Total Phenolics and Total Flavonoids in Selected Indian Medicinal Plants

C. T. SULAIMAN* AND INDIRA BALACHANDRAN
Phytochemistry Division, Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Malappuram-676 503, India

Sulaiman and Balachandran: Total Phenolics and Total Flavonoids in Medicinal Plants

Plant phenolics and flavonoids have a powerful biological activity, which outlines the necessity of their determination. The phenolics and flavonoids content of 20 medicinal plants were determined in the present investigation. The phenolic content was determined by using Folin-Ciocalteu assay. The total flavonoids were measured spectrophotometrically by using the aluminium chloride colorimetric assay. The results showed that the family *Mimosaceae* is the richest source of phenolics, (*Acacia nilotica*: 80.63 mg gallic acid equivalents, *Acacia catechu* 78.12 mg gallic acid equivalents, *Albizia lebbeck* 66.23 mg gallic acid equivalents). The highest total flavonoid content was revealed in *Senna tora* which belongs to the family *Caesalpiniaceae*. The present study also shows the ratio of flavonoids to the phenolics in each sample for their specificity.

Key words: Flavonoids, gallic acid, phenolics, quercetin

Phenolic compounds are plant substances which possess in common an aromatic ring bearing one or more hydroxyl groups. There are about 8000 naturally occurring plant phenolics and about half of this number are flavonoids[1]. Phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic as well as ability to modify the gene expression[2]. Phenolics are the largest group of phytochemicals that account for most of the antioxidant activity in plants or plant products[3].

***Address for correspondence**
E-mail: slmnct@gmail.com

Flavonoids are the largest group of naturally occurring phenolic compounds, which occurs in different plant parts both in free state and as glycosides. They are found to have many biological activities including antimicrobial, mitochondrial adhesion inhibition, antiulcer, antiarthritic, antiangiogenic, anticancer, protein kinase inhibition, etc[4]. The flavonoids have two benzene rings separated by a propane unit. The flavones and flavonols are the most widely distributed of all the phenolics[5]. Flavonoids are particularly beneficial, acting as antioxidants and giving protection against cardiovascular disease, certain forms of cancer and age-related degeneration of cell components. Their polyphenolic nature enables them to scavenge injurious free radicals such as super oxide and hydroxyl radicals[6]. A variety of dietary plant flavonoids inhibits tumour development in experimental animal models[7]. The biflavonoids possess pharmacological effects like ability to inhibit the release of histamines, the adhesion of blood platelets and the action of lens aldose reductase, to block the inflammatory effects of hepatotoxins, and to act as a heart-stimulant[8].

Based on the strong evidence of biological activities of phenolic compounds, the study was focused on determination of total phenolics and flavonoids in selected medicinal plants of various species. A total of 20 medicinal species was screened for the estimation of total phenolics and flavonoids.

The plant materials were collected from the herb garden of Arya Vaidya Sala (AVS), Kottakkal, and authenticated by the Taxonomy division of Centre for Medicinal Plants Research (CMPR), AVS, Kottakkal. The voucher specimens are deposited in CMPR herbarium.

Five grams of each of the shade-dried plant material was pulverised into coarse powder and subjected to hydro-alcoholic extraction using soxhlet apparatus. The extracts were concentrated to dryness in a rotary evaporator under reduced pressure. The dried residues were then dissolved in 100 ml of 80% methanol. The extracts were used for total phenolic and flavonoid assay.

The total phenolics content was determined by using the Folin-Ciocalteu assay[9]. An aliquot (1 ml) of extracts or standard solution of gallic acid (20, 40, 40, 60, 80 and 100 μg/ml) was added to a 25 ml volumetric flask, containing 9 ml of distilled water. A reagent blank was prepared using distilled water. One millilitre of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. The volume was then made up to the mark. After incubation for 90 min at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV/Vis spectrophotometer. Total phenolics content was expressed as mg gallic acid equivalents (GAE).

Total flavonoid content was measured by the aluminium chloride colorimetric assay[10]. An aliquot (1 ml) of extracts or standard solutions of quercetin (20, 40, 60, 80 and 100 μg/ml) was added to a 10 ml volumetric flask containing 4 ml of distilled water. To the flask, 0.30 ml of 5% $NaNO_2$ was added and after 5 min, 0.3 ml of 10% $AlCl_3$ was added. After 5 min, 2 ml of 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE). The results for total phenolic and total flavonoid content and the ratio of total flavonoids/total phenolics in the studied plant extracts are presented in Table 1.

The results show that the family Mimosaceae is the richest source of phenolics, (*Acacia nilotica*: 80.63 mg GAE, *Acacia catechu* 78.12 mg GAE, *Albizia lebbeck* 66.23 mg GAE). The second group with high phenolic content is the family Caesalpiniaceae (*Senna tora* 65.54 mg GAE, *Saraca asoca* 38.15 mg GAE and *Caesalpinia sappan* 24.52 mg GAE). The family Caesalpiniaceae possesses a significant F/P ratio which indicates that the family is rich in flavonoids. The bark of *Oroxylum indicum* shows least flavonoid content (Flavonoids 0.08 mg QE). *Embelia ribes*, *Aerva lanata, Biophytum sensitivum* show an average value of phenolic content with flavonoids/phenolic 0.05, 0.30 and 0.13, respectively. *Ficus microcarpa* and *Ficus racemosa* belonging to the family *Moraceae* show comparatively good phenolics, 24.47 mg GAE and 12.36 mg GAE.

