International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 16-01-2022, Accepted: 31-01-2022, Published: 16-02-2022
Volume 7, Issue 2, 2022, Page No. 322-328

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

B C Jayatha, R Sumathi
Department of Botany, Mount Carmel College, Bengaluru, Karnataka, India

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, Phytochemical analysis. The total ash value, water soluble and acid insoluble value of the authentic sample and was found be 5.82%, 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*.

Keywords: pharmacognostic, *Aerva lanata (L.)*, amaranthaceae

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 (Hassan, 2012) [3]. According to World Health Organization (WHO) 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. 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. These secondary metabolites are often used by the plants for protecting themselves against the biotic and abiotic stress a plant undergoes. These secondary metabolites are used as a source of drug, flavors, fragrances, insecticides and dyes by human beings (Pagare *et al*., 2016) [8]. 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. Herbal medicines off late have 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* (L.) Juss. 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* (L.) Juss. 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

Materials and Methods
Collection of Plant Material
Aerva lanata (L.) Juss. 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*., 2015 [8] was followed. The shade dried samples were moderately fine powdered using mortar and pestle and sieved through 355 µm 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 (L.) Juss. 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.

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

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: 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

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.

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 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.

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 (Sharma *et al*., 2008) [9].

DPPH [Diphenyl picryl hydrazyl] radical scavenging assay (Mensor *et al*., 2001) [6]

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

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

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* (L.) Juss. 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, Zimbabwe, 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

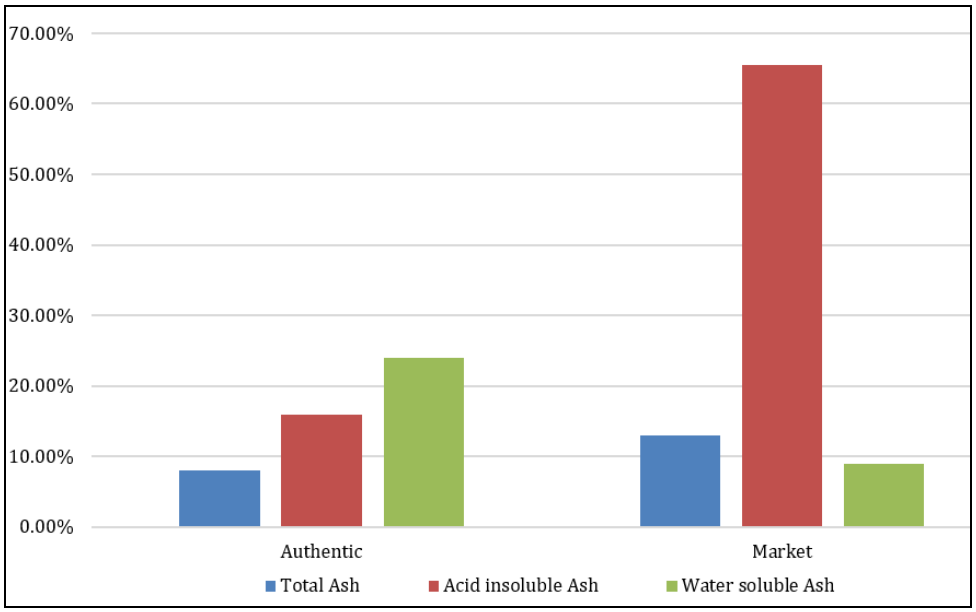
Ash analysis

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 %	12.6 %
Acid insoluble Ash	16 %	65.5 %
Water soluble Ash	24 %	9 %

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.



