Asian Journal of Chemistry; Vol. 28, No. 3 (2016), 497-500

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2016.19370

Evaluation of Phenolic Compounds and Development of Chromatographic Profiles in *Spathodea campanulata* Inflorescence by HPTLC

JYOTI V. VASTRAD* and GIRIDHAR GOUDAR

DBT Project, All India Co-ordinated Research Project on Home Science (Clothing and Textile) Department, University of Agricultural Sciences, Dharwad-580 005, India

*Corresponding author: E-mail: jyotivastrad@gmail.com

Received: 25 May 2015; *Accepted*: 15 July 2015; *Published online*: 5 December 2015; AJC-17638

In the present work, an investigation of phenolic constituents in flower petals of *Spathodea campanulata* was carried out. Ethyl alcohol, 50 % v/v ethyl alcohol and distilled water were used as solvents for the optimization of extraction process. The extracts obtained were subjected to phytochemical screening and quantified for total phenolic content and total flavonoid content. Total phenolic content of the extracts obtained were in the sequence: distilled water (25.75) > 50 % ethyl alcohol (15.12) > ethyl alcohol (2.37), which was expressed in terms of mg/g gallic acid equivalent (GAE) of sample. Whereas total flavonoid content of the extracts were in the sequence: distilled water (17.65) > 50 % ethyl alcohol (10.25) > ethyl alcohol (2.90) which was expressed in terms of mg/g rutin equivalent (RE) of sample. High performance thin layer chromatography analysis of the sample extracts was performed to obtain chromatographic profiles, scanning was performed at 270, 320 and 370 nm and the R_f values obtained for the sample extracts were compared with the R_f values of the flavonoid compounds *viz*., rutin, catechin and quercetin.

Keywords: Phytochemical, HPTLC, *Spathodea campanulata*, Flavonoid.

INTRODUCTION

Spathodea campanulata belongs to the family Bignoniaceae and is commonly known as the Fountain tree, African Tulip tree, *Pichkari* or *Nandi Flame*. The generic name comes from the Ancient Greek words referring to the spathe-like calyx. It is much appreciated for its very showy reddish-orange or crimson (rarely yellow), campanulate flowers. Pigments produce the colours that we observe at each step of our lives, because pigments are present in each one of the organisms in the world and plants are the principal producers in the form of leaves, fruits, vegetables and flowers. Natural and synthetic pigments are used in medicines, foods, clothes, furniture, cosmetics and in other products [1]. Flower is an important part of plant which contains a great variety of phenolic compounds, such as phenolic acids, flavonoids, anthocyanins *etc.* [2,3].

Phenolic compounds are the most abundant secondary metabolites of plants. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8000 phenolic structures currently known. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colours [4]. Phenolic acids also have pharmacological activity, for example cholagogue, diuretic, immunostimulating, antiseptic and antiinflammatory, *etc*. [5].

In flavonoids, the anthocyanins are the most important pigments. They produce colours from orange to blue in petals, fruits, leaves and roots. Flavonoids also contribute to the yellow colour of flowers, where they are present with carotenoids or alone in 15 % of the plant species [6]. The most studied secondary metabolites are the flavonoids. These are phenolic compounds with two aromatic rings bonded by a C3 unit (central pyran ring) and divided in 13 classes based on the oxidation state of the pyran ring and on the characteristic colour. Flavonoids are also known for their anti-allergic, antitumor, antibacterial, spasmolytic and estrogenic effects [7-9]. Secondary metabolites, other than providing plants with unique survival or adaptive strategies, are of commercial significance to human kind. They have been used as dyes, fibers, glues, oils, waxes, flavouring agents, drugs and perfumes and are viewed as potential sources of new natural drugs, antibiotics, insecticides and herbicides [10,11].

Growing consciousness about organic value of eco-friendly products has generated renewed interest of consumers towards use of textiles (preferably natural fibre product) dyed with eco-friendly natural dyes. Natural dyes produce very uncommon, soothing and soft shades as compared to synthetic dyes. On

the other hand, synthetic dyes, which are widely available at an economical price and produce a wide variety of colours, sometimes causes skin allergy and other harmfulness to human body, produces toxicity/chemical hazards during its synthesis, releases undesirable, hazardous and toxic chemicals [12].

