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Achyranthes aspera ethanolic extract study on CNS depressants and behavioral

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Abstract

In present study, CNS depressant activity of EEAA was evaluated by Rota rod test, which has clearly demonstrated the CNS depressant activity evidenced by decreased fall off time. Another important step in evaluating drug action on CNS is to observe its effect on locomotor activity of the animal. The activity is a measure of the level of excitability of the CNS; decreased activity results from depression of the central nervous system. The extracts significantly decreased the locomotor activity as observed in the results of the Actophotometer test. The fresh, healthy, mature plants were collected from farm of Dhule away from pollution. The plant materials were identified using the Flora of Dhule and in India and herbariums were also preserved. The leaves, stem and roots were washed and used for the present study. The macroscopic observations of the mature plants, leaves, inflorescences and other parts were noted down. Plants contain many bioactive phytochemical substances that produce definite physiological and biochemical action on the human body. The phytochemical research approach is considered to be effective in discovering bioactive profile of plants for therapeutic importance. The total ash was 9.59% and percentage yield of water-soluble extract was 20.91%. This experiment shows the evaluation of qualitative phytoconstituents of water extract of whole plant excluding root of *Achyranthes aspera* and *Achyranthes aspera*. The phytochemical analysis of water extract of whole plant excluding root of *Achyranthes aspera* and *Achyranthes aspera* 30 shows that various bioactive phytochemicals such as carbohydrate, protein, alkaloid and phenolic compounds are retained even with dilution in Homoeopathic 30 potency of *Achyranthes aspera*. The foam test of *Achyranthes aspera* 30 is negative whereas of water extract is positive.

Keywords: *Achyranthes aspera*, CNS depressant activity.

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Introduction

The Plants have been used in traditional medicine for several thousand years. The use of traditional medicine in most developing countries is a normative basis for the maintenance of good health. The plant kingdom has been the best source of remedies for curing a variety of disease and pain. This is why medicinal plant has played a key role in the worldwide maintenance of health. Current advancements in drug discovery technology and search for novel chemical diversity have intensified the efforts for exploring leads from Ayurveda the traditional system of medicine in India [1]. World Health Organization has tried to identify all medicinal plants used globally and listed more than 20,000 species [1]. According to the WHO more than 80 % of the world's population relies on traditional herbal medicine for their primary health care [2]. Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active

constituents of immense therapeutic value. In the present era of drug development and

discovery of newer drug molecules many plant products are evaluated on the basis of their traditional uses. The secondary metabolites of the plants are the major sources of pharmaceutical, food additives and fragrances. In the present era of drug development and discovery of newer drug molecules, many plant products are evaluated on the basis of their traditional uses. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different compositions which occur as secondary metabolites [2-3]. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic etc. that are responsible for protecting the plants from microorganisms, insects and other natural pests. In the present era of drug development and discovery of newer drug molecules many plant products are

evaluated on the basis of their traditional uses. One of the many plants which are being evaluated for their therapeutic efficacies is *Achyranthes aspera* Linn. Belongs to the family *Amaranthaceae*. It is an annual, stiff erect herb and found commonly as a weed throughout India and is one of the important medicinal plants having many therapeutic uses. It has been used all the parts in traditional systems of medicines. Seeds, roots and shoots are the most important parts which are used medicinally. The present article gives an account of updated information on its phytochemical and pharmacological properties. The review reveals that wide numbers of phytochemical constituents have been isolated from the plant which possesses activities like antiperiodic, diuretic, purgative, laxative, antiasthmatic, hepato-protective, anti-allergic and various other important medicinal properties [3].

