International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 3, 2016

Original Article

HPLC DETERMINATION OF PHENOLIC ACIDS, FLAVONOIDS AND ASCORBIC ACID IN FOUR DIFFERENT SOLVENT EXTRACTS OF *ZANTHOXYLUM ACANTHOPODIUM*, A WILD EDIBLE PLANT OF MEGHALAYA STATE OF INDIA

TAPAN SEAL

Plant Chemistry Department, Botanical Survey of India, AJC Bose Indian Botanic Garden, Shibpur, Howrah, West Bengal, India
Email: kaktapan65@yahoo.co.in

Received: 26 Nov 2015 Revised and Accepted: 13 Jan 2016

ABSTRACT

Objective: A high-performance liquid chromatographic (HPLC) method using photodiode array (PDA) detector with gradient elution was developed for the quantification of ascorbic acid, free phenolic acids such as gallic acid, methyl gallate, caffeic acid, syringic acid, ferulic acid, para (*p*)-coumaric acid, sinapic acid and flavonoids (catechin, rutin, quercetin, myricetin, apigenin and kaempferol), in four different solvent extracts like chloroform, methanol, 80% aqueous (aq.) ethanol and 1% aqueous (aq.) acetic acid of wild edible leaves of *Zanthoxylum acanthopodium* collected from Meghalaya state of India.

Methods: The chromatographic separation was carried out on Acclaim™120 C 18 column (5 µm particle size, 250 x 4.6 mm), Dionex Ultimate 3000 liquid chromatography and detection were carried out at three different wavelengths (272, 280 and 310 nm) using a mobile phase of acetonitrile and 1% aq. the acetic acid solution with gradient elution.

Results: The experimental results showed a high concentration of ascorbic acid (16.8 mg/g of dry plant material) and gallic acid (0.952 mg/g of dry plant material) in 1% aq. acetic acid extract as compared to the 80% aq. ethanol extract of the plant. The high percentage of recovery (96-103%), low coefficient of variation (R^2>0.99) and low limit of detection (LOD) and limit of quantitation (LOQ) confirm the suitability of the method for simultaneous quantification of ascorbic acid and all phenolic compounds in the plant under investigation.

Conclusion: The method can be applied for the simultaneous determination of phenolic acids and flavonoids in different plants and also for the isolation of several bioactive components for the use in pharmaceutical and nutraceutical sector.

Keywords: Phenolic acids, Flavonoids, Ascorbic acid, Different solvent extract, *Z. acanthopodium*, Gradient HPLC

INTRODUCTION

Phenolic compounds are ubiquitous in plants, and these are secondary metabolites which shield the plants against ultraviolet radiation or resist the pathogenic aggression. The phenolic acids with the common biogenetic precursor, shikimic acid, are mostly found in the bound form and are classified into three main groups *viz.* benzoic acid derivative, hydroxycinnamic acid derivative and depside. These groups are well-known for their analgesic, antipyretic, cholagogic, sedative and antibiotic properties [1]. The commonest is a hydroxycinnamic acid which consists mainly of ferulic, *p*-coumaric, caffeic, sinapic acid, etc. These acids occur chiefly in the form of an ester of quinic acid or glucose, *e. g.*, chlorogenic acid. The derivatives of hydroxyl benzoic acid include vanillic, protocatechuic, *p*-hydroxy benzoic acid, etc. which are found predominantly as glycosides. Phenolic acids play a potential protective role against different kinds of oxidatively damaged diseases through consumption of fruits and vegetables. The amazing antioxidant cum nutraceuticals properties of phenolics attracted global attention over the past decades. The biological activities like anti-mutagenicity, anti-bacterial action, anti-viral activity, anti-inflammatory traits, apoptotic actions, etc. can only be rationalized by detecting and quantitating such compounds [2]. It is worthy to be noted that only long-term ingestion leads to mitigation.

