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Yellow and White Flowered Variants of Aerva Lanata: A Phytochemical Variation Study

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ABSTRACT *White and yellow flowered variants of Aerva lanata were analyzed for various classes of compounds in order to study the extent and cause of variation between them. Cold extraction of the plant material was performed using methanol to extract free phenolics while hot extraction in mild alkaline condition was done to extract bound phenolics. Total Phenolic Content assayed using Folin-Ciocalteau method showed similar phenolic content. Methoxy kaempferol has been identified for the first time in the flowers of both variants. The yellow variant showed slightly higher flavonoid content than the white variant, especially when subjected to mild alkaline extraction using Na2CO3 solution. Phenolic acids identified were similar except that melilotic acid was found only in the yellow variant. Chlorophyll content estimated in the white variant was very high as compared to the yellow counterpart. HPLC fingerprint comparison of the methanolic extracts revealed phytochemical variation in the two samples.*

Introduction

Aerva lanata Juss ex Schultes, occurs as a common weed found in all plains districts and upto 900 metres elevation. It is widespread in the drier parts of the tropics and subtropics of Africa and Asia (Sivarajan & Indira, 1994). Plant decoction is diuretic and is given to remove bladder and kidney stones. It is also used to treat diarrhoea (Warrier, Nambiar & Ramankutty, 1994). Leaf paste is mixed with gingelly oil and given to treat piles. Leaf and root paste is applied to treat pimples and skin infections. Leaf decoction is anthelmintic and demulcent, while root and flower decoction is given to treat headache. Root decoction is used as an antidote for snakebite. Root powder is used as tooth paste to treat toothache. Whole plant is used to treat cough, boils, lithiasis (calculus formed by inorganic salts) and pus.

The plant is known to contain β-sitosterol, α-amyrin, hentriacontane, campesterol, stigmasterol, stigmasterol acetate, daucosterol, β-sitosterol palmitate, ergosterol, lupeol, β-amyrin, olean-12-en-28-oic acid-3,16-dioxymethyl ester (Aiyer *et al*, 1973; Aboutabl, 1996; Chandra & Sastry, 1990; Wassel & Amnar, 1987), kaempferol, kaempferol-3-galactoside, kaempferol–rhamnogalactoside, starch, free sugars such as fructose, galactose, rhamnose and sucrose (Afaq, Tajuddin & Afridi, 1991), alkaloids like canthin-6-one, β-carboline-1-propionic acid, 10-methoxy-canthin-6-one, 10-hydroxy-canthin-6-one, 10-O-β-glucopyranosyloxy canthin-6-one, 6-methoxy-β-carboline 1-propionic acid, aervoside, aervolanine, flavonols like aervitrin, narcissin and a flavone chrysin (Zapesochnaya *et al*, 1991; 1992a; 1992b; Zadorozhnii, 1986, Pervykh; 1992). The plant also shows the presence of saponins and phenolic acids such as vanillic and syringic acids (Mangalan, 1988).

Certain plants of *Aerva lanata* showed variation in colour of the flowers during winter and summer. Though normally this plant has white inflorescence, some of the plants growing in close vicinity of them possessed deep yellow coloured flowers. The difference in colour of the flowers was evident in visible light but the difference was more pronounced when observed under ultraviolet light. The yellow flowers exhibited bright yellow fluorescence under the ultraviolet light (365 nm), while the white flowers appeared dull under the same light (**Plate 1**). The yellow colour was observed both in visible light as well as under UV light indicating the presence of compounds that have absorbance in both these regions of light. The flowers could have some phytochemical that absorbs in visible range and another that absorbs in ultraviolet range, or they could possess some compounds that have absorbance in both regions.

Materials and methods

Aerva lanata collected was identified and authenticated at Botanical Survey of India, Pune. The voucher specimen of this plant (No.BSI/WC/Tech/2007/734) is deposited at the Herbarium, B.S.I., Pune. The flowers were shade dried for a day and then dried completely in an oven at 38°C. The dried flowers were coarsely powdered using a rotary grinder and stored in airtight plastic containers to be used for all analysis.

Five grams each of both samples of the plant were subjected to soxhlet extraction using methanol. The methanolic extracts were distilled *in vacuo* and the residue was reconstituted in distilled water. These extracts were hydrolyzed using 7% HCl in a water bath. Hydrolyzed components such as flavonoids and other phenolics were extracted using diethyl ether (25x3). The three aliquots of diethyl ether were pooled and the solvent was removed *in vacuo*. The dry residue was reconstituted upto 100 mL in a volumetric flask using methanol, and further used for determination of Total Phenolic Content and Total Flavonoid Content.

Fifty grams of the powders of both variants were subjected to extraction in methanol, using Soxhlet's apparatus. The methanolic extracts were further analyzed for identification of flavonoids and phenolic acids using ascending paper chromatography techniques (Harborne, 1984; Mabry, Markham &Thomas, 1970; Ibrahim & Towers, 1960).