Thin layer chromatography analysis is used to carry out a preliminary evalution of plants containing flavonoids (flavonols, flavones, flavanones, chalcones/aurones, anthocynanins), hydroxycinnamic acids, tannins and anthraquinones, which are the phylo-compounds (colour compounds) found in the plants [13]. Identification of dyes in historic textiles through chromatographic and spectrophotometric methods as well as by sensitive colour reactions is highlighted [14] and further the retention of carminic acid, indigotin, corcetin, gambogic acid, alizarin flavanoid, anthraquinone and purpurin are also studied [15].

The objective of the present work is to identify the phenolic compounds and to evaluate the selected flavonoid compounds in flowers of *S. campanulata* by developing a simple, economical, precise and accurate method by HPTLC.

EXPERIMENTAL

The inflourescence petals from plant of *S. campanulata* were collected from the campus of University of Agricultural Sciences, Dharwad, India.

All the chemicals *viz*., ammonia, chloroform, hydrochloric acid, sulphuric acid, ferric chloride, sodium hydroxide, sodium carbonate, sodium chloride, sodium sulphate, lead acetate, gelatin were purchased from Rankem chemicals, Bangalore which were of analytical grade. Ethyl alcohol, acetone, ethyl acetate, methanol, formic acid solvents were of analytical grade from Thomas Baker Chemicals, Mumbai. Gallic acid, rutin, catechin and quercetin standards were procured from Sigma-Aldrich Co. (St.Louis, USA). HPTLC plates, Silica gel 60 F_{254} (0.25 mm) were purchased from Merck KGaA, (Darmstadt, Germany). All the solvents used for HPTLC analysis were of HPLC grade.

The chromatographic analysis was carried out by the analytical instrument high performance thin layer chromatography (Camag, Muttenz, Switzerland). Comprising of Linomat V automated spray-on band applicator, Vertical twin trough development chamber (10 cm × 10 cm, with metal lids) in which chromatograms were developed at room temperature (27 ± 3 °C), UV-visible documentation cabinet and the zones obtained were quantified by scanning at 270, 320 and 370 nm with TLC Scanner III with a deuterium source in the reflection mode, slit dimension settings of length 6 and width 0.1, monochromator bandwidth 20 nm, a scanning rate of 10 mm s^{-1}. The peak height and areas of chromatograms were determined using winCATS TLC software (version 4.X, Camag TLC, Software).

Preparation of standard stock solution: Preparation of standard solutions was carried out with little modification [16]. The stock solutions of rutin, quercetin and catechin (1,000 μg mL^{-1}) standards were prepared by dissolving 25 mg of accurately weighed standards in methanol and diluting to 25 mL with methanol in a standard volumetric flask. A series of working standard solutions of concentrations 20, 40 and 60 μg mL^{-1}) were prepared by the dilution of aliquots 0.2, 0.4 and 0.6 mL of stock solution in a 10 mL standard volumetric flask with methanol.

Sample preparation: The collected petals of the flowers were washed and dried at 40 ± 2 °C in hot air oven, homogenized with a laboratory blender and sieved through an 80-mesh (BSS) size (180 microns). Extraction process was optimized by three solvents *viz*., ethyl alcohol, ethyl alcohol (50 %v/v) and distilled water. In plant preparations (extracts, decoctions), the content and composition of phenolic compounds depend on extraction technique, its conditions (extraction time and temperature) and solvents. Solvents such as methanol, ethanol, water, acetone and their combinations are used often for the extraction of phenolics from plant materials [17]. One gram of accurately weighed powder was extracted with 50 mL of each solvent, incubated under agitation at 25 °C for 3 h. Centrifuged at 10,000 rpm at 4 °C for 15 min (Remi Elektrotechnik, C24 Plus, Mumbai). The supernatant obtained was separated and residue was re-extracted with fresh 50 mL of solvents, the process was repeated and the supernatant were pooled and stored under refrigeration (4 °C) till further analysis within a week.

Screening of phytochemicals: Qualitative phytochemical screening of plant extracts was carried out for the identification of various classes of active chemical constituents like alkaloids, flavonoids, tannins, saponins and terpenoids using different methods as previously described [18,19].

Total phenolic content (TPC): Total phenolic content in the extracts was determined using Folin-Ciocalteu reagent assay method [20,21] with little modification using gallic acid as the reference standard. Briefly, all the solvent extracts were diluted to appropriate volumes and were mixed with 100 μL of Folin-Ciocalteu reagent, incubated at room temperature for 3 min. Then 2 mL of 10 % Na_2CO_3 solution was added to the mixture. The resulting solution was incubated for 60 min at room temperature under dark, the absorbance was measured at λ_{max} 765 nm using UV-visible spectrophotometer (Cary 50, Varian, Middelburg, Netherlands). The total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per g sample.

