Pharmacognostic, preliminary phytochemical and pharmacological investigations on leaves of *Aerva lanata*

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Abstract

The present paper deals with pharmacognostic, preliminary phytochemical and pharmacological investigations of *Aerva lanata*. The leaves of the plant were studied for pharmacognostic evaluation which includes the study of organoleptic, microscopic and physical parameters. The dried leaves were subjected to successive soxhlation using petroleum ether, chloroform, ethyl acetate and methanol, and the extracts thus obtained were studied for preliminary phytochemical screening for detection of presence of various classes of chemical principles viz., carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, tannins and phenolic compounds. The extracts of leaves were evaluated for CNS depressant, analgesic, anti-inflammatory and diuretic activities, in animal models, and antimicrobial activity against bacteria. The results obtained in the present investigation reveal that one or more extracts of the herb have shown significant CNS depressant, analgesic, anti-inflammatory, diuretic and antibacterial activities.

Keywords: *Aerva lanata*, Analgesic, anti-inflammatory, CNS depressant, diuretic, pharmacognosy, phytochemistry

Introduction

Nature has proved a complete store house of remedies to cure all ailments of mankind and the plants are indispensable to man for his life. Herbal medicines are the oldest form of health care known to mankind and they are notable source of phytopharmaceuticals. Plant materials or their extracts has been utilized by all cultures through out history but India has one of the oldest, richest and most diverse cultural living traditions associated with the use of medicinal plants. It is remarkable that many plants that can be identified from the old sources are still known and used in some form today as herbals. Over the centuries, societies around the world have developed their own traditions to make sense of medicinal plants and their uses. *Aerva lanata* (Syn. *Achyranthes lanata* L) is a common weed in cultivated and waste lands belonging to family Amaranthaceae and commonly known as Polpala. The plant is widespread in the dry parts of the tropics and subtropics of the world. The plant was found to be distributed throughout the warmer parts of India, Sri Lanka, Tropical Africa, Java and Philippines. The literature survey revealed that *Aerva lanata* is endowed with various chemical principles such as alkaloids, steroids, terpenoids, flavonoids and their glycosides, and carbohydrates (Yuldashev et al., 2002; Chandra and Sastry, 1990; Wassel and Ammar, 1987; Pervykh et al., 1993; Aboutabl, 1996-97; Zapesochnaya et al., 1992a; Zapesochnaya et al., 1992b; Zapesochnaya et al., 1991; Zidorozhnyi et al., 1986; Aboutabl et al., 1997).

Aerva lanata has been used as a medicine for very long time. According to literature, one of the largest tribes of India, has used since 1890's to treat diarrhea, dysentery and snake bite. An infusion of the leaves is used to treat sore-eyes. A decoction of leaves is used as a wash for babies who become unconscious from malaria. Smoke from the plant is used as an inhalant. Roots are used in the treatment of headache. A decoction of root is prescribed in

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strangury, antidote in cases of poisoning by arsenic. The plant is said to possess diuretic and demulcent properties. A decoction of the plant is considered of great value in lithiasis and diabetes. It is also used for cough and as a vermifuge in children. The herb is steeped in hot water and used to bathe swelling. The plant is also administered in bladder troubles and gonorrhoea. This herb is described as one of the best known remedies for bladder and kidney stones. Ayurvedic practitioners recommend a decoction of the plant to be taken internally for a few days to dissolve the stones and to clear the urinary path. The plant was scientifically proved to be useful in diabetes, urolithiasis, acute renal failure and to treat solid tumors (Selvan et al., 2001; Shirwaikar et al., 2004; Nevin and Vijayammal, 2003; Vetrichelvan and Jagadeesan, 2002).

The present study was aimed at investigating the pharmacognostic parameters of the leaves, and phytochemical and pharmacological screening of different extracts of leaves of *A. lanata*. The leaves were studied with respect to organoleptic, microscopic and physical parameters. The petroleum ether, chloroform, ethyl acetate and methanol extracts were studied for preliminary phytochemical screening for detection of presence of various classes of chemical principles. The extracts were evaluated for CNS depressant, analgesic, anti-inflammatory and diuretic activity in animal models. The extracts were also studied for antibacterial activity. The results obtained in the present investigation reveal that one or more extracts have shown significant CNS depressant, muscle relaxant, analgesic, anti-inflammatory, antibacterial and diuretic activities.

