Anti-Inflammatory Activity of Achyranthes Aspera Linn.: An Experimental Evaluation

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Abstract- In this study, Achyranthus aspera was screened for various phytochemicals and proved to cherish with various phytochemicals such as alkaloids, glycosides, saponins, tannins, steroids and carbohydrates. Various fractionation of the plants is studied for detailed phytochemical profiling and reported to possess a good amount of phenolic content, flavonoid content and total alkaloidal content. The antioxidant activity of Hydro alcoholic extract of A. aspera was screened in various in-vitro methods such as DPPH assay, reducing power assay, and total antioxidant assay. The results suggest that the polyphenolic contents present in the whole plant of A. aspera are responsible for the reported antioxidant activity. Further, Hydro alcoholic extract of Achyranthes aspera exhibited promising anti-inflammatory activity, which is attributed to phenols, flavonoids, alkaloids and saponin phytoconstituents found in extracts. This abundantly available plant may serve as an important addition in therapeuti armamentarium of chronic inflammatory diseases such as arthritis.

I. INTRODUCTION

Knowledge of herbs has been handed down from generation to generation for thousands of years [1]. Herbal drugs constitute a major part in all traditional systems of medicines. Herbal medicine is a triumph of popular therapeutic diversity. Plants above all other agents have been used for medicine from time immemorial because they have fitted the immediate personal need, are easily accessible and inexpensive [2]. *Achyranthes aspera* as a rough flowered stalk is described as in Sanskrit synonyms. It is described in 'Nighantas' as pungent, purgative, digestive, and a remedy for inflammation of the internal organs, itch, piles, abdominal enlargements and enlarged cervical glands. The diuretic property of the plant was well known to the natives of India and European physicians. Various plant parts form ingredients in many native prescriptions were used in combination with more active remedies. The plant is globally available as a medicinal weed in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America. In tnis current study, anti inflammatory property was screened using carragenen model.

II. EXPERIMENTAL METHODOLOGY

Whole plant of *Achyranthes aspera* Linn was collected from the Hyderabad rural areas and authenticated by Dr. P.V. Prasanna, Scientist "G",Botanical survey of India, Hyderabad, Telangana. The whole plant was subjected to shade drying process and powdered mechanically into coarse form.
Extraction was carried using Microwave irradiation using various solvents based on polarity from non polar to polar region. Extraction vessel was placed in the microwave oven and irradiated with microwave energy at a controlled power level (e.g., 300-600 W) and time (e.g., 30-90 seconds). Extraction vessel was removed from the microwave oven and allowed to cool, later, the extract was filtered using muslin cloth to separate the solvent from the plant material. Evaporated to get crude extracts.

III. PHYTOCHEMICAL SCREENING:

The preliminary phytochemical screening of all the extracts of *Achyranthes aspera* Linn were performed according to the standard procedures.

IV. ESTIMATION OF TOTAL PHENOLIC, FLAVONOID AND ALKALOID CONTENT

Estimation of Total phenolic content:
The total phenolic content (TPC) for the Hydro alcoholic extract of Achyranthes aspera (EEAA) was determined by using Foline- Ciocalteau phenol

reagent method [27] Briefly, 1.0 mL of the extract at various concentrations was mixed with 2.5 mL of 10% Foline- Ciocalteau reagent and 2.5 mL of 7.5% sodium carbonate. The contents were thoroughly mixed and allowed to stand for about half an hour (30 minutes). The absorbance was read at 750 nm in a spectrophotometer. The total phenol content was expressed as gallic acid equivalents in milligram per gram of the extract.

Estimation of Total Flavonoid Content:

The flavonoid content (TFC) for Hydro alcoholic extract of Achyranthes aspera was screened using aluminium chloride colorimetric method[3]. Quercetin was used as a reference standard for plotting a calibration curve.TFC is determined by the following procedure 0.5mL of the extract at various concentrations was mixed with 3mL of 95% methanol, 0.1mL of 10% (weight/volume) aluminium chloride, 0.1mL of 1M potassium acetate, and 2.8mL of distilled water. Let the reaction mixture to stand at room temperature for about 30 minutes and absorbance was read at 415 nm against the blank sample. A calibration curve was generated using quercetin in methanol. The flavonoid content was expressed as Quercetin equivalents in milligram per gram of the extract.

Estimation of Total Alkaloid Content:

The total alkaloid content for Hydro alcoholic extract of Achyranthes aspera was determined by a slightly modified Bromocresol green (BCG) method. Atropine was used as the reference standard for plotting the calibration curve.

