

# Evaluation of Antioxidant, Antimicrobial, and Antiuro lithiatic Potential of Different Solvent Extracts of *Aerva lanata* Linn Flowers

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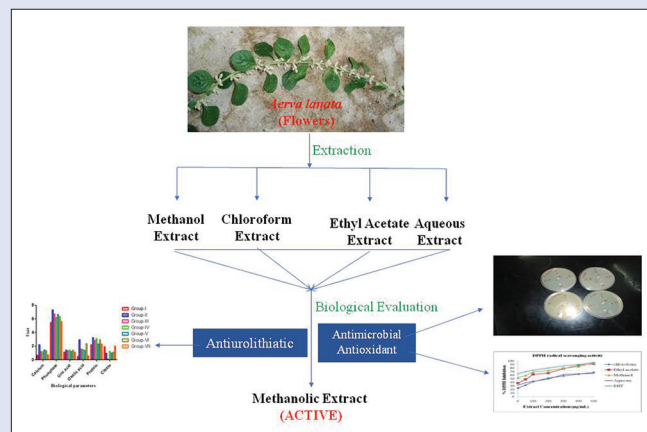
## ABSTRACT

**Introduction:** *Aerva lanata* (Linn) of family Amaranthaceae is an important and commonly used plant for its medicinal and pharmacological properties and proving the traditional uses of flowers of *A. lanata* Linn. **Objective:** All extracts of *A. lanata* were further evaluated for antioxidant, antimicrobial, and antiuro lithiatic potential to scientifically prove the traditional uses. **Materials and Methods:** In the present investigation, different solvent extracts of flowers were obtained using a Soxhlet extractor. Microorganisms were obtained from IMTECH, Chandigarh. Antiuro lithiatic study was carried out in Albino Research and Training Centre, Hyderabad. **Results:** Regardless of the antioxidant studied, the methanolic extract presented the highest antioxidant activity and the aqueous extracts offered the lowest, following the order: methanolic extract > ethyl acetate > chloroform > aqueous. The results of this antimicrobial study indicate that methanolic extract of *A. lanata* could be used as antimicrobial agents. Overall, the methanolic flower extract of *A. lanata* (Linn) was significantly more promising as antiuro lithiatic spectrum. This result also suggested the potential usefulness of the methanolic extract as an antiuro lithiatic agent. **Conclusion:** Henceforward, this research can be acknowledged as a prime new report that focuses on the application of *A. lanata* (Linn) as an antioxidant, antimicrobial, and antiuro lithiatic agent.

**Key words:** *Aerva lanata*, antimicrobial, antioxidant, antiuro lithiatic activity, flower

## SUMMARY

- Overall, methanolic flower extract of *Aerva lanata* Linn showed promising antioxidant activity
- Additionally, methanolic flower extract of *A. lanata* Linn exhibited remarkable antimicrobial and antiuro lithiatic potential.



**Abbreviations used:** IMTECH Chandigarh: Institute of Microbial Technology, Chandigarh; IMMT: Institute of Mineral and Material Technology; CSIR: Council of Scientific & Industrial Research; DPPH: 1,1-diphenyl-2-picrylhydrazyl; MTCC: Microbial Type Culture Collection; BHT: Butylated Hydroxyl Toluene.

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## INTRODUCTION

Due to infectious diseases, the expected mortality rate count is 50-75% in hospitals.<sup>[1]</sup> Nowadays, growing bacterial resistance to conventional drugs is a major public health-care problem for the therapy of infectious diseases. For the treatment of bacterial diseases, antimicrobial drugs are frequently being used by the practitioners against infectious diseases and multiple drug resistance diseases among the human pathogenic microorganisms. It has been increased worldwide, thus limiting therapeutic options. These microbes develop resistance for their survival by several mechanisms to different antimicrobials;<sup>[2]</sup> most of them suffer from severe side effects and toxicity. Subsequently, development of drug resistance, side effects and toxicity associated with these modern system of medicaments; there is a need to move to an alternative therapy that may be more or equally effective besides being at cost-effective price. To reduce this drug resistance and increase its efficacy, there is an urgent need to move to plants or ayurvedic-based therapy as a best alternative therapy.