Materials and Methods

Streptomycin injection (Sarabhai Piramal Pharmaceuticals, Vadodara, Gujarat) was procured from local market. Diclofenac sodium and furosemide were obtained from Mangalam Drugs and Pharmaceuticals Ltd, Wapi, Gujarat. Diazepam was procured from Ranbaxy Laboratories Ltd, Gurgaon, HP. Microbial cultures, *Bacillus subtilis* NCIM 2063, *Staphylococcus aureus* NCIM-2079 and *Escherichia coli* NCIM 2065, *Proteus vulgaris* NCIM-2027 were obtained from Department of Microbiology, Vaagdevi College of Pharmacy, Hanamkonda. Nutrient agar and carrageenan were obtained from Himedia labs, Mumbai. Acacia was purchased from Loba Chemie, Mumbai. All the solvents were procured from E. Merck, Mumbai. Albino rats and mice were procured from Mahaveer Enterprises, Hyderabad.

Procurement of plant material

For the present investigation, the leaves of *A. lanata* were collected, after proper identification by taxonomist, Dr. V.S. Raju, Department of Botany, Kakatiya University, Warangal. The collected plant material was thoroughly checked for foreign organic matter and a part of the plant material was dried under shade.

Pharmacognostic evaluation

Organoleptic evaluation

In organoleptic evaluation, various sensory parameters of the plant material were observed with sense organs. Color, odor and taste of the leaves and leaf powder were recorded.

Microscopic evaluation

In this study, stomatal index was determined for fresh leaves and powder analysis was performed for dried leaf powder. The stomatal index, studied by using *camera lucida*, is of diagnostic significance and is used for the authentication of leaf drugs or for the detection of their adulterants. The type of stomata present in the leaves was also recorded. The presence of various diagnostic characters of leaf powder was studied by microscopical analysis with or without staining.

a) Powder analysis of leaf

A little quantity of powder was taken onto a microscopic slide. To this powder, 1-2 drops of 0.1% phloroglucinol solution and a drop of concentrated hydrochloric acid were added and covered with a cover slip. The slide preparation was mounted in glycerol and examined under microscope. Presence of starch grains was studied by addition of 2-3 drops of 0.01M iodine solution and detected by the formation of blue color.

b) Determination of stomatal index

Leaf fragments of about 5 x 5 mm in size were taken in a test tube containing 5 ml of chloral hydrate solution and boiled on water bath for about 15 min until the fragments became transparent. These fragments were transferred onto microscopic slide and mounted in glycerol. The slide was examined with 40 x objective and 6 x eye piece to which a *camera lucida* was attached. On the drawing paper epidermal cells and stomata were drawn which lied within a selected area. Stomatal index was calculated as the percentage of number of stomata present per number of epidermal cells, each stoma being counted as one cell.

Physical evaluation

In physical evaluation, the total ash, alcohol, water and ether soluble extractive values were determined. The determinations were performed in triplicate and the results are expressed as mean ± SD. The percentage w/w values were calculated with reference to the air-dried drug.

a) Determination of total ash

Two gm of accurately weighed powder of leaves was taken separately in a pre-weighed ash-less filter paper and incinerated at 400°C for about 3-4 min or until the vapors completely ceased. The temperature was gradually reduced and the apparatus was cooled. The contents/ash was collected and weighed.

b) Determination of alcohol soluble extractive

Five gm of accurately weighed powder of leaves was taken and macerated with 100 ml of 95% alcohol for 24 hrs. The contents were frequently shaken during 6 hours and allowed to remain for 18 hrs. After 24 hours, the extract was filtered and 25 ml of the filtrate was evaporated. The extract was dried at 105°C to constant weight, cooled in a desiccators and weighed.

c) Determination of water soluble extractive

Water soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that chloroform water was used for maceration.

Preliminary phytochemical screening

The leaf powder was extracted by successive soxhlation using petroleum ether (60-80°C), chloroform, ethyl acetate and methanol, and the resultant extracts were evaporated to dryness at room temperature. The extracts thus obtained were weighed; percentage yields were calculated and were used for preliminary phytochemical screening by performing various chemical tests to detect the presence of carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, tannins and phenolics.