VI..IN-VITRO ANTIOXIDANT ACTIVITY

DPPH Antiradical capacity:

The free radical potential of Hydro alcoholic extract of *Achyranthes aspera* was determined spectrophotometrically[4]. A stock solution of DPPH was prepared by dissolving 33mg of DPPH in 1 liter of methanol. Five different concentrations of Hydro alcoholic extract of Achyranthes aspera (100, 200, 400, 600 and 800 µg/ml) were mixed with 100 µL of DPPH radical solution in a 96-well microplate and incubated for 20 min at room temperature. The resultant mixture was read spectrophotometrically at 517 nm against a methanol blank and the following equation was used to calculate the % inhibition of each extract:

$$\% \text{ inhibition} = (Ao - As)/ (Ao) \times 100$$

Where, A o is the absorbance of the control, and As is the absorbance of the test sample. The IC_{50} represented the concentration of the extract that inhibited 50% of radical

Fe Reducing power Determination:

The reducing ability of a compound generally depends on the presence of reductants which exhibits antioxidant potential by breaking the free radical chain, donating a hydrogen atom. The Fe 2+ reducing power of plant extract was determined by the method [5]. Hydro alcoholic extract of *Achyranthes aspera* (0.75mL) at various concentrations was mixed with 0.75 ml of phosphate buffer (0.2 mole, pH 6.6) and 0.75 mL of potassium ferricyanide K 3 Fe(CN) 6 (1%w/v), followed by incubating at 50°C for 20 mins. The reaction was stopped by adding 2.5mL of 10% (w/v) trichloroacetic acid followed by centrifugation at 3000 rpm for 10min. Finally, 1.5mL of the upper layer was mixed with 1.5mL of distilled water and 0.5mL of $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm. Higher the absorbance of reaction mixture indicated greater the reducing power. Ascorbic acid is used as reference compound.

Total Antioxidant Activity:

Total antioxidant activity was estimated by phosphomolybdenum assay [6]. Hydro alcoholic extract of Achyranthes *aspera* in different concentration ranging from 100µg/ml to 800µg/ml were added to each test tube individually containing 3ml of distilled water and 1ml of molybdate reagent solution. These tubes were kept incubated at 95ºC for 90 min. After incubation, these tubes were normalized to room temperature for 20- 30 min and the absorbance of the reaction mixture was measured at 695nm.Mean values from independent samples were calculated for each extract ascorbic acid was used as positive reference standard.

Total antioxidant = OD of test X concentration of standard in µg X made up volume of sample

V. EVALUATION OF IN-VIVO ANTI - INFLAMMATORY ACTIVITY

Animals:

This study's an ethical approval was acquired from the "Institutional Animal Ethical Committee" with an Approval no: MRCP/CPCSEA/IAEC/2025/1/13. Albino rats with average body weight from 150 to 250 g were utilized for conducting this study. They were procured from Sanzyme Bio-analytical lab, Plot no. 8 Sys.No.542, Kothur (V), Shameerpet, RR dist. Polypropylene cages were used for housing rats as well as standard conditions (12h dark and light cycles at 35-60 % humidity and 25±3°C) were used for their maintenance. Standard tap water and pellet feed were allowed adlibitum.

Anti inflammatory Activity: Anti inflammatory Activity of Hydro alcoholic extract of Achyranthus aspera was estimated by carrageenan - induced paw edema in Wistar Albino Rats [7].

Carrageenan-induced paw edema: The rats were randomly assigned into 5 different groups (n=6). An injection (subcutaneously) was made of 0.1 mL of 1% carrageenan into the right hind paw of each rat under the sub plantar region.

Group I : Carrageenan (0.1ml of 1%)

Group II : Diclofenac (5 mg/kg, P.o)

Group III: Hydro alcoholic extract of Whole plant of Achyranthus aspera Linn (HAAA) (100 mg/kg), P.o

Group IV: Hydro alcoholic extract of Whole plant of Achyranthus aspera Linn (HAAA) (200 mg/kg),

Group V: Hydro alcoholic extract of Whole plant of Achyranthus aspera Linn (HAAA) (400 mg/kg),

After the treatment the paw volumes were measured after 30min, 1hr, 3hr, 6hr and 24hr of induction using plethysmograph and percentage inhibition in paw volume is determined using the formula.

Percentage inhibition (%) = (control – treated)/control x 100

Parameters to be estimated:

1. Volume of paw

The volume of the paw up to the ankle joint was measured by a plethysmometer.The percentage inhibition of edema was calculated using these paw volumes, with respect to their controls.

