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QUALITATIVE AND QUANTITATIVE ANALYSIS OF THE PHYTOCHEMICALS IN THE PLANT *AERVA LANATA*

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ABSTRACT

The current study's objective was to use both qualitative and quantitative screening techniques to determine whether phytochemicals were present in the methanolic, ethanolic, and aqueous extracts of *Aerva lanata*. Standard techniques were used to analyze the phytochemical compounds present in crude extracts, including steroids, terpenoids, phlobatannins, reducing sugar, triterpenoids, alkaloids, phenolic compounds, flavonoids, saponins, quinones, oxalates, tannins, cardiac glycosides, and anthraquinones. The methanolic extracts showed positive results for the maximum number of phytochemical compounds. In quantitative analysis, the essential secondary metabolites such as phenolic compounds, flavonoids, tannins, carbohydrates, alkaloids, saponins, phenols, and terpenoids were tested in all three solvent extracts of the leaf. The methanolic extract of the leaf showed the highest amounts of phytochemicals when compared with other solvent extracts.

Keywords: *Aerva lanata,* Phytochemicals, Solvent extracts, Qualitative analysis, Quantitative analysis

INTRODUCTION :

India is a marvelous location for medicinal plants, and plant extracts have been used in Ayurvedic and Siddha medicine to treat many diseases. An organic component

from traditional medicinal herbs promotes health and alleviates diseases. Research into various pharmacological effects of medicinal plants has grown in recent years all around the world **[1]**. These are due to the complex combinations of many bioactive molecules that make up the herbal constituents found in plants, which can vary significantly based on genetic and environmental variables **[2]**. According to the World Health Organization, the majority of people in underdeveloped nations are dependent on traditional treatments and botanical medicines for necessary social health because the medicinal plant has fewer adverse effects than synthetic medications, and medicinal plant use is becoming more widespread **[3]**. Phytochemicals are physiologically active, naturally occurring chemical substances that are present in plants. They have a more significant positive impact on human health than macro- and micronutrients **[4]**. Phytochemicals add color, perfume, and flavor to the plant and shield it from diseases and harm. Phytochemicals, in general, refer to plant compounds that shield plant cells from environmental dangers such as pollution, stress, dehydration, UV exposure, and pathogenic assault **[5, 6]**.

Phytochemicals build up in a variety of plant tissues, including the roots, stems, leaves, flowers, fruits, and seeds **[7]**. These phytochemicals, which are classified as primary and secondary metabolites, accumulate in plant tissue. Secondary metabolites are compounds found in plants that have biological characteristics such as antioxidant activity, antibacterial action, regulation of detoxification enzymes, immune system activation, reduction of platelet aggregation, and modification of hormone metabolism. There are about a thousand phytochemicals, both known and undiscovered. Although recent studies have shown that several phytochemicals can also protect humans against disease, it is widely known that plants create these substances to protect the plants themselves **[8]**. In the *Aerva lanata* plant some biological activities like Antioxidant properties **[9, 10, 11]** Anticancer **[12, 13]** Anti-inflammatory **[14, 15]** Hepatoprotective effect **[12, 16]** Diuretic, Anturolithic activity **[14, 17, 18]** and Anti-diabetic property **[19, 20, 21]** has been already reported.

Primary metabolites are organic substances that contribute to the formation and development of the human organism. These substances include glucose, starch, polysaccharides, protein, lipids, and nucleic acids. Alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, volatile oils, and other secondary metabolites are produced by plants **[22, 23]**. Secondary metabolites have a crucial part in the treatment of many diseases

because of their therapeutic efficacy, which includes phytochemicals. Phytochemicals possess different biological activities such as

(a) Alkaloids have antispasmodic, antimalarial, analgesic, and diuretic properties.

(b) Terpenoids, have anti-inflammatory, anti-cancer, anti-malarial, antiviral, and anti-anthelmintic activities.

(c) The antifungal and antibacterial effects of glycosides are well established.

(d) Flavonoids and phenols are said to have antibacterial, anti-allergic, and antioxidant activities.

(e) Saponins have anti-viral, anti-inflammatory, and plant defense characteristics **[24, 25]**.

