



Comparative Pharmacognostic Study of Two Species of *Aerva* Juss

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Accepted on: 13-06-2013; Finalized on: 31-08-2013.

ABSTRACT

Aerva javanica Juss. and *Aerva lanata* Juss. belongs to family Amaranthaceae and is being used in the indigenous systems of medicine as a ailment for Kidney stone, diarrhoea, cholera and snakebite. These species are commonly known as pashanbhedi. The drug part is usually used as the roots. The present studies include the macroscopic, microscopic characters, histochemistry and phytochemistry. The phytochemical screening is also confirmed by HPTLC analysis for Quercetin.

Keywords: *Aerva*, HPTLC, Pharmacognosy, Phytochemical analysis.

INTRODUCTION

A *erva javanica* Juss. and *Aerva lanata* Juss. belong to family Amaranthaceae. In India, it is found in open forests, on mountain slopes, on waste and disturbed ground, deserted cultivation and coastal scrub and found at altitudes from sea level to 900metres.¹ *Aerva lanata* Juss. is an erect or prostrate shrub with a long tap root and many woolly- tomentose branches. The roots are cylindrical in shape ranges the length from 1-1.5cm in diameter and *Aerva javanica* Juss is a perennial upright herbaceous plant or small shrub and grows to a height of 30-160cm tall occasionally up to 1.6m in height. The roots are cylindrical and are measuring 10-12cm long and 1-1.8cm in diameter.² The roots of both the species are medicinally important and are commonly known as 'BUI' in indigenous system of medicine. It is used as diuretic, anti inflammatory, hypoglycemic, antidiabetic,³⁻⁶ antiparasitic, anti-microbial, hepatoprotective, anti-urolithiasis, antiasthmatic and antifertility.⁷⁻⁹ As both the drugs are used against the above mentioned disease, it is important to define specifications that will allow the correct botanical identification of the plant. In addition, there are 14 species of *Aerva* recorded in India of which this 2 species of *Aerva* are found to be growing in Maharashtra.¹⁰ Hence, *A. javanica* Juss. and *A. lanata* Juss choose for the present investigation as it is being sold widely in the market under the common name Buikallan or Boor.

MATERIALS AND METHODS

Collection and identification of plant materials

The plant materials were collected from in and around Pune district of Maharashtra for correct botanical identification. Efforts were made to collect the plants in flowering and fruiting condition for the correct botanical identification. It was identified with the help of Flora of The Presidency of Bombay.²

Microscopic and macroscopic evaluation

Thin (25 μ) hand cut sections were taken from the fresh tuberous roots, permanently double-stained and finally mounted in Canada balsam as per the plant micro techniques method of Johansen.¹¹ The macroscopic evaluation was studied by the method of Trease and Evans¹² and Wallis.¹³

Histochemical study

The thin transverse sections of fresh root were taken (about 25 μ). It was treated with respective reagent for the detection and localization of chemicals in the tissues as per the method of Krishnamurthy.¹⁴

Phytochemical evaluation

Some roots were dried under the shade so as to avoid the decomposition of chemical constituents, powdered in a blender and finally stored in dry air tied containers for phytochemical screening. Ash and percentage extractive content was measured by following the standard Pharmacopoeial techniques.¹⁵ Fluorescence analysis was carried out as per Chase and Pratt.¹⁶ Qualitative phytochemical tests were carried out by standard methods of Harborne¹⁷ and Trease and Evans.¹² Quantitative phytochemical analysis was determined for proteins, carbohydrates and flavonoid by the methods of Lowry *et al.*¹⁸, Nelson¹⁹ and Boham and Kochipal²⁰ respectively. The phytochemical screening was also done by the High Performance- Thin Layer Chromatography (HPTLC). HPTLC study was carried out on Linomat 5 for application using Densitometer-TLC Scanner 3 with "WINCATS" software (Camag, Switzerland). These studies were carried out on pre-coated aluminum fluorescent plates (E. Merck). For HPTLC studies, an extract of methanol (25% GR) solvent system was used and after development, plate was scanned at 254 and 366 nm.^{21,22}



Macroscopic evaluation

The details of the macroscopic examination are mentioned and illustrated in Figures 1 (a & b).