% edema inhibition = (Vt − V0) control − (Vt − V0) treated/(Vt − V0) control × 100.

2. Motility Test

The motility pattern of the rats was observed for a period of 5 minutes and scored 0, if the rat walked with difficulty and avoided touching the toes of the inflamed paw to the floor; 1, if the rat walked with little difficulty, but with toe touching the floor; 2, if the rat walked easily.

VII.RESULTS AND DISCUSSION

Percentage Yield: The percentage yield with all the extracts was calculated and depicted in Table 1. The Ethanol yielded more quantity, followed by extraction with water.

Table 1: Percentage yield of various extracts of Achyranthes aspera

Solvent	% yield (gm)
Petroleum ether	3.17
Chloroform	3.35
Ethyl acetate	0.57
Ethanol	5.15
Hydro alcoholic	6.04
Aqueous	4.05

Phytochemical screening was carried out for all extract of Achyranthes aspera by preliminary tests. From the results it was evident that, carbohydrates, Alkaloids, steroids, Glycosides, Flavonoids, Saponins, Phenols, Tannins. Results were shown in Table 2. '+' indicates the presence and '-' indicates the absence of phytochemical constituents.

Table 2: Phytochemical analysis of various extracts of Achyranthes aspera

Phyto Constituent	Pet Ether	Chloroform	Ethyl Acetate	Ethanol	Hydro alcoholic	Aqueous
Alkaloids	-	+	+	+	+	-
Saponins	-	-	+	+	+	+
Carbohydrates	-	-	-	+	+	+
Phenols	-	-	+	+	+	+
Flavonoids	-	-	+	+	+	+
Steroids	+	+	-	-	-	-
Glycosides	+	+	-	-	-	-
Terpenoids	+	+	-	-	-	-
Quinones	-	-	-	+	+	+
Tannins	-	+	-	+	+	-

Estimation of Total Phenolic Content:

The total phenolic content in various fractions of the whole plant of Achyranthes aspera was determined using the Folin-Ciocalteau method. The results were

expressed in terms of milligram per gram equivalents of gallic acid. Among the Six extracts, Hydro alcoholic extract (91.22 ± 0.22) and ethyl acetate extract (15.87 ± 0.25) has shown the higher and lower amount of phenolic compounds for *Achyranthes aspera* Linn.

Estimation of Total Flavonoid Content:

Flavonoids scavenge the excess free radicals that cause cellular stress. Flavonoids are polyphenolic substances obtained from a large number of plants. They exhibit various biological activities such as antioxidants, antimicrobials, anti-inflammatory, antiallergic and antiviral. The total flavonoid content profile of the plant extracts was established through the colorimetric method using AlCl3. Among the Six extracts, Hydro alcoholic extract (84.28 ± 0.22) and ethyl acetate extract (18.87 ± 0.25) has shown the higher and lower amount of phenolic compounds for *Achyranthes aspera* Linn.

Estimation of total alkaloid content:

The determination of total alkaloids using a visible spectrophotometric method with Bromocresol Green (BCG) is a simple and sensitive technique that requires no special equipment. BCG can react with certain alkaloids, i.e., the ones that have nitrogen inside their structure, but not with amine and amide alkaloids. The reaction of alkaloids with BCG forms a yellow-coloured product.Among all extracts, Hydro alcoholic extract (81.22 ± 0.22) ethanolic extract (70.12 ± 0.32), Chloroform (57.27 ± 0.3) and ethyl acetate extract (11.47 ± 0.35) were shown the presence of alkaloids. Hydro alcoholic extract has shown the higher and ethyl acetate extract has shown lower amount of alkaloidal compounds for *Achyranthes aspera* Linn

In-VITRO ANTI-OXIDANT POTENTIAL:

DPPH Assay:

DPPH is a relatively stable free radical and the assay determines the ability of Hydro alcoholic extract of Achyranthes aspera Linn reduced DPPH free radicals to the corresponding hydrazine by converting the unpaired electrons to paired ones. Antioxidant can act by converting the unpaired electron to paired one. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance at 517nm and also for visible deep purple colour and the IC50 value was calculated as 187.09 µg/mL using GraphPad Prism 8.3.1 in Figure.1