The Amaranthaceae family includes *Aerva lanata* (Linn.) Juss. ex Schult is 0.6 - 1 m tall, prostate-shaped, and woolly undershrub that is commonly found during the rainy season in all the states, India. The vernacular name of *Aerva lanata* is Hindi: Gorakhganja, English: Mountain knotgrass, Oriya: Paunsia, Bengali: Chaya, Sanskrit: Bhadra, Tamil: Sirupulai, Cerupulai, Malayalam: Cherula, Cheruvula, Cerupula, Kannada: Bilihindisoppu, Telugu: Pindiconda, Pindicettu, Marathi: Kapur-madura, Gujarati: Gorakhaganjo, Punjabi: Buikallan, Chhattisgarhi: Pithauri bhaji **[26]**. The *Aerva lanata* is a perennial semi-shrubby plant that grows in dry tropical and subtropical climates. The *Aerva lanata* is covered with densely matted hairs on stems and leaves. It is much branched with a vigorous round stem. The flowers are small and whitish and arranged in dense, woolly terminal panicles **[27, 28]**.

MATERIALS AND METHODS:

(a) Collection of plant samples: The plant materials were collected in the month of November-December 2022 from Village- Bicharpur, Lormi Block, Mungeli District, Chhattisgarh. After collection, the plant was washed with tap water and then rinsed with distilled water. After washing each part of the plant was separated i.e., leaves, stems and flowers were collected separately and dried in the shade for 30 days. When the plant became dried it was powdered to get a coarse powder using a mixture grinder stored in an air-tight container and used for further successive extraction **[29]**. The collected plant was identified by the Botanical Survey of India central region centre Allahabad.

(b) Preparation of Extracts: (By Maceration method) [30]

I Aqueous Extraction: 2 gm of each air-dried leaf, stem, and flower powder was taken in three different 150 ml conical flasks, and 100 ml de-ionized water was added in each

conical flask, plugged with cotton wool, and then shaken for 6 hours at 150 rpm in room temperature using a mechanical shaker. After shaking the plant sample was macerated for another 48 hours at room temperature. The solvents were filtered using Whatman No. 1 filter paper, collected, and stored at 4°C temperature in air-tight bottles.

II **Methanolic Extraction:** 2 gm of each air-dried leaf, stem, and flower powder was taken in three different 150 ml conical flasks, and 100 ml methanol was added in each conical flask, plugged with cotton wool, and then shaken for 6 hours at 150 rpm in room temperature using a mechanical shaker. After shaking the plant sample was macerated for another 48 hours at room temperature. The solvents were filtered using Whatman No. 1 filter paper, collected, and stored at 4°C temperature in air-tight bottles.

III **Ethanolic Extraction:** 2 gm of each air-dried leaf, stem, and flower powder was taken in three different 150 ml conical flasks, and 100 ml ethanol was added in each conical flask, plugged with cotton wool and then shaken for 6 hours at 150 rpm in room temperature using a mechanical shaker. After shaking the plant sample was macerated for another 48 hours at room temperature. The solvents were filtered using Whatman No. 1 filter paper, collected, and stored at 4°C temperature in air-tight bottles.

(c) Phytochemical screening: Qualitative analysis

The qualitative analysis of the plant *Aerva lanata* is done by the standard method of Harbon. Preliminary screening for the secondary metabolites was carried out as per standard methods eg. alkaloids (Wagner's Test**)**, carbohydrate (Molisch test), cardiac glycoside (Keller Kiliani's test), flavonoids (Lead acetate test), phenols (Ferric chloride test), phlobatannins (Precipitate test), amino acids and proteins (Ninhydrin test), saponins (Foam test), sterols (Libermann-Burchard test), tannins (Braymer test), terpenoids (Swalkowski test), quinones (Hydrochloric acid test), oxalate (Acetic acid test) **[31]**.

(d) Phytochemical Screening: Quantitative analysis

The quantitative analysis is performed in all three extracts of the leaf of *Aerva lanata* .

I. **Determination of Total Alkaloids:** 25 ml of the plant

extracts are taken in a beaker and 200 ml of 10% CH_3COOH in C_2H_5OH is added. The mixture is covered and allowed to stand for 4 hours. The mixture is then filtered and the extract is allowed to become concentrated in a water bath until it reaches 1/4 of the original volume. Concentrated NH_4OH is added until the precipitation is complete. The whole solution is centrifuged for 1 hour at 2000 rpm and after this is allowed to settle down, the precipitate is collected and washed with dilute NH_4OH and then filtered. The residue is alkaloid, which is then dried and weighed **[32]**.

% of alkaloid = weight of alkaloid/ weight of sample × 100

II. **Determination of Total Phenols:** The quantity of phenols is determined using the spectrophotometer method. The 25 ml of the plant extracts are boiled with 50 ml of $(CH_3CH_2)_2O$ for 15 minutes. 5 ml of the boiled sample is then pipetted into 100 ml flask, and 10 ml of distilled water is added. After the addition of distilled water, 2 ml of NH_4OH solution and 5 ml of concentrated $CH_3(CH_2)_3CH_2OH$ are added to the mixture. The sample is made up to the mark and left for 30 min to react for color development and measured at 505 nm wavelength using a spectrophotometer **[33]**.