The flavonoids are a large family of polyphenolic compounds synthesized by plants and structurally derived from the parent substance flavone. Flavonoids present in fruits and leafy vegetables are thought to provide potential and versatile health benefits through radical scavenging and chelating activity. The *in-vitro* antioxidant activities of the flavonoids are due to their ability to reduce the free radical formation and hence exhibit several biological activities. Many studies have suggested that flavonoids like rutin, kaempferol, quercetin, apigenin are well-known for its anti-inflammatory, anti-allergic, anti-thrombotic, hepato-protective, anti-spasmodic and anticancer properties [3]. Each different fruits and leafy vegetables are capable of displaying the different extent of antioxidant activities owing to the presence of a varied amount of free phenolic and flavonol contents.

Ascorbic acid, a water soluble vitamin is an essential nutrient in human diets and found mainly in fruits and vegetables. Due to the remarkable antioxidant properties of this compound, it is widely employed in pharmaceutical and cosmetic industry and also exerted several biological activities.

Z. acanthopodium DC belongs to the family Rutaceae, known as 'Jaiur-khlaw' in Khasia language and wildly grown at Meghalaya, a tiny North-Eastern state of India. The fruits, leaves and seeds of this plant are consumed by the tribal people as food and used in traditional folk medicine to alleviate fever, dyspepsia and cholera [4, 5]. The leaves containing volatile essential oils find wide use as an insect repellent [6]. The species is alarmingly dwindling and has managed to secure its position in Red Data Book of Indian Plants [7].

The leaves of this plant are characterized by high protein content (28.06 %), low-fat content (1.99 %) and substantial mineral content (Na, K, Ca etc.). The methanol extract of the leaves are reported to show high phenolic content (61.19 mg Gallic acid equivalent (GAE)/g of dry extract) and strong 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity (IC_{50}0.24 mg/g of dry extract) [8]. Thus, the presence of an appreciable amount of ascorbic acid, flavonoids and phenolic acids in the plant are inferred. The antioxidant activities of the extractive solution represent an important parameter to evaluate the biological property of the plant. Therefore, it is necessary to characterize and quantify the important compounds present in the plant and also to validate the method of separation and identification of active constituents. The use of the plant in folk medicine and its nutraceuticals role provide unequivocal testimony to the fact. The extraction of polyphenolic compounds from the plant highly depends on the polarity of the solvent because the polar compound is easily extracted using polar

solvent. Thus, the solvent used for the extraction of bioactive compounds must be critically chosen because it will influence the quantity and quality of the final extract [9].

Since literature survey does not yield corroborative data on the flavonoid and phenolic acid contents of the leaves of *Z. acanthopodium*, the authors envisaged the present study to detect and quantify flavonoids (catechin, methyl gallate, rutin, myricetin, quercetin, apigenin, kaempferol), phenolic acids (gallic acid, caffeic acid, syringic acid, *p*-coumaric acid, sinapic acid, ferulic acid) and ascorbic acid extracted from the leaves of *Z. acanthopodium* in four solvents of different polarity *viz.,* chloroform, methanol, 80% aq. ethanol, 1% aq. acetic acid using reversed phase HPLC with diode array detection.

MATERIALS AND METHODS

Plant material

The leaves of *Z. acanthopodium* were collected from the local market of Meghalaya state of India. It was duly authenticated, and a voucher specimen was kept at the Department of Plant Chemistry of Botanical Survey of India under the Registry No. BSITS 1 for future reference. The plant part was shed-dried, made a coarse powder and stored in an air-tight container for extraction.

Chemicals

The standards chemicals like ascorbic acid, phenolic acids (Gallic, caffeic, syringic, *p*-coumaric, ferulic, and sinapic), flavonoids (catechin, rutin, myricetin, quercetin, apigenin and kaempferol) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and the HPLC-grade solvents such as chloroform, methanol, water and acetic acid were purchased from Merck (Germany).

HPLC equipment

HPLC analyses were performed with Dionex Ultimate 3000 liquid chromatograph (Germany) with four solvent delivery system quaternary pump (LPG 3400 SD) including a diode array detector (DAD 3000) with 5 cm flow cell, a manual sample injection valve equipped with a 20μl loop and Chromeleon 6.8 system manager as data processor. The separation was achieved by a reversed-phase Acclaim ™ 120 C18 column (5 μm particle size, i.d. 4.6 x 250 mm).