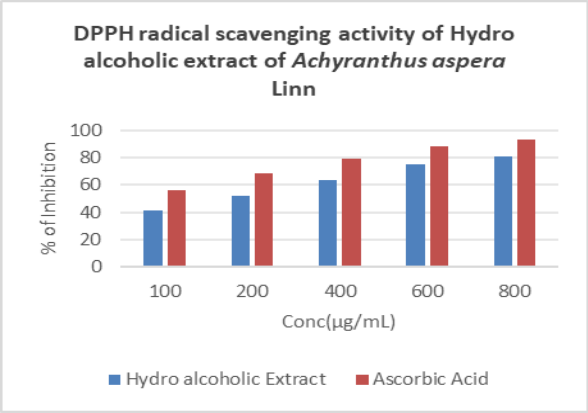

Figure 1: DPPH free radical scavenging activity of Hydro alcoholic extract of *Achyranthus aspera* Linn

Fe Reducing power assay:

Reducing power experiment is a good reflector of antioxidant activity of the plant. The plant having high reducing power generally reported to carry high antioxidant potential too. In this experiment, Ferric ions are reduced to ferrous ions, identified by colour change from yellow to bluish green. The results for ferric reducing power activity of hydro alcoholic extract of Achyranthes aspera Linn in comparison with ascorbic acid are reported in Figure 2. Reducing power potential of extracts increase with the dose, however the extract exhibited low reducing power than that of ascorbic acid.

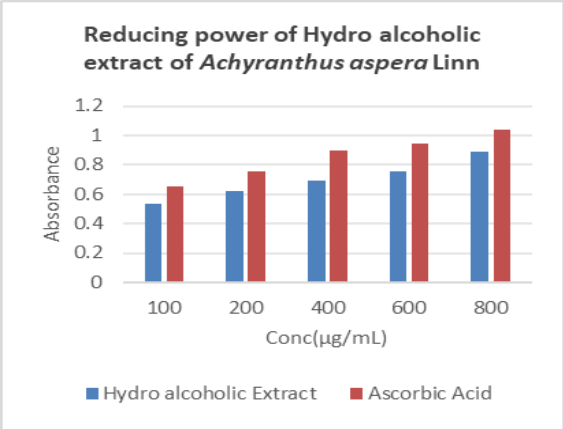

Figure 2: Reducing power of Hydro alcoholic extract of *Achyranthus aspera* Linn

Total Antioxidant Activity:

The Hydro alcoholic extract of *Achyranthes aspera* Linn at various concentrations were assayed for their

antioxidant potency by the formation of green phosphomolybdenum complex. The total anti-oxidant capacity was measured by taking ascorbic acid as standard. IC50 was calculated by using GraphPad Prism 8.3.1 and was 160.98µg/ml

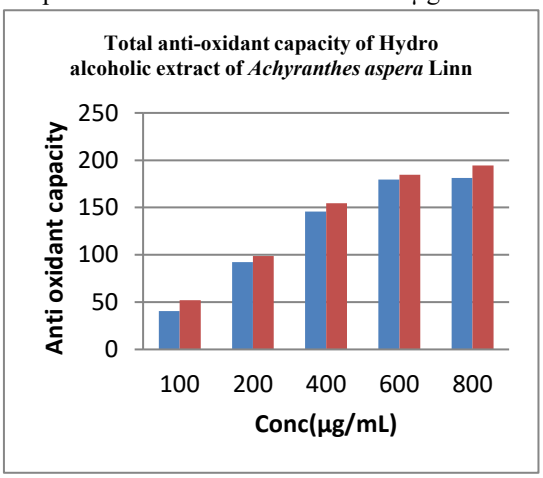

Figure 3: Total Antioxidant Activity of Hydro alcoholic extract of *Achyranthus aspera* Linn

In-Vivo Anti-inflammatory activity of hydro alcoholic extract of Achyranthus aspera linn:

The Anti-inflammatory effects of orally administered Hydro alcoholic extract of Achyranthus aspera Linn whole plant was evaluated by carrageenan-induced paw edema in rats. Paw volume (Table 3, figure 4,) and % oedema inhibition (Table 4, figure 10) were calculated for assessing the efficacy of the Hydro alcoholic extract of Achyranthus aspera Linn. The three doses of plant extract (Group III, IV and V), started suppression of oedema significantly ($p < 0.05$) after 3 h of carrageenan injection as compared to control showing significant inhibition of oedema, respectively. Lower doses of plant extract (Group III and IV) suppresses edema formation when compared to control but unable to reach significant level as high dose of plant extract (Group V). The group which received the Indomethacin showed significant ($p<0.01$) inhibition of inflammation starting from 3 h post carrageenan injection. The extracts showed dose dependent inhibition of paw edema in rats

Table:3Anti-inflammatory effects of orally administered Hydro alcoholic extract of Achyranthus aspera Linn (paw volume measurement).

