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Pharmacognostic, antibacterial and anti-oxidant activity of *Aerva lanata* (L.) A.L. Juss ex Schultes (Amaranthaceae)

Abstract

Aerva lanata (L.) A.L. Juss ex Schultes, commonly known as 'Pashanabeda', is known to have high medicinal value. The different parts of the plant were used since antiquity in the indigenous systems of medicine. The present investigation deals with the pharmacognostic studies of market and authentic sample of *Aerva lanata* (L.) A.L. Juss ex Schultes (Amaranthaceace). Pharmacognostic studies include Fluorescence analysis, Ash values, and phytochemical analysis. The total ash value, water soluble and acid insoluble value of the authentic sample and was found by 5.82%, 1 6%, and 24% respectively whereas for market sample showed 12.6%, 65.5% and 9 % respectively. Fluorescence analysis was carried out at visible and UV spectrum. Phytochemical analysis of authentic sample showed the presence of alkaloids, coumarin, quinol and tannin whereas market sample showed the presence of tannin, coumarin and quinol. The anti-oxidant value in authentic sample was found to be 42.17% in Petroleum ether, where as in market sample it showed 17.65%. Petroleum ether extract of the plant was found to be effective active against the *Escherichia coli*.

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Introduction

Alternative medicine is a common word used now days to promote plant based medicinal systems across the world. The herbal plants include various types of plants used in herbalism or ingredients which can be used in drug development and synthesis. From the herbal plants we get raw materials for preparation of Herbal medicine.[1] According to World Health Organization 80% of the population is dependent on plants for primary health care and worldwide it is estimated that around 21,000 plants are used for primary health care (Santosh *et al.,* 1995).

Generally, it is not that the plants are directly used for treatment of an illness but it is the plant extracts with the phytochemical constituents that are significantly used for therapeutic purposes. The phytochemical constituents are nothing but the secondary metabolites present in the plants which are practically useless for the plants. This secondary metabolites are often used by the plants for protecting themselves against the biotic and abiotic stress a plant undergoes. This secondary metabolites are used as a source of drug, flavors, fragrances, insecticides and dyes by human beings.[2] Because of such high potential of the secondary metabolites in recent years a lot work is being carried out by different countries to prove the efficacy of plant secondary metabolites (Al Magboul *et al.,* 1985).

Herbal medicines off late has known play a critical role in the modern health care management system as well as traditional systems of medicine. In order to ensure that herbal medicines are safe for use in public domain, it necessary to establish quality standards to ensure safety and efficacy of the herbal medicine. If such a safety protocols are established right at the collection stages of the raw materials, it will ensure that the world does not question the efficacy of any recorded system of medicine.

With this background for the present study we choose *Aerva lanata* (L.) Juss. (Amaranthaceae) for pharmacognosy studies. *Aerva lanata* is a woody, prostrate perennial herbs which is traditionally known as Pashana beda. It has well recorded in Ayurvedic history and has been used in various Indian systems of medicine right from Ayurveda, Siddha, Unani, Homeopathy etc. for various pathological conditions (Plate 1).

Pashanabeda are highly controversial drugs, at least 8 different plants are equated with Pashanabeda because of their alleged ability to dissolve renal and vesicle calculi. Pashanabheda in Sanskrit means any material which is capable of breaking stones. There are many plants that are known by this name due to dissolving ability, to break stones of kidney and urinary bladder. *Aerva lanata* is commonly found in wastelands throughout India in waste places. The authentic sample of the plant was compared to the market sample and both the samples were subjected to Qualitative and Quantitative comparison for Secondary metabolites.

Customary uses

Traditionally the plant is used for arresting hemorrhage during pregnancy, used as an anti-inflammatory drug, used in headache, skin disease but majorly it is used to dissolve kidney and gall bladder stones. It is also used to increase lactation after delivery. In ethnic medicine plant extract is commonly used for treating nasal bleeding, cough, scorpion sting, fractures and spermatorrhoea. There has been well recorded use of it being used as anti-helminthic and for medication that soothes inflamed and injured skin. The plant is also used in treatment of diarrhea, cholera and dysentery. The roots are used for diuretic and demulcent and are credited with healing properties (Rajesh *et al.,* 2011).

Objectives

The authentic samples of *Aerva lanata* were collected from Tirunelveli during the month of August, 2019. Market samples were procured from Flipkart. Both the samples were subjected to pharmacognostical, physiochemical, phytochemical and fluorescence analysis.

The following parameters are the objectives of the present study:

- Pharmacognostical evaluation of authentic and market samples
- Phytochemical evaluation of authentic and market samples
- Fluorescence analysis of authentic and market samples with various solvents.
- To assess the Anti- microbial activity of the plants against gram negative bacteria.
- Evaluation of antioxidant activity by DPPH radical scavenging method.