Plants have been an incredible reservoir of remedy for multitudinous of times. It has been recognized by researchers as an ancient form of medicine to cure common population.<sup>[3]</sup> About four billion population of the world, i.e., 80% of the total world population presently use the traditional system of medicine or herbal drugs for primary health care.<sup>[4]</sup> In India, >65% of the total population use herbal medicinal products for the treatment of diseases.<sup>[5]</sup> Over the eras, many plant species have been investigated

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for possible medical applications. It has been trusted in all cultures and communities throughout the world due to their lesser side effects, higher safety, and efficacy against health illness. Extensive research from the last decades has revealed the applications of *Aerva lanata* (Linn) for the treatment of urinary disorders. It is a natural plant belonging to the family Amaranthaceae and grows in the warmer parts of India ascending to 1000 m. In Sanskrit, *A. lanata* is known as pashanabheda, gorakshaganjaa, satkabhedhi, and aadaanpaak. It is commonly known as sirupeelar in Tamil or Siddha.<sup>[5]</sup> The plant is extensively used in urinary dysfunctions such as Ashmari (urinary calculi), Mootrakrichra (dysuria), and Mootravikara by most of the Ayurveda and Siddha practitioners in South India, in the name of pashanabheda. The plant bears almost all the characteristics similar to that of the source of pashanabheda.<sup>[6]</sup> The primary phytoconstituents reported from this plant are flavonoids, tannins, anthraquinones, alkaloid, phenol, proteins, amino acids, and carbohydrates.<sup>[7]</sup> These phytoconstituents have been reported to show action against urinary disorders. Though there have been no information on antibacterial activity of flowers of *A. lanata*, the aim of the existing research was to investigate the antibacterial activity of ethyl acetate extract of *A. lanata* (flower). The antimicrobial activities were determined by employing disc diffusion assay and minimal inhibitory concentration values.<sup>[7]</sup>

## MATERIALS AND METHODS

### Plant materials

*A. lanata* flowers were obtained from Araku Valley, Vishakhapatnam, India. The plant was identified with the help of local flora and authenticated by Dr. N.K. Dhal, senior principal scientist cum taxonomist, Institute of Mineral and Material Technology (CSIR), Bhubaneswar, Odisha. A voucher specimen (12853/2012) was deposited in Institute of Mineral and Material Technology (CSIR), Bhubaneswar, Odisha, for future reference.

### Processing and extraction of plant materials

Freshly collected flower samples of *A. lanata* were dried in shade, and then coarsely powdered. The powdered flowers (500 g) after defatting with petroleum ether (60°C–80°C) for 48 h were successively extracted with methanol (35 g), ethyl acetate (5 g), chloroform (3 g), and aqueous (12 g) for 48 h in a Soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. The dried crude extracts were subjected to preliminary phytochemical screening for the presence or absence of various phytoconstituents. The crude extract was used for evaluation of antioxidant, antimicrobial, and antiuro lithiatic properties.<sup>[8]</sup>

### Experimental animals

Forty-two adult Wistar albino rats (male) with weights ranging 180–200 g were obtained from the Institutional Animal Ethics Committee of Albino Research and Training Institute, Hyderabad, India. The animals were allowed to acclimatize under standard environmental conditions. The animals were housed in the animal house in groups of five animals each in clean polyacrylic cages and maintained for 12 h/d and light cycles at an average ambient temperature of 25°C ± 2°C and 60% ± 10% relative humidity. The study protocol was approved by the Institutional Animal Ethics Committee, Albino Research and Training Institute vide approval number 1172/po/a/13CPCSEA/IAEC/Exp-42.

### Assessment of extracts of *Aerva lanata* flowers for antioxidant activity

#### 1,1-diphenyl-2-picrylhydrazyl scavenging activity

Free radical scavenging activities of the different extracts were measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method described by G.

Miliauskas *et al.* briefly. 0.1 mM solution of the DPPH in ethanol was prepared. One milliliter of the solution was added to 3 ml of different extracts (aqueous, methanol, EtOAc, and chloroform) in methanol at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min and absorbance was measured at 517 nm using ultraviolet spectrophotometer. The percentage of DPPH scavenging activity is =  $A_0 - A_1/A_0 \times 100$  ( $A_0$  = absorbance of control reaction,  $A_1$  = absorbance in the presence of standard or sample).<sup>[9]</sup>

### Antimicrobial activity

The test organisms included for the study were Gram-negative bacteria such as *Acinetobacter* (MTCC-8530), *Escherichia coli* (MTCC-739), *Raoultella planticola* (MTCC-530), and Gram-positive bacteria such as *Micrococcus luteus* (MTCC-1538) and *Staphylococcus aureus* (MTCC-3160). All the bacterial strains were obtained from Microbial Type Culture Collection and Gene Bank, an International Depository Authority, Chandigarh, India, maintained in nutrient broth at –20°C. Three hundred milliliter of each stock-culture was added to 3 mL of nutrient broth.<sup>[10]</sup>