RESULTS AND DISCUSSION

Macroscopic examination

Aerva javanica Juss.

Characters

Herb: It is a perennial upright herbaceous plant or small shrub and grows to a height of 30-160cm tall but occasionally reaches up to 1.6 m in height.

Roots: The root is cylindrical in shape. Their length ranges from 1-1.5 cm in diameter.

Leaves: Leaves are simple (2-7 cm long) are alternately arranged along the stems and are quite variable in shape. They can range from being long and narrow to being almost round. Their upper surfaces are thinly covered in whitish hairs and are greenish in colour, while their undersides are more densely covered in these hairs and have a whitish appearance.

Inflorescence: The inflorescence is terminal spikes.

Flower: The whitish flowers are borne in elongated clusters at the tips of the branches. Both types of flowers also have five small 'petals' (i.e. perianth segments or tepals). The petals on the female flowers are larger than those on the male flowers. Female flowers also have a small ovary and a slender style with two very elongated stigmas at its tip, while male flowers have five delicate yellowish-colored stamens

Bracts: Bracts are narrow or egg-shaped in outline (i.e. lanceolate to ovate) and are at least partly covered in a dense layer of white woolly hairs. They can have rounded or pointed tips (i.e. obtuse or acute apices) and remain on the plant.

Fruit: The fruit is very small (1-1.5 mm long) capsule-like. They are rounded in shape, but somewhat flattened, consisting of a single dark brown to black colored seed.

Distribution: This species is widespread usually in the drier parts of tropical and sub-tropical Africa and Asia and also in the Indian sub-continent (i.e. in Afghanistan, India, Pakistan, Myanmar and Sri Lanka).

Aerva lanata Juss

Characters

Herb: It is an erect or prostrate woody herb found to be usually growing near sea coasts as it requires saline soil.

Roots: The root is cylindrical in shape. Their length ranges from 1-1.5 cm in diameter.

Leaves: Leaves simple alternate, 2-2 x 1-1.6 cm on the main stem, 6-10 x 5-6 mm on the branches, elliptic or obovate, or sub orbicular, obtuse or acute, entire,

pubescent above, more or less white with cottony hairs beneath; petioles 3-6 mm long, often obscure.

Inflorescence: The inflorescence is axillary heads or spikes.

Flower: Flowers are greenish white, very small, sessile, often bisexual, in small dense sub sessile axillary heads or spikes 6-13 mm long, often closely crowded and forming globose clusters; Perianth 1.5-1.25 mm long; tepals oblong, obtuse, sometimes apiculate, silky-hairy on the back.

Bracts: Bracts are 1.25 mm, long, membranous, broadly ovate, concave, apiculate.

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

Distribution

This species is widespread in the parts of Northeast Tropical Africa, Kenya, West-Central Tropical Africa: Zaire, Zimbabwe, Southern Africa, Western Indian Ocean, Madagascar, Arabian Peninsula and Saudi Arabia.

Microscopic characters

In both the species, transverse section of the roots had a circular outline. It shows 5-7 layers of cork cells, upper 2-3 layers filled with brownish content; secondary cortex a wide zone consisting of circular to oval, elongated, thin walled parenchymatous cells, most of the cells containing rosette crystals of calcium oxalate; endodermis not distinct; pericycle present in the form of interrupted ring of pericyclic fibres; anomalous secondary growth present; secondary xylem and phloem tissues in form of 3 or 4 alternating rings; medullary bundles present; phloem consisting of sieve tubes, companion cells and phloem parenchyma; xylem consists of vessels, tracheids, fibres, and xylem parenchyma; vessels circular to oval having simple pits; pith cells circular in shape containing rosette crystals of calcium oxalate. (Figure: 2(a & b) respectively).

