



Evaluation of Flavonoid Contents

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Research Paper—Botany

Presented investigation describes of the isolation, identification and quantification estimation of flavonoid contents from leaves and fruits of *Eclipta alba* and *Petalium mures*.

Materials and Methods-Leaves and fruits *Eclipta alba* and *Petalium murex* were collected from study area. These were washed with tap water to remove dust, wiped off with cotton and separately cut to small pieces. The plant parts were dried at 100 oC for 15 min. to inactivate enzymes followed at 60 oC till the constant weight was achieved in each case.

Extraction Procedure-The various dried samples were separately soxhlet extracted (Subramanian and Nagarajan, 1969) in 80% ethanol (100 ml/gm dry weight) on a water bath for 24 hours. Each of the extract was concentrated and the concentrate re-extracted in petroleum ether (40 – 600 c: fraction I), ethyl ester (fraction II) ethyl acetate (fraction III) in succession. Each of the steps was repeated three times to ensure complete extraction in each case. Fraction I was rejected due to its being rich in fatty substances where as fraction II was analyzed for free flavonoids and fraction III for bound flavonoids in each of the sample. Fraction III of each of the test sample was hydrolyzed by relaxing with 7% sulphuric acid (mg/gm residue) for two hours. The mixture was filtered extracted with ethyl acetate in separating funnel.

Chromatography- a. Thin layer chromatography for flavonoids: The glass plates (20 x 20 cm) coated (0.2 – 0.3 mm thick) with silica gel G (30 gm/60 ml) were dried at room temperature. The dried activated at 100 oC for thirty min. in an

oven and cooled at room temperature. Ethyl ether and ethyl acetate fraction from each of the test sample were separately applied 1 cm above the edge of the plates along with the standard reference compound (Apigenin, Kaempferol, Luteolin, Quercetin and Vitexin).

Several other solvent mixture such as ethyl acetate saturated with water, acetic acid (6:4); forestall system (acetic acid, conc. HCl and water; 10:3:30) were also tried. The solvent mixture of n-butanol, acetic acid, water (4:1:5) gave the best results in all the cases examined. The developed plates were air dried and visualized under UV light which showed two fluorescent spot in both the fractions II and III in all the instances co-including with those of the standard sample of Quercetin (blue, Rf 0.82) and Kaempferol (Bright yellowish blue, Rf 0.93). The plates were placed in a chamber saturated with ammonia vapors to observe the color of the spots (Quercetin, Deep-yellow; Kaempferol, light yellow). On spraying the developed [plates with 5% ethanolic FeCl₃ solution which also showed only one spot (in both the fraction II and III) in which fraction was considered with that of the reference of Quercetin (blueish gray) and fraction III with Kaempferol (brownish). The Rf values were calculated an average of the 5 replicate.

b. Preparative thin layer chromatography-The glass plates (20 x 20 cm) thickly coated (0.4 – 0.5 mm) with silica gel (45 gm/ 80 ml water) and activated at 100 oC for 30 min and cooled at room temp. were used for preparative thin layer chromatography (PLC). The extract of both the fraction (II and III) were applied on separate

plates and developed plates were air-dried and visualized under UV light. Each of the florescent spot coinciding with those of the standard reference compounds of Quercetin and Kaempferol were marked.

Quantitative Estimation- Quantitative estimation of the identified flavonoids was carried out calorimetrically following the method of Kariyone et al. (1953) and Naghhski et al. (1975) in case of quercetin; and Mebry et al. (1970) in case of kempferol. Stock solution (25 micro gm/ml) of querceting and kempferol were separately prepared by dissolving the authentic sample in methanol. Six conc., (25 micro gm/ml – 150 micro gm/ml) of each of the standard samples were spotted on silica gel coated and activated plates. Separate plates of each of the conc. Of quercetin and kaempferol were used and these chromatograms were developed in the same solvent system as used for the quantitative method (n-butanol; acetic acid; water, 4:1:5, upper layer). Such developed chromatograms were air dried and visualized under UV light. The fluorescent spots were marked and collected along with the adsorbent in separate test tubes the mixture shaken vigorously, centrifuged and supernatants collected separately. The volume of the replicates was made up to 10 ml by adding spectroscopic methanol. To each of these sample 3 ml of 0.1 mole AlCl₃ was added, stopper tightly and the mixture shaken vigorously. Such tubes were kept at room temp. for 20 min. Five such replicates were prepared in each case and optical density is measured using spectronoic – 20 colorimeter set at 440 nm for quercetin and 423 nm for kaempferol against a blank (10 ml spectroscopic methanol + 3 ml 0.1 mole AlCl₃).