Preparation of standard solutions

The stock solution of concentration 1 mg/ml was prepared by dissolving 1 mg ascorbic acid in 0.5 ml HPLC-grade methanol followed by sonication for 10 min and the resulting volume was made up to 1 ml with the solvent for the mobile phase (acetonitrile and 1% aq. acetic acid 1: 9). The same method was followed to prepare the stock standard solutions of the phenolic acids and the flavonoids. The working solutions of the sample under investigation were prepared by further dilution of the standard solution with the mobile phase solvent system. The standard and working solutions were filtered through 0.45 µm PVDF-syringe filter and the mobile phase was degassed before the injection of the solutions.

Extraction of plant samples using four solvents of different polarity

One gram of coarsely powdered leaf was extracted using 5 ml chloroform with constant stirring for 24 h at the ambient temperature. The extract so prepared was filtered and the plant residue so left was macerated with the same volume of fresh solvent, stirred and filtered. The process was repeated thrice, and the extracts were combined. The extracts were finally filtered through 0.45µm PVDF membrane, and the volume was made up to 10 ml using the same solvent and stored. The same processes were followed for the preparation of sample extract in methanol, 80% aq. ethanol and 1% aq. acetic acid solution.

Chromatographic analysis of ascorbic acid, phenolic acids and flavonoids

The mobile phase contains 1% aq. acetic acid solution (Solvent A) and acetonitrile (Solvent B), the flow rate was adjusted to 0.7 ml/min, the column was thermostatically controlled at 28 °C and the injection volume was kept at 20 µl. A gradient elution was performed by varying the proportion of solvent B to solvent A. The gradient elution was changed from 10 % to 40% B in a linear fashion for the duration of 28 min, from 40 to 60 % B in 39 min, from 60 to 90 % B in 50 min. The mobile phase composition back to the initial condition (solvent B: solvent A: 10: 90) in 55 min and allowed to run for another 10 min, before the injection of another sample. Total analysis time per sample was 65 min.

HPLC Chromatograms were detected using a photodiode array UV detector at three different wavelengths (272, 280 and 310 nm) according to absorption maxima of analysed compounds. Each compound was identified by its retention time and by spiking with standards under the same conditions.

The quantification of the sample was done by the measurement of the integrated peak area and the content was calculated using the calibration curve by plotting peak area against the concentration of the respective standard sample. The data were reported with convergence limit in triplicate.

Validation of the method

According to the USP and ICH guidelines [10], there are various parameters to validate the reproducibility of the method *viz.* the effectiveness, the limit of detection (LOD), the limit of quantitation (LOQ), the linearity, the precision and the accuracy.

The effectiveness of the HPLC method was detected with the standard solutions of ascorbic acid, phenolic acids and the flavonoids. Generally, methanol of diverse composition is used as eluent, but solvents like acetonitrile, acetic acid, formic acid are also reported in the literature. In this study, different proportion of acetonitrile and 1% aq. acetic acid was used to achieve the best resolution.

To ascertain the linearity, the stock solution of the standard (1 mg/ml) was diluted to six different concentrations (5, 10, 20, 30, 40, 60 µg/ml) which were fed individually in triplicate to the HPLC system and the calibration curve so obtained by plotting peak area versus concentration for each sample where the square of the correlation coefficient R^2>0.99 is indicative of the measure of linearity.

The accuracy of the method was determined by application of the standard addition method. The leaves extract of *Z. acanthopodium* was spiked with two known concentration of calibration solutions (20 µg/ml and 40 µg/ml). The amounts of phenolic acids and flavonoids present in the investigated leaves were previously determined. For each standard compound, the percentage of recovery was calculated as follows

Recovery (%) = (amount found-amount contained)/amount added × 100

The high recovery rate in the range of 96–103% for the samples is indicative of efficacy and consistency.

Limit of detection and limit of quantification were calculated using the following formula

$$LOD = 3.3\ (\sigma)/S$$

$$LOQ = 10\ (\sigma)/S,$$

Where (σ) = standard deviation of response (peak area) and 5 = slope of the calibration curve.

The precision refers to the degree of proximity of the results expressible as % relative standard deviation (RSD) of the retention time and the peak area. The repeatability of the retention time and peak areas (Pa) were checked by injecting the mixed standard solutions at two concentration levels (20 µg/ml and 40 µg/ml) into the HPLC system. The RSD of retention time and peak areas were calculated for five replicate determinations.