Histochemical screening

Histochemical screening showed the presence of starch, protein, fat, saponin, tannin, sugars and alkaloids (Table 1).

Phytochemical studies

The roots had a total ash acid insoluble ash content is more in *A. lanata* as compare to *A. javanica* (Table 2a). The values of percentage extractives were higher in Petroleum ether and lower in acetone solvent (Table 2b). Fluorescence analysis was carried out to check the purity of the drug. The powder drug was observed in visible light, and then powder was treated with nitrocellulose, 1N sodium hydroxide, 1N sodium hydroxide in nitrocellulose and dried for 30 min. After this it was observed under ultraviolet light and it emits the color as shown in (Table 2c) for both the species. Qualitative analysis of the roots indicated the presence of proteins, reducing and non-reducing sugars, saponins, fats, tannin, glycoside and alkaloids (Table 2d).



Figure 1a: Habit of *A. javanica* Juss.



Figure 1b: Habit of *A. lanata* Juss.

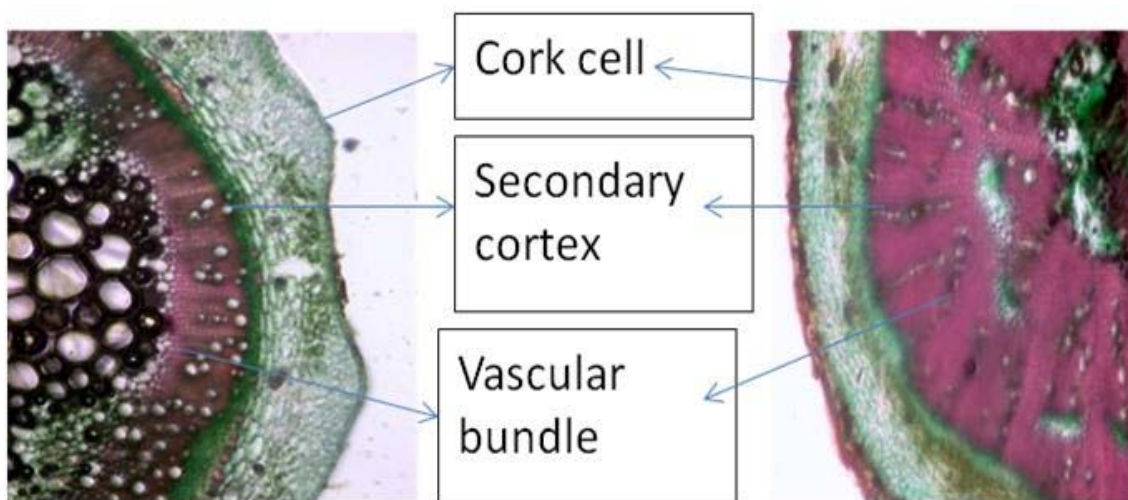


Figure 2a & 2b: Roots of *A. javanica* & *A. lanata* Juss.

Table 1: Histochemical study of *Aerva* sps.