Graph 1: Ash analysis of authentic and Market samples

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

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

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:

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

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)

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

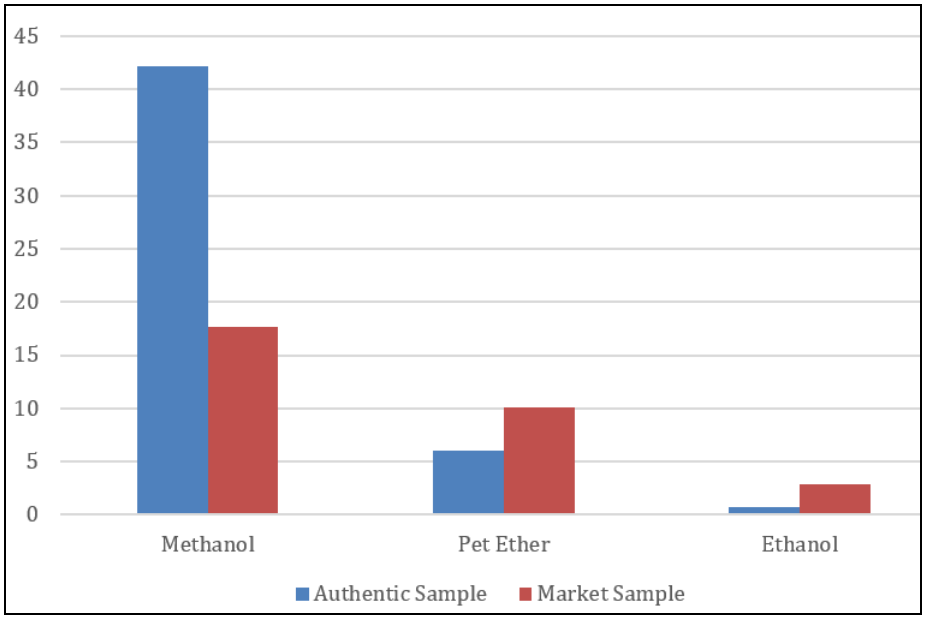
Microorganism	Authentic sample			Market sample		
	Methanol	Ethanol	Petroleum Ether	Methanol	Ethanol	Petroleum Ether
Klebsiella pneumonia	-ve	-ve	-ve	-ve	-ve	-ve
Escherichia coli	-ve	-ve	+ve	-ve	-ve	-ve

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: 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 be 24% and market sample 9%. The acid insoluble ash for authentic was found be 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.

References

1. Al-Ansari Mysoon, L.A.Al – Humaida, Vijayaraghavan P. Identification of phytochemical components from *(L.) A.L. Juss ex Schultes* medicinal plants and its *in-vitro* inhibitory activity against drug resistant microbial pathogens and antioxidant properties, 2019.
2. Behera Padma Charan, Manik Ghosh. Evaluation of antioxidant, antimicrobial, and antiurolithiatic potential of different solvent extracts of *(L.) A.L. Juss ex Schultes*,2018:14(53):53-57.
3. Rassol Hassan BA. Medicinal Plants (Importance and Uses). Pharmaceut Anal Act,2012:3(3):e139. doi:10.4172/2153-2435.1000e139
4. Kokate CK, Gokhale SB. Practical Pharmacognosy. 12th ed. Nirali Prakashan, 2008.
5. Kritikar KP, Basu BD, Mahaskar. Indian Medicinal plants. International Book Distributor, Allahabad, 1987.
6. Mensor LI, Pargare Saurabh, Manila Bhataia, Niraj Tripathi, Bansal YK. Secondary metabolites of plants and their role overview. Current trends in Biotechnology and Pharmacy,2015:9(3):293-304.
7. WHO. The promotion and development of Traditional system of medicine. WHO technical report series no, 1978, 622.
8. Raphael R Marandi, John Britto S, Prahabat K Soreng. Phytochemical Profiling, antibacterial screening and Antioxidant properties of sacred Tree (*Shorea robusta* Gaertn.) of Jharkhand. International Journal of Pharmaceutial Sciences and Research, 2016.
9. Sharma G, Vivek I, Ashish Kumar Gupta. Phytochemical composition, antioxidant and antibacterial potential of underutilized parts of some fruits. International Food Research Journal,2017:24(3):1167-1173.