RESULTS AND DISCUSSION

Validation of HPLC method

A typical HPLC chromatogram of the all standard mixture recorded at 272 nm is presented in fig. 1. As shown in the chromatogram, all

investigated compounds had responses at 272 nm, where they were successfully separated. The constituents under investigation were also identified by the recorded absorption spectra, which were comparable both for leaf extracts of *Z. acanthopodium* and standard substances. The regression coefficient together with LOD and LOQ values are shown in table 1. The high value of R^2>0.9906 in the range of analyzed concentrations at 272 nm is indicative of responsive linearity.

The repeatability of the retention time for all the standard samples and that for the peak areas two standards *viz*., 20µg/ml and 40 µg/ml was found to be below one percent. The significant high rate of recovery of the standard phenolics and the flavonoids worth's mention. It follows that the method under consideration is characterized by precision, accuracy, meticulousness and can be used for the qualitative as also quantitative assay of ascorbic acid, the phenolics and the flavonoids in the leaf extract of *Z. acanthopodium*.

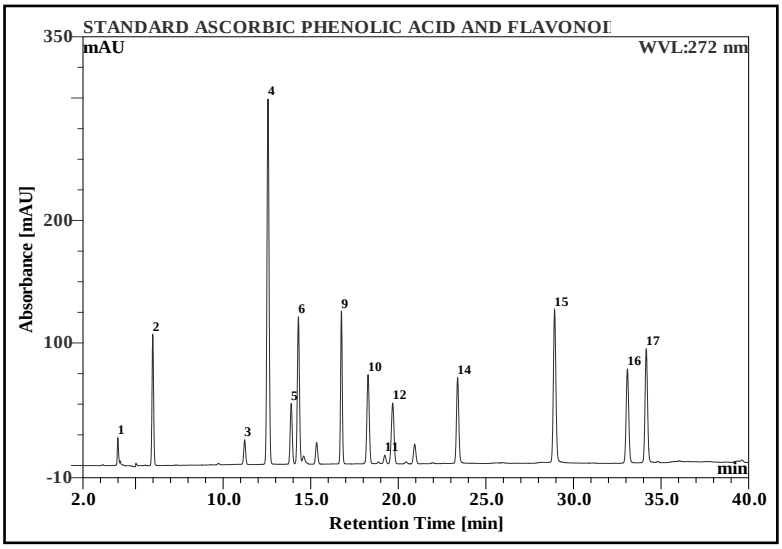

Fig. 1: HPLC chromatogram of standard ascorbic acid, phenolic acids and flavonoids

1. Ascorbic acid 2. Gallic acid 3. Catechin 4. Methyl gallate 5. Caffeic acid 6. Syringic acid, 9. Rutin 10. p-Coumaric acid 11. Sinapic acid 12. Ferulic acid 14. Myricetin 15. Quercetin 16. Apigenin 17. Kaempferol

Table 1: Retention time and parameters of calibration curve, precision, repeatability, LOD, LOQ and percent recovery study of standard ascorbic acid, phenolic acids and flavonoids for HPLC method validation

Name of the Standard	Detected at wavelength λ nm	Retention time	RSD (%) of the retention time	RSD (%) of the peak area at 20 µg/ml	RSD (%) of the peak area at 40 µg/ml	Regression coefficient R^2	LOD µg/ml	LOQ µg/ml	Percentage of recovery (%)
Ascorbic acid	272	4.02	0.437	0.069	0.550	0.9981	0.045	0.136	96.05
Gallic acid	272	6.01	0.178	0.014	0.011	0.9956	0.009	0.027	98.76
Catechin	272	11.28	0.124	0.144	0.077	0.9970	0.089	0.270	97.45
Methyl gallate	272	12.60	0.107	0.018	0.011	0.9949	0.012	0.035	98.02
Caffeic acid	272	13.96	0.060	0.018	0.009	0.9969	0.011	0.033	96.40
Syringic acid	272	14.33	0.085	0.313	0.018	0.9966	0.192	0.582	100.92
Rutin	272	16.80	0.061	0.037	0.016	0.9970	0.023	0.070	97.27
p-coumaric acid	272	18.37	0.051	0.079	0.005	0.9967	0.048	0.146	98.69
Sinapic acid	272	19.29	0.059	0.148	0.019	0.9967	0.093	0.280	97.65
Ferulic acid	272	19.77	0.050	0.100	0.010	0.9970	0.062	0.187	97.76
Myrecetin	272	23.49	0.053	0.253	0.090	0.9947	0.153	0.464	103.40
Quercetin	272	29.02	0.050	0.223	0.122	0.9957	0.130	0.395	98.12
Apigenin	272	33.16	0.050	0.032	0.012	0.9921	0.018	0.053	98.95
Kaempferol	272	34.23	0.046	0.025	0.008	0.9906	0.013	0.041	97.93

