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Phytochemical screening and antimicrobial activity of shade dried and sun-dried *Aerva lanata* (L.) juss. ex schult.

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Aerva lanata (L.) Juss. ex Schult. is an important medicinal plant widely used in the treatment of several human ailments in the southern part of India including as a source of *Pashana Bheda* (Stone Breaker). The plant materials were extracted in different solvent extracts and analyzed to know the composition of minerals, secondary metabolites and the efficacy of solvent extracts against pathogenic bacteria and *Candida* species. Ethyl acetate extract of sun-dried plant inhibited the growth of *S. typhie* and *K. pneumoniae.* The anticandidal activity displayed in ethyl acetate and methanol extracts was very effective against *C. glabrata, C. albicans* and *C. haemulonii* respectively. A maximum of 11.834 ppm calcium content was recorded, followed by potassium (6.87 ppm) and magnesium (6.5 ppm). Similarly, amongst the secondary metabolites, saponins (1.987 mg/g) content was maximum, followed by flavonoids (1.85 mg/g) and sterol (0.85 mg/g) in sundried plant material. In shade dried plant material, phenol content was more (1.14 mg/g). No significant difference was observed in the occurrence of secondary metabolites, minerals, and antimicrobial activity between sundried and shade dried plant materials.

Keywords: *Aerva lanata*, Antimicrobial activity, Elemental analysis, Phytochemical constituents.

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Introduction

Herbal products from traditional medicine are gaining importance all over the world due to no side effects, hence, it is necessary to investigate their medicinal property for scientific validation and promoting their proper use in the preparation of new drugs[1]. The knowledge obtained from the chemical constituents of plants is not only desirable for the discovery of therapeutic agents but also considered to be valuable in disclosing the new sources of economic materials such as tannins, oils, gums, precursors for the synthesis of complex chemical substances. Alkaloids, flavonoids, tannins, and phenolic compounds are important plant constituents that are mainly responsible for medicinal properties and valuable by products[2].

Aerva lanata (L.) Juss.ex Schult (*Amaranthaceae)* is native to tropical Africa and Phillippines and grows at the altitude of 3000 m[3]. It is one of the important medicinal plants localized throughout the plains of India[4], Sri Lanka, Arabia, Egypt, and Java[5]. In India, it is present especially in the states of Tamil Nadu, Andhra Pradesh and Karnataka[6]. In the traditional system of medicine, the plant is used as a diuretic and anthelmintic, antidiabetic, antihyperglycemic, antimicrobial, expectorant agent, and in the treatment of Lithiasis and inflammatory symptoms[7]. The plant *A. lanata* has been documented for its pharmacologic properties such as anti-asthmatic[8], urolithiasis[9], and antioxidant[10]. The flowers are used to treat gonorrhoea as well as stones in the kidney and stomach[7]. The herb has the remarkable properties of curing skin diseases like bedsores wounds, rashes, and rheumatism[11], the decoction of the whole plant is also given to cure Pneumonia, Typhoid, and other prolonged fevers[12]. The leaves of *A. lanata* is used as a sap for eye complaints and an infusion is given to cure diarrhea[13]. The decoction of leaves is used for sore throat and in various complex treatments against Guinea-worm[14]. The roots have various medicinal uses. In Rajasthan, roots are used for liver congestion, jaundice, biliousness and dyspepsia[15]; in Madhya Pradesh, for skin diseases, snake bites and headache[16]. In view of these valuable traditional medicinal applications, the present study was undertaken to analyze the secondary metabolite and mineral composition of *A. lanata* and determine their

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antimicrobial property against pathogenic bacteria and *Candida sp*.

Materials and Methods

Collection and identification of the plant

The whole plant of *A. lanata* was collected during the period from June to October 2018 from different localities of Kalaburagi, Karnataka. The plant was identified with the help of local flora and authenticated by 'Flora of Gulbarga District' (Ref. No HGUG7). The specimen has been deposited in "Herbarium center", Department of Botany, Gulbarga University (Voucher specimen number HGUG 5233). The collected plant was washed thoroughly, cut into small pieces and then dried under shade for about two weeks in a tray at a controlled temperature at 35 °C and also under direct sunlight. The dried plant material was coarsely powdered by a mechanical grinder and stored in a closed vessel for further use.