Groups	Time (Hr)				
	0.5	1	3	6	24
Control	0.82±0.33	1.92±0.28	1.73±0.3	1.38±0.21	1.31±0.22
Diclofenac(5mg/kg)	0.46±0.4	0.44±0.3	0.34±0.5	0.26±0.6	0.21±0.2
HAAA 100mg/kg	0.50±0.15	0.45±0.2	0.39±0.15	0.34±0.08	0.30±0.05
HAAA 200mg/kg	0.48±0.05	0.41±0.08	0.36±0.2	0.35±0.15	0.25±0.04
HAAA 400mg/kg	0.49±0.15	0.55±0.18	0.40±0.09	0.33±0.04	0.21±0.05

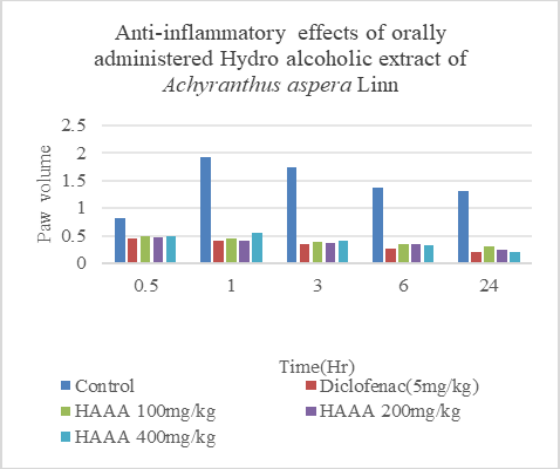

Figure: 4 Anti-inflammatory effects of orally administered Hydro alcoholic extract of Achyranthus aspera Linn (paw volume measurement).

Groups	%Inhibition of inflammation at time (Hr)				
	0.5	1	3	6	24
Control	43.90	77.08	80.35	81.16	83.97
Diclofenac (5mg/kg)	39.02	71.35	77.46	85.36	99.10
HAAA 100mg/kg	40.24	71.35	76.88	76.09	82.81
HAAA 200mg/kg	42.68	51.04	68.61	76.81	91.39
HAAA 400mg/kg	51.46	58.65	79.19	82.64	98.92

Table: 4. Anti-inflammatory effects of orally administered Hydro alcoholic extract of Achyranthus aspera Linn on carrageenan-induced mice paw oedema. (% oedema inhibition)

Motility: Walking ability of the rats to climb the staircase at the time of peak inflammation was checked by motility score. Animals administered with higher dose of each plant extract were showing the highest score when compared with Diclofenac received group. Score for carrageenan rats was found to be 0.14 ± 0.244. This was found to be the lowest when comparing all groups. (Figure 4)

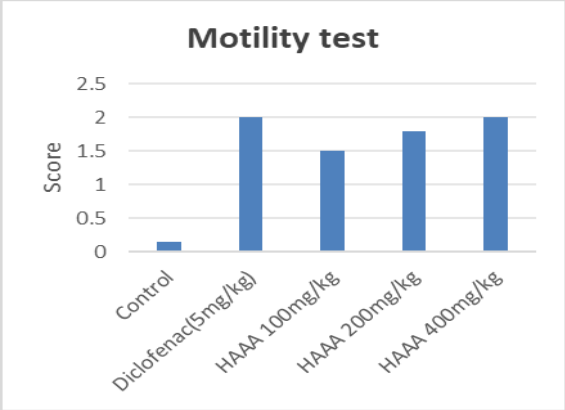

Figure :4 Effects of orally administered Hydro alcoholic extract of *Achyranthus aspera* Linn on Motility of carrageenan induced experimental animals

VIII.SUMMARY & CONCLUSION

To conclude the results, A. aspera was screened for various phytochemicals and proved to cherish with various phytochemicals such as alkaloids, glycosides, saponins, tannins, steroids and carbohydrates. Various fractionation of the plants is studied for detailed phytochemical profiling and reported to possess a good amount of phenolic content, flavonoid content and total alkaloidal content. The antioxidant activity of Hydro alcoholic extract of A. aspera was screened in various in-vitro methods such as DPPH assay, reducing power assay, and total antioxidant assay. The results suggest that the polyphenolic contents present in the whole plant of A. aspera are responsible for the reported antioxidant activity. Further, Hydro alcoholic extract of Achyranthes aspera exhibit promising anti-inflammatory activity, which is attributed to phenols, flavonoids, alkaloids and saponin phytoconstituents found in extracts. This abundantly available plant may serve as an important addition in therapeutic armamentarium of chronic inflammatory diseases such as arthritis.

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