Review of literature

Akanji et al.,[3] in their work on leaf extracts of *Aerva lanata* which was known to inhibit the activities of Type 2 diabetes-Related enzymes and possess Antioxidant properties. It also showed inhibitory effects on alpha amylase and alpha glucosidase. The anti-diabetic and free radical scavenging properties were investigated using in vitro model whereas alpha amylase and alpha glucosidase were investigated by DPPH scavenging agent.

Al-Ansari et al.,[4] have worked on phytochemical components from *Aerva lanata* and its in vitro inhibitory activity against drug resistant microbial pathogens and antioxidant properties. They prepared extracts with chloroform, acetone, methanol and water for phytochemical analysis. Kirby- Bauer method for antimicrobial activity and DPPH Reagent for Antioxidant activity. The extracts such as ethano chloroform, acetone, water and methanol showed the presence of flavonoids, glycosides, tannins, steroids. The methanol and ethyl acetate extracts of the *Aerva lanata* revealed antimicrobial activities. The water and ethyl acetate extracts showed the effective percentage of antioxidant properties.

Athira et al.,[5] have worked on Pharmacognostic review on *Aerva lanata* and have explained the morphology, cultivation, microscopic characters of the plant. The plant contains chemical constituents such as alkaloids, flavonoids, minerals and saponins. The alcoholic extracts of the plant showed diuretic activity, anti-ulcer activity, antidiarrheal activity, it was found to have hepato-protective activity and anti-inflammatory effect.

Beher et al.,[6] in their work on evaluation of antioxidant, antimicrobial and antiurolithiatic activity have used different solvent extract of the flowers. The aqueous extract of the flower of *Aerva lanata* showed the least antioxidant activity whereas the methanolic extract showed the highest antioxidant activity. The methanolic extract of the flower showed significant anti-urolithic and antimicrobial activity.

Dinnimath et al.,[7] assessed the natural constituents from *Aerava lanata* and worked on Antiurolithiatic activity. Two compounds quercetin and betulin were isolated from *Aerva lanata* by modern analytical techniques. Two isolated compounds were screened for antiurolithiatic potentials in induced calculi. It was found that both quercetin and betulin were equally potent and their antiurolithiatic activity was found to be associated with the diuretic activity.

Gosh[8] worked on antioxidant, antimicrobial and antiurolithiatic potential using different solvents. The phytochemical screening of Methanolic extracts revealed the presence of phenolic compounds such as flavonoids and terpenoids. The presence of certain combination secondary metabolites in the extracts can be responsible for the maximum therapeutic properties. Anti – microbial activity was carried out by well diffusion method. Methanolic extract showed antimicrobial activity against gram negative bacteria such as *Escherichia coli* and gram positive bacteria such as *Micrococcus luteus and Staphylococcus aureus.* Anti-oxidant activity of the plant extract was carried out by DPPH scavenging radical. The methanolic extract had the highest antioxidant activity compared to the other plant extracts. Methanolic extract of the plant showed significant antiurolithiac activities.

Goyal et al.,[9] in their work on phytochemistry and pharmacological aspects showed the presence of alkaloids and flavonoids. The ethyl acetate and methanol extracts showed interesting antimicrobial activities against *Bacillus cereus, Eshcherichia coli*. The ethanolic extract of *Aerva lanata* leaves showed effectiveness against parasites. The petroleum ether extract of *Aerva lanata* was found to be protective against liver damage. The aqueous extract showed significant anti-diarrheal activity.

Goyal et al.,[9] worked on phytochemistry and pharmacological aspects of *Aerva lantata*. Qualitative phytochemical analysis of this plant confirmed the presence of alkaloids and flavonoids. Leaves of Aerava showed high nutritive content in carbohydrates, crude protein and minerals. In-vitro activity of methanol and ethyl acetate extracts of the plant against activities of bacteria. The ethanolic extract of seed and leaf show anti-parasitic activities against tapeworms and earthworm. The alcoholic extract of *Aerva lanata* at a dose of 800 mg/kg acted as a diuretic. Ethanolic extracts from aerial parts of *Aerva*, in goat demonstrated antiastmatic activity at 100µg/ml.

Gujjeti et al.,[10] worked on phytochemical screening and thin layer chromatography of Root extracts from *Aerava lanata*. The percentage yield of the root of *Aerva lanata* with different solvents such as hexane, chloroform, ethyl acetate, methanol and acetone was calculated. The highest yield was reported in hexane and least in acetone. The qualtitative phytochemical screening of root extracts revealed the presence of alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, proteins, amino acids, flavanoids, quinones and terpenoids shows different types of results in different solvents extracts.