Extraction of crude drugs

Exactly 500 mg powdered plant material was extracted with 100 mL of solvents like ethyl acetate, petroleum ether, chloroform, methanol and water and filtered through Whatman no.1 filter paper. These extracts were used further for qualitative analysis of phytochemicals.

Preliminary screening of secondary metabolites

The preliminary phytochemical tests have been performed by adopting standard methods described by Harborne[17].

Quantitative estimation of secondary metabolites

The quantitative estimation of secondary metabolite for alkaloids, flavonoids[18], glycosides[19], phenols and polyphenol[20], saponins[21] Triterpenoids[22], Sterols[23], and tannins[24], were carried out by using standard methods.

Elemental analysis

The minerals were determined by the dry ash extraction method using atomic spectrometry. one gram of ash was weighed and in a conical flask, it was mixed with 25 mL of double distilled water and 25 mL of concentrated H_2SO_4. The total volume was adjusted to 50 mL and stirred gently. Then the solution was filtered in Whatman filter paper. Then 950 mL of double-distilled water was added to make the final volume of 1000 mL. With an accurate concentration of ash, the solution was read at a different wavelength with respect to its metal and it was then analysed by Atomic Absorption Spectrophotometer and the concentrations were calculated in mg/L[25].

Antimicrobial activity

Exactly 500 mg powdered plant material was extracted with 100 mL of solvents like ethyl acetate, petroleum ether, chloroform, methanol and water and filtered through Whatman no.1 filter paper. These extracts were further used for antimicrobial activity.

Antimicrobial activity of different solvent extracts against pathogenic microorganisms namely *Staphylococcus aureus (*MTCC96), *Escherichia coli (*MTCC45), *Klebsiella pneumoniae*, *Candida haemulonii* (MTCC1966), *Candida glabrata* (MTCC3814), *Candida albicans* (MTCC1637), *Candida tropicalis* (MTCC2795), *Candida tropicalis* (MTCC230), *Candida haemulonii* (MTCC8303) was done using the well diffusion method (The microbes are procured by Microbial Type Culture Collection (MTCC) and Gene Bank, Chandigarh-India). For antibacterial activity, nutrient agar plates were used whereas for anticandidal activity YPDA (Yeast extract-1g, peptone-2g, dextrose-2g, agar-1.5/100 mL of distilled water) medium was poured into sterilized Petri plates and freshly grown 24 h old bacterial and *Candida* cultures were seeded on respective medium. Wells of 5 mm diameter were made using cork borer. 20 µL (40 mg/mL) of the crude solvent extract was added into each well in all the plates by using sterile micropipette. After incubation at 35 °C for 24 hours, the zone of inhibition was measured[26].

Results and Discussion

The result of preliminary phytochemical screening (Table 1, 2) of *A. lanata* extracts reveals the presence of saponin, flavonoid, sterol, terpenoid, and phenol in both shade dried and sundried plant materials. The chloroform extract of *Aerva* plant has high polarity and is an effective solvent to isolate biologically active agents. Saponins are noted in chloroform, ethyl acetate, and aqueous plant extracts, while flavonoids were detected in chloroform and aqueous extracts. Correspondingly, all solvent extracts tested positive for phenolic and sterol compounds.

