WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 12, 118-127. Research Article ISSN 2277–7105

COMPARATIVE EVALUATION OF THE FLAVONOIDS CONSTITUENTS IN SOME VERBENAEUS SPECIES CULTIVATED IN EGYPT.

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Article Received on
14 August 2017,
Revised on 03 Sept. 2017,
Accepted on 24 Sept. 2017
DOI: 10.20959/wjpr201712-9621

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ABSTRACT

Phytochemical screening of *Tectona grandis* (1), *Verbena bipinnatifida* (2), *Duranta repens* (3), *Duranta lorentzii* (4), *Caryopteris incana* (5), *Gmelina arborea* (6), *Gmelina hystrix* (7) and *Verbena hybrida* (8) plants showed the presence of flavonoid compounds. Quantitative analysis of total flavonoid compounds using Aluminium chloride reagent revealed the presence of (42.80., 74.10, 67.20, 96.20, 112.80, 27.40, 32.40 and 57.60 µg Quercetin Equivalent (QE) per 100 g dried sample weight), respectively. HPLC analysis of flavonoids revealed the presence of 24 peaks all of them were qualitatively identified and quantitatively estimated. The flavonoids were represented as five C -glycosyl flavones, four flavones, eight flavonol glycosides, five flavanones and two 2,3 dehydro flavone. Luteolin-6-arabinose-8-glucose was a major component of plants 3,4,5 and 6 (1206.81, 2487.41, 1464.29 and 1570.66), While Apigenin-6-arabinose-8-galactose was a major component of plants 4 and 5 (403.95, 223.59), Apigenin-6- rhamnose-8- glucose was a major component of plant 3 (785.24), Apigenin-6-glucose-8-rhamnose was a major component of plant 8 (480.46), Luteolin-7-O- glucose was a major component of plant 4 (299.89), Narengin was a major component of plant 5 (5787.49), Hesperdin was a major component of plants 2,3, 4,5,7 and 8 (3826.90, 1024.50, 1568.32, 1501.16, 1113.65 and 1104.40), Rutin was a major component of plants 3 and 4 (636.30, 886.74), Kaempferol - 3,7 - dirahmnoside was a major component of plant 1 (283.87), Quercetrin was a major

component of plant 3 (505.12), Quercetin was a major component of plant 4 (332.82), Kaempferol-3-(3-*p*-coumaroyl) glucose was a major component of plant 8 (967.52), Hespertin was a major component of plant 5 (341.38), and Acacetin was a major component of plant 2 (1005.04).

KEYWORDS: Verbenaceae, Flavonoid, $AlCL_3$, HPLC.

1-INTRODUCTION

Medicinal plants have a promising future because there are approximately half million plants around the world, and most of the medicinal activities has not been investigated yet. In recent times, focus on plant research has increased all over the world, and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems.[1] Family verbenaceae contains 31 genera and nearly 920 species, which are distributed mostly in tropical and subtropical South America and Africa. Other places of origin include central Asia, a worldwide but mainly tropical grouping of 30 genera and some 1,100 species, of mainly tropical flowering plants. It contains trees, shrubs, and herbs some of which are important for their flowers. Verbenaceae represented in Egypt, by five genus.[2] The chemical investigation of the verbenaceae species in different countries revealed that their main chemical constituents are flavonoids.[3] iridoids,[4],[5], [6], [7] Phenylethanoids,[8],[9],[10] Verbena chalcone.[11] Essential oil.[12], [13] Triterpenes.[14] Medicinal plants belonging to family verbenaceae are reported to be used as tonic, diaphoretic, sedative, diuretic and expectorant remedies.[15], [16] In addition verbenaceae species have antioxidant, antifungal antibacterial, parasiticide, immunostimulant, anti-depressant, hypotensive, hepato-protective, neuro-protective and hypoglycemic effects.[17], [18], [19] Topical preparations showed both anti-inflammatory and analgesic activities.[20] Based on the available literature there is no data concerning either the comparative studies on flavonoides constituents of some verbenaceaus plants.

II-MATERIAL AND METHODS

II-1-Plant materials

Eight species of the plant family used for this study were collected from the Garden of Faculty of Agriculture , Al-Azhar University and Al-Orman Garden, Giza, Egypt at their flowering stage (April 2015) and were kindly authenticated by Agricultural Engineer Terese Labib, El Orman Botanical Garden. Voucher specimens are deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy Al-Azhar University, Cairo, Egypt.

These species were air-dried, powdered and kept in tightly closed amber colored glass containers and protected from light at low temperature as possible.

II-2-Material for determination of total flavonoid content

1-All chemicals, solvents and reagents used were of analytical, HPLC and pure grade.