Jaswanth et al.,[11] worked on anthihelmenthic activity of whole plant extract *Aerava lanata* in naturally infected sheep. The effect of ethanolic extract from whole plant of *Aerva lanata* was studied on natural acute/sub-acute parasitic gastro-enteritis due to mixed nematode species in sheep. The *Aerva lanata* extract significantly reduced the egg production by the helminthic dose. The extract reversed this and produced a decrease in WBC counts to normal values indicating the anthelmintic potential of the plant through elimination of the worms in the injected sheep. *Aerva* plant can be used as medicine for anthelmintic by isolation of its phyto-constituents for pharmacological screening.

Karthikeyan et al.,[12] worked on phytochemical screening and anti-bacterial activity of *Aerava lanata.* The phytochemical analysis of the plant extract revealed the presence of alkaloids, terpenoids, cardiac glycosides, tannins, saponins and flavonoids. Anti-bacterial activity was carried out by disc diffusion method. The methanolic extract of Aerva lanata showed antimicrobial activity against both gram positive bacteria such as *Staphylococcus aureus* and *Enterococcus fecalis* and gram negative bacteria *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The presence of the phytochemical constituents suggests that the plant is pharmacologically active and can be used for further medicinal purposes.

Kumar et al.,[13] worked on the phytochemical composition and in vitro antioxidant properties of aqueous extract of *Aerva lanata* stem. The aqueous extract was screened for phytochemical screening. It

showed the presence of phenolic compounds, saponins, flavonoids, tannins,phytosterols as major phytochemical groups. Antioxidant activity of the extract was determined by 2, 2-diphenyl1-picrylhydrazyl radical scavenging activity, metal chelating activity, reducing power activity and DNA damage inhibition activity. The aqueous extract of *Aerva* exhibited high 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity, metal chelating activity, reducing power activity and DNA damage inhibition efficiency. The extract also showed the presence of high amount of total phenolic content and were identified as gallic acid apigenin-7-O-glucoside, quercetin-3-O-rutinoside and myricetin by high performance liquid chromatography analysis. The extract was found nontoxic towards human erythrocytes in the hemolytic assay.

Malar et al.,[14] worked on pharmacognostic characters and antimicrobial activity of *Aerva lanata.* The physicochemical, phytochemical and fluorescence analysis of powdered aerial and root parts of *Aerva lanata* was performed. The physicochemical parameters such as total ash, acid insoluble ash, water-soluble ash showed that the plant has less amount of carbonates, silicates. The various colors obtained by fluorescence were analyzed, and the moisture content of aerial and root parts were found to be 37.5% and 35.33% respectively. The phytochemical screening of the plant revealed that the maximum constituents in chloroform, ethyl acetate, and water extract. The minimum phytochemical constituents were found in acetone extract.

Manokaran et al.,[15] in their work on *Aerva lanata* have worked on Hepatoprotective activity against Paracetamol induced Hepatotoxicity in Rats, the ethanol and aqueous extract of the plant material showed the presence of alkaloids, flavonoids, glycosides, steroid and tannins. The hydro-alcoholic extract of *Aerva lanata* showed significant action towards the normal functioning of the liver and its prominent hepatoprotective activity.

Murugan et al.,[16] worked on phytochemical, FT-IR and antibacterial activity of whole plant extract of *Aerva lanata*. Petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of whole plant of *Aerva lanata* were examined for the phyto-constituents and antibacterial activity against the human pathogens. The phytochemical screening of methanol and ethanol plant extracts revealed the presence of alkaloid, anthraquinone, catechin, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid and sugar. The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. IR analysis of the whole plant powder of *Aerva lanata* showed the presence of different functional groups. Antibacterial activity was carried out by the disc diffusion method against the pathogens. The maximum zone of inhibition was in methanol extract against gram negative bacteria i.e *Serratia marcescens.*

Nagarathna et al.,[17] worked on pharamacological, microscopy and macroscopy characters of the plant. Macroscopy characters explains about the root, stem and leaves of the plant. Powder microscopy reveals about the presence of rosette crystals of calcium oxalate, scalariform vessels with adjacent tracheid's and fibre and fragments of upper epidermis and lower epidermis contains anomocytic stomata. The phytochemical screening of whole plant showed the presence of glycosides, starch, free sugars, alkaloids, phenolic compounds, phytosterols, carbohydrates, proteins, amino acids, flavonoids and quinones. It also showed the presence of nutrients such as Carbohydrate, crude protein moderately in ash form.