Total flavonoid content (TFC): Total flavonoid content was determined by a colorimetric method [21,22] with minor modification. Aliquots (1 mL) of appropriately diluted extracts or standard solutions were pipetted into 15 mL polypropylene conical tubes containing 2 mL double distilled H_2O and mixed with 0.15 mL of 5 % $NaNO_2$. After 5 min, 0.15 mL of 10 % $AlCl_3 \cdot 6H_2O$ solution was added and the mixture was allowed to stand for another 5min and then 1 mL of 1 M NaOH was added. The reaction solution was well mixed, kept for 15 min and the absorbance was determined at λ_{max} 765 nm using UV-visible spectrophotometer (Cary 50, Varian, Middelburg, Netherlands). Total flavonoid content was calculated using the standard rutin curve and expressed as rutin equivalent (RE) in milligrams per gram of sample.

RESULTS AND DISCUSSION

The phytochemical screening of the *S. campanulata* flower extracts using absolute ethyl alcohol, 50 % (v/v) ethyl

alcohol and deionized distilled water is presented in Table-1. The results revealed that aqeous and 50 % ethanolic extracts of *S. campanulata* flower exhibited positive results for ferric chloride test for phenolic compounds and tannins whereas negative results were obtained for absolute ethanolic extract. Gelatin test for phenolic compounds revealed positive results for absolute ethanolic and 50 % ethanolic extracts, whereas negative results were obtained for aqueous extract. Further the test for lead acetate for phenolic compounds and tannins showed positive results for all the extracts with varrying concentrations.

TABLE-1
PHYTOCHEMICAL SCREENING OF *S. campanulata* FLOWER EXTRACTS

Phytochemical group	Chemical test	Extracts of *S. campanulata* flower		
		A*	B*	C*
Phenolic compounds & tannins	Ferric chloride	++	++	–
	Gelatin	–	+	+++
	Lead acetate	+++	+	++
Flavonoids	Ammonia	+++	++	+
	Sodium hydroxide	++	++	+++
Alkaloids	Dragendorff's	++	+	+
	Wagner's	++	+++	+
Saponins	Foam	++	+	–
Terpenoids	Salkowski	++	+++	++

A* = Aqueous extract, B* = 50 % ethyl alcohol extract, C* = Ethyl alcohol extract; '+'= presence of compound, '–'= absence of compound, '++' = Dark colour, '+++' = Dark colour with precipitate

Flavonoid compounds were screened by ammonia and sodium hydroxide tests. Both the tests revealed positive results in all the extracts with varrying concentrations. Also the tests for alkaloids were Dragendorff and Wagner, which revealed positive results for all the solvent extracts with varying concentrations.

It is further observed from Table-1 that aqueous and 50 % ethanolic extracts depicted the presence of saponins (foam test) while negative results were obtained for absolute ethanolic extract. Further screening for terpenoids by Salkowski test exhibited positive results for all the extracts with varying concentrations.

Total phenolic content: Total phenolic content of the solvent extracts of *S. campanulata* tree flower is depicted in Table-2. Total phenolic content was found to be high in aqeous extract 25.75 mg/g compared to absolute ethanolic and 50 % ethanolic extracts *i.e.*, 2.37 and 15.12 mg/g, respectively.

TABLE-2
TOTAL PHENOLIC CONTENT (TPC) AND TOTAL FLAVONOID CONTENT (TFC) OF DIFFERENT SOLVENT EXTRACTS OF *S. campanulata* FLOWER EXTRACTS

Solvent extract	TPC mg/g (GAE)*	TFC mg/g (RE)*
Absolute ethyl alcohol	2.37 (± 0.23)	2.90 (± 0.29)
50 % Ethyl alcohol	15.12 (± 0.47)	10.25 (± 0.15)
Distilled water	25.75 (± 0.37)	17.65 (± 0.28)

*GAE = Gallic acid equivalent, RE = Rutin equivalent.
Data are mean ± standard deviation of triplicate determinations.