Note: RSD Relative standard deviation, LOD Limit of detection, LOQ limit of quantification

Identification and quantification of different phenolic acids and flavonoids in four different extracts of the plant

The HPLC chromatogram of the chloroform extract of the leaves of this plant showed the presence of rutin and apigenin as presented in fig. 2.

The methanol extract of the leaves of this plant revealed the presence of ascorbic acid, methyl gallate, caffeic acid, syringic acid, rutin, *p*-coumaric acid, ferulic acid and kaempferol at 272 nm as depicted in the HPLC chromatogram in fig. 3. The chromatogram of the extract in 80 % aq. ethanol indicated the presence of ascorbic acid, gallic acid, methyl gallate, caffeic acid, syringic acid, rutin, *p*-coumaric acid, ferulic acid and kaempferol in varying amounts as shown in fig. 4.

However, ascorbic acid, gallic acid, caffeic acid, rutin, p-coumaric acid and kaempferol were present in the chromatogram of the in 1% aq. the acetic acid extracts as shown in fig. 5.

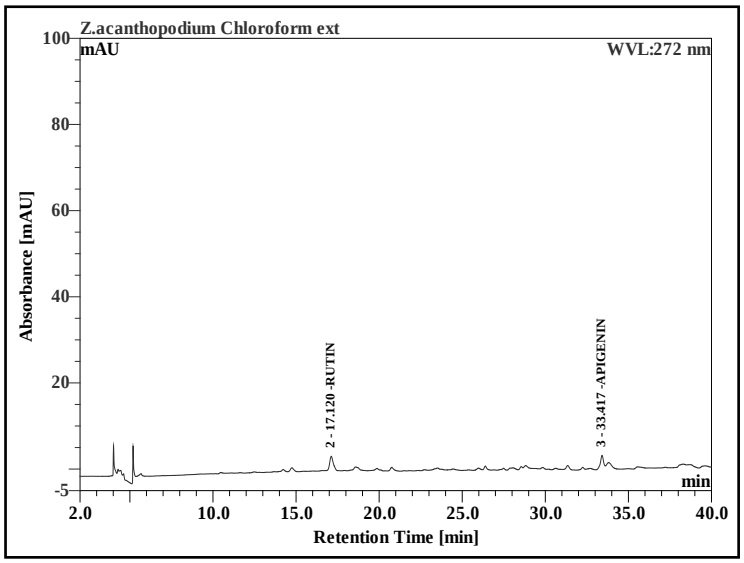

Fig. 2: HPLC chromatogram of the chloroform extract of *Z. acanthopodium*

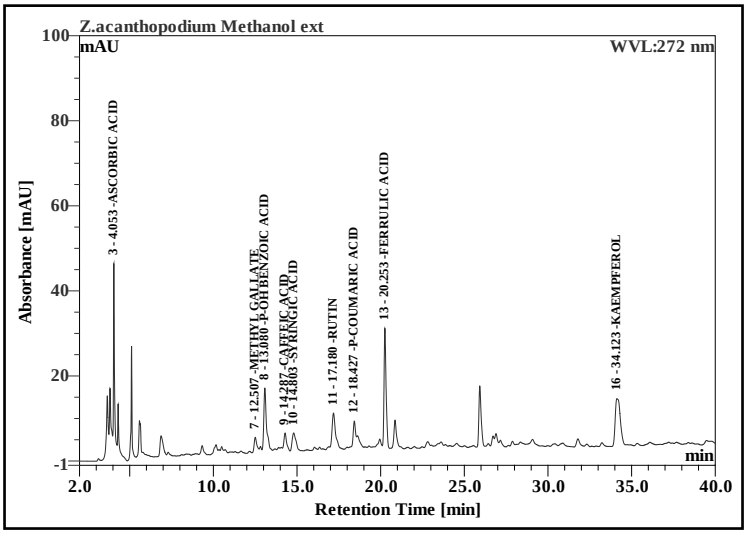