Payal et al.,[18] worked on phytochemistry and biological activities of *Aerva*. Powder microscopy of leaf, root and flower revealed the presence of fibres, xylem vessels, calcium oxalate crystals, starch grains, cork cells, secondary phloem, parenchyma cells. The phytochemical analysis showed the presence of Alkaloids, phenolic compounds, phytosterols, carbohydrates, proteins, amino acids, flavonoids and quinones were identified in different solvents extracts. The hydro-alcoholic extracts of *Aerva lanata* from flowers showed significant diuretic activity in rats.

Priyadarshini[19] in her work on evaluation of antimicrobial activity and preliminary screening on *Aerva lanata* has explained about the presence and absence of the phyto-constituents and inhibitory action on bacteria. The phytochemical screening showed the presence of tannin, anthraquinone, alkaloid, saponin, etc. The aqueous extract of the plant showed negligible antimicrobial activity whereas methanol, ethanol and hexane extracts have shown the maximum inhibition.

Ramalingam et al.,[20] worked on antifungal activity of *Aerva lanata* against fungal pathogens. The root, flower and leaf of *Aerva* with different solvents like acetone, aqueous, benzene and ethylacetate extracts were carried out against *Candida parapsilosis, Aspergillus flavus, Trichosporon asa*hii and *Mucor indicus.* Antifungal activity was carried out by agar well diffusion method. Benzene extract of leaf of *Aerva lanata* showed maximum zone of inhibition against *Aspergillus flavus*. The acetone extract of root showed antifungal activity against *Candida parapsilosis, Aspergillus flavus, Trichosporon asahii* and *Mucor indicus.* The root extract from *Aerva lanata* with all the solvents showed the best antifungal activity against all fungal pathogens compared to that of flower and leaf extracts. There is in need of study to isolate and purify the active phytocompounds of *Aerava lanata* which possess antifungal activity against fungal species and it can be used in the treatment of fungal diseases.

Silvia et al.,[21] on pharmacognostic and phytochemical screening of *Aerva lanata.* Flower samples of *Aerva lanata* were studied for its macroscopical, microscopical characters. Physicochemical, phytochemical, and fluorescence analysis of powder of the plant were performed. Macroscopy characters revealed that the flowers are small, actinomorphic, and solitary or aggregated in cymes. Powder microscopy of flower revealed the presence of trichomes, calcium oxalate crystals, starch grains, oil globules and pollen grains. The fluorescence analysis of the powder sample was performed. Physiochemical parameters such as total ash, acid insoluble ash sulfated ash and water soluble ash was formed.

Materials and methods

Collection of plant material

Aerva lanata was collected from Tirunelveli during the month of August, 2019. The plant was identified and authenticated using Flora of Madras Presidency with voucher specimens being deposited Mount Carmel College herbarium. The plants were shade dried and stored in an air tight container for further study. The dried plants were used for pharmacognostical, physiochemical, phytochemical, Fluorescence, antimicrobial and antioxidant paraments.

Pharmacognostical evaluation

Morphological features: For external morphology the following details for the plants were recorded namely, plant height, stem surface, leaf characters–arrangement, shape, size, upper and lower surface Inflorescence length, flowers–color, morphometric measurement, surface feature, bract and bracteole, sepal, corolla, androecium.

Powder microscopy

For powder microscopy the technique described by Raphael et al.,[22] was followed. The shade dried samples were moderately fine

powdered using mortar and pestle and sieved through 355µm sieve mesh. The powder was dusted on glass slide and cover slip was placed and observed under Labomed microscope under 4x, 10x and 40x to detect minute details of the samples and the observations were photographed.

Vein clearing: For vein clearing the technique prescribed by Bob Harms (bioscieutexas.edu/prc) was followed. Leaves are collected and placed in Petri plates followed by addition of basic Fuchsin 1% aqueous solution. Covered Petri plates were left for overnight. The next day 10gms of sodium hydroxide was added and left overnight. Sodium hydroxide erodes most of the mesophyll tissue except the veins. The next day excess of sodium hydroxide was removed and washed multiple times with tap water. This is followed by placing the specimens in 50% ethanol for several days and followed by gradually replacing ethanol with xylene until the veins are clear. The specimen was then observed under a microscope for vein characters.

Physicochemical evaluation

Aerva lanata was analyzed through physicochemical parameters i.e., loss of drying, total ash value, acid insoluble ash, water soluble ash. The procedure for the same is described below:

Determination of total ash

About 2g of accurately weighed, ground plant sample was taken in a previously weighed silica dish that was ignited in a muffle furnace by gradually increasing the temperature. The ground dry sample was scattered in a fine even layer on the bottom of the dish. Incinerated by gradually increasing the heat not exceeding dull red heat (450°C) until it was freed from carbon. It was cooled and weighed. The percentage of ash with reference to the air dried plant sample was calculated.

$$\text{Total ash\%} = \frac{\text{Weight of total ash}}{\text{Weight of sample taken}} \times 100$$