Quantitative estimation of phytochemicals exhibits saponin, flavonoid, and phenol as major plant constituents whereas, sterol and terpenoid are recorded in moderate quantity (Table 3). Shade dried plant material serves as a good source for the extraction of secondary metabolites such as saponins and phenols. In contrast, sundried plants recorded to contain a high amount of flavonoids and sterol. Among all phytochemicals, Saponins is recorded as a major constituent with a concentration of 2.289 and

Table 1 — Qualitative screening of secondary metabolites of *Aerva lanata* (L.) Juss. ex Schult. extract dried under sunlight

Phytochemical test	Test	Petroleum ether extract	Chloroform	Ethylacetate	Methanol	Aqueous
Alkaloids	Mayer's	+	+	-	-	+
	Dragendroff's	-	-	-	-	-
	Wagners	-	-	-	-	-
Flavonoids	Pew	-	-	-	-	-
	Shinoda	-	-	-	-	-
	NaOH	+	+	-	-	+
	Lead acetate	-	+	-	-	+
	$FeCl_3$	+	+	+	-	-
Glycosides	Kellar-Killiani	-	-	+	+	+
	H_2SO_4	+	-	-	-	-
	Legal test	+	-	-	+	+
Phenols	Ellagic acid	+	+	+	+	+
	Phenol	+	+	+	+	-
	Hot water test	+	+	+	+	+
Saponins	Foam	-	+	+	-	+
Terpenoids	Libermann-Burchard test	+	+	+	+	+
	Salkowski's Test	-	+	-	-	+
Sterols	Libermann-Burchard test	+	+	+	+	-
	Salkowski's test	+	-	-	-	-
	Sulphur test	+	+	+	+	-
Tannins	$FeCl_3$	-	+	-	+	-

Note: The table showing the results of present (+) and absent (-) of secondary metabolites.

Table 2 — Qualitative screening of secondary metabolites of *Aerva lanata* (L.) Juss. ex Schult. extract dried under shade.

Phytochemical test	Test	Petroleum ether extract	Chloroform extract	Ethylacetate extract	Methanol extract	Aqueous extract
Alkaloids	Mayer's	-	-	-	-	+
	Dragendroff's	-	-	-	-	-
	Wagners	-	-	-	-	-
Flavonoids	Pew	-	-	-	-	-
	NaOH	-	-	-	-	-
	Lead acetate	+	+	-	-	+
	$FeCl_3$	-	+	-	-	+
		-	+	+	+	-
Glycosides	Kellar-Killiani	+	-	+	+	+
	H_2SO_4	-	-	-	-	+
	Legal test	+	-	+	-	+
Phenols	Ellagic acid	+	+	+	+	+
	Phenol	+	+	+	+	-
	Hot water test	+	+	+	+	+
Saponins	Table 0 Foam	-	+	+	-	+
Triterpenoids	Libermann-Burchard test	+	+	+	+	+
	Salkowski's Test	+	-	-	-	+
Sterols	Libermann-Burchard test	+	+	+	+	-
	Salkowski's test	-	-	-	-	+
	Sulphur test	+	+	+	+	-
Tannins	$FeCl_3$	-	+	-	+	-

Note: The table showing the results of present (+) and absent (-) of secondary metabolites.

Table 3 — Estimations of secondary metabolites of sundried and shade dried extract of *Aerva lanata* (L.) Juss. ex Schult.

Phytoconstituents	Sun dried (mg/g)	Shade dried (mg/g)
Alkaloid	0.005	0.006
Flavonoid	1.85	1.26
Glycoside	0.028	0.026
Phenol	0.48	1.14
Saponin	1.987	2.289
Terpenoid	0.53	0.50
Sterol	0.86	0.551
Tannin	0.08	0.10
Polyphenol	0.32	0.30