### Bacterial media

Muller-Hinton Agar Media was mixed with distilled water and then sterilized in autoclave at 15 lb pressure for 15 min. The sterilized media were poured into Petri dishes and allowed for solidification. The solidified plates were bored with 6 mm diameter cork borer. The plates with wells were used for the antibacterial studies.<sup>[11]</sup>

### Antibacterial activity of the plant extracts

Different extracts of *A. lanata* at a concentration of 50 µl from stock solution (1 mg/ml) were tested against the Gram-negative bacteria such as *Acinetobacter* (MTCC-8530), *E. coli* (MTCC-739), and Gram-positive bacteria such as *M. luteus* (MTCC-1538) and *S. aureus* (MTCC-3160) by well diffusion method.<sup>[12]</sup>

### Well diffusion method

Antibacterial activity of the flower extract was tested using well diffusion method. The prepared culture plates were inoculated with different selected strains of bacteria using streak plate method. Wells were made on the agar surface with 6 mm sterilized cork well borer. Each extract was taken 50 µg/mL from stock solutions (1 mg/mL) and poured inside the wells using a sterile syringe. The plates were incubated at 37°C for 24 h for bacterial activity. The plates were observed for the zone clearance around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.<sup>[13]</sup> The readings were taken in three different fixed directions in all three replicates and the average values were tabulated.

### Assessment of antiuro lithiatic activity

#### Experimental design

All the animals were weighed accurately and randomly divided into seven groups containing six animals in each group. Group I served as control and received regular diet and drinking water *ad libitum*. Ethylene glycol (0.75% v/v) in drinking water was fed to Group II to Group VII for induction of renal calculi till the 28<sup>th</sup> day. Group II served as disease control. Group III received aqueous extract. Group IV received chloroform extract, Group V received ethyl acetate extract, and Group VI received methanolic extract from 15<sup>th</sup> day till the 28<sup>th</sup> day. Group VII received the standard antilithiatic drug, cystone (750 mg/kg body weight) from 15<sup>th</sup> day till 28<sup>th</sup> day. The cystone was suspended in distilled water using 5% w/v gum acacia and given after recovery once daily by oral route (5 ml/kg body weight) for 7 days.

### Collection and analysis of urine

All the experimental animals were kept separately in metabolic cages and samples of the urine were collected on the 28<sup>th</sup> day of the 24 h completion of the treatment. A drop of concentrated hydrochloric acid was added to the urine which will act as a preservative. It was being stored at 4°C. Urine samples were analyzed for calcium, phosphate, uric acid, oxalic acid, citrate, and protein contents.<sup>[14]</sup>

### Serum analysis

After completion of 1 h of the last dose of treatment, blood sample was collected. All rats were sacrificed under thiopentone anesthesia (50 mg/kg I.P). Blood samples were collected by cardiac puncture. Serum was separated by centrifugation at 10,000 rpm for 10 min. After completion of 10 min, it was taken for creatinine analysis.<sup>[15]</sup> Calcium, citrate, oxalate, uric acid, phosphorus, and protein levels were assessed using auto-analyzer and diagnostic kits (Beacon Diagnostic Pvt. Ltd., India).

### Statistical analysis

All the results were expressed as mean ± standard error of mean. The statistical significance was calculated using one-way analysis of variance followed by Dunnett's comparison test and *P* < 0.05 was considered statistically significant [Figure 1 and Table 1].

## RESULTS

### Preliminary phytochemical screening

The results of the preliminary phytochemical screening revealed that methanolic extract was enriched with the presence of phenolic compounds such as flavonoids and terpenoids. Phytoconstituents of different extracts were listed in Table 2. The presence of certain combinations of phytochemicals (secondary metabolite) in the extracts of plant materials may be responsible for the maximum therapeutic properties.

### 1,1-diphenyl-2-picrylhydrazyl scavenging activity

In basic process of oxidation, the most important step is the formation of free radicals (having unpaired electrons) in living systems, drugs, and even food. It is reported that antioxidants prevent the oxidative damage which is caused by reactive oxygen species and free radicals. It was assessed in terms of free radical scavenging activity by DPPH assay, and the result was expressed in terms of percentage. Antioxidants act by interfering in the oxidation process reaction with the chelating, catalytic metals and free radicals as oxygen scavengers. Methanolic extract showed 59.0% inhibition in concentration of 50 ppm and 95.0% inhibition in concentration of 500 ppm. Percentage scavenging activity of standard (butylated hydroxyl toluene [BHT]) was found to be 96.0% at 500 ppm. This shows that the free radical scavenging activity of methanolic extract is comparable with BHT, a standard antioxidant.