Determination of acid insoluble ash

The ash obtained in the process described under determination of total ash was boiled for 5 minutes with 25ml of dilute hydrochloric acid. The insoluble matter was collected on an ashless filter paper. It was washed with hot water and ignited at 550°C to 600°C. It was weighed and the percentage of acid insoluble ash was calculated with reference to the air dried sample.

$$\text{Acid insoluble ash\%} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of sample taken}} \times 100$$

Determination of Water insoluble Ash

2 grams of sample was boiled for 5 minutes with 25ml of water then the insoluble matter was collected on an ashless filter paper, hot water washings were given and ignited for 15 minutes at 450°C temperature. The difference in weight of ash and the insoluble matter represents the water soluble ash. The percentage of water soluble ash was calculated with reference to air dried drug.

$$\text{Water soluble ash value \%} = \frac{\text{Weight of water soluble ash}}{\text{Weight of sample taken}} \times 100$$

Phytochemical screening

Phytochemical screening for carbohydrate, Protein, Alkaloid, Steroid, Glucose etc., has been carried about extraction of active phytochemicals in solvents such as Methanol, Ethanol and Petroleum Ether.

Preparation of extracts for preliminary phytochemical screening

Crude plant extract was prepared by the Soxhlet extraction method. 5 gm of powdered material was uniformly packed into a thimble and extracted with Methanol (150 ml), Petroleum ether (150ml) and ethanol (150ml). The process of extraction was continued for 18–24 hours. The extracts were concentrated by keeping in a water bath set at 55°C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4°C for their future use in phytochemical analysis (Table 1).

Table 1 Procedure for phytochemical screening

Sl No.	Secondary Metabolite	Name of the test	Methodology and confirmation of compounds
1	Alkaloid	Mayer' test	To 2 -3 ml of filtrate, fe2 drops of dilute Hydrochloric acid and Mayer's reagent were added and shook well. Formation of yellow precipitate shows the presence of Alkaloids.
2	Anthraquinones	Borntrager's test	To the test solution, a few drops of Magnesium acetate solution (1%) were added. Formation of pink coloration indicates the presence of Anthraquinones
3	Coumarin	Alcoholic sodium hydroxide test	To the 2 ml of extract add a few drops of alcoholic sodium hydroxide and shake for 5 minutes, the appearance of yellow colour indicates the presence of Coumarins.
4	Flavones	Shinoda Test	To the test solution, add a few fragments of magnesium ribbon and a few drops of concentrated hydrochloric acid and boil for five minutes. Appearance of red and orange or red color indicates the presence of flavones.
5	Phenol	Ferric chloride test	To the test solution, add a few drops of ferric chloride solution (1%). Appearance of bluish green or red color indicates the presence of phenol.
6	Quinone	Sulphuric acid test	The 2 ml of test solution adds a few drops of concentrated Sulphuric acid. Formation of red colour indicates the presence of Quinone
7	Saponin	Frothing/Foam test	To 0.5ml of test solution 5ml of distilled water was added and shaken vigorously. Persistent lather formation indicates the presence of Saponin.
8	Tannin	Ferric chloride test	To the test solution, 5 drops of ferric chloride (5%) was added. Formation of green or brown color indicates the presence of tannins.
9	Terpenoids	Noller's test	The test solution was warmed with a piece of tin and a few drops of thionyl chloride. Violet or purple coloration indicates the presence of terpenoids.
10	Xanthoprotien	Nitric acid test	To the test solution, a few drops of concentrated nitric acid and a few ml of ammonia were added. Appearance of a reddish precipitate indicates the presence of xanthoprotein.
11	Sugar	Fehling's Test	To the 2ml test solution equal volumes of Fehling's solution A and B and heated. Formation of red color indicates the presence of sugars

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Fluorescence analysis

The fresh plant material was collected and were shade dried and powdered into small fragments until fracture was uniform and smooth. The powders were subjected to successive extraction with different organic solvent. The fluorescent property of the powdered drug extracts taken in different solvent systems were analyzed under Visible and UV long light (365 nm) according to the procedure described by Chase and Pratt. Specimens were recorded either as fluorescent (with color and intensity) or not fluorescent and their responses were tabulated in Table 3.

Antimicrobial activity

The test organisms *Klebsiella pneumoniae and Escherichia coli* were isolated from previously isolated, identified and stored collections in the Botany Department laboratory of Mount Carmel College. The micro-organisms were grown in the Mueller-Hinton broth medium. The assay for antibacterial activity was carried by a well diffusion method. 20 ml of Mueller Hinton agar (Appendix 1&2) medium was poured into sterile Petri plates and left to solidify. After solidification of the medium 120µml of bacterial broth was spread evenly. After inoculation, plates were dried for 15 minutes and 6 to 8 mm holes were punched using sterile borers. Once wells were formed, they were filled with different dilution of plant extracts were introduced into well and blanks. Plates were incubated for 24 hours at 37°C to allow the leaf extract to diffuse through agar media to form zones of inhibition. The diameters of zone of inhibition for different extracts against different bacteria were measured in millimeter for further analysis.