| Test | Reagent | <i>A. javanica</i> Juss. | <i>A. lanata</i> Juss. |
|-------------|--|--------------------------|------------------------|
| Starch | I ₂ KI | Cork, Sec.cort. | Cork, Sec.cort. |
| Protein | Potassium Ferrocyanide + water + acetic acid + 60% alcohol + FeCl ₃ | Cork, Sec.cort. | Cork, Sec.cort. |
| Tannin | Acidic FeCl ₃ | Cork, Sec.cort, Xy. | Cork, Sec.cort, Xy. |
| Saponins | Conc. H ₂ SO ₄ | Cork, Sec.cort. | Cork, Sec.cort. |
| Fat | Sudan III | Cork, Sec.cort, Xy. | Cork, Sec.cort, Xy. |
| Sugar | 20% aq. NaOH | Cork, Sec.cort, Xy. | Cork, Sec.cort, Xy. |
| Glycosides | Guignard's Test | Cork, Sec.cort, Xy. | Cork, Sec.cort, Xy. |
| Alkaloids | Mayer's Reagent | Cork, Sec.cort, Xy. | Cork, Sec.cort, Xy. |
| | Wagner's Reagent | Cork, Sec.cort, Xy. | Cork, Sec.cort, Xy. |
| | Dragendorff's Reagent | Cork, Sec.cort. | Cork, Sec.cort. |
| Tannic acid | Hager's Reagent | Cork, Sec.cort. | Cork, Sec.cort. |
| | 10% FeCl ₃ | Cork, Sec.cort, Xy. | Cork, Sec.cort, Xy. |

Abbreviations: I₂KI: Potassium iodide; FeCl₃: Ferric chloride; Conc. H₂SO₄: Concentrated sulphuric acid; NaOH: Sodium hydroxide; Sec. Cort: Secondary Cortex; Xy: Xylem; Phlo: Phloem.

Analytical studies (Quercetin)

The HPTLC analysis showed that the flavonoid quercetin is confirmed from *A. javanica* Juss. and from *A. lanata* Juss. root samples. The plates were scanned at 254 and 366

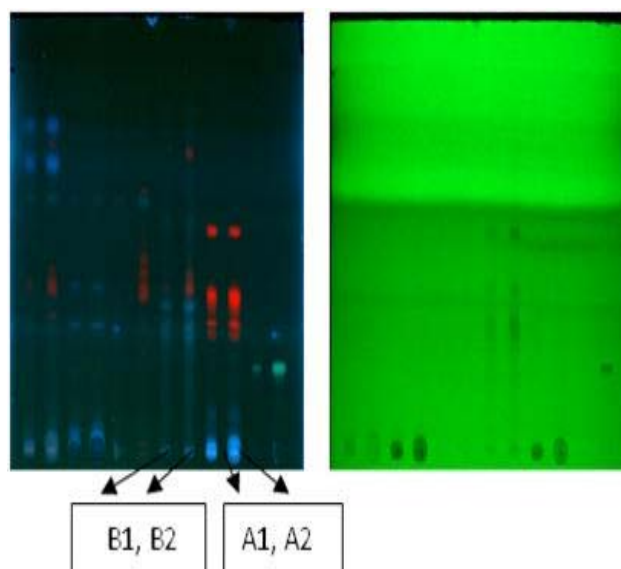
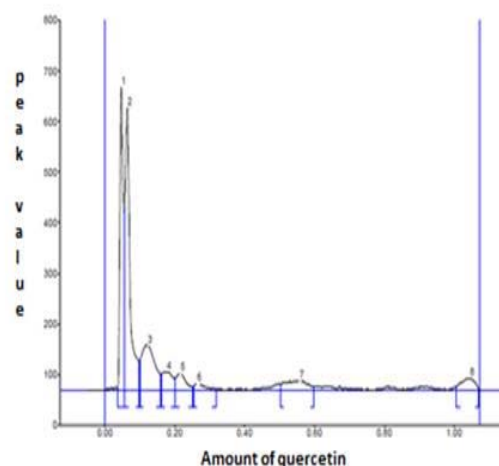
nm. When images were compared with the graph and table values, it showed maximum area at 366 nm after derivatization. The table also indicates the R_f values (Figure 3 & 4 Table 4).