The crushed plant is used in pneumonia and infusion of the root is used as mild astringent in bowel complaints. Decoction of powdered leaves with honey or sugar candy is useful in early stages of diarrhoea and dysentery. For the last few decades or so, extensive research work has been done to prove its biological activities and pharmacology of its extracts. Saponins, oleonic acid, dihydroxy ketones,

INTRODUCTION OF ACHYRANTHES ASPERA

Chaff flower (*Achyranthes aspera*) is an annual herb belonging to *Amaranthaceae* family. It has been used for thousands of years in medicinal practices and to some extent as food constituent also. The genus *Achyranthes* contain 8 to 15 species. The exact number of species of the genus *Aspera* is uncertain because of huge variability among the constituent species. Variability is prevalent in morphology, growth habit, flower color, leaves, stems and chemical composition. Chaff flower can cross pollinate easily and they also reproduce by self-pollination. The name of this plant is different in different regions of the world. It is called as "Chaff Flower" in English. *Achyranthes aspera* is known by different names in different parts of the world. In English it is called "Chaff Flower", "prickly chaff flower" and "devil's horsewhip", it is called "snake's tail" in Kenya, "Adhoghanta", "Adhvashalya", "Aghamargava", "Aghata", "Apamarga", "Apangaka", "Chamatkara", "Dhamargava" in Sanskrit, "Apang", "Chirchra", "Chirehitta", "Latjira" and "Onga" in Hindi, "Kharevazhun" in Persian, "Kutri" in Punjabi, "Jarong" in Indonesia and "Grootklits" in African countries. Probably the most common *Achyranthes aspera* is chaff

flower; however this has a wide range of varieties and cultivars which vary in flavour and uses. There are more than 10 cultivars available. Popular example include *Achyranthus aspera* [4] *aspera*, *Achyranthus aspera borbonica*, *Achyranthus aspera pubescens*, *Achyranthus aspera rubrofusca*, *Achyranthus aspera velutina*, *Achyranthus aspera late ovata*, *Achyranthus aspera nigro olivacea*, *Achyranthus aspera porphyristachya* and *Achyranthus aspera villosior*. Scents and flavour can vary from *borbonica*, *pubescens* and *rubrofusca* to *villosior*. *Achyranthes aspera* can be herbaceous, rarely can it be shrubby and its height ranges from 1m to 2m depending upon the species. Leaves can be thick, ovate, elliptic or obovate. Flower colour ranges from greenish to white. Most common varieties of chaff flower are treated as perennial and some varieties are annual. The essential oil content of chaff flower is equally variable between cultivars and species and is thought to be related to growing conditions, geographic origins, harvesting time and difference in nutritional status of plants. Huge variation was found in characteristics of chaff flower growing in different parts of the world. This variability was found when 10 cultivars of chaff flower were examined in different investigations. The chemical analysis of the cultivars also shows some similarities in morphological characters. One cultivar is *Achyranthes aspera red* with limonene chemo types. The majority of essential oil was concentrated in stem and seeds, while small amounts of essential oil were present in leaves and roots too [5,23].

History/Origin :

Achyranthes aspera is native to South-east Asia where it has been grown from ancient times. The generic name, *Achyranthes* comes from two ancient Greek words "Achyro" meaning "chaff or husk" and "anthas" meaning "flower". The word *aspera* also comes from Greek language and the meaning of *aspera* is "rough", as the stem and leaves of this herb are very rough so it is called "aspera". The suggested origin for the name of this herb is uncertain but some authors stated that herb has been given this name because its symmetry is above the plan. The history of chaff flower is steeped in legend. It is thought to have been brought to Greece by Alexander the Great (356-323 B.C.E). The *aspera* was brought to France and USA in 1700s from South Asia. Culpeper, Gupta and Sharma mention *aspera* in the respective herbals. Sharma praised chaff flower as a remedy for many disorders but

Baraik warned that excessive use of chaff flower is not safe for health. Pradeep Singh claimed that chaff flower can cure snake bite. Garima Mishra reported that chaff flower can be used to cure insect stings [6].