% of phenols = weight of phenols/ weight of sample × 100

III. **Determination of Total Tannins:** The quantity of tannins is determined by using the spectrophotometer method. 25 ml of plant extracts are taken into a 100 ml in 150 ml conical flask. 50 ml of distilled is added and stirred for 1 hour. The sample is filtered into a 100 ml volumetric flask and made up to mark. 10 ml of the filtered sample is then pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M $K_4Fe(CN)_6.3H2O$. The absorbance is measured with a spectrophotometer at 395 nm wavelength within 10 minutes **[34]**.

% of tannins = weight of tannins/ weight of sample × 100

IV. **Determination of Total Saponins:** 25 ml of plant extracts are taken into a 150 ml conical flask and 100 ml of 20% C_2H_5OH is added. The sample is heated over a hot water bath for 4 h with

continuous stirring at about 55°C. The mixture is then filtered and the residue is re-extracted with another 200 ml of 20% C_2H_5OH. The combined extracts are reduced to 40 ml over a water bath at about 90°C. The concentrated is then transferred into a 250 ml separator funnel and 20 ml of $(CH_3CH_2)_2O$ is added to the extract and shaken vigorously. The aqueous layer is recovered while the $(CH_3CH_2)_2O$ layer is discarded and the purification process is repeated. 60 ml of n-C_4H_9OH is added and the combined n-C_4H_9OH extracts are washed twice with 10 ml of 5% NaCl. The remaining solution is then heated in a water bath and after evaporation; the samples are dried in the oven to a constant weight **[35]**.

% of saponins = weight of saponins/weights of sample × 100

V. **Determination of Total Flavonoids:** 25 ml of plant extracts are mixed with 100 ml of 80% aqueous methanol at room temperature. The whole solution is then filtered through filter paper Whatman No. 1 and the filtrate is later transferred into a water bath and the solution is evaporated into dryness. The sample is then weighed until a constant weight **[32]**.

% of Flavonoid = Weight of Flavonoid/Weight of sample × 100

VI. **Determination of Total Terpenoids:** 25 ml of plant extract was taken in a 150 ml beaker and soaked in 10 ml of C_2H_5OH overnight. The extract after filtration, was extracted with 50 mL of petroleum ether using a separating funnel. The ether extract was separated and dried in a water bath and weighed **[36]**.

% of terpenoids = weight of terpenoids/weight of sample × 100

VII. **Determination of Total Carbohydrates:** 25 ml of extracts are taken boiling tube and add 2.5 N HCl solution and boiled in a water bath for 5 minutes. After that cool it and neutralize with Solid Na_2CO_3. The mixture is then centrifuged for 1 hour at 2500 rpm. The float was prepared to 100 ml by using deionized water. 1 ml Phenol and 5 ml Concentrated H2SO4 are added to the solution and heated at 30°C for 20 minutes. Then cool the solution and the absorbance is measured with a

spectrophotometer at 395 nm wavelength **[37]**.

% of carbohydrates = weight of carbohydrates/ weight of sample × 100

RESULT and DISCUSSIONS:

The present study carried out on the *Aerva lanata* revealed the presence of medicinally active constituents. Preliminary phytochemical screening shows the presence of Alkaloids, flavonoids, cardiac glycosides, Phenol, Tannins, Saponins, and the minute amount of terpenoids as shown in **Table 1**.

Total Alkaloid Content

The results of total Alkaloid content are shown in **Table 2**. Alkaloids are reported as an immunity buster, Anti-fungal, and anti-bacterial activity **[25]**.

Total Carbohydrate Content

The results of total Carbohydrate content are shown in **Table 3**. Carbohydrates possess antioxidant properties **[25]**.

Total Flavonoid Content

The results of total flavonoids are shown in **Table 4**. Flavonoids are naturally occurring phenols with a wide range of biological properties, such as potent anti-inflammatory, anti-allergic, antithrombotic, and vaso-protective actions **[38]**.

Total Phenolic Content

The results of total Phenolic content are shown in **Table 5**. Because of their hydroxyl groups, phenolic substances are known to be potent antioxidants that may scavenge free radicals [39].

Total Saponin Content

The results of total Saponin content are shown in **Table 6**. The saponins contain antibacterial, antifungal, and anticarcinogenic properties **[40]**.

Total Tannin Content

The results of the total Tannin content are shown in **Table 7**. Tannins have antibacterial, antiviral, and antiparasitic properties. Tannins have also been investigated recently for their possible role in the prevention of cancer through a variety of pathways **[41]**.