Total flavonoid content: Total flavonoid content of the solvent extracts of *S. campanulata* tree flower is also depicted in Table-2. Total flavonoid content was found to be high in aqeous extract 17.65 mg/g compared to absolute ethanolic and 50 % ethanolic extracts *i.e.*, 2.90 and 10.25 mg/g, respectively.

The chromatographic profiles obtained after scanning at λ_{max} 270 nm are depicted in Table-3. The total number of peaks obtained for aqueous, 50 % ethyl alcohol and absolute ethanol extracts of *S. campanulata* were 5, 9 and 6, respectively, the R_f values of the obtained peaks were identified by comparing the R_f values obtained for the flavonoid standards. Rutin and catechin peaks were obtained in aqueous and 50 % ethyl alcohol extracts, with the area 488.0 and 731.3 AU in aqueous extract and 179.9 and 1588.1 AU in 50 % ethyl alcohol extract, whereas only catechin was identified in absolute ethanol extract with 475.4 AU area at λ_{max} 270 nm. Chromatographic profiles developed after scanning at λ_{max} 320 nm are given in Table-4, the number of peaks obtained for aqueous, 50 % ethyl alcohol and absolute ethanol extracts of *S. campanulata* were 4, 6 and 4, respectively. Rutin and catechin peaks were obtained in aqueous and 50 % ethyl alcohol extracts with the area 1638.7 and 923.1 AU for aqueous extract and 241.0 and 1088.3 AU for 50 % ethyl alcohol extract, whereas only catechin was identified in absolute ethanol extract 323.4 AU area at λ_{max} 320 nm. The profiles of λ_{max} 370 nm are depicted in Table-5, the total number of peaks obtained for aqueous, 50 % ethyl alcohol and absolute ethanol extracts of *S. campanulata* were 4, 3 and 1, respectively, rutin and catechin peaks were obtained in aqueous extracts with the area 1593.1 AU for aqueous extract and 631.3 AU for 50 % ethyl alcohol extract, whereas only rutin was identified with the area 351.5 AU in 50 % ethyl alcohol extract and none of the three flavonoids selected were identified in absolute alcohol extract when scanned at λ_{max} 370 nm.

TABLE-3
HPTLC CHROMATOGRAPHIC PROFILES (R_f VALUES AND AREA AT λ_{max} 270 nm)

S. campanulata flower extracts								
Aqueous			Ethyl alcohol (50 % v/v)			Ethyl alcohol		
Peak No.	R_f value	Area (AU)	Peak No.	R_f value	Area (AU)	Peak No.	R_f value	Area (AU)
1(Rutin)	0.06	488.0	1(Rutin)	0.05	179.9	1	0.11	487.2
2	0.17	1252.6	2	0.11	735.2	2	0.18	351.2
3(Catechin)	0.47	731.3	3	0.17	926.8	3	0.38	631.8
4	0.56	315.9	4	0.21	298.7	4(Catechin)	0.50	475.4
5	0.76	4304.3	5	0.35	766.1	5	0.56	2142.8
			6	0.44	225.9	6	0.78	2954.6
			7(Catechin)	0.47	1588.1			
			8	0.53	4955.3			
			9	0.76	5066.2			

TABLE-4
HPTLC CHROMATOGRAPHIC PROFILES (R_f VALUES AND AREA AT λ_{max} 320 nm)

S. campanulata flower extracts								
Aqueous			Ethyl alcohol (50 % v/v)			Ethyl alcohol		
Peak No.	R_f value	Area (AU)	Peak No.	R_f value	Area (AU)	Peak No.	R_f value	Area (AU)
1(Rutin)	0.05	1638.7	1(Rutin)	0.04	241.0	1	0.45	701.9
2	0.18	1500.9	2	0.42	582.1	2	0.61	583.8
3	0.57	599.0	3	0.58	1590.8	3(Catechin)	0.68	323.4
4(Catechin)	0.68	923.1	4(Catechin)	0.64	1088.3	4	0.79	295.7
			5	0.76	306.3			
			6	0.90	121.9			

TABLE-5
HPTLC CHROMATOGRAPHIC PROFILES (R_f VALUES AND AREA AT λ_{max} 370 nm)

S. campanulata flower extracts								
Aqueous			Ethyl alcohol (50 % v/v)			Ethyl alcohol		
Peak No.	R_f value	Area (AU)	Peak No.	R_f value	Area (AU)	Peak No.	R_f value	Area (AU)
1(Rutin)	0.05	1593.1	1(Rutin)	0.04	350.5	1	0.61	351.5
2	0.18	1503.8	2	0.58	1234.1			
3	0.57	309.6	3	0.90	87.7			
4(Catechin)	0.68	631.3						

Conclusion

The developed HPTLC method is precise, specific and accurate for the determination of flavonoid compounds (rutin, catechin, quercetin) in *S. campanulata* flower extracts. The proposed method can be used for qualitative as well as quantitative analysis of flavonoids. An attempt has been made here to give scientific overview of phenolic compounds (flavonoids) responsible for colour in natural dyes, which can be used as antimicrobial finishes of fabrics in textiles.