Botany/Morphology/Ecology :

Achyranthes aspera is an erect herb, 0.2m-2.0m high with square stems and branches often with tinged purple colour. Leaves are elliptic, ovate or broadly rhombate and are simple and oppositely arranged on stem. They are 5cm-22cm long, 2cm-5cm broad and adpressed pubescent. The inflorescences are 8-30cm long, with many single, white or red flowers, 3-7mm wide. The petiole is 1.2cm-3cm long. The leaves have numerous oil glands which exude strongly scented volatile oil. The inflorescence is usually long upright; the terminal spikes are usually much longer than the lateral

Class	Mangoliophsida
Kingdom	Plantae
Sub Kingdom	Tracheobinta
Super Division	Spermatophyta
Subclass	Caryophyllidae
Order	Caryophyllales
Family	Amaranthaceae
Genus	<i>Achyranthes</i>
Species	<i>Aspera</i>

ones. The bracts are stalked, shorter than the calyx, ovate and acute. Calyx is 4mm long, enlarging

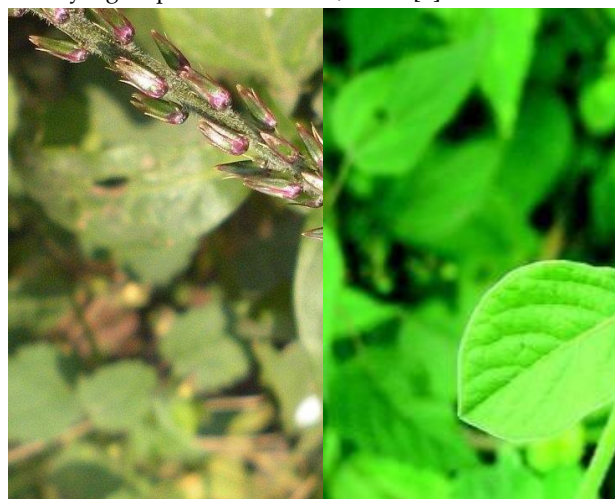
in fruit. The fruit has a short pedicel. The calyx lower lip has two central teeth and is longer than the upper lip. *Achyranthes aspera* does not produce corolla but produces scale like tepals. Nutlets are about 2 mm long,

ellipsoid, black and pitted. Sepals of flower are five and remain fused into a two-lipped calyx. Ovary is superior and fruit consists of four achenes. *Achyranthes aspera* requires mediterranean conditions. Optimum temperature for germination is 20°C, with growing temperatures of 10-30°C. The plant develops best in long day and full sun conditions. *Achyranthes aspera* like moist conditions but it can also withstand drought. *Achyranthes aspera* grows in disturbed lands having good organic matter content. It grows well in soil with pH ranging from 4 to 8.2 and an optimum pH of 6.2. *Achyranthes aspera* has long, deep roots and a high-water requirement [7].

Geographical Distribution:

The plant is widespread in the world as a weed, in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America. In India, it is easily found anywhere on

roadsides or on the edges of field and waste places as a weed throughout up to an altitude of 2100 m and in South Andaman Islands. In the northern part of India it is known as a medicinal plant in different systems of folk medicine. Collection and Authentication of Plant: Fresh, healthy and young seeds (3 to 6 month old) of *A. aspera* were collected from their natural habitat of GUNTUR in Guntur district, Andhrapradesh, India and authenticated by professionals in Department of Botany, St. Mary's group of institutions, India [8].



TAXONOMIC CLASSIFICATION

DEPRESSION

Major depressive disorder, otherwise known as depression, is a disorder that is characterized by a pervasive and persistent low mood that is accompanied by low self-esteem and by a loss of interest or pleasure in normally enjoyable activities [9,24].

What is CNS depression?

The central nervous system (CNS) consists of the brain and the spinal cord. The brain is command central. It orders your lungs to breathe and your heart to beat. It rules virtually every other part of your body and mind, including how you feel about and

interact with the world around you. The spinal cord handles nerve impulses, allowing your brain to communicate with the rest of your body. When CNS functions slow down, it's called CNS depression. Slowing down a little isn't necessarily dangerous. In fact, sometimes it's even helpful. But if it slows down too much, it can quickly become a life-threatening event. Continue reading to learn more about CNS depression and the warning signs of trouble [10].

What are the symptoms of CNS depression?

As slowing of the CNS may make you feel less anxious and more relaxed. That's why CNS depressants (sedatives) are used to treat anxiety and insomnia.

In some cases, you may also exhibit:

1. lack of coordination
2. slowed or slurred speech
3. drowsiness
4. If the CNS slowing worsens, symptoms may include:
5. poor judgment
6. slowed breathing
7. slowed heart rate
8. confusion
9. lethargy

A severely depressed CNS can lead to delirium or coma. With prompt treatment, this is potentially fatal [11,25].