Pharmacological evaluation of *A. lanata*

The petroleum ether, ethyl acetate and methanolic extracts of leaves of *A. lanata* were studied for acute toxicity test followed by CNS depressant, anti-inflammatory, analgesic and diuretic activities in animal models. The extracts were also studied for antibacterial activity. Institutional Animal Ethical Committee (IAEC) approved the animal study protocols. The animals were kept in the animal house for one week prior to experimentation in order to acclimatize them to the laboratory conditions.

Acute toxicity test

Albino mice weighing between 20 - 25 gm were selected and kept overnight fasting for 18 hrs prior to the test. For each extract, the animals were divided into 4 groups with each group containing 6 animals. The test extracts were given orally in the form of a 2-5% suspension in acacia. The four groups of animals received the doses of extracts at 200, 400, 800 and 1000 mg/kg. The mice were then continuously and carefully observed for 2 hrs followed by occasionally for further 4 hrs. The behavior and mortality of mice was observed up to 24 hrs.

CNS depressant activity

The locomotor/CNS depressant activity of the extracts was measured using actophotometer according to the method reported by Dews (1953) with slight modification. Albino mice of either sex weighing 20 - 28 gm. were divided into 8 groups with each group containing 6 animals. The animals were fasted overnight and orally fed with control,

standard and test extracts in the form of a 2% suspension in acacia. One group received 2% acacia suspension which served as control. The six groups received 200 and 400 mg/kg body weight of the extracts (petroleum ether, ethyl acetate and methanolic extracts). The 8th group received 4 mg/kg body weight of diazepam as a standard. After oral feeding, the animals were placed individually in actophotometer for 5 min and the number of interruptions by animals was noted. The locomotor activity was observed for 5 min after 0.5, 1, 2, 4 and 6 hrs.

Anti-inflammatory activity

Anti-inflammatory activity of the extracts was assessed by rat paw edema method by using the method described by Winter et al. (1962). Young adult male Wistar rats weighing 180-220 gm were fasted for 12 hrs prior to the experiment, while allowing access to water at all times. The rats were divided into 8 groups with each group containing 6 animals. The control group received 2% acacia suspension whereas the standard group was administered with 150 mg/kg body weight of diclofenac sodium. The six groups of animals received 200 and 400 mg/kg of petroleum ether, ethyl acetate and methanolic extracts of leaves.

After 1 hr of administration of control, test and standard samples, 0.05 ml of 1% carrageenan suspension (in normal saline) was injected into the dorsal region of subplantar surface of hind paw of rat, subcutaneously with the help of 26 G needle. The paw volumes were measured at the end of 0.5, 1, 2, 3 and 4 hrs using plethysmometer. The change in paw volume of rats was obtained by subtracting test and standard paw volumes from the control paw volume at different time intervals.

Analgesic activity

The analgesic activity of the extracts was assessed by tail flick method based on the procedure reported by D'Amour & Smith (1941). Albino mice weighing between 20 - 30 gm were taken and divided into 8 groups with each group consists of 6 animals. Before giving the drugs, basal reaction time to radiant heat was taken by placing the tip of the tail (1-2 cm) in the heat source (water bath at 55°C). The tail withdrawal from the heat source (tail flicking response) was taken as the end point. A cut off period of 15 sec was taken for preventing injury to the tail of animal. Three basal reaction times for each mouse at a gap of 5 min. were taken for the confirmation of the result.

After observing the basal reaction time, the test extracts and standard drug were given orally in the form of 5% suspension in gum acacia. Test extracts were studied at doses of 200 and 400 mg/kg each. 150 mg/kg of diclofenac sodium was used as standard. The reaction time was noted at 15, 30, 60, 120 and 180 minutes after giving the drug/extract.

Diuretic activity

The diuretic activity of the extracts was assessed by the measurement of urine output according to the method described by Kagawa & Kalm (1962). Wistar rats weighing between 200 – 225 gm were divided into 11 groups, with each group containing 6 animals. Control group animals were administered with normal saline. Test group were given with extracts of leaves and the standard group were administered with furosemide (20 mg/kg). All the extracts were studied at the doses of 200, 400 and 600mg/kg body weight. All the doses were administered orally in the form of 2% suspension in gum acacia. After giving the dosages, the animals were placed individually in indigenously fabricated metabolic cages. The urine output was measured using measuring cylinder attached to the cages. After 5 hr of administration of drugs, the total volume of urine collected was measured. The effect of different doses of extracts on the output of urine in test animals was compared with the control and the standard.