2-All reference standers were purchased from Sigma chemical Co., St. Lewis, USA or Fluke chemical company (Switzerland).

II-3-Preparation of plant extract

Dried and finely powdered of the studied plants were completely extracted with 70% methanol in a Soxhlet apparatus. The extracts were concentrated under reduced pressure in a rotary evaporator, diluted with water, filtered, purified and partitioned into fractions according to a general procedure elaborated for flavonoids.[20]

III-Spectrophotometric determination of the Total flavonoids in samples

Aluminum chloride colorimetric method is used for flavonoids determination. Two milliliters of 2% $AlCl_3$ in ethanol is added to 2 ml of the test sample. The UV absorption is measured at 425 nm after 1 h at room temperature. Concentration of 0.05 mg/ml sample solution is used while Quercetin concentrations of 0.01 to 0.09 mg/ml are used to obtain a calibration curve. Determinations were performed in triplicates. Total flavonoid contents were obtained from the regression equation of the calibration curve of Quercetin (Y=0.0103+0.0102, r2= 0.9966).[21],[22]

IV- Determination of Flavonoid compounds by HPLC

Flavonoid compounds were determined by HPLC according to the method of[23] as follow: 5 g of sample were mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtrated through a 0.2 µm Millipore membrane filter then 1-3 ml was collected in a vial for injection into HPLC Hewllet Packared (series 1050) equipped with autosamplling injector, solvent degasser, ultraviolet (UV) detector set at 330 nm and quarter HP pump (series 1050). The column temperature was maintained at 35˚ C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml⁄ min. Flavonoid acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis of Hewllet Packared software.

RESULTS AND DISCUSSION

From the standard calibration curve, and the absorbencies obtained for each sample, the total flavonoids content was calculated as Quercetin Equivalent (QE) in the plant sample under investigation. Results are recorded in table (1).

Table (1): Total flavonoids measured by spectrophotometric and HPLC methods in some verbenaeus species cultivated in Egypt.

No.	Plant name	Total flavonoids (µg/100g) Using UV method	Total flavonoids (µg/100g) Using HPLC method
1	*Tectona grandis*	42.8	43.5
2	*Verbena bipinnatifida*	74.1	76.01
3	*Duranta repens*	67.2	68.76
4	*Duranta lorentzii*	96.2	96.94
5	*Caryopteris incana*	112.8	113.72
6	*Gmelina arborea*	27.4	28.32
7	*Gmelina hystrix*	32.4	33.06
8	*Verbena hybrida*	57.6	57.93

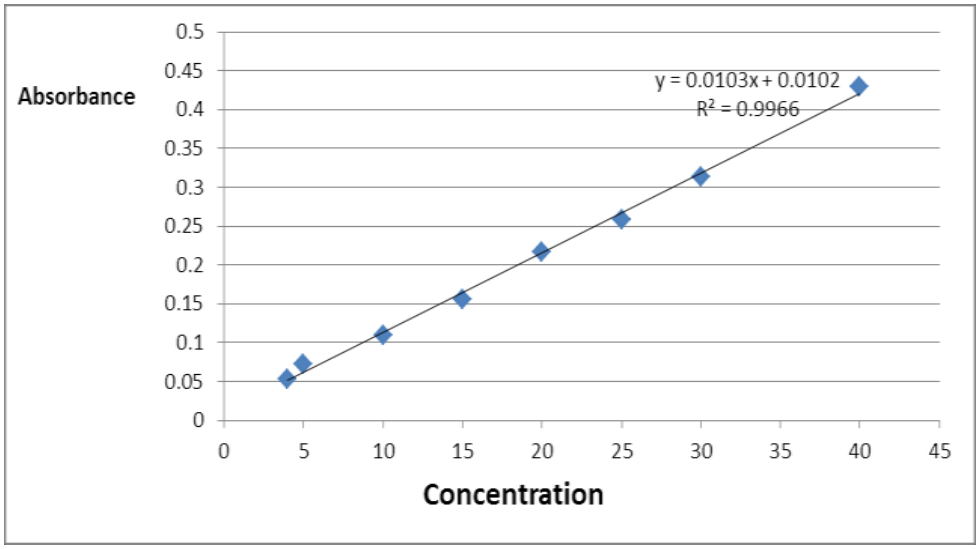

Figure (1): The calibration curve of Quercetin with different concentrations.

Table (2): Results of HPLC analysis of flavonoid in the studied plants.