What causes CNS depression?

Certain drugs affect the neurotransmitters in your brain, causing brain activity to slow. That, in turn, makes your breathing slower and shallower. It also makes your heart beat slower. Common causes of CNS depression include the use of medications, drugs, or alcohol. Initially, they may cause a mild stimulant effect or even a feeling of euphoria. But make no mistake about it, these substances are CNS depressants. Some specific depressant drugs include

University. The collected electric grinder and stored in an air-tight container at 25°C. stems and leaves were washed, shade dried and milled into coarse powder using [13,14].

Preparation of extracts

Dried powdered materials were placed in the Soxhlet thimble to obtain sequential extracts of different solvents ranging from nonpolar to polar - petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distilled water by placing them in 250 ml round bottom flask. The materials were refluxed with each solvent for 12- 14 hours at 40-70° C. Extracts were collected and cooled at room temperature and poured in glass Petri dishes & then evaporated at 40°C using hot air oven.



Dried extracts were kept in desiccators for two days and stored at 5°C in air tight containers. Chemicals and Reagents Ferric chloride, folin-ciocalteu's reagent, gelatin, HCl, dragendorff's reagent, methanol, gallic acid, H₂SO₄, Na₂CO₃, vanillin, tannic acid, acetic anhydride, fehling solutions were all purchased from Merck, USA. All other unlabelled chemicals and reagents were of analytical grade and of high purity [15,21].

Qualitative

ACHYRANTHES ASPERAPLANT

MATERIALS AND METHODS

Material

Achyranthes aspera was selected for the current work. The stems and leaves of the plant were collected from the roadsides of University campus, Banasthali University, Banasthali, Banasthali, Tonk District, Rajasthan, India. Botanical identification was performed by the Botanist of Krishi Vigyan Kendra of this

Experimental animals

Adult cyclic virgin female Wistar rats weighing 185-230g were used in various experiments. Swiss albino male and female mice weighing 27-34g were used for acute toxicity testing. All animals were housed in standard cages with uniform conditions of lighting (12h dark: 12h light cycle) and at room temperature. Animals were fed on pellet and tap water ad libitum. Animals were handled in this study as per the International Guidelines for handling experimental animals [16].

PREPARATIONS OF EXTRACTS: HOT WATER EXTRACTION [18,20]

5mg of dried finely powdered plant material mixed with 200 ml of distilled water



Heated on hot plate with continuous stirring at 30c – 40 c for 20 min



Filtered through filter paper
SOLVENT EXTRACTION [17]

20 mg powdered plant material packed into thimble and extraction with 250 ml of solvents



Extraction continues for 24 hours or till the solvent in sphon tube of an extractor become colourised



Kept on hot plate and heated at 30 -40 c

Extraction yield of A.aspera leaves

Solvents	Yield of extract of [mg\50 g] leaves {yield (m± sd)}	Yield %
Aqueous	4240±0.07	8.48
Ethanol	4118±0.03	8.2
Chloroform	800±0.05	1.6
Ethyl acetate	730.±0.78	1.46
Benzene	5840±0.13	11.68
Ether	7210±0.02	14.42
Aqueous	4240±0.07	8.48

Methodology

Procurement of plant:

Achyranthes aspera plant was collected from Guntur , Andhra Pradesh, Achyranthes aspera 30 was procured from . All other used chemicals have been procured from Metropolitan Scientific Company, Delhi.

Processing of plant material :

Parts of *Achyranthes aspera* plant were dried under shade for 15 days. Dried parts of the whole plant excluding root were homogenised to fine powder using mortar and pestle. The fine powder is stored in an amber colour glass bottle to carry out further studies [19].