Appendix 1 Composition of nutrient media Agar medium

Sl no	Constituents	Quantity
1	Beef extract	3.0 gms
2	Peptone	5.0 gms
3	Water	1000 ml
4	NaCl	5.0 gms
5	Agar	15 gms

Ph is adjusted to neutral (7.4) at 25°C.The agar plates are prepared by pouring 20ml of molten agar into sterile petriplates.

Appendix 2 Composition of nutrient broth medium

Sl no	Constituents	Quantity
1	Beef extract	3.0 gms
2	Peptone	5.0 gms
3	Water	1000 ml
4	NaCl	5.0 gms

Antioxidant activity by DPPH radical scavenging method

The various in vitro assays which are commonly used to evaluate antioxidants potential of a compound includes Oxygen Radical Absorbance Capacity (ORAC), Ferric Reducing Ability of Plasma (FRAP), Xanthine Oxidase Methods, β-Carotene-Linoleate Model System, Total Radical Trapping Antioxidant Parameter (TRAP) method, Microsomal Lipid Peroxidation or Thiobarbituric Acid (TBA) Assay, Superoxide Radical Scavenging Activity, Hydroxyl Radical Scavenging Activity, Nitric Oxide Radical Inhibition Activity, Reducing Power Method, Phosphomolybdenum Method, Peroxynitrite Radical Scavenging Activity, Trolox Equivalent Antioxidant Capacity Assay (TEAC), DMPD(N, N-dimethyl-p-phenylene diamine dihydrochloride) Method, Conjugated Diene Assay, Scavenging of the Stable Radical 2,2' diphenyl-1-2-picryl Hydrazyl (DPPH) method, Lipid Peroxidation through Thiobartbituric (TBA) Reactive Substances Assay and others.[23]

DPPH [Diphenyl picryl hydrazyl] radical scavenging assay:[24]

DPPH is commercially available stable free radical and is purple in colour. Antioxidant molecules when incubated with DPPH neutralize it to Diphenyl hydrazine. The completion of neutralization is determined by the change in the DPPH colour to either colourless or pale yellow. The degree of neutralization was measured at 520 nm, which is a measure of scavenging potential of antioxidant in the plant extracts. The results were calculated using the formula

$$\text{DPPH Scavanged}(\%) = \frac{(\text{A conc} - \text{A test})}{\text{A conc}} \times 100$$

Where, A conc is the absorbance of the control reaction

Results and discussion

In the changing scenario of health issues, lot of attention is being given to herbal medicines and study on medicinal plants are gaining importance in the recent years in India and abroad. For the present study authentic sample of *Aerva lanata* was compared with the market sample and subjected to various qualitative phytochemical analysis and also the anti-oxidant, anti-microbial activity for its therapeutic applications.

Pharmacognostical evaluation

External Morphology

Habit: It is an erect or prostrate woody herb to sub shrub growing in sandy soil.

Roots: The roots are cylindrical in shape. Their length ranges from 1–1.8 cm in diameter.

Leaves: Leaves simple, alternate, 2-2 x 1–1.8 cm on main stem, elliptic or obovate or sub orbicular, obtuse or acute at apex, entire along margin, hairy above and shows the presence of more or less white cottony hairs beneath; petiole 3-8 mm long.

Inflorescence: The flowers are borne on axillary or terminal heads or spikes.

Flower: Flowers are very small greenish, sessile, bisexual. Perianth 1.5–1.75mm in length, tepals oblong, obtuse sometimes apiculate, silky on the back.

Bracts: Bracts are 1.25 x 1.01mm, membranous, ovate, concave, apiculate.

Fruits: Fruits are ovoid and acute consisting of seed which is black and bean shaped.

Distribution: This species is widespread in parts of Northeast tropical Africa, Kenya, West-Central Tropical Africa, Zaire, Zimbabww, Southern Africa, India, Madagascar, Arabian peninsula and Saudi Arabia.

Powder microscopy (Plate–2)

Leaf: The powder microscopy of shows trichomes, starch grain, calcium oxalate crystals. Trichomes are uniseriate with spiulated surface, tapered at the end and multiarticulate.

Root: Lignified and nonlignified fibers which are cylindrical in shape were observed. Xylem vessels were lignified with bordered pits, scattered parenchyma cells, calcium oxalate crystals were also observed. The starch grains were simple, oval or rounded without any striations.