Result and discussion- Presence of quercetin (Rf 0.82, bluish yellow) and kaempferol (Rf 0.93, bright yellowish blue) was confirmed in all the selected samples. The presence of quercetin and kaempferol was confirmed by superimposable IRE spectra of the isolated and reference compound. Among the plant samples tested the flavonoid contents were found to be maximum (6.30

mg/g d.w.) in leaves of *Eclipta prostrata* and minimum (3.30 mg/g d.w.) in the fruit of *Pedaliium murex*. The maximum quercetin (0.95 mg/g d.w.) in the leaves of *Eclipta prostrata*, while minimum (0.08 mg/g d.w.) in the leaves of *Pedillium murex*.

The maximum amount of kaempferol (5.20 mg/g d.w.) was found in the leaves of *Eclipta prostrata* and minimum (3.20 mg/g d.w.) in fruits of *Pedaliium murex*.

Table 4.1 Flavonid Contents (in mg/g d.w)

Plants	Plants Parts	Quercetin	Kaempferol	Total
				Contents
Eclipta alba	Leaves	0.10	5.20	6.30
	Fruits	0.95	5.10	6.05
Pedaliium murex	Leaves	0.08	3.60	3.60
	Fruits	0.10	3.20	3.30

EVALUATION OF STEROL CONTENTS-The present investigation describes the isolation, identification and quantitative estimation of sterol contents from various plant parts of *Eclipta prostrata* and *Pedaliium murex* collected from study area.

Material and Method-Fully matured and healthy plant parts of selected plant species were collected from study area.

Material and Method-Each of the dried samples were hydrolyzed with 30% HCl (2 gm/20 ml) for 4 hours on a water bath. The hydrolyzed test samples were filtered and washed with distilled water till the filtrate-attained pH 7.0. Test samples so obtained were dried at 60 oC for eight hours and Soxhlet extracted in benzene (200 ml) for 24 hrs (Nag et al., 1979) separately.

Thin layer chromatography (TLC)-Each of the crude extract applied separately on the silica gel G coated and activated thin glass plates along with standard reference samples of sterols (-sitosterol, campesterol, cholesterol, lanosterol and stigmasterol). The plates were developed in an organic solvent which is a mixture of benzene and ethyl acetate (85:15) (Heble et al., 1968), air-dried sprayed with 50% H₂SO₄ and subsequently heated at 100 oC. Two colored coinciding with those of standard samples of the -sitosterol (Rf. 0.52) and stigmasterol (Rf. 0.49) were observed

in the plant samples. A few other solvent system (hexane: acetone, 80:20; benzene : ethyl acetate, 3 : 1) were also tried but benzene and ethyl acetate solvent mixture (85:15) gave excellent result in the present investigation.

Preparative thin layer chromatography-

Each of the extract along with standard reference sterols were applied separately on thickly (0.3 mm – 0.4 mm) silica gel G coated and activated glass plates.

Quantitative estimation-Quantitative estimation of β -sitosterol and stigmasterol in each of the test samples was carried out using the method of Das and Banerjee (1980). A stock solution of β -sitosterol and stigmasterol in chloroform (500 micro gm/ml) was separately prepared, from this 6 conc. (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6) were prepared and spotted on silica gel G coated and activated glass plates. The plates were developed in a solvent system of benzene and ethyl acetate (85 : 15). Such developed chromatographs were air dried and exposed to iodine vapors. Iodine positive spots were marked and heated to evaporate excess of iodine. The spots were scrapped along with silica gel and each was eluted with 5 ml of chloroform in test tubes. Each of the tube was centrifuged, supernatants taken, solution evaporated to dryness and processed further. To each of the dried samples 3 ml of glacial acetic acid was added and shaken on a vortex mixture at room temp. for 1 min. and then immersed in crushed ice. To this frozen sample, 2 ml freshly prepared chromogenic reagent (0.5 ml of 0.5% anhydrous ferric chloride in glacial acetic acid and 100 ml of conc. Sulphuric acid; Klyne, 1965) was added drop wise at 0°C and mixed thoroughly. Each of the reaction mixture was incubated at 40°C for 30 min. Optical density of each of the test sample was taken in a Spectronic – 20 colorimeter set at 540 nm against a blank (3 ml glacial acetic acid & 2 ml of chromogenic reagent). Five such replicates run for each of conc. to minimize the standard deviation and average optical density was plotted against their respected conc. to complete a regression curve, which followed

Beer's law.