1.98 mg/g in shade dried and sundried plant materials whereas flavonoids are noted as the second-highest constituent with a concentration of 1.85 and 1.26 mg/g in sundried and shade dried plant respectively. Saponin has been recorded as a bioactive antibacterial agent[27] and utilized in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory, and weight loss etc. It is also known to have anti-fungal properties[28]. Flavonoids exist widely in the plant kingdom and display a positive correlation between increased consumption of flavonoids and reduced risk of cardiovascular and cancer diseases[29]. Flavonoids belong to the polyphenolic group and are typically known for their health-promoting properties such as antiallergic, anti-inflammatory, antimicrobial, antioxidant and anticancer properties[30]. Phenolic and sterol compounds are shown to be present in the concentration of 1.14 and 0.86 mg/g in the shade and the sundried materials, respectively. The phenolic compounds are aromatic secondary metabolites that impart colour, flavour, and are associated with health benefits such as the reduced risk of heart and cardiovascular diseases, antidiabetic, anti-carcinogenic, antimicrobial, anti-allergic, anti-mutagenic and anti-inflammatory, Furthermore, phenolic compound accounts for most of the antioxidant activities in plants[31]. The chloroform extract appeared as a rich source of phytochemicals as compared to the other extracts. Sterols and terpenoids were determined to be present in lesser amounts (0.86 and 0.53 mg/g). The total polyphenol content was observed to be 0.32 mg/g in sundried and 0.30 mg/g in shade dried plant materials. Polyphenol constitutes one of the most numerous and widely distributed groups of natural products in the plant kingdom and serves as a defence agent against physiological and environmental stimulators[32].

Table 4 — Elemental analysis of *Aerva lanata* (L.) Juss. ex Schult.

Elemental analysis	Sun dried (ppm)	Shade dried (ppm)
Calcium (Ca)	11.795	11.834
Pottasium (K)	6.811	6.8706
Magnesium (Mg)	6.2362	6.501
Iron (Fe)	0.5147	0.4822
Copper (Cu)	0.0514	0.0314
Zinc (Zn)	0.0479	0.0469
Chromium (Cr)	0.0006	0.03
Cadmium (Cd)	-0.0092	-0.006

Additionally, it possesses antibacterial, antifungal, anti-inflammatory, and antitumor activities[33].

Elemental analysis revealed the presence of calcium (11.795 & 11.834 ppm), potassium (6.811 & 6.87 ppm), and magnesium (6.236 & 6.5 ppm) in sundried and shade dried samples (Table 4). The higher calcium diet is associated with a lower kidney stone formation[34]. Hence, the present research supports the traditional use of the plant as a *Pashana Bheda* (Stone Breaker).

The antimicrobial activity of different solvent extracts against pathogenic bacteria and different *Candida* species are shown in Table 5 & 6. The antibacterial activities of ethyl acetate extract of sundried plant against *Klebsialla pneumoniae* and *S. typhie* displayed at 40 mg/mL were almost similar to the standard drug, while the chloroform, pet ether, and aqueous extracts showed no activity. Among the tested *Candida* sp, *C. haemulonii*, *C. albicans*, *C. glabrata* and *C. albicans* were found susceptible to ethyl acetate and methanol extract at 40 mg/mL. *Candida glabrata*, *C. haemulonii*, and *C. albicans* are major causative agent for urinary tract infection[35]. Antifungal susceptibility profile of clinical isolates of these *candida* species were found to be resistant to fluconazole[36]. Inhibition of candida sp. by the *A. lanata* extract indicate that plant contain novel antimicrobial compound that would be used as an alternative drug against these drug resistant *Candida* species. The ethyl acetate and methanol in the present research proved to be good solvent for recovering active biomolecules from the plant. However, *Candida tropicalis* and *Candida haemulonnii* are resistant to all other extracts. The antimicrobial activity of different extracts is due to the presence of bioactive compounds such as alkaloids, glycosides, flavonoids, steroids, saponins etc[37]. Flavonoids have been reported to have antibacterial and antifungal properties[38]. Similarly, tannins have antimicrobial properties[39].