Microscopic properties

Venation pattern: Veinlets are uniformly thin and straight forming wide, vein islets of variable shape. Veins islets have to vein termination that are simple and unbranched or branched. Stomata are anomcytic with no distinct subsidiary cells.

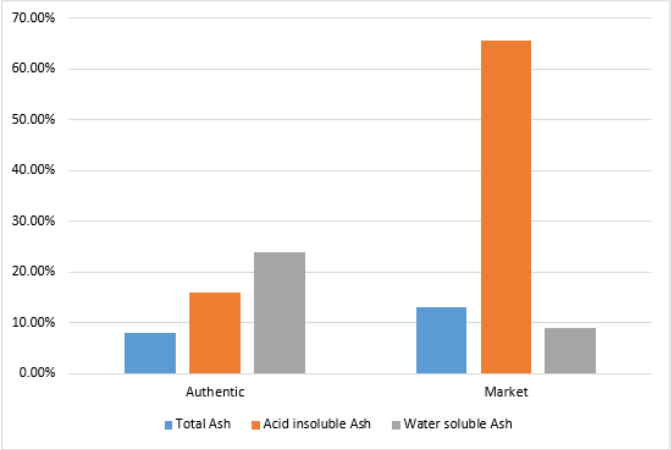
Physiochemical evaluation

Ash analysis

This parameter is mainly employed to assess the quality of harvest of plant material. The ash value helps us in in determining how well the plant material has been harvested, high percentage of inorganic and siliceous matter in plant material is reflected as high percentage of ash values. For determining the ash value we studied three parameters namely the total ash, water soluble ash and acid soluble ash the results of which is depicted in Table 2 and Graph 1.

Table 2 Ash value of authentic and market samples

Type of Ash	Percentage of Ash (Authentic)	Percentage of Ash (Market)
Total Ash	5.82%	12.60%
Acid insoluble Ash	16%	65.50%
Water soluble Ash	24%	9%



Graph 1 Ash analysis of authentic and market samples.

High amount of water soluble ash in the authentic sample is attributed to greater fluid content of the materials. The high percentage of acid insoluble ash in the market sample is an indicative of high contamination of the sample with silica and contamination with earthy particles especially from sand and soil adhering to the surface of the plants.

Phytochemical screening

Bioactive compounds present in plant are very useful for human beings not only as nutrients but also provide as with dietary supplements which protect human beings against various diseases. This secondary metabolites also known as non-nutritive compounds have antioxidant properties and also reduce the risk of many diseases, hence it is very important to do preliminary screening of plant for secondary metabolites. The details of phytochemical screening of both authentic and market samples are depicted in Table 3.

Flourescence analysis

The use of fluorescence can be very useful adjunct to botanical pharmacognosy since it is an easy method which can be employed on day to day basis for authentication of the plant material. Normally the fluorescence study of the material is done at two wavelengths namely under Visible and UV long light (365 nm). Specimens were recorded either as fluorescent (with color and intensity) or not fluorescent and their responses are tabulated in Table 4.

Antimicrobial activity

Antibacterial activity deals with anything that destroys bacteria or suppresses their growth or their ability to reproduce themselves. Heat, chemical and antibiotic drugs all have antibacterial properties. Result of antibacterial activity of isolated extract using different solvent (Petroleum Ether, Ethanol & Methanol) is shown in the Table 5. Petroleum Ether extract of authentic sample showed a considerable activity against *Escherichia coli* (Plate-3).

Antioxidant activity by DPPH radical scavenging method

DPPH radical scavenging activity was done with Methanol, Petroleum Ether and Ethanol with required concentrations. After 30 minutes of incubation in room temperature, absorbance was measured at 517 nm and the percentage of free radical scavenging was calculated (Plate-4) (Table 6, Graph 2).

Table 3 Phytochemical screening of authentic and market samples of *Aerva lanata*

S No.	Metabolites	Authentic sample			Market sample		
		Pet Ether	Ethanol	Methanol	Pet Ether	Ethanol	Methanol
1.	Alkaloid	+ve	-ve	+ve	+ve	-ve	-ve
2.	Anthraquinones	-ve	-ve	-ve	-ve	-ve	-ve
3.	Coumarin	+ve	+ve	+ve	-ve	+ve	-ve
4.	Flavones	-ve	-ve	-ve	-ve	-ve	-ve
5.	Phenol	-ve	-ve	+ve	-ve	-ve	-ve
6.	Quinone	+ve	+ve	+ve	-ve	-ve	-ve
7.	Saponin	-ve	-ve	-ve	-ve	-ve	-ve
8.	Tannin	+ve	+ve	+ve	+ve	-ve	-ve
9.	Terpenoids	-ve	-ve	-ve	-ve	-ve	-ve
10.	Xanthoprotien	-ve	-ve	-ve	-ve	-ve	-ve
11.	Sugar	-ve	-ve	-ve	-ve	-ve	-ve