Each of the extract dissolved was spotted along with β -sitosterol and stigmasterol on silica gel G and activated glass plates and developed in benzene and ethyl acetate (85 : 15). The two spots coincided with those of the authentic of β -sitosterol and stigmasterols were marked in all the samples of selected plant species. Each of the elute was dried, taken up in 5 ml of chloroform and processed as described above, conc. of β -sitosterol and stigmasterol were calculated (mg/g d.w.) by comparing the optical density of the experimental samples with regression curve of standard β -sitosterol and stigmasterol separately. Five such replicates were examined in each case and mean values calculated. Result and Discussion- β -sitosterol and stigmasterol were conformed by Co-TLC (Rf. β -sitosterol, 0.52 and stigmasterol, 0.49), M.Pt. (β -sitosterol, 139 – 140°C and stigmasterol 132 – 133°C) and superimposable IR spectra of isolated and the authentic samples of sterol. Maximum sterol contents were observed in fruits of *Eclipta prostrata* (3.04 mg/g d.w.) when compared with other plant parts of *Pedalium murex*, whereas, it was minimum in root of *Pedalium murex* (1.73 mg/g d.w.)- β -sitosterol contents were maximum in shoots of *Eclipta prostrata* (2.14 mg/g d.w.) where as it was minimum in root of *Pedalium murex* (1.034 mg/g d.w.) stigmasterol contents were maximum in fruits of *Eclipta prostrata* (0.92 mg/g d.w.) where as it was minimum in shoots of *Pedalium murex* (0.08 mg/g d.w.).

This investigation indicates that sterols can be isolated from intact part of medicinal plant species growing at Kalanaur area of Rohtak district. The medicinal plants of this zone have sufficient amount of sterol content, which can be a good source of phytosterol.

FINDINGS AND CONCLUSIONS-The xerophytes type of flora dominates in the district. The district is inadequately wooded and some parts are practically bare of trees. Weeds plants found in the district are indirain, asgandha, glo, kharanthi, bhakra and datura santi chenopodium, amaranthus,

argemone, asphodelus, convolvulus, calotropis, orobranche, citrullus, opium, achyranthus, colnum surrettence etc.

Table – 4.2 Sterol Concentration (mg/g d.w.)

Name of Sterol	Eclipta Prostrata			Pedalium Murex		
	Roots	Shoots	Fruits	Roots	Shoots	Fruits
-Sitosterol	1.76	2.14	2.12	1.04	1.26	1.32
Stigmaststerol	0.87	0.67	0.92	0.69	0.48	0.72
Total Sterol contents	2.63	2.81	3.04	1.73	1.74	2.04

Climatological Studies: Rohtak area have dry climate with large variation in temperature and has scanty rainfall. In Rohtak scanty rainfall occur because only when the westward moving monsoon depression reaches. During the period from January 2007 to November 2007, the maximum (142.6 mm) rainfall was recorded in June 2007 while minimum 0.0 mm in November 2007.

Temperature-The average maximum and minimum temperature (oC) was taken. During the period from January 2007 to November 2007 the average maximum temp. (40.7 oC) was recorded in May, while average minimum temp. (4.03 oC) was recorded in the month of January as in Table –3.2.

Relative Humidity-Data on relative humidity during the period January, 2007 to November, 2007 (Table – 3.3) reveal that the highest values were observed in the month of January, February, October and November. After the rainy season, the value of relative humidity decreases in September to November and then increases in December to February.

Soil analysis-The present investigation deals with various mechanical, physical and chemical characteristics of soil of Rewari area. Soil samples were collected from three different sites i.e. Bawal, Bolni, Dharuhera. Soil samples at 0 – 22 cm (superficial) and 22 – 45 cm (deep) were taken and packed in polythene bags and brought to the laboratory for analysis. The mechanical analysis of soil is to determine the percentage of various size particles-Sand, Silt and Clay present in the soil. The maximum percentage of sand was observed in Kalanaur (82%) at 0 – 22 cm depth. The water holding capacity was comparative high in Kalanaur area at 22 – 45 cm depth.

The amount of bulk density was observed maximum in Meham soil (2.16 g/cm³) at 0 – 22 cm depths. The maximum pH value was estimated in Meham (8.75) at 22 – 45 cm depth. The result of soil pH analysis indicates that the soils of the sites are alkaline. Maximum electrical conductivity was found in Kalanaur soil (0.86 mmhos/cm) at 22 – 45 cm depth (Table – 4.3). CaCO₃ percentage was found to be maximum in Meham (14.05%) at 22 – 45 cm depth. Semi-arid zone soils are characterized by low organic content for two reasons – scanty vegetation and high temperature for its rapid oxidation.

In the present study, organic carbon content was found to be maximum in Meham (0.16) at 0 – 22 cm depth. The amount of available Nitrogen was found to be maximum in soil of Meham (25 kg/hac) at 22–45 cm depth. The value of available phosphorous was observed maximum in Rohtak soil (65 kg/ha) at 22 – 45 cm depth.

The quantity of available potassium was maximum (700 kg/ha) in the soil of Rohtak at 22 – 45 cm depth (Table – 4.3). The quantity of micro nutrients like Zinc and Manganese was observed higher (0.72 ppm) in Kalanaur soil at 0 – 22 cm depth, but the quantity of Copper was comparatively higher (0.72 ppm) in Bachod at 0 – 22 cm depth (Table – 4.3).

