloroform, Ethyl acetate, n-Butanol, and Ethanol in a soxhlet extractor at a temp 45^0 - 50^{0}c up to 40 cycles[11].

Qualitative Chemical Investigation of Extract

It shows the presence of Sterols, Carbohydrates, Alkaloids, Glycoside and Saponins in all extracts[12].

Identification of Active Principle by Thin Layer Chromatography

Adsorbent – Silica gel G (activated)

Plate size – 20 cm x 8 cm

Plate thickness – 0.2 mm

Solvent system – Chloroform: Methanol (85:15)

Spraying agent – Anisaldehyde- sulphuric acid

Developing time – At 110^{0}c for 10 min

The spot were visualized as blue and pink[13].

The ethanolic extract revealed the presence of sterols.

Column Chromatography of Ethanolic Extract

Adsorbent : Silica gel G activated at 105^{0}c. for 1 hour

Length of column : 41cm.

Diameter of column Outer : 3 cm.

Inner : 2.8 cm

Rate of elution : 10-15 drops/min.

Volume of each Eluent collected : 25 ml each

Total Volume of Eluent collected : 100 ml.

Elution: Acetone

Acetone:Methanol

Methanol

Totally eluents were collected and each eluent was subjected to thin layer chromatography as described above for identification of sterols. The R_f value has been calculated.

Analgesic Activity

Animal Selection

Female albino mice weighting between, 20-25gm were used for acute toxicity study of various extracts.

Acute Toxicity Study

Acute toxicity study was carried out according to OECD guidelines[14].

Preparation and Administration of Doses

The dose of 1 ml/100gm b.w. of all test materials was given to the mice in stepwise procedure using little doses of 5,50,300 and 2000mg/kg b.w. food was given to the mice 3 to 4 hr. after administering the test materials. Signs and symptoms of toxicity were observed at 2000mg/kg in single animal for all extracts in singing study. The same dose was given to three animals for main toxicity study.

Tail Flick Method

Group of 6 albino rats of both sexes with a weight between 150-200gm were used for each dose. Before administration of the test compound or the standard the normal reaction time was determined. The animal was pet into small cage with an opening for the tail at the rear wall. The tail was held gently by the investigator. By opening of shutter light beam exerting radiant that is directed to the proximal third of the tail. For about 6 s. the investigator observes the reaction of the animals. The rat tries, to pull the tail away and turns the head. With a switch a shutter was closed as soon as the investigator notices the reaction. Rat with reaction time of more than 6 s. were not used in the test. The escape reaction which was the end point of the test can be regarded as a complex phenomenon medicated as a spinal relax. The test compound and standard were administered orally or subcutaneously. Animals were submitted to the same testing procedures after

30, 60 and 120 min. for each individual reaction time.

Eight groups were made each containing 6 animals for both methods.

Group I - Control (200 mg/kg)

Group II - Aspirin as Standard (150 mg/kg)

Group III - Petroleum ether extract (200 mg/kg)

Group IV - Benzene extract (200 mg/kg)

Group V - Chloroform extract (200 mg/kg)

Group VI - Ethyl acetate extract (200 mg/kg)

Group VII - n-Butanol extract (200 mg/kg)

Group VIII- Ethanol extract (200 mg/kg)

Writhing Test Method

Albino rats of either sex weight between 150-200 gm were used. 1 ml of 0.6% (v/v) acetic acid was injected intraperitonially. Test animals were administered the drugs at various pretreatment times prior to acetic acid administration. The rats were placed individually into glass beakers and 5 min. were allowed to elapsed. The rats were then observed for a period of 10 min. and the no. of writhes was recorded for each animal. For scoring purpose, a cortile was indicated by stretching of the abdomen with simultaneous stretching of at least 1 hind limb. The formulas for computing percentage inhibition was average writhes in the control group minus cortiles in drug group divided by writhes in the control group times 100%. The time period with the greatest percentage of inhibition was considered the peak time. A dose range was reserved for interesting compounds of those which inhibit more than 70% compounds with less than 70% inhibition are considered to have minimal activity[15].

STATISTICAL ANALYSIS

Data generated during the above investigations were subjected to appropriate statistical tests to decide the significance of the differences between the groups. $P<0.05$ was considered significant in all cases.

RESULT AND DISCUSSION

Successive Extraction

Table: 1 Percentage Yield of Various Leaves Extracts of *Achyranthus Aspera* Linn.

Extracts	Weight	% yield
Petroleum ether(60-80)	25 gm	2.5%
Benzene	33.5 gm	3.35%
Chloroform	36.8 gm	3.68%
Ethyl acetate	28.9 gm	2.89%
n-Butanol	37.1 gm	3.71%
Ethanol	46.2 gm	4.63%

Phytochemical Investigation and Qualitative Chemical Test

The results of qualitative chemical investigation of leaves of *Achyranthus aspera* linn has as follows

Petroleum ether (40-60) extract - Sterols, Carbohydrates.

Benzene extract - Carbohydrates.

Chloroform extrac - Carbohydrates.

Ethyl acetate extract - Alkaloids, Glycoside.

n-Butanol extract - Carbohydrates

Ethanol extract - Sterols, Alkaloids, Glycoside, Saponins.

Identification of Active Principles by Thin Layer Chromatography

Presence of sterols in ethanolic extract was identified by TLC profile. The ethanolic extract on TLC revealed the presence of 2 spots. The R_f value of which were found to be as 0.52 (blue) and 0.92 (Pink) respectively in Chloroform: Methanol, (85:15) solvent system.

Table: 2 R_f Value of Ethanolic Leaves Extracts of *Achyranthus Aspera* Linn.