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Table 4 Fluorescence properties of the extract of *Aerva lanata* (Authentic) in various solvents

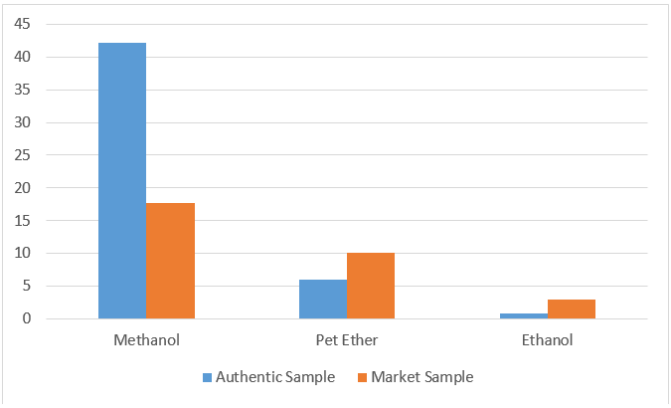
S No.	Treatment	Visible light		UV light (365 nm)	
		Authentic	Market	Authentic	Market
1.	*Aerva lanata* Powder	Green	Green	Pale Yellow	Dark Green
2.	1 N HCl	Pale Green	Pale Green	Dark Green	Dark Green
3.	Concentrated H_2SO_4	Black	Blackish Green	Purplish black	Purplish green
4.	50% H_2SO_4	Pale Green	Light Green	Dark Green	Pale Green
5.	Concentrated HCl	Brownish green	Brownish green	Black	Brownish green
6.	Acetic Acid	Pale green	Light green	Pale Green	Purplish green
7.	Petroleum Ether	Light Green	Light Green	Dark violet	Dark Green
8.	Acetone	Light Green	Light Green	Violet	Purplish green
9.	Chloroform	Green	Dark Green	Violet Green	Purplish green
10.	Methanol	Light green	Pale Green	Green	Purplish green
11.	Ethanol				
12.	Ammonia	Light Green	Dark Green	Dark Green	Purplish green
13.	Nitric acid + Ammonia	Dark Green	Light Green	Purplish black	Dark Green
14.	1N Ferric chloride	Blackish green	Dark Green	Dark Green	Purplish green
15.	40% Sodium hydroxide + 10% Lead acetate	Green	Dark Green	Dark Green	Purplish Green
16.	50% Nitric Acid	Dark Green	Orange green	Brownish green	Dark Green
17.	Concentrated Nitric acid	Brownish Green	Orange green	Purplish Green	Blackish green
18.	1N Sodium hydroxide (Alocholic)	Light Green	Dark Green	Dark Green	Purplish green
19.	1N Sodium hydroxide (Aqueous)	Light Green	Dark Green	Dark Green	Purplish green

Table 5 Antibacterial activity of *Aerva lanata* of authentic and market samples

Microorganism	Authentic sample			Market sample		
	Methanol	Ethanol	Petroleum Ether	Methanol	Ethanol	Petroleum Ether
Klebsiella pneumonia	-ve	-ve	-ve	-ve	-ve	-ve
Escherichia coli	-ve	-ve	+ve	-ve	-ve	-ve

Table 6 Anti-oxidant activity of authentic and market samples of *Aerva*

Sample	Authentic sample			Market sample		
Solvent	Methanol	Pet Ether	Ethanol	Methanol	Pet Ether	Ethanol
Percentage	42.17%	0.75%	5.97%	17.65%	2.86%	10.06%



Graph 2 Antioxidant activity of authentic and marker samples of *Aerva lanata.*

Summary

The present study is mainly focused on the Pharmacognostic, anti-bacterial activity and anti-oxidant activity of *Aerva lanata* (L.) A.L. Juss ex Schultes of market and authentic sample. The total ash content of authentic sample was found to be 5.82% and market sample 12.6%. The water soluble as was found by 24% and market sample 9%. The acid insoluble ash for authentic was found by 16% and market sample 65.5%. Fluorescence analysis was carried out for both authentic and market samples with various solvents. Phytochemical screening of authentic plant sample tested positive for alkaloids, coumarin, quinol, tannin. The market samples were tested positive for alkaloids, tannin and coumarin. The authentic plant sample extracted with petroleum ether showed inhibitory action against the gram negative bacteria *Escherichia coli.* The anti-oxidant activity was found to be highest in the methanol plant extract of the authentic sample.

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Conflicts of interest

Author declares that there is no conflict of interest.

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