The *A. lanata* extract in chloroform (68%), ethyl acetate (92%), and aqueous (65%) produced comparable free radical scavenging activities both in low and high concentrations versus standard BHT. The order of free radical scavenging of test samples (0 and 500 ppm) and standard is: BHT > methanolic extract > ethyl acetate > chloroform > aqueous [Figure 2 and Table 3].

Our findings confirm reports that flowers of *. lanata* are extremely abundant with significant phenolic contents such as flavonoids, phenolic acids, and anthocyanins. Due to the presence of abundant amount of phenolic compound, it possesses antioxidant potential, anti-inflammatory and anticancer properties compared with BHT as a standard. Based on the findings of antioxidant potential, the importance of phenolic components may be attributed to the next level of findings, i.e., isolation of antioxidant components from the plants. Results of the antioxidant

potential suggested that the methanol extract of this plant possesses the strongest ability to scavenge DPPH radical as compared to other extracts.

### Antimicrobial activity

The antimicrobial activity of plant extracts and its phytoconstituents was evaluated with antibiotic susceptible and resistant microorganisms. The antimicrobial activities of the extracts of *A. lanata* were studied in concentration of 50 µg/ml against four pathogenic bacterial strains. The antimicrobial activities of different extracts of plants were assessed in terms of zone of inhibition of bacterial growth. The results were summarized in Table 4. The methanolic extract showed good antibacterial activity against the Gram-negative bacteria such as *Acinetobacter* (MTCC-8530), *E. coli* (MTCC-739), *R. planticola* (MTCC-530), and Gram-positive bacteria such as *S. aureus* (MTCC-3160).

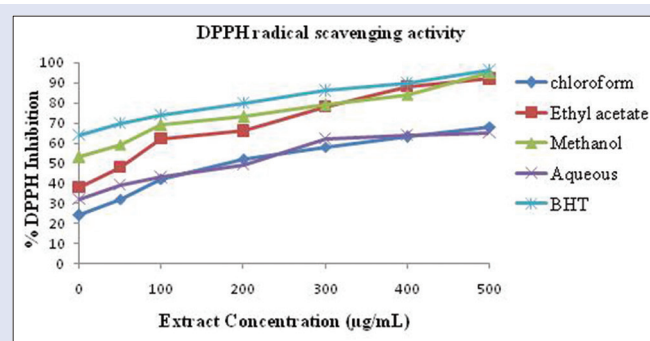


Figure 1: 1,1-diphenyl-2-picrylhydrazyl scavenging activity of different extracts

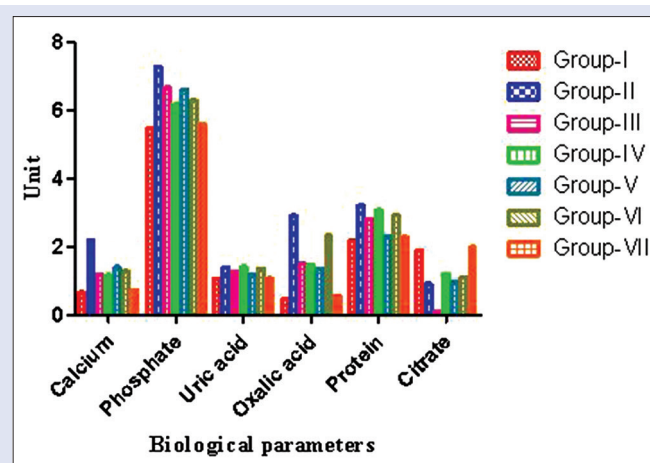


Figure 2: Antiuro lithiatic activity of flower extracts

Table 1: Phytochemical screening of different extracts of *Aerva lanata*

	Chloroform	Ethyl acetate	Methanol	Aqueous
Alkaloids	-	-	+	+
Glycosides	-	-	+	-
Tannins	-	+	+	+
Saponins	-	-	+	+
Flavonoids	-	+	+	+
Phytosterols	+	-	+	-
Terpenoids	-	-	+	+
Phenols	-	-	+	+
Steroids	-	+	-	-

-: Absent; +: Present

The above antimicrobial results showed that the activity of methanolic extract of *A. lanata* has a significant role in bacterial diseases. This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index. The result of phytochemicals in the present investigation showed that the plant contains more or less same components such as saponin, triterpenoids, steroids, glycosides, anthraquinones, flavonoids, proteins, and amino acids. Results show that plants rich in phenolic compounds have been shown to possess antimicrobial activities against a number of microorganisms [Table 4].