Antibacterial activity

The antibacterial activity of a drug is generally expressed as its inhibitory effects towards to growth of bacteria in nutrient agar media. Antibacterial activity of different extracts of *A.lanata* leaves were tested against Gram-positive bacteria (*Bacillus subtilis* NCIM 2063 *Staphylococcus aureus* NCIM-2079) and Gram-negative bacteria

(*Escherichia coli* NCIM 2065, *Proteus vulgaris* NCIM-2027) by cup plate method. Streptomycin was used as standard drug (100 µg/ml). The extracts of leaves were dissolved in dimethyl formamide to get final concentrations of 1000, 1500 and 2000µg/ml.

The cup plate assay of drug potency is based on the measurement of diameter of zones of inhibition of microbial growth surrounding cylinders (cups) containing various dilutions of test compounds. The petriplates containing sterile solidified media were taken and the test samples were placed in 6mm diameter wells. Then the plates were incubated at 37°c for 24 hours and observed for zone of inhibition. Zones of inhibition was measured for all extracts and recorded.

Results and Discussion

Pharmacognostic evaluation

Organoleptic and microscopic evaluation

The leaves and leaf powder were studied for organoleptic properties. The leaves were found to be simple, alternate, short-petioled, densely tomentose, rounded and apiculate to acute at the apex. The leaves and leaf powder were found to be green in color and odorless and tasteless.

The leaves and leaf powder were also studied for various microscopic characteristics. The powder analysis of the leaf powder indicates the presence of rod and square shaped calcium oxalate crystals: simple as well as compound starch grains; multicellular, long and lignified trichomes; and xylem and phloem fibers. Both lower and upper epidermises of the leaf have shown the presence of anisocytic stomata with stomatal index 20 and 18 respectively.

Physical evaluation of leaves

The leaf powder was evaluated for physical parameters, total ash, ethanol and water soluble extractive values and the values were found to be 10 ± 0.2, 12 ± 0.2 and 16 ± 0.2 %w/w respectively

Preliminary phytochemical evaluation

The leaf powder of *A. lanata* was extracted with petroleum ether, chloroform, ethyl acetate and methanol by successive soxhlet extraction. Extraction of the leaf powder with petroleum ether has yielded 3.8% of brownish green color solid mass. Chloroform extract is of blackish green solid mass in nature with a yield of 1.9%. The ethyl acetate yielded 4.4% of a blackish green colored resinous extract. Similarly methanol yielded 5.6% of brownish green colored resinous extract. The leaf extracts obtained by various solvents were tested for the presence of different phytochemicals viz., carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, tannins and phenolics. The chemical nature of the extracts was presented in **table 1.**

Pharmacological evaluations

Acute toxicity studies

The results of the acute toxicity studies indicate that none of the extracts studied were either showed any toxic symptoms or caused death of mice even after 24 hrs at all the doses of the extracts of leaf studied. The experimental observations lead to the conclusion that the LD_{50} value of all the extracts could be above 1000 mg/kg, in mice after oral administration.

CNS depressant activity

Perusal of **table 2** clearly indicates that all extracts of *A. lanata* could show CNS depressant activity up to 4 hrs after administration which is analogous to that of the standard. The extent of CNS depressant activity of the extracts could not be compared at all with that of the standard in view of a vast difference in the dosage, i.e., 4 mg/kg body weight of the standard against 200 and 400mg/kg body weight of the extract. Among the different extracts, methanol extract was found to be superior in CNS depressant action then followed by Ethyl acetate at dose level of 400mg/kg body weight.

Analgesic activity

The result presented in **table 3** indicate that the mice in the control group could readily respond to heat stimuli by withdrawing the tail within 3-4 sec. Administration of diclofenac sodium at 150mg/kg body weight showed a significant increase in analgesic activity after 30 min. The mice treated with diclofenac sodium were able to withstand heat up to 6.16, 8 and 9.5 sec respectively after 30, 60, and 120 min of drug administration. Interestingly all the extracts studied could show good analgesic activity after 30 min of their administration, followed by decrease in activity after 120 min. The analgesic activity of methanol extract of the leaves was superior to that of other extracts. Methanol extract at 400mg/kg body weight has shown good analgesic activity that is similar to that of the standard. In the preliminary phytochemical screening, the methanol extract was found to contain carbohydrates, proteins, amino acids, tannins, phenolics and flavonoids, one or more of which are responsible for the analgesic activity.