Fig. 3: HPLC chromatogram of the methanol extract of *Z. acanthopodium*

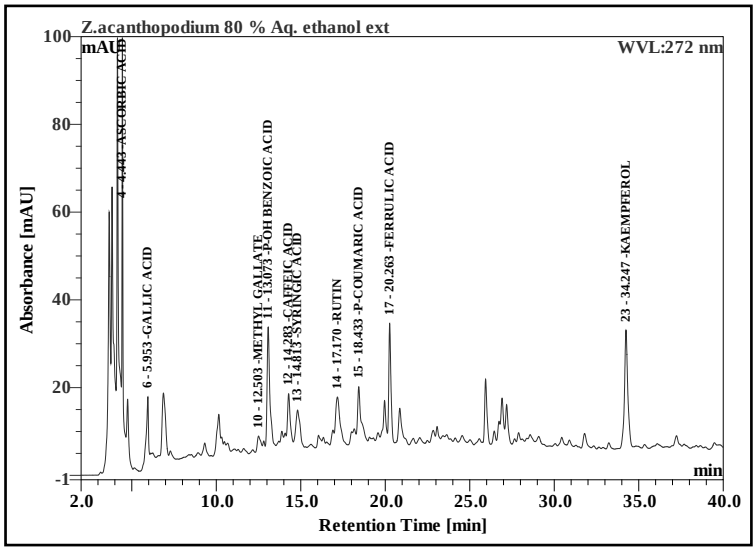

Fig. 4: HPLC chromatogram of the 80% aq. ethanol extract of *Z. acanthopodium*

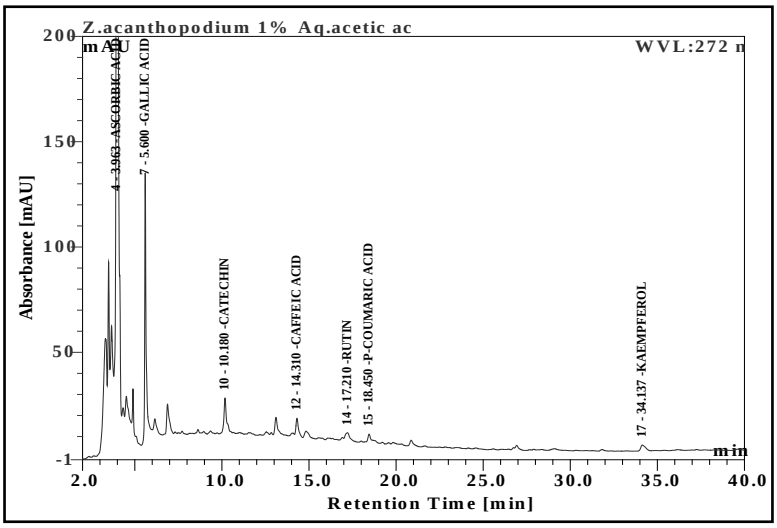

Fig. 5: HPLC chromatogram of the 1% aq. acetic acid extract of *Z. acanthopodium*

It is noteworthy that the flavonoids, being comparatively less polar, were found mainly in the less polar media and that their quantity was found to be improved to some extent in polar media accounting for the synergistic effect of neighbouring molecular architecture imposing restrictions on the freedom of-OH groups in the complex bio-matrix. Contrariwise, the elevated affection of the solvent polarity together with the concomitant cleavage of the ester linkage (as in depsides, for example) resulted in the increase in the content of the phenolic acids came to a compromise and the 80% aq. ethanol containing a maximum number as also the extent of the bio-active components was found to be the solvent of choice. The presence of huge amount (16.8 mg/g) of ascorbic acid in the 1% aq. acetic acid extract could be related to the oxidative stress, increased level of photosynthesis and the climatic animosity.