ACKNOWLEDGEMENTS

The authors thank Department of Biotechnology (DBT), New Delhi, India for the financial support for under-taking the research work.

REFERENCES

1. F. Delgado-Vargas, A.R. Jiménez and O. Paredes-López, *Crit. Rev. Food Sci. Nutr.*, **40**, 173 (2000).
2. G. Kaur, M.S. Alam, Z. Jabbar, K. Javed and M. Athar, *J. Ethnopharmacol.*, **108**, 340 (2006).
3. Z. Youwei, Z. Jinlian and P. Yonghong, *LWT–Food Sci. Technol.*, **41**, 1586 (2008).
4. J. Dai and R.J. Mumper, *Molecules*, **15**, 7313 (2010).
5. Z. Sroka and W. Cisowski, *Food Chem. Toxicol.*, **41**, 753 (2003).
6. R.E.K. Koes, F. Quattrocchio and J.N.M. Mol, *BioEssays*, **16**, 123 (1994).
7. J.B. Harborne and C.A. Williams, *Phytochemistry*, **55**, 481 (2000).
8. G. Di Carlo, N. Mascolo, A.A. Izzo and F. Capasso, *Life Sci.*, **65**, 337 (1999).
9. J. Robak and R. Gryglewski, *Pol. J. Pharmacol.*, **48**, 555 (1996).
10. R. Croteau, T.M. Kutchan and N.G. Lewis, in eds.: B. Buchanan, W. Gruissem and R. Jones, Natural Products (Secondary Metabolites), In: Biochemistry and Molecular Biology of Plants, American Society of Plant Physiologists, Rockville, MD, p. 1250 (2000).
11. P.M. Dewick, Medicinal Natural Products: A Biosynthetic Approach. In: The Shikimate Pathway: Aromatic Amino Acids and Phenylpropanoids, Wiley, Chichester, edn 2, p. 149 (2002).
12. A.K. Samanta and A. Konar, in ed.: . In: Emriye Akcakoca Kumbasar, Dyeing of Textiles with Natural Dyes, In: Natural Dyes, Intech Publisher, Croatia, Europe, p. 29 (2011).
13. P. Guinot, A. Roge, A. Gargadennec, M. Garcia, D. Dupont, E. Lecoeur, L. Candelier and C. Andary, *Colouration Technol.*, **122**, 93 (2006).
14. R. Blanc, T. Espejo, A. López-Montes, D. Torres, G. Crovetto, A. Navalón and J.L. Vílchez, *J. Chromatogr. A*, **1122**, 105 (2006).
15. B. Szostek, J. Orska-Gawrys, I. Surowiec and M. Trojanowicz, *J. Chromatogr. A*, **1012**, 179 (2003).
16. V.L. Suryavanshi, P.A. Sathe, M.M. Baing, G.R. Singh and S.N. Lakshmi, *Chromatographia*, **65**, 767 (2007).
17. Š. Sona, M. Ladislava and M. Ludmila, Advances in Food and Nutrition Research, Oxford University Press, New York, Vol. 67, Chap. 3, p. 75 (2012).
18. R. Chulet, P. Pradhan, S.K. Sharma, M.K. Jhajharia, *J. Chem. Pharm. Res.*, **2**, 476 (2010).
19. I.A. Ajayi, O. Ajibade and R.A. Oderinde, *Res. J. Chem. Sci.*, **1**, 58 (2011).
20. V.L. Singleton and J.A. Rossi, *Am. J. Enol. Vitic.*, **16**, 144 (1965).
21. J.V. Vastrad, S.A. Byadgi, G. Goudar and R. Kotur, *Forest Products J.*, **64**, 259 (2014).
22. Y. Shen, L. Jin, P. Xiao, Y. Lu and J. Bao, *J. Cereal Sci.*, **49**, 106 (2009).