Anti-inflammatory activity

The anti-inflammatory activity of various extracts was studied in rats and the results obtained are presented in **table 4**. From the table, it could be observed that the standard drug, diclofenac sodium has protected to an extent of 29.16, 44.7, 50.7, 60.8 and 51.8% against inflammation induced by carrageenan at 0.5, 1, 2, 3 and 4 hrs. Some of the extracts studied have produced promising results for their anti-inflammatory activity. Ethyl acetate extract showed highest activity at dose level of 400 mg/kg at 2 hr. Petroleum ether extract has shown an inhibition up to 2 hr and then the effect was decreased. It showed maximum activity at dose of 400 mg/kg at 2 hr. Methanol extract has shown significant activity at 30 min compare to standard. The results indicate that leaves contain anti-inflammatory principles, the activity of which is comparable to that of diclofenac sodium at the studied concentration.

Diuretic activity

The diuretic activity of various extracts of *A. lanata* was studied in rats and the results obtained are shown in **table 5**. The results presented in the table reveal that the extracts of *A. lanata* possess a significant diuretic activity when compared to the standard. When compared to all the extracts, methanolic extract could show a significant activity in dose dependent manner.

Antibacterial activity

The results obtained in this study are shown in **table 6**. The results reveal that the extracts possess antibacterial activity in a concentration dependent manner against the test organisms. The gram negative bacteria were observed to be more susceptible than gram positive bacteria towards all plant extracts. It is also observed that the methanol extract at a dose of 2000ìg/ml has shown highest zone of inhibition, followed by ethyl acetate and petroleum ether.

Conclusion

The output of the present study was interesting, particularly with respect to pharmacological evaluation. Significant analgesic, anti-inflammatory, diuretic, CNS depressant and antibacterial activities were found with various extracts of *A. lanata*. However, there was a significant difference in their potency when compared to standard drugs employed to assess various activities. In the present investigation, crude extracts of the plants were used and this might be the reason for their low potency of activity. Thus, it is worthwhile to take-up further studies for isolating the active constituents responsible for activity. Bioactivity guided fractionation techniques could be helpful to produce promising principles from *A. lanata*.

Table 1. Preliminary phytochemical examination of *A. lanata* leaf extracts

Specific Test	Petroleum ether	Ethyl acetate	Chloroform	Methanol
I. Tests for Carbohydrates				
1. Molish's Test	-	+	+	+
2. Fehling's Test	-	+	+	+
3. Benedict's Test	-	+	+	+
4. Barfoed's Test	-	-	+	+
II. Tests for Proteins				
1. Biuret Test	-	-	-	+
2. Million's Test	-	-	-	+
III. Tests for Amino Acids				
1. Ninhydrin Test	-	-	-	+
IV. Tests for Steroids				
1. Salkowski Reaction	+	+	+	-
2. Liebermann – Burchard reaction	+	+	+	+
3. Liebermann's reaction	+	+	+	-
V. Tests for Alkaloids				
1. Dragendorff's test	-	-	-	-
2. Mayer's test	-	-	-	-
3. Hager's test	-	-	-	-
4. Wagner's test	-	-	-	-
VI. Tests for Tannins & Phenolics				
1. 5% $FeCl_3$ solution	-	-	-	+
2. Lead acetate solution	-	-	-	+
3. Potassium dichromate solution	-	-	-	+
4. Dilute iodine Solution.	-	-	-	-
VII. Flavonoids				
Shinoda test	-	+	-	+

Table 2: CNS depressant activity of leaf extracts of *Aerva lanata*

Treatment	Dose (mg/kg)	MEAN LOCOMOTOR ACTIVITY				
		30min	1hr	2hr	4hr	6hr
Control	-	272.5±3.5	247.8±8.1	210.3±7.0	177.3±6.7	165.1±7.0
Standard (diazepam)	4	178.2±2.8	138.4±3.6	62.1±3.1	36.2±5.8	105.3±6.8
Petroleum ether	200	135.0±6.7	146.6±8.2	138.8±6.1	109.6±10.4	161.6±7.8
	400	110.8±8.5	136.0±3.0	128.5±8.7	85.0±5.4	140.5±6.9
Ethyl acetate	200	149.8±5.6	131.8±5.5	117.6±5.3	113.1±6.8	142.5±10.0
	400	139.0±4.7	114.5±4.7	99±4.9	75.0±6.0	102.8±8.03
Methanol	200	125.3±6.7	116.8±3.1	85.6±2.4	66.5±2.0	125.5±7.4
	400	114.3±4.2	70.3±3.0	66.0±3.4	43.5±7.6	107.8±7.5