Table 5 — Antimicrobial activity of shade-dried *Aerva lanata* (L.) Juss. ex Schult. extract

Organisms	Conc.	Zone of Inhibition (mm)				
		Methanol	Ethyl acetate	Chloroform	Petroleum ether	Aqueous
S. typhie	20	3	1	1	1	1
	40	3	3	1	1	2
	Std	11	3	12	6	7
K. pneumonie	20	3	1	1	1	1
	40	2	2	1	2	1
	Std	5	4	9	1	1
S.aureus	20	3	1	1	1	1
	40	3	2	1	2	1
	Std	6	3	11	1	9
E. coli	20	3	4	5	1	1
	40	4	5	8	1	2
	Std	10	6	15	6	7
C. glabrata (3019)	20	2	3	1	1	1
	40	3	4	2	2	1
	Std	2	3	4	9	6
C. haemulonii (1966)	20	2	3	2	2	1
	40	4	4	2	3	2
	Std	16	18	6	8	6
C. haemulonii (8303)	20	1	3	1	2	1
	40	3	5	1	2	1
	Std	2	4	7	6	5
C. glabrata (3814)	20	8	5	2	6	2
	40	8	6	3	6	1
	Std	8	7	9	9	5
C. tropicalis (2795)	20	2	2	2	2	2
	40	3	3	3	3	2
	Std	1	12	8	9	6
C. albicans (183)	20	7	5	2	2	1
	40	8	5	3	2	1
	Std	7	5	8	8	5
C. tropicalis (230)	20	4	4	2	4	2
	40	5	4	2	4	2
	Std	7	4	7	8	6
C. albicans (1637)	20	2	4	1	2	2
	40	3	5	1	2	2
	std	2	4	3	7	6

Table 6 — Antimicrobial activity of sun-dried *Aerva lanata* (L.) Juss. ex Schult. extract

Organisms	Conc.	Zone of inhibition (mm)				
		Methanol	Ethyl acetate	Chloroform	Petroleum ether	Aqueous
S. typhie	20	4	2	1	1	1
	40	3	3	2	1	1
	Std	5	2	9	5	6
K. pneumonie	20	3	2	2	1	1
	40	3	2	1	2	1
	Std	5	2	11	1	8
S. aureus	20	3	1	1	1	1
	40	0	1	1	1	1
	Std	7	3	12	9	8

(*Contd*.)

Table 6 — Antimicrobial activity of sun-dried *Aerva lanata* (L.) Juss. ex Schult. extract (*Contd*.)

Organisms	Conc.	Zone of inhibition (mm)				
		Methanol	Ethyl acetate	Chloroform	Petroleum ether	Aqueous
E. coli	20	1	3	1	1	1
	40	2	2	1	1	1
	Std	4	4	1	7	7
C. glabrata (3019)	20	2	5	02	2	1
	40	3	5	3	2	1
	Std	4	4	3	7	5
C. haemulonii (1966)	20	3	4	1	2	1
	40	3	4	1	3	1
	Std	14	19	5	9	6
C. haemulonii (8303)	20	2	5	1	2	1
	40	4	5	2	2	2
	Std	2	6	5	7	6
C. glabrata (3814)	20	2	6	3	0	1
	40	4	8	3	5	1
	Std	5	8	1	8	6
C. tropicalis (2795)	20	2	2	2	3	3
	40	3	2	2	3	3
	Std	5	1	8	7	5
C. albicans (183)	20	5	3	2	2	1
	40	5	2	3	2	1
	Std	6	3	9	7	6
C. tropicalis (230)	20	3	3	2	4	2
	40	4	4	2	4	2
	Std	5	3	8	7	4
C. albicans (1637)	20	2	3	1	2	2
	40	1	4	2	3	2
	std	3	3	5	9	5

Conclusion

A. lanata is one of the most important medicinal plants used in Ayurveda, Siddha, and Unani for treating different human ailments. The detection of phytochemicals such as flavonoids, saponins, triterpenoids, sterols, tannins in plant extract truly indicates their pharmacological efficacy. Rich calcium levels in the plant serve as dietary content to support its established use against kidney calculi. A negligible difference was recorded in the contents of phytochemicals, minerals, and antimicrobial action in shade dried and sundried plants. The studies support the traditional use of the plant in therapeutic applications as a *Phashana Bheda* and strongly recommend the plant as an important source for the development of novel antimicrobial agents against multidrug-resistant *Candida* species, a causative agent of urinary tract infection.

Conflict of interest

Nil.

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