Table 2: Phytochemical evaluation of *Aerva* sps.

| Parameter | Results % dry wt. | |
|---|---------------------------------|--------------------------------------|
| | <i>A. javanica</i> Juss | <i>A. lanata</i> Juss |
| a) Total Ash | 14.4% | 17.9% |
| Acid Insoluble Ash | 13.1% | 16.7% |
| b) Percentage extractives | | |
| Solvent used | | |
| Distilled water | 8% | 23.6% |
| Absolute alcohol | 10% | 17.8% |
| Petroleum ether | 4% | 30.6% |
| Benzene | 18% | 25.4% |
| Chloroform | 2% | 26.8% |
| Acetone | 0.5% | 17.4% |
| c) Fluorescence Analysis | | |
| Treatments | | |
| Powder as such. | Greenish yellow | Greenish brown |
| Powder as such in UV-light. | Greenish grey | Grayish brown |
| Powder + Nitrocellulose | Grayish black | Grayish black |
| Powder + 1N NaOH in Methanol. | Greenish black | Greenish black |
| Powder + 1N NaOH in Methanol dry for 30min. + Nitrocellulose. | Olive green with brownish tinge | Olive green with reddish brown tinge |
| d) Phytochemical test | | |
| Water extract | | |
| Starch | +ve | +ve |
| Protein | +ve | +ve |
| Tannin | +ve | +ve |
| Saponin | +ve | +ve |
| Fat | +ve | +ve |
| Sugar | +ve | +ve |
| Alcohol extract | | |
| Mayer's reagent | +ve | +ve |
| Wagner's reagent | +ve | +ve |
| Dragendorffs Reagent | +ve | +ve |
| Tannic acid | +ve | +ve |

Table 3: Quantitative estimation of *Aerva* sps.

| Quantitative estimation | <i>A. javanica</i> Juss. | <i>A. lanata</i> Juss. |
|-------------------------|--------------------------|------------------------|
| Protein | 0.653 | 1.30325 |
| Reducing Sugar | 1.8293 | 1.73210 |
| Non - Reducing Sugar | 2.62400 | 2.46210 |
| Starch | 3.15079 | 3.43128 |
| Flavonoid | 0.0691 | 0.0797 |

**Figure 3:** Detection of quercetin by HPTLC techniques**Figure 4:** Peak for Quercetin.

CONCLUSION

The plant *A. javanica* and *A. lanata* showed the correct taxonomy which is helpful for the standardization of drug. The morphological characters and histochemical study with double staining of the root, percentage extractives, fluorescence and ash analysis and the phytochemical screening of the plants. As in case of Quercetin the peaks are denoted by the Rf values. These investigations will be useful for the correct botanical identification and authentication of the drug. After getting the overall results of *A. javanica* and *A. lanata* and if data is comparable with the other species of *Aerva*, it can be used as a substitute for them.

Acknowledgements: Both the authors would like to express a sincere thank to Head, Department of Botany, University of Pune, Pune-411 007 for encouragement and necessary laboratory facilities and The first author is grateful to authorities of Pune University for providing financial support in the form of research stipend.

Table 4: Showing the value of peak for Quercetin

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max% | End Rf | End Height | Area | Area% |
|------|----------|--------------|--------|------------|-------|--------|------------|--------|-------|
| 1 | 0.04 | 1.5 | 0.05 | 599.9 | 43.84 | 0.05 | 351.8 | 4208.4 | 26.91 |
| 2 | 0.06 | 354.4 | 0.06 | 558.4 | 40.81 | 0.10 | 58.0 | 5750.8 | 36.77 |
| 3 | 0.10 | 58.6 | 0.12 | 88.1 | 6.44 | 0.16 | 31.8 | 2477.5 | 15.84 |
| 4 | 0.16 | 32.0 | 0.18 | 36.0 | 2.63 | 0.20 | 23.2 | 791.0 | 5.06 |
| 5 | 0.20 | 23.6 | 0.22 | 32.6 | 2.38 | 0.25 | 7.4 | 654.2 | 4.18 |
| 6 | 0.25 | 7.5 | 0.26 | 11.9 | 0.87 | 0.32 | 1.4 | 278.0 | 1.78 |
| 7 | 0.50 | 11.9 | 0.56 | 18.7 | 1.36 | 0.60 | 6.2 | 838.6 | 5.36 |
| 8 | 1.01 | 6.5 | 1.05 | 22.8 | 1.67 | 1.07 | 1.8 | 641.1 | 4.10 |