Table 3. Analgesic activity of leaf extracts of *A. lanata*

Treatment	Dose (mg/kg)	MEAN REACTION TIME IN SECONDS				
		15min	30min	60min	120min	180min
Control	-	3.33±0.22	4.33±0.21	4.0±0.25	4.16±0.16	3.5±0.22
Standard	150	3.66±0.21	6.16±0.30	8.0±0.26	9.5±0.22	5.33±0.33
Petroleum ether	200	2.66±0.06	3.0±0.25	4.66±0.33	4.33±0.33	3.16±0.16
	400	3.33±0.07	4.5±0.22	5.66±0.42	7.66±0.43	3.66±0.33
Ethyl acetate	200	3.16±0.30	4.16±0.16	5.5±0.22	5.5±0.42	3.5±0.22
	400	3.83±0.40	4.83±0.40	7.5±0.42	8.5±0.22	4.16±0.16
Methanol	200	3.0 ±0.26	4.83±0.40	6.33±0.2	7.66±0.33	3.32±0.21
	400	4.33±0.42	6.33±0.21	8.0±0.25	9.66±0.34	4.5±0.34

Standard: Diclofenac sodium

Table 4. Anti-inflammatory activity of leaf extracts of *A. lanata*

Treatment	Dose (mg/kg)	MEAN OEDEMA VOLUME(ml)				
		30min	1hr	2hr	3hr	4hr
Control	-	0.24±0.022	0.38 ±0.025	0.63 ±0.055	0.74 ±0.021	0.72±0.033
Standard	150	0.17 ±0.036	0.21±0.033	0.31 ±0.036	0.29 ±0.042	0.35 ±0.036
Petroleum ether	200	0.20 ±0.021	0.30±0.033	0.47±0.056	0.67±0.047	0.65±0.042
	400	0.18±0.020	0.27±0.034	0.40±0.025	0.57±0.033	0.61±0.036
Ethyl acetate	200	0.19±0.016	0.28 ±0.030	0.44 ±0.036	0.58±0.057	0.62±0.066
	400	0.17 ±0.030	0.25 ±0.047	0.39±0.034	0.52±0.047	0.58 ±0.049
Methanol	200	0.21±0.021	0.29±0.034	0.48 ±0.036	0.58 ±0.042	0.60±0.030
	400	0.16±0.036	0.22 ±0.042	0.35 ±0.037	0.43 ±0.040	0.52±0.054

Standard: diclofenac sodium

Percentage protection against oedema formation

Treatment	Dose (mg/kg)	% Protection				
		30min	1hr	2hr	3hr	4hr
Standard	150	29.16	44.7	50.7	60.8	51.8
Petroleum ether	200	16.6	21.0	25.6	16.2	11.1
	400	25.0	28.9	36.5	22.9	15.2
Ethyl acetate	200	20.8	26.3	30.1	21.6	13.8
	400	29.1	34.2	38.0	29.7	19.4
Methanol	200	12.5	23.6	23.8	21.6	16.6
	400	33.3	42.1	44.4	41.8	27.7

Standard: diclofenac sodium

Table 5. Diuretic activity of leaf extracts of *A. lanata*

Treatment	Dose (mg/kg)	Mean volume of urine (ml) after 6hrs SEM
Control	-	0.51±0.20
Standard	20	2.4±0.09
Petroleum ether	200	0.50±0.2
	400	1.0±0.31
	600	0.66±0.22
Ethyl acetate	200	1.15±0.56
	400	1.61±0.50
	600	1.08±0.17
Methanol	200	1. 02±0.21
	400	1.22±0.41
	600	2.26±0.13

Standard: furosemide

Table 6. Zone of inhibition of leaf extracts of *A. lanata*

Extract		Gram +ve bacteria		Gram –ve bacteria		Standard (streptomycin)
		Bacillus substilis	Staphylococcus aureus	E.coli	P.vulgaris	
Petroleum ether	1000µg/ml	05	-	-	-	18
	1500µg/ml	08	04	03	04	22
	2000µg/ml	10	06	10	11	29
Ethyl acetate	1000µg/ml	07	08	12	13	17
	1500µg/ml	12	11	19	16	22
	2000µg/ml	20	18	21	19	25
Methanol	1000µg/ml	06	05	10	12	18
	1500µg/ml	11	09	14	16	20
	2000µg/ml	15	14	22	21	28

References

[1] Aboutabl EA, Phytochemical profile and bioactivity validation of traditional use of *Aerva lanata* and *Aerva javanica*. Phytomedicine, 3(1), 1996-97, 131-132.