The plant material remaining after soxhlet extraction using methanol was further subjected to hot extraction using 1M Na_2CO_3 solution in order to extract the bound phenolics under alkaline condition. The flowers were boiled in 100 mL of 1M Na_2CO_3 solution in a beaker for an hour, cooled and filtered. The filtrate was neutralized to pH 2 using 2N HCl solution. The neutralized solution was subjected to acidic hydrolysis in HCl in a water bath. The hydrolyzed components were extracted using diethyl ether, solvent removed, and dry residue reconstituted in methanol in 100mL volumetric flasks. Total Phenolic and Total Flavonoid Contents were determined for the two flower samples.

Chlorophyll estimation: Determination of Total Chlorophyll, Chlorophyll a, and Chlorophyll b were determined spectrophotometrically (Harborne, 1984). One gram each of fresh yellow and white *Aerva lanata* flowers was ground in a mortar and pestle in presence of 10ml acetone, and 0.5g of $CaCO_3$

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to prevent pheophytin formation. The extract was filtered using Buchner funnel. The plant residue was treated with another aliquot of acetone and filtered again. Extraction and filtration were repeated till colourless. All the washings were pooled and volume made upto 100ml in a volumetric flask. Absorbance of the samples was measured at 663nm and 646nm in 1cm cells. The concentrations of chlorophyll were calculated using the following formulae.

Total chlorophyll (mg L^{-1}) = 17.3 A_{646} + 7.18 A_{663}

Chlorophyll a (mg L^{-1}) = 12.21 A_{663} – 2.81 A_{646}

Chlorophyll b (mg L^{-1}) = 20.13 A_{646}– 5.03 A_{663}

Total Phenolic Content: Total Phenolic Content was measured using the Folin Ciocalteau method (McDonald, Prenzler, Autolovich & Robards, 2001). Dilute methanolic extracts of the samples and different concentrations of gallic acid standard (0.5 ml each) were mixed with Folin Ciocalteau reagent (5ml, diluted 1:10), and aqueous sodium carbonate (4ml, 1M solution). The mixture was allowed to stand for 15 minutes and the absorbance read at 765nm on a Shimadzu Lambda'25 spectrophotometer against reagent blank. Each sample was analysed in triplicate. Standard curve was prepared using 0, 10, 50, 100, 200, 300 mg L^{-1} solutions of gallic acid in methanol: water (50:50 v/v). Total Phenolic Content is expressed in terms of gallic acid equivalents (mg per gram of dry mass).

Total Flavonoid Content: Total Flavonoid Content was estimated by the aluminium chloride chelation method (Chang, Yang, Wen & Chern, 2002). To each sample (0.5 ml), 1.5 ml of methanol was added, followed by addition of 0.1 ml of 10% aluminium chloride solution in methanol, 0.1 ml of 1M potassium acetate solution and 2.8 ml of distilled water. After being allowed to stand for 30 minutes the absorbance was recorded at 415nm on a Shimadzu Lambda'25 spectrophotometer against reagent blank. Calibration curve was prepared using 0, 50, 125, 250, 500 mg L^{-1}solutions of quercetin standard in methanol.

HPLC analysis: Methanolic extracts of the flowers of the two variants were subjected to HPLC analysis on a Shimadzu LC 20AT instrument equipped with a photodiode array detector. The samples were analyzed on a GraceSmart RP C18 column (250x4.6mm, 5µ), with the detection wavelength set at 420 nm. The mobile phase consisted of acetonitrile as Solvent A and 2% acetic acid in water (v/v) as Solvent B. The flow rate was kept at 1 ml per minute for a total run time of 30 minutes and the gradient program was as follows: 0% B for 15 minutes, 0%B to 10%B in 5 minutes, 10% B to 25% B in 5 minutes, 25% B to 50% B in 5 minutes, 50% B to 0% B in 10 minutes. Post run reconditioning time for the column was done for 10 minutes between successive runs. Injection volumes were 20µl and the samples were analyzed in duplicate. All samples were filtered using 0.45µm Acrodisc syringe filter (Pall Corporation, India) prior to analysis.

Results and Discussion

Comparative account of the various parameters studied for the two variants of *A. lanata* flowers are shown in **Table 1**. Dimensions of the white flowers are found to be higher than those of the yellow flowers. However, the leaves show completely converse results wherein the leaf of the yellow variant are observed to grow longer and broader than the white counterpart. Total Chlorophyll Content in the white variant is observed to be almost 5 times in amount as compared to the yellow variant. Similar results are shown in the Chlorophyll a and Chlorophyll b Contents of the two flowers. Total Phenolic Content did not vary much except that the values in the yellow flower extracts were slightly higher than the white ones, but the difference is not very significant. Total Flavonoid Content in the methanolic extracts showed similar values. However, its value in the Na_2CO_3 extract of the yellow variant was more than twice the value of that of the white variant. Melilotic acid identified in the yellow variant was found absent in the white variant. Other phytochemicals identified were similar in both samples. HPLC chromatograms of both samples showed 18 peaks in the white variant, and 52 peaks in the yellow variant (**Figure 1**). Peaks at retention times 9.67, 11.26 and 24.20 minutes were significant in the yellow variant, but absent in the white variant. Ultraviolet spectra of all the three peaks showed maximum absorption at 400nm, pertaining to the yellow region of the visible range (**Figure 2**). All other peaks were similar in both samples, except for some quantitative differences.