Extract	Color	R_f Value
Ethanol	Blue	0.52
	Pink	0.92

Isolation of Active Principles by Column Chromatography

The elution's of ethanolic extract by a column chromatography, on TLC profile revealed, the presence of two spot. The R_F value of which were found to be as 0.49 (blue) and 0.89 (Pink) in Chloroform: Methanol, (85:15) solvent system.

extract has good significantly increased reaction time at various time intervals when compared to pretreatment reaction time. The n-butanol, chloroform and pet. ether extract have significantly increased reaction time. However, the benzene and ethyl acetate extracts have increased slightly reaction time at various time intervals when compared to pretreatment reaction.

Writhing Test Method

It indicated that Ethanolic extract has good significant decrease in abdominal writhes to the extent of 47.05%, when compared to standard group was 63.52 %.

Table: 3 Isolation of Active Principle of Ethanolic Leaves Extract of *Achyranthus Aspera* Linn

Solvents	Concentration	No. of Eluent	No. of spot	Color of spot	Avg. R_f Value
Acetone	100	61 to 64	1	Blue	0.49
Acetone: Methanol	80 : 20	65 to 68	1	Pink	0.89
	60 : 40	69 to 72	1	Pink	0.82
	40 : 60	73 to 76	1	Pink	0.89
	20 : 80	77 to 80	1	Pink	0.90
Methanol	100	81 to 84	1	Violet	0.15

Pharmacological screening

Acute Toxicity Study

The acute toxicity study of various extracts of *Achyranthus aspera* Linn. leaves was showed signs of toxicity like tremour, convulsion and deep breathing at 2000mg/kg b.w. $1/10^{th}$ of the same dose for all these exracts were taken as therapeutic dose i.e. 200mg/kg.b.w.

Tail Flick Method

It indicated that there was no significant difference in the mean pretreatment reaction time between the different groups. The ethanolic

The n-butanol extract, ethyl acetate, and chloroform extract have significance decrease in abdominal writhes, to the extent of 39.05%, 32.94% and 31.29% respectively, when compared to standard group 63.52%.

However, pet-ether extract and benzene extract have failed to decrease abdominal writhes to the extent of 11.05%, 24.70%, when compared with standard group was 63.52% (Table 5 and Fig. 1).

Table: 4 Leaves Extract of A*chyranthus Aspera* Linn. on Tail Flick Method

Group (Dose mg/kg)	**Pretreatment reaction time (In sec)**	**Post treatment reaction time in sec**		
		30 min	**60 min**	**120 min**
Control (200 mg/kg)	3.3 ± 0.21	3.42 ± 0.23	3.67 ± 0.16	3.93 ± 0.31
Pet-ether (200 mg/kg)	3.6± 0.25	4.12 ± 0.49	4.76 ± 0.28	5.04 ± 0.13
Benzene (200 mg/kg)	3.7 ± 0.23	3.85 ± 0.0.27	3.96 ± 0.16	4.32 ± 0.18
Chloroform (200 mg/kg)	3.3 ± 0.23	4.03 ± 0.24	4.62 ± 0.36	4.92 ± 0.12
Ethyl acetate (200 mg/kg)	3.8 ± 0.17	3.78 ± 0.41	3.89 ± 0.31	4.14 ± 0.15
n-butanol (200 mg/kg)	3.5 ± 0.19	4.20 ± 0.35	4.83 ± 0.23	5.10 ± 0.17
Ethanol (200 mg/kg)	3.6 ± 0.22	4.74 ± 0.19	5.24 ± 0.24	5.63 ± 0.14
Aspirin (150 mg/kg)	3.8 ± 0.26	4.90 ± 0.17	5.35 ± 0.16	5.75 ± 0.41

Table: 5 Leaves Extract of *Achyranthus Aspera* Linn. on Writhing Test Method

Group	**Dose (mg/kg)**	**No. of writhing per 10 min**	**% Inhibition**
Control	200 mg/kg	85 ±1.581	-
Pet.ether	200 mg/kg	75.6 ±2.302	11.05 %
Benzene	200 mg/kg	64 ±2.550	24.70 %
Chloroform	200 mg/kg	58.4 ±2.510	31.29 %
Ethyl acetate	200 mg/kg	57.4 ±2.191	32.94 %
n-butanol	200 mg/kg	51.8 ±1.924	39.05 %
Ethanol	200 mg/kg	45 ±1.871	47.05 %
Aspirin	150 mg/kg	31 ±1.581	63.52 %

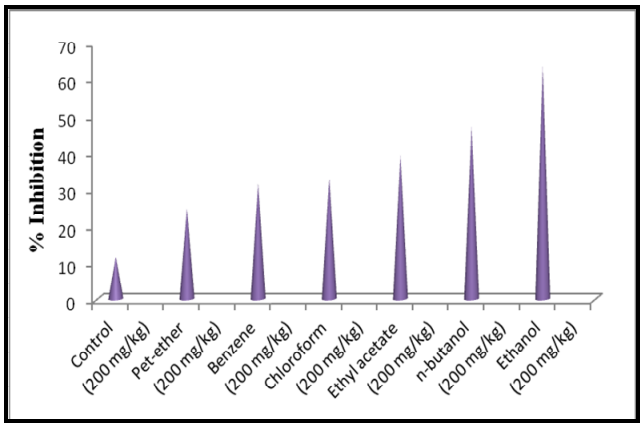

Figure: 1 Persentage Inhibition of All Extract of *Achyranthus Aspera* Linn. on Writhing Test Method

CONCLUSION

Hence, to put into nutshell, the active principle/s of leaves of *Achyranthus aspera* Linn. like glycoside, sterols, carbohydrates, saponins may be responsible for the analgesic activity. However, it needs isolation, structural elucidation and screening of any of the above mention active principle/s to pin point of the activity of drug.

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