There is a positive correlation between phenolic content and free-radical scavenging activity[11]. The antioxidant potential of *Acacia nilotica* has been reported[12]. The high phenolic content of *Acacia nilotica* (80.63 mg GAE) shows the linear correlation

TABLE 1: TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT

Plant	Family	Part investigated	Total phenolics mg GAE/100 g	Total flavonoids mg QE/100 g	Flavonoids/phenolics (F/P ratio)
Acacia catechu (L.f.) Willd	*Mimosaceae*	Bark	78.12	8.54	0.19
Acacia sinuata (Lour.) Merr.	*Mimosaceae*	Bark	43.06	03.65	0.08
Acacia nilotica (L.) Willd. ex Del.	*Mimosaceae*	Bark	80.63	07.86	0.10
Albizia lebbeck (L.) Benth.	*Mimosaceae*	Bark	66.23	06.87	0.10
Caesalpinia sappan L.	*Caesalpiniaceae*	Bark	24.52	7.58	0.40
Senna tora (L.) Roxb.	*Caesalpiniaceae*	Bark	65.54	21.58	0.33
Cassia fistula L.	*Caesalpiniaceae*	Bark	22.83	09.56	0.42
Saraca asoca (Roxb.) de Wilde	*Caesalpiniaceae*	Bark	38.15	11.36	0.30
Embelia ribes Burm. f	*Myrsinaceae*	Bark	26.59	01.35	0.05
Aerva lanata (L.) Juss. ex Schult.	*Amaranthaceae*	Whole plant	22.7	6.99	0.30
Biophytum reinwardtii (Zucc.) Klotzsch.	*Oxalidaceae*	Whole plant	13.52	01.86	0.13
Jasminum grandiforum Linn.	*Oleaceae*	Whole plant	7.80	01.23	0.16
Holoptelea integrifolia (Roxb.) Planch.	*Ulmaceae*	Bark	4.71	01.08	0.22
Gmelina arborea Roxb.	*Verbenaceae*	Bark	29.43	02.65	0.09
Plumbago indica L.	*Plumbaginaceae*	Root	7.76	0.65	0.08
Justicia adhatoda L.	*Acanthaceae*	Whole plant	42.36	02.65	0.06
Oroxylum indicum (L.) Benth. ex Kurz	*Bignoniaceae*	Bark	17.12	0.08	0.01
Pseudarthria viscida (Linn) W and A	*Fabaceae*	Root	6.66	0.25	0.04
Hygrophila schulli (Buch.-Ham.) M. R. and S. M. Almeida	*Acanthaceae*	Whole plant	07.56	0.35	0.04
Ficus racemosa L.	*Moraceae*	Bark	12.36	0.86	0.069
Ficus microcarpa L. f.	*Moraceae*	Bark	24.47	01.65	0.07

The absorbance against the reagent blank was determined at 550 and 510 nm with an UV-Visible spectrophotometer for phenolics and flavonoids, respectively. Total phenolics content was expressed as mg Gallic acid Equivalents (GAE) and total flavonoid content was expressed as mg quercetin equivalents (QE).

between phenolic content and antioxidant activity. Phenolic acids have repeatedly been implicated as natural antioxidants in fruits, vegetables and other plants. Phenolic compounds contribute to quality and nutritional value in terms of modifying colour, taste, aroma and flavour and also in providing health-beneficial effects. The general assessment of the analytical results for the plant extracts definitely shows the individual specificity of each sample and a rich diverse spectrum of phenolic compounds differing from flavonoid group.

ACKNOWLEDGEMENTS

The authors are thankful to the authorities of Arya Vaidya Sala, Kottakkal for extending the facilities and TATA Trust Mumbai for the financial support.

REFERENCES

1. Harborne JB, Herbert H. Phytochemical dictionary: a handbook of bioactive compounds from plants. London: Taylor and Francis; 1993.
2. Marinova D, Ribarova F, Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J Univ Chem Tech Metall 2005;40:255-60.
3. Okpuzar J, Ogbunugafor H, Kareem GK, Igwo-Ezikpe MN. *In vitro* investigation of antioxidant phenolics compounds in extract of *Senne alata*. Res J Phytochem 2009;3:68-76.
4. Bhat SV, Nagasampagi BA, Sivakumar M. Chemistry of natural products. New Delhi: Narosa Publishing House; 2005.
5. Kaufman PB, Cseke LJ, Warber CS, James AS, Brielmann HL. Natural Products from Plants. London: CRC Press; 1999.
6. Dewick PM. Medicinal natural products: A biosynthetic approach. England: John Wiley and Sons; 2001.
7. Arnason JT, Mata R, Romeo JT. Recent trends in phytochemistry. London: Plenum Press; 1995.
8. Harborne JB. The flavonoids: Advances in research since 1980. New York: Chapman and Hall Ltd.; 1988. p. 121.
9. Singleton VL, Rossi JA Jr. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Amer J Enol Viticult 1965;16:144-58.
10. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effect on superoxide radicals. Food Chem 1999;64:555-9.
11. Oki T, Masuda M, Furuta S, Nishibia Y, Terahara N, Suda I. Involvement of anthocyaninsand other phenolic compounds in radical-scavenging activity of purple-fleshed sweet potato cultivars. Food Chem Toxicol 2002;67:1752-6.
12. Singh BN, Singh BR, Singh RL, Prakash D, Sarma BK, Singh HB. Antioxidant and antiquorum sensing activities of green pod of *Acacia nilotica* L. Food Chem Toxicol 2009;47:778-86.

Accepted May 15, 2012
Revised May 8, 2012
Received November 11, 2010
Indian J. Pharm. Sci., 2012, 74 (3): 258-260