Flavonoids	R_t		(% of flavonoids (µg/100g)							
	St	Test	1	2	3	4	5	6	7	8
Luteolin-6-arabinose-8-glucose	9.48	9.44	643.61	628.20	1206.81	2587.41	1464.29	1570.66	542.79	865.48
Luteolin-6-glucose -8-arabinose	10.81	10.82	928.40	82.97	56.63	78.96	56.50	33.41	31.15	34.33
Apigenin-6-arabinose-8-	11.37	11.36	94.45	27.30	24.34	403.95	223.59	18.09	49.38	168.42

galactose										
Apigenin-6-rhamnose-8-glucose	11.81	11.83	92.52	310.47	785.24	352.64	100.33	136.25	440.74	272.49
Apigenin-6-glucose-8-rh	12.19	12.18	150.63	79.77	105.03	297.80	240.81	17.95	29.99	480.46
Luteolin-7-glucose	12.30	12.28	---	39.31	206.25	399.89	----	23.11	51.19	---
Narengin	12.35	12.37	301.40	99.76	289.45	352.03	5787.49	173.98	67.57	381.69
Hesperdin	12.48	12.48	768.70	3826.90	1024.5	1568.32	1501.16	400.48	1113.65	1104.40
Rutin	12.61	12.57	134.30	360.82	636.30	886.74	---	91.89	87.25	166.39
Quercetin-3-*O*-glucoside	12.51	12.52	---	---	---	---	141.52	---	---	---
Apigenin-7-*O*-neohisperoside	13.14	13.41	75.16	74.33	95.61	122.03	194.03	11.07	24.49	63.10
Kaempferol-3,7-dirahmnoside	13.21	13.26	283.87	54.75	202.82	180.06	83.07	12.13	170.88	88.31
Quercetrin	13.45	13.42	107.86	127.11	505.12	347.32	434.02	71.74	47.93	116.81
Rosmarinic	13.88	13.92	4.27	7.91	49.16	46.60	143.39	8.82	19.41	25.57
Quercetin	14.90	14.91	44.46	92.98	261.89	342.82	44.68	10.34	49.18	150.37
Naringenin	15.03	15.05	15.52	15.14	104.10	63.99	64.67	2.90	14.48	48.96
Acacetin-neo rutinoside	15.10	15.12	---	---	---	---	---	---	---	---
Kaempferol-3-3-	15.16	15.18	304.56	235.57	522.60	527.78	416.20	131.43	316.10	967.52
Hespertin	15.35	15.3	152.72	110.88	115.34	326.14	341.38	15.40	90.14	238.09
Kaempferol	16.24	16.26	11.52	141.28	169.06	79.10	45.76	11.39	19.51	29.79
Rhamnetin	16.44	16.49	11.60	129.45	167.13	53.44	10.65	8.48	8.27	29.14
Apigenin	16.56	16.64	17.67	100.02	102.55	34.57	36.95	3.34	19.84	155.16
Apigenin-7-glucose	17.24	17.26	16.28	50.84	---	7.55	15.79	30.52	33.04	180.78
Acacetin	18.82	18.86	190.61	1005.04	246.14	254.77	226.09	48.07	78.92	225.56
Total Flavonoids			4350.11	7600.80	6876.07	9693.91	11372.37	2831.45	3305.90	5792.82

1-*Tectona grandis* 2-*Verbena bipinnatifida* 3-*Duranta repens* 4-*Duranta lorentzii*

5-*Caryopteris incana* 6-*Gmelina arborea* 7-*Gmelina hystrix* 8- *Verbena hybrida*

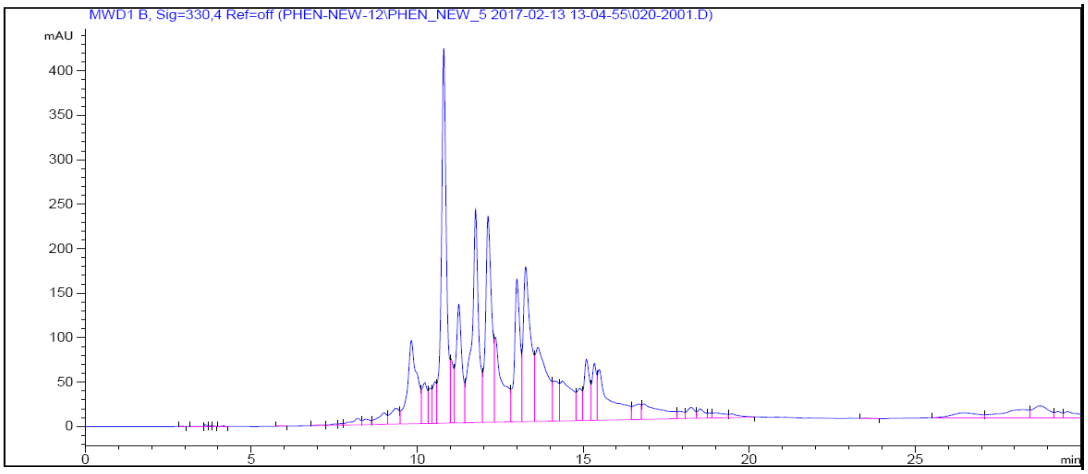

Fig. (2) HPLC chromatogram of Flavonoid *Tectona grandes* plant.