Conclusion

The two variants observed in *Aerva lanata* displayed morphological variation with respect to dimensions of flowers and leaves. Two methods of extraction of phenolic compounds did not yield very significant difference in results except that the yellow variant showed slightly higher flavonoid content than the white variant. Melilotic acid identified in the yellow flowers were absent in the white flowers. Other compounds identified were similar. HPLC chromatograms of the two samples showed variation, indicating difference in chemical constitution. The most significant observation was that of the immense variation in the chlorophyll content in the white variant, which could be responsible for masking the yellow colour of the flavonoids. This makes the flowers appear greenish white in normal light and completely pink under UV 365nm light. This is supported by the fact that chlorophyll exhibits bright pink fluorescence under ultraviolet light. The chlorophyll content being quite less in the yellow variant, the flowers show bright yellow fluorescence under ultraviolet light.

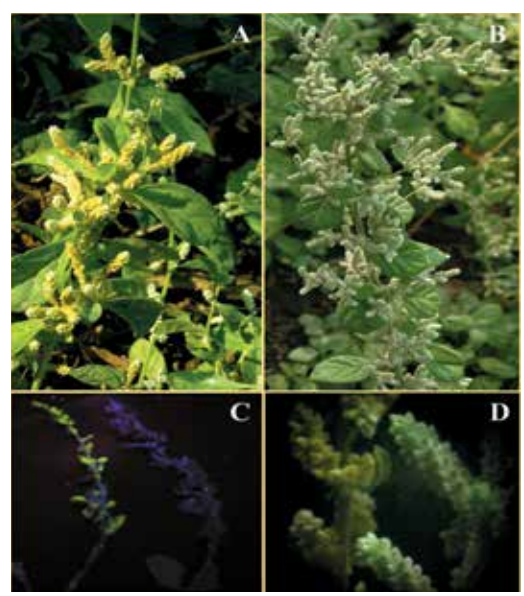

Plate 1: *Aerva lanata* **showing yellow inflorescence (A), white inflorescence (B) in habitat. Both the variants as observed under UV 365nm light (C), and under dissection microscope (D).**

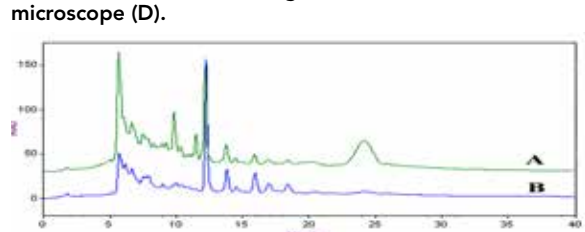

Figure 1: HPLC chromatograms of the methanolic extracts of yellow flowers (A), and white flowers (B).

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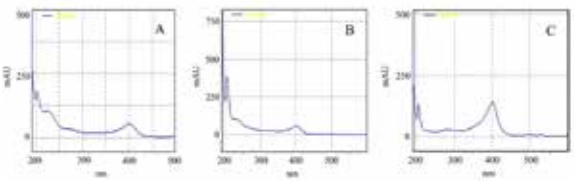

Figure 2: Ultraviolet Spectra of peaks detected at 9.67 min (A), 11.26 min (B), and 24.20 min (C) during HPLC chromatographic run of yellow variant of *A. lanata*.

Table 1: Parameters studied to locate variation in the variants of *Aerva lanata*

Parameter analyzed	Yellow variant	White variant
Physical Parameters (mm)		
Flower Length	120	200
Flower Breadth	25	35
Leaf Length	280	210
Leaf Breadth	160	120
Chlorophyll Content (mg L^{-1})		
Total Chlorophyll Content	3.819	19.431
Chlorophyll a Content	2.031	8.469
Chlorophyll b Content	1.791	10.978
Phenolics identified		
Flavonols	Kaempferol, 4'-Methoxy kaempferol	Kaempferol, 4'-Methoxy kaempferol
Phenolic acids	Vanillic, syringic, *p*-hydroxy benzoic, *p*-coumaric, ferulic and melilotic acids	Vanillic, syringic, *p*-hydroxy benzoic, *p*-coumaric and ferulic acids
Total Phenolic Content (mg g^{-1})		
Soxhlet methanolic extract	0.958	0.878
Na_2CO_3 treated extract	0.456	0.366
Total Flavonoid Content (mg g^{-1})		
Soxhlet methanolic extract	5.726	5.752
Na_2CO_3 treated extract	3.443	1.314

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