[2] Aboutabl EA, Wassel GM, Abdel Wahab SM, Ammar NM, Yassin N, Afifi M, Study of different carbohydrates in *Aerva lanata* Juss Ex Schult and *A. javanica* Burm. Fil. growing in Egypt and evaluation of hypoglycaemic effect of their polysaccharides. Egyptian Journal of Pharmaceutical Sciences, 38(1-3), 1997, 33-42.

[3] Chandra S, Sastry MS, Chemical constituents of *Aerva lanata*. Fitoterapia, 61(2), 1990, 188-189.

[4] Dews PB, The measurement of the influence of drugs on voluntary activity in mice. Brtish Journal of Pharmacology, 8, 1953, 46-48.

[5] D'Amour FE, Smith DL, A method for determining loss of pain sensation. The Journal of Pharmacology and Experimental Therapeutics, 72, 1941, 74-79.

[6] Janssen PAJ, Jageneau AH, Niemegeers CJE, Effects of various drugs on isolation-induced fighting behavior of male mice. The Journal of Pharmacology and Experimental Therapeutics, 129, 1960, 471-475.

[7] Kagawa CM, Kalm MJ, Diuretic properties of steriospecific 3-imidomethyloxazolidines in laboratory animals. Arch Int Pharmacodyn Ther, 137, 1962, 241-249.

[8] Nevin KG, Vijayammal PL, Effect of *Aerva lanata* on solid tumor induced by DLA cells in mice.Fitoterapia, 74(6), 2003, 578-582.

[9] Pervykh LN, Karasartov BS, Zapesochnaya GG, A study of the herb *Aerva lanata*. IV. Flavonoid glycosides, Chemistry of Natural Compounds, 28(5), 1993, 509-510.

[10] Selvan R, Kalaiselvi P, Govindaraj A, Balamurugan V, Sathish kumar AS, Effect of *Aerva lanata* leaf extract and vediuppu chunnam on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria. Pharmacological Research, 43(1),2001, 89-93.

[11] Shirwaikar A, Issac D, Malini S, Effect of *Aerva lanata* on cisplatin and gentamicin models of acute renal failure. Journal of Ethnopharmacology, 90(1), 2004, 81-86.

[12] Vetrichelvan T, Jagadeesan M, Anti-diabetic activity of alcoholic extract of *Aerva lanata*(L) juss.ex Schultes in rats. Journal of Ethnopharmacology, 80(2), 2002, 103-107.

[13] Wassel GM, Ammar NM, Phytochemical study of *Aerva lanata*. Fitoterapia, 58, 1987, 367-368.

[14] Winter CA, Risely EA, Nuss GW, Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med, 111, 1962, 544-547.

[15] Yuldashev AA, Yuldashev MP, Abdullabekova VN, Components of *Aerva lanata*. Chemistry of Natural Compounds, 38(3), 2002, 293-294.

[16] Zapesochnaya G, Kurkin V V, Okhavov, Hiroshnikov A, Canthin-6-one and beta-carboline alkaloids from *Aerva lanata*. Planta Medica, 58(2), 1992a, 192-196.

[17] Zapesochnaya GG , Kurkin VA , Okhanov VV, Perzykh LN, Miroshnilov A I, Structure of the alkaloids of *Aerva lanata*. Chemistry of Natural Compounds, 27(6), 1992b,725-728.

[18] Zapesochnaya GG, Pervykh LN, Kurkin VA, A study of the herb *Aerva lanata* III. Alkaloids.Chemistry of Natural Compounds, 27(3), 1991, 336-340.

[19] Zidorozhnyi AM, Zapesochnaya GG, et al, Study of the aboveground part of *Aerva lanata:* 1. Flavonoid o-acylglycosides. Khimiko Farmatsevticheskii Zhurnal, 20(7),1986,855-858.

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