Maceration technique



washed, shade dried and powdered



The water extract was prepared by taking 10 g of fine powder in 100 ml of water,



stir for 6 h and leave it overnight then filtered it through What's man
Powdered material mixed with 150 ml distilled water for 1 hr



Extract was filtered using muslin cloth & whatsmann filter paper



Concentrated by evaporation on water bath
The extract was dried & used as powder

RESULTS & DISCUSSION:

s.no	Parameter	Range	Mean
1	Stomatal frequency (lower surface)	73-113	93
2	Stomatal frequency (lower	162-190	176

	surface)		
3	Stomatal index (upper surface)	7.3-11.3	9.3
4	Stomatal index (lower surface)	162-19.0	17.65
5	Veinlet number	10.1 -13.1	11.1
6	Vein termination	8.5 -13.1	10.8
7	Palisade ratio	1.13 -1.18	1.16

Quantitative microscopy of the leaves of *Achyranthes aspera*

s.no	Test	Leaves extraction	
		Ethanol	Methanol
1	Protein	++	++
2	Glycosides	-	-
3	Alkaloids	++	++
4	Tannins and phenolic compounds	+	++
5	Steroid	+	+
6	Saponin glycosides	++	+
7	Reducing sugar	+++	+++

Qualitative phytochemical of *Achyranthes aspera*

(+++) appreciable amount; (++) moderate amount; (+) trace amount and (-) completely absent; Enol= Ethanol; MnoI=Methanol; Ext=Extract

PHYTOCHEMICAL STUDIES

Phytochemical Screening

The Phytochemical screening of all six extracts was performed by the standard methods

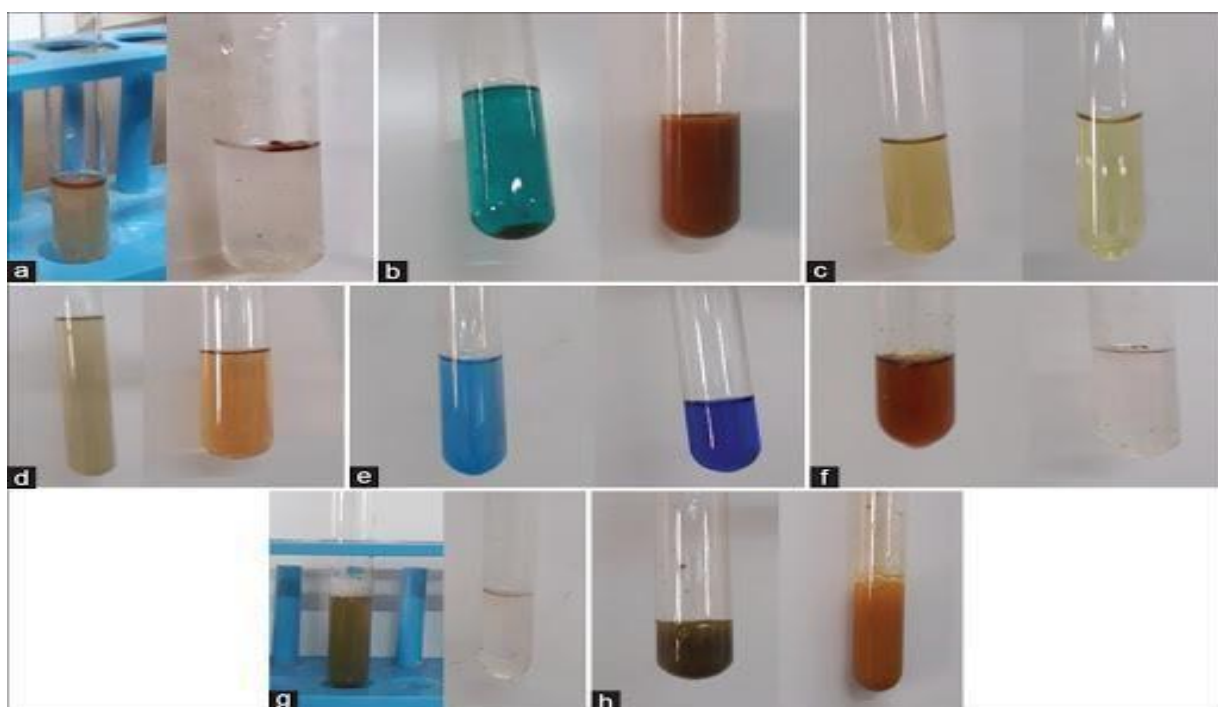
Cardiac glycosides	Keller – killiani test	+++	++	+++	++	-	-
	Salkowski test	+	++	+	-	-	+
	Baljet test	++	-	+	-	+	+++
Steroids	Liebermannbuchard test	++	-	++	-	-	-
Flavonoids	Alkaline reagent test	-	-	-	-	+	++
	Lead acetate test	-	-	+	-	-	+
Terpenoids	Salkowski test	+	++	+	-	-	+
Proteins	Ninhydrin test	-	-	-	-	-	-
Reducing sugars	Fehling’s test	+++	++	++	+	++	+++
Saponins	Froth test	-	+	+	+	+	+