REFERENCES

- Hara H, The Flora of Eastern Himalaya, Japan, Tokyo University Press, 1966, 407.
- Cooke T, Flora of Presidency of Bombay, B.S.I., Calcutta, 3, 1958, 280-289.
- Nadkarni AK, K. M. Nadkarni's Indian Materia Medica, Popular Prakashan Ltd, Bombay, 3, 1927, 208-209.
- Chopra RN, Nayer SL, Chopra IC, Glossary of Indian medicinal Plants, CSIR, New Delhi, 1956, 218.
- Chandra S, Sastry MS, Chemical constituents of *Aerva lanata*, Fitoterapia, 61, 1990, 188.
- Govindarajan R, Vijayakumar M, Pushpangadan P, Antioxidant approach to disease management and the role of 'Rasayana' herbs of Aired, Jour. Ethnopharmacol, 99, 2005, 165-178.
- Anonymous, Medicinal Plants more on *Aerva*, Georgia: Agriculture and Industry Survey, 2011, 38-39.
- Kirtikar, KR, Basu BD, The Indian Medicinal Plants, Lalit Mohan Basu, Publ. Allahabad, 1933, 73.
- Kirtikar KR, Basu BD, Amaranthaceae *Aerva*. In: Kirtikar KR, Basu BD, editors, Indian Medicinal Plants, Allahabad, India, L.M. Basu Publishers, 1975, 2508-2509.
- Anonymous, *Aerva javanica* (Burm. f) Juss. ex Schult., Amaranthaceae, Pacific Island Ecosystems at Risk (PIER): plant threats to Pacific ecosystems, 2005, 34-35.
- Johansen DA, Plant Micro technique, New York, McGraw-Hill Book Co. Inc., 1940, 151-154, 182-203.
- Trease GE, Evans WC, Trease and Evans Pharmacognosy, 15th ed. W. B. Saunders Edinburgh London, New York, Philadelphia St. Louis Sydney Toronto, 2002, 3-4, 528-33, 538-547.
- Wallis TE, A Text Book of Pharmacognosy, Reprinted edition, London, Churchill, Livingstone, 1967, 578-617.
- Krishnamurthy KV, Methods in the Plant Histochemistry, Madras, Viswanadhan Pvt. Limited, 1988, 1-77.
- Anonymous, Pharmacopoeia of India, Government of India, 1st ed. Delhi, Ministry of Health Manager Publications, 1955, 370 & 864.
- Chase CR, Pratt R, Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, Jour. Am Pharm Asso, 38, 1949, 324-330.
- Harborne JB, Phytochemical Methods, London, Chapman and Hall International Edition, 2, 1973, 5-8.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, Protein measurement with the Folin-Phenol reagent, Jour. Biol. Chem, 193, 1951, 265-275.
- Nelson N, A photometric adaptation of the Somogyi method for the determination of Glucose, Jour. Biol. Chem, 153, 1944, 375-380.
- Boham AB, Kocipai AC, Flavonoid and condensed tannins from Leaves of *Hawaiian vaccinium*, *vaticulum* and *vicalycinium*, Pacific Sci, 48, 1994, 458-463.
- Wagner H, Baldt S, Plant Drug Analysis, A Thin Layer Chromatography Atlas, Berlin, Springer – Verlag, 1996, 129, 144, 155, 157, 176, 178, 206.
- Reich E, Schibii A, High Performance- Thin Layer Chromatography for the analysis of medicinal plants, Germany, Thieme medical publishers Inc, 2007, 129-160, 206-210, 224-240.

Source of Support: Nil, **Conflict of Interest:** None.