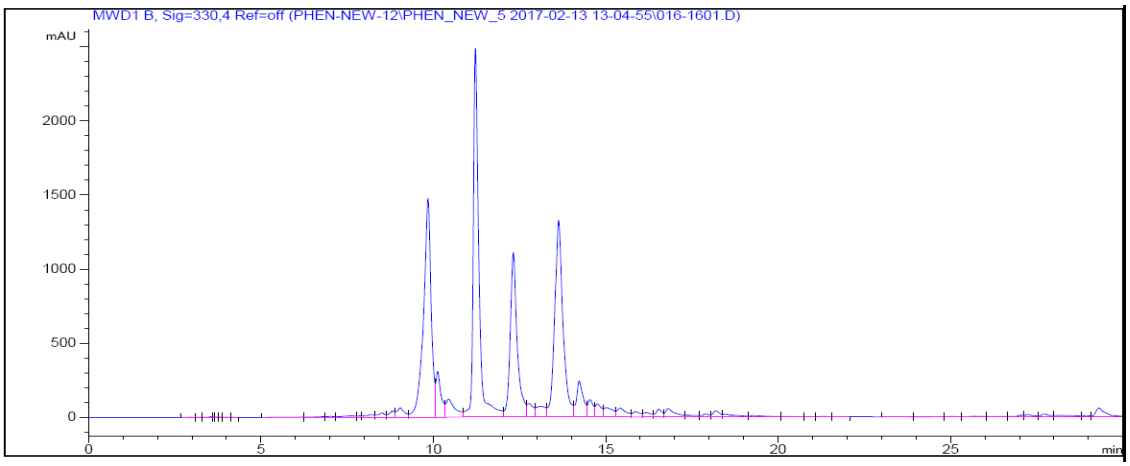

Fig. (3) HPLC chromatogram of Flavonoid *Caryopters incana* plant.

Quantitative analysis of total flavonoid compounds (table 1) of *Tectona grandis, Verbena bipinnatifida, Duranta repens, Duranta lorentzii, Caryopteris incana,Gmelina arborea, Gmelina hystrix* and *Verbena hybrida* plants using colourmetric method (Aluminium chloride reagent) revealed that the concentration of flavonoids were (40.87,72.1, 67.2, 101.2, 112.8, 20.4, 50.4 and 57.6 µg QE per 100 g dried sample weight, respectively. Flavonoids are known to be associated with reduced risk for certain chronic diseases.[24] High flavonoid content increases the antioxidant activity.[25], [26], [27] From the flavonoids point of view HPLC analysis of both species {Table (1,2) and Fig.(2,3) }have the highest concentrations of *C*-glycosyl pattern; *Caryopteris incana* is characterized by the highest contents of *C*-glycosyl Luteolin and Apigenin (Luteolin-6-arabinose-8-glucose, Luteolin-6- glucose -8-arabinose, Apigenin-6-arabinose-8-galactose, Apigenin-6-glucose-8-rhamnose, Luteolin-7- glucose, Apigenin-7-*O*-neohisperoside), Narengin, Hesperdin Rutin, Kaempferol-3,7-dirahmnoside, Quercetrin, Naringenin, Kaempferol-3-(3-*p*-coumaroyl, Hespertin),[28], [29] While *Tectona*

grandis is characterized by the highest contents of Apigenin-6- rhamnose-8- glucose, kaempferol and its derivatives (reported for the first time). Kaempferol-3, 7-dirahmnoside, Kaempferol, Rhamnetin.[30], [31], [32], [33] *Gmelina arbore* is characterized by the highest contents of Kaempferol-3-(3-*p*-coumaroyl).[34], [35] All compounds ware represented in the plant species in different concentrations. Quercetin-3-*O*-glucoside represented only in *Caryopteris incana*, (141.52 µg/100g). Thus, the biological activity of the plants under investigation may be attributed to their flavonoids content. This suggests that, these plants are good sources of natural biological substances.

CONCLUSION

Pants are known for their medicinal property since ancient times. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. In conclusion, the present study demonstrated that eight species belonging to verbenaceae family were studded according to flavonoid content using colorimetric and HPLC analysis. HPLC analysis of flavonoids revealed the presence of 24 peaks all of them were qualitatively identified and quantitatively estimated. The flavonoids were represented as five C-glycosyl flavones, four flavones, eight flavonol glycosides, five flavanones and two 2,3 dehydro flavone.

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