Total Terpenoid Content

The results of total Terpenoid content are shown in Table 8. The plant's high total terpenoid content is utilized therapeutically to treat fatal conditions including cancer and Alzheimer's. Terpenoids also have antimicrobial, antioxidant, neuroprotective, and chemoprotective effects when present **[36]**.

Table 1: Qualitative phytochemical screening of *Aerva lanata*

S. No.	Phytochemicals	Method	Solvents								
			A	B	C	D	E	F	G	H	I
1	Alkaloids	Wagner's test	-	-	+	-	-	+	-	+	+
2	Carbohydrates	Molisch test	+	+	+	+	+	+	+	+	+
3	Cardiac glycosides	Keller Kiliani's test	-	-	+	-	-	+	-	+	+
4	Flavonoids	Pb(OAc)2 test	+	+	+	+	+	+	+	+	+
5	Amino acids + Proteins	Ninhydrin test	-	+	-	+	+	-	-	+	+
6	Oxalate	Acetic acid test	+	+	+	+	+	+	+	+	+
7	Phenols	FeCl3 test	-	-	+	-	-	+	-	-	+
8	Phlobatanins	Precipitate test	-	-	-	-	-	-	-	+	+
9	Quinones	HCl test	+	+	-	+	+	-	-	-	-
10	Saponins	Foam test	+	+	+	+	+	+	+	+	+
11	Sterols	Libermann Burchard test	-	-	-	-	-	-	-	+	+
12	Steroids and Triterpenoids	Layer test	-	+	+	-	+	+	-	-	-
13	Tannins	Braymer's test	-	-	+	-	-	+	-	-	+
14	Terpenoids	Salkowski's test	+	+	+	+	+	+	+	+	+

where Solvent A= Ethanolic extract of Stem, Solvent B= Ethanolic extract of Flower, Solvent C= Ethanolic extract of Leaf, Solvent D= Methanolic extract of Stem, Solvent E= Methanolic extract of Flower, Solvent F= Methanolic extract of Leaf Solvent G= Aqueous extract of Stem, Solvent H= Aqueous extract of Flower, Solvent I= Aqueous extract of Leaf, + = test is positive, - = test is negative.

Table 2: Total Alkaloid Content

S. No.	Extracts	Total Alkaloid Content (mg/g)	%
1	Ethanol	53 mg	5.3
2	Methanol	78 mg	7.8
3	Deionized Water	46 mg	4.6

Table 3: Total Carbohydrate Content

S. No.	Extracts	Total Carbohydrate Content (mg/g)	%
1	Ethanol	11 mg	1.1
2	Methanol	62 mg	6.2
3	Deionized Water	84 mg	8.4

Table 4: Total Flavonoid Content

S. No.	Extracts	Total Flavonoid Content (mg/g)	%
1	Ethanol	51 mg	5.1
2	Methanol	43 mg	4.3
3	Deionized Water	26 mg	2.6

Table 5: Total Phenol Content

S. No.	Extracts	Total Phenol Content (mg/g)	%
1	Ethanol	41 mg	4.1
2	Methanol	57 mg	5.7
3	Deionized Water	32 mg	3.2

Table 6: Total Saponin Content

S. No.	Extracts	Total Saponin Content (mg/g)	%
1	Ethanol	54 mg	5.4
2	Methanol	28 mg	2.8
3	Deionized Water	52 mg	5.2

Table 7: Total Tanin Content

S. No.	Extracts	Total Tanin Content (mg/g)	%
1	Ethanol	78 mg	7.8
2	Methanol	66 mg	6.6
3	Deionized Water	34 mg	3.4

Table 8: Total Terpenoid Content

S. No.	Extracts	Total Terpenoid Content (mg/g)	%
1	Ethanol	38 mg	3.8
2	Methanol	48 mg	4.8
3	Deionized Water	70 mg	7.0

CONCLUSION:

The *Aerva lanata* is a significant medicinal plant utilized in a variety of pharmaceutical-related fields and for the creation of medicines. The various portions of *Aerva lanata 's* studied ethanolic, methanolic, and aqueous extracts revealed the presence of chemical elements such as flavonoids, alkaloids, saponins, carbohydrates, phenols, tannins, and terpenoids. There is no question that this plant is a source of chemical compounds with the potential to be beneficial as medications as well as fresh leads and hints for contemporary drug creation. Future study on *Aerva lanata* has huge potential because of its various therapeutic characteristics. The unexploited potential of this plant should be investigated through more clinical and pharmacological investigations.

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