ETHNOBOTANICAL ASPECT OF SELECTED WEEDS SPECIES-The name of plant, its family, local name, habitat, morphological characteristics, flowering and fruiting, economic as well as medicinal uses have been described. 18 medicinal plant species selected from study area which have great medicinal values are given below:

1. Argemone maxicana
2. Capparis deciduas
3. Citrullus colocynthis
4. Convolvulus microphyllus
5. Datura innoxia
6. Calotropis procera
7. Boerhaavia diffusa
8. Solanum surattense
9. Tephrosia purpurea
10. Withania somnifera
11. Eclipta prostrata
12. Padiium murex
13. Achyranthes aspera
14. Momordica dioica
15. Cynodn dactylon
16. Saccharum spontanium
17. Chenopodium muralae

EVALUATION OF FLAVONOID

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REFERENCES

- * Abhichandani, C.T.; Krishnana, A.; Bhatt, P.N. and Rakhecha, P. (1967). Some observations on soil moisture changes in arid zone soils. *J. Ind. Soil Sci.* 15 (1) : 7 – 15. * Acharya, J. (1999). Ecophysiological and phytochemical studies of some arid zone plants, Ph.D. Thesis, M.D.S. University, Ajmer (India). * Aggarwal, R.K. and Lahari, A.N. (1981). * Evaluation of soil fertility status of stabilized and unstabilised dunes of the Indian desert *Agrochemica.* 25 : 54 – 60. * Anonymous. (1985). *The Wealth of India: Raw Materials.* Vol. 1 (Revised edition), CSIR, New Delhi. * Anonymous. (1988). *The Wealth of India: Raw Materials.* Vol. 2 (Revised edition), CSIR, New Delhi. * Anonymous. (1992). *The Wealth of India: Raw Materials.* Vol. 3 (Revised edition), CSIR, New Delhi. * Bakshi, T.S. (1954). The vegetation of Pilani and its neighbourhood. *J. Bombay Nat Hist. Soc.* 52 : 484 – 514. * Bakshi, T.S. and Bhandari, M.M. (1974). * Famine foods in the Rajasthan Desert. *Econ. Bot.* 28 : 73 – 81. * Batesmith, E. (1969). * Flavonoid patterns in the mono cotyledons. In: prospective in photochemistry (Eds.) Harborne, J.B. and T. Swamin. Academic Press, London. * Bhanari, M.M. (1978). *Flora of Indian Desert.* Scientific publishers, Jodhpur. * Bhanari, M.M. (1990). *Flora of Indian Desert.* M.P.S. publishers, Jodhpur. * Bhanari, M.M. (1995). *Flora of Indian Desert.* II ed. M.P.S. publishers, Jodhpur. * Biswas, K. (1952). Desert vegetation. *Bull. Nat. Inst. Sci., India,* 1 : 247. * Biswas, K. and Rolla. Rajputana desert vegetation. *Proc. Nat. Inst. S.R. Sci., India.* 19 B : 411 – 421. (1953). * Harsh, M.L. (1982). * Primary and Secondary products from medicinal plants of Indian arid Zone in vitro and in vivo tissue cultures. Thesis, University of Rajasthan, Jaipur, India. Jain, S.C. and Khanna, (1973). * Production of sterol from *Sesamum indicum* P. linn., tissue cultures, *Indian J. Exp. Biol.* 35 : 163 – 164. * Jain, S.C.; Khanna, Quartering from the seeds and in vitro tissue R. and Khanna, P. (1975). Cultures of *Datura* spp. *Indian J. Exp. Biol.* 13 : 83 – 84. * Jhja, B. (2000). Implications of intensive agriculture on soil and water resources: some evidence from Kurukshetra district. *Indian J. Agri. Eco.* 55 (2): 182 – 193. * Joshi, D.C. and Dhir, R.P. (1981). * Distribution of different forms of copper and zinc in the soils of extremely arid part of western Rajasthan. *J. Indian Sci. Soil.* 29; 379 – 381. * Kapoor, L.D.; Kapoor, S.L.; Srivastava, S.N.; Singh, A. and Sharma, P.C. (1971). * Survey of Indian Plants for saponins, alkaloids and flavonoids. *Lloydia* 3

BOOKS—Merrill. A. Ross; Carole. A. lembi Applied Weed Science. 2nd Ed. UNI. West Lafayette, Indiana. * Rao. V.S. Principle of weed Science. 2nd Ed. International consultant; Weed Science Santaclava California U.S.A. * Holm Leroy; Doll Zerry; Holm Eric : World Weeds-Natural Histories and Distribution John Will & Sons Inc. * Singh M.P.; Dey Soma Indian medicinal plants Satish serial publishing house.