(+++) appreciable amount; (++) moderate amount; (+) trace amount and (-) completely absent. PEAA- Petroleum ether extract of *Achyranthes aspera*; BEAA- Benzene extract of *Achyranthes aspera*; CEAA- Chloroform extract of *Achyranthes aspera*; EAEEA- Ethyl acetate extract of *Achyranthes aspera*; EEAA- Ethanolic extract of *Achyranthes aspera*; AEAA- Aqueous extract of *Achyranthes aspera*.

phytochemical	PEAA	BEAA	CAA	EAEEA	EEAA	AEAA
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Alkaloids	Dragendroff''s test	-	+	++	+++	+++	+++
	Tannin acid t –test	+	+	-	-	-	++
Tannins	Ferric chloride test	-	+	++	-	++	++
	Gelatin test	-	-	-	-	++	++
	Vanillin – hcl test	+	++	++	++	++	+

The traditional healers or practitioners make use of water primarily as a solvent, according to this study showed that ethyl acetate extract of these plant parts were certainly much better. This may be due to the better solubility of the active compound in organic solvents. Preliminary phytochemical screening of the various extracts of *Achyranthes aspera* leaves revealed the presence of cardiac glycosides, reducing sugars, alkaloids, and tannins were the most prominent. Terpenoids, reducing sugars, cardiac glycosides were prominently found in sequential extracts of stems. These compounds may be responsible for antioxidant activity and antimicrobial activity and may serve as a substitute for synthetic drugs. For leaves, flavonoids and terpenoids were found to be present in moderate amounts. Flavonoids were present in ethanolic and aqueous sequential extracts of leaves and terpenoids were found to be present in petroleum ether, chloroform, benzene and aqueous extracts. Steroids were also present in petroleum ether and chloroform extract. The results of phytochemical screening of leaves are tabularized



Phytochemical analysis by colour reactions. (a) Molisch test, (b) Fehling's test, (c) Seliwanoff's test, (d) Xantoprotein test, (e) Biuret test, (f) Wagner's test, (g) foam test, (h) ferric chloride test (first figure under each heading is of water extract and second is of dilution).

s.no	Chemical constituents	Test	EEAA
1	Test for alkaloids	Mayer's test	+
		Wagner's test	+
		Hager's test	-
		Dragendroff's test	+
2	Test for carbohydrates	Molisch's test	+
3	Test for saponins	Foam test	+
		Haemolytic test	-
4	Test for triterpenoids/steroids	Salkowski's test	+
		Liebermann Burchard test	+
5	Test for flavonoids	Shinoda test	-
6	Test for tannins	Ferric chloride test	-
		Gelatin test	-

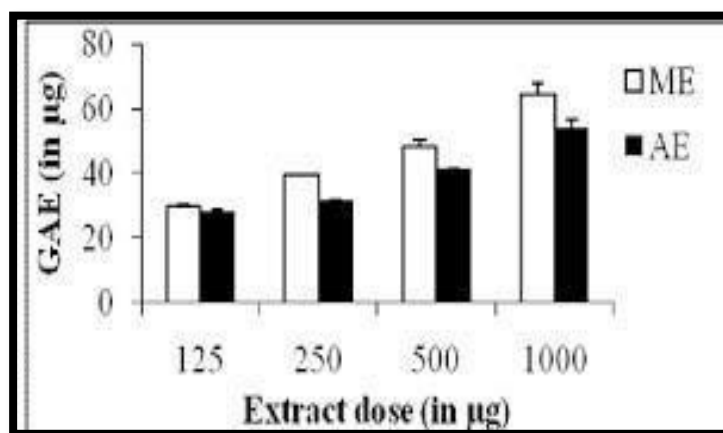
Activity score in Actophotometer method and mean fall off time using Rota-rod method:

Drugs (n=6)	Mean score in 5min (% reduction)			Mean fall off time in secs. (%reduction)	
	Basal	30 mins	60 mins	Basal	30 mins
Group-I (5% gum acacia)	350±12.5	341.7±12.1	344.8±12.8	223.3±18.9	226.7±22.3
Group-II (Diazepam 2 mg/kg)	372±57.7 *	*244.2±36.8	*108.8±19.7 (70.8%)	229.7±22.9	86.3±2.4 (62.4%)
Group-III (A. Aspera extract 400 mg/kg)	86.3±2.4 (62.4%)	*244.8±15	*258.8±9.9 (24.8%)	230±185	*139.3±20.1 (39.4%)

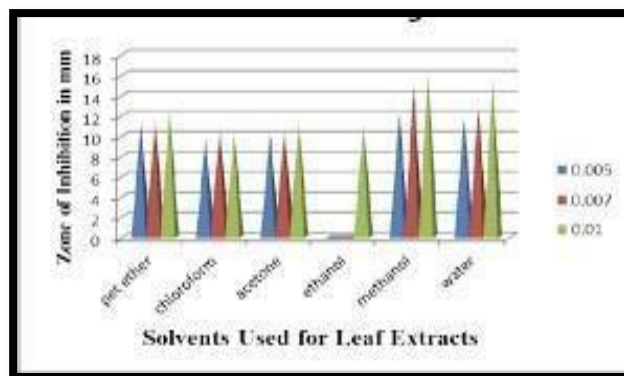
n=6, The percent inhibition for each group was calculated by comparison with the control group. Values indicate Mean±S.E.M. (ANOVA test followed by Dunnett's t-test). Significance variation against control at, * p less than . percent reduction in parenthesis calculated with references to basal score
Mean score using open field performance method:

Drugs (n=6)	Mean scores and SEM				
	Ambulation (numbers) Peripheral squares	Central squares	Rearing (counts)	Preening (counts)	Defecation (counts)
Group-I (5% gum acacia)	25.2±3.2	0.5±0.34	5.7±1.4	1.3±0.80	2.8±0.31
Group-II (Diazepam 2mg/Kg)	101.7±31**	9±4.3*	33.2±8.6*	9±0.82**	1.3±0.49*
Group-III (A. Aspera Extract 400 mg/kg)	97±9.5**	35.2±5.6*	31.7±3.8*	3.7±0.42*	1±0.52**

n=6, The percent inhibition for each group was calculated by comparison with the control group. Values indicate Mean±S.E.M. (ANOVA test followed by Dunnett's t-test). Significance variation against control at, *p<0.001.



Extraction Profile



Solvent Leaf Extract Profile

SUMMARY AND CONCLUSION

Summary Chaff flower (*Achyranthes aspera*) is an annual to perennial herb belonging to Amaranthaceae family. It has been used throughout the world from ancient times due to its medicinal importance. Phytochemical and pharmacological investigations carried out in the plant reveals its multidisciplinary usage. Presence of wide range of phytochemicals reveals its importance in medicine. Mostly

chaff flower contains linalool, arachidic and behenic contents. The extent of these chemical constituents varies depending upon the type of species or cultivars. It is an essential component of pharmaceutical industry. More uses and applications of chaff flower are continuously being added. Further research on extraction method of chaff flower oil and on its preservation method is needed, particularly in the developing world where it's harvesting and post-harvest methods are traditional. The pharmacological investigations performed on chaff flower must be extended to next level of clinical trial. From the results obtained, we can conclude that EEAA possesses considerable CNS depressant and anxiolytic activity which is comparable with the standard. Triterpenoid, saponins may be the phytochemicals responsible for this activity. Central depressant and anxiolytic activity along with strong analgesic effect as reported in our earlier studies may complement each other and thus, may be used in variety of painful and excitatory conditions.

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