### Antiuro lithiatic activity

Table 1 depicts the urinary biochemical data that were obtained at the end of the experiment in each group of animals. The calcium, phosphate, uric acid, oxalic acid, citrate, and protein levels were significantly elevated in kidney homogenate of the calculi-induced animal group compared to the animals of standard treatment given group. Methanolic extract reduced the calcium, phosphate, uric acid, oxalic acid, protein, and citrate levels significantly on comparison with disease control group (Group II) and prevented the risk of stone formation. The methanolic extract and cystone treatment significantly ( $P < 0.05$ ) diminished the levels of all parameters mentioned. Low dose of extract failed to exhibit significant reduction in calcium, phosphate, uric acid, oxalic acid, protein, and citrate in kidney homogenate.

### DISCUSSION

Methanolic extract shows good antimicrobial activity against both Gram-positive as well as Gram-negative bacteria [Table 4]. The antioxidant activity of the extract was found to be in the order of methanolic > ethyl

acetate > chloroform > aqueous [Table 3]. Calcium and oxalate excretion significantly increased in ethylene glycol-induced urolithiatic rats when compared with normal control rats. Serum phosphorus, uric acid, and protein levels were significantly elevated in urolithiatic-treated rats as compared with control group rats. Different flower extracts of *A. lanata* dissolved the calcium oxalate stone and restored the renal structure in ethylene glycol-induced urolithiatic rats. Uric acid is known to promote calcium oxalate crystal growth. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggest its primary role in stone formation. In the present study, higher concentration of uric acid was observed in ethylene glycol-induced urolithiatic rats [Table 1 and Figure 1].

### CONCLUSION

There are very few literatures available about this plant and its applications. Only very few studies are documented in literature about chemical compositions and its biological potentials. The present study provides scientific proof for the traditional claim of *A. lanata* as an antioxidant, antimicrobial, and antiuro lithiatic plant. Sound to excellent antioxidant and antimicrobial activities of the methanolic extracts of the plant were obtained. Apart from these activities, methanolic extracts of the plant showed magnificent antiuro lithiatic activities. Future works on the plant would hopefully lead to the development of serviceable medical attention for its medicinal applications.

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**Table 2:** The percentage inhibition of 1,1-diphenyl-2-picrylhydrazyl by extracts of *Aerva lanata*

Extract concentration	Percentage DPPH inhibition				
	Chloroform	Ethyl acetate	Methanol	Aqueous	BHT
0	24	38	53	32	64
50	32	48	59	39	70
100	42	62	69	43	74
200	52	66	73	49	80
300	58	78	79	62	86
400	63	88	84	64	90
500	68	92	95	65	96

DPPH: 1,1-diphenyl-2-picrylhydrazyl; BHT: Butylated hydroxytoluene

**Table 3:** Antimicrobial activity of different extracts of *Aerva lanata*

Plant extract (1 mg/mL)	Pathogen (zone size in mm)			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Acinetobacter</i>	<i>Proteus mirabilis</i>
Chloroform	8	-	-	-
Ethyl acetate	8	-	8	8
Methanol	7	9	8	8
Aqueous	7	-	-	-

**Table 4:** Antiuro lithiatic activity of flower extracts

Treatment	Calcium	Phosphate	Uric acid	Oxalic acid	Protein	Citrate
Group I	0.6650±0.008	5.478±0.013	1.080±0.005	0.466±0.012	2.175±0.013	1.897±0.012
Group II	2.183±0.019	7.282±0.018	1.395±0.013	2.917±0.022	3.205±0.030	0.915±0.020
Group III	1.185±0.014	6.673±0.022	1.270±0.013	1.523±0.018	2.808±0.018	0.097±0.004
Group IV	1.155±0.019	6.177±0.019	1.402±0.021	1.473±0.016	3.080±0.012	1.210±0.001
Group V	1.393±0.034	6.605±0.018	1.205±0.007	1.347±0.015	2.303±0.011	0.971±0.006
Group VI	1.283±0.023	6.300±0.021	1.363±0.011	2.333±0.032	2.928±0.021	1.107±0.008
Group VII	0.725±0.014	5.608±0.022	1.080±0.010	0.576±0.008	2.292±0.014	1.998±0.015

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### Conflicts of interest

There are no conflicts of interest.

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