Quantification of ascorbic acid, phenolic acids and flavonoids in four different extracts of the plant

The present study indicated the presence of a large amount of ascorbic acid and gallic acid in the extract of leaves in the 1% aq. acetic acid in comparison to other extractive media as in table 2.

Table 2: Quantification of ascorbic acid, phenolic acids and flavonoids in the four different solvent extracts of *Z. acanthopodium*

Ascorbic acid/phenolic acids/flavonoids	Amount of ascorbic acid, phenolic acids and flavonoids (mg/g) in the four different solvent extracts of *Z. Acanthopodium*			
	1% Aq. acetic acid extract	80% Aq. ethanol extract	Methanol extract	Chloroform extract
Ascorbic acid	16.8±0.21	3.727±0.04	1.602±0.005	Not detected
Gallic acid	0.952±0.005	0.095±0.003	Not detected	Not detected
Catechin	Not detected	Not detected	Not detected	Not detected
Methyl gallate	Not detected	0.141±0.003	0.074±0.003	Not detected
Caffeic acid	0.141±0.002	0.086±0.003	0.034±0.002	Not detected
Syringic acid	Not detected	0.076±0.002	0.040±0.003	Not detected
Rutin	0.212±0.004	0.306±0.002	0.258±0.002	0.112±0.003
p-coumaric acid	0.085±0.002	0.136±0.003	0.064±0.003	Not detected
Ferulic acid	Not detected	0.231±0.004	0.231±0.004	Not detected
Myrecetin	Not detected	Not detected	Not detected	Not detected
Quercetin	0.288±0.003	0.125±0.003	0.199±0.004	0.122±0.004
Apigenin	Not detected	0.034±0.002	Not detected	0.029±0.004
Kaempferol	0.110±0.003	0.266±0.004	Not detected	Not detected

Each value in the table was obtained by calculating the average of three experiments, and data are presented as mean±SEM

Ascorbic acid is ubiquitous in fruits and vegetables and the boon of this acid as a nutraceutical in human nutrition dates back from time immemorial. An appreciable amount of the acid (16.8 mg/g) is present in the leaf of the plant as compared to that in commonly available plants, such as, *Allium officinalis* (1.90 mg/g), *Allium vineale* (1.30 mg/g), *Chenopodium album* (1.30 mg/g), green peppers (1.28 mg/g), lettuce (0.18 mg/g), spinach (0.51 mg/g), tomato (0.23 mg/g) etc [11]. This can be traced to the phylogenetic origin of the plant.

In the complex phyto-matrix, gallic acid remains either in the Free State or in the combined form as ester and acts as a powerful antioxidant. The gallic acid content in this plant is well compared to that in fruits such as, chilli pepper (3.33 mg/g), lemon (2.03 mg/g), spinach (1.82 mg/g), onion bulb (1.55 mg/g), cabbage (0.49 mg/g) etc.[12].

The extract in the 1% aq. the acetic acid solution contained high amount of caffeic acid (0.136 mg/g) and quercetin (0.29 mg/g). The polarity of these compounds made their appearance in 80% aq. ethanol extract too. The acid is one of the major hydroxycinnamic acid components found in wine and it is a well-known antioxidant which boosts immunity, controls lipid levels in blood and anti-mutagenic. The acid is found mainly in the form of its ester (as in chlorogenic acid) in fruits, vegetables and herbs. The caffeic acid content is compatible with the same in cauliflower (0.058 mg/g), carrot (0.09 mg/g), lettuce (1.57 mg/g) and potato (2.80 mg/g) [13].

Syringic acid with hydroxybenzoic acid skeleton is found in fruits and is well known for its anti-cancer, anti-proliferative, sedative, decongestant and hepatoprotective actions [14]. The content of the acid (0.08 mg/g) in the extract of the leaf in 80% aq. ethanol was higher than that reported for common leafy vegetables, such as,

cauliflower (0.0113 mg/g) [15], *Salvia officinalis* (0.0335 mg/g), *Organum vulgare* (0.0375 mg/g) [16].

One of the important phenolics, ferulic acid, is well-known for its physiology functions, such as anti-microbial, anti-microbial, anti-inflammatory, anti-cancer activities, etc. It also lowers cholesterol level in serum and increases sperm viability [17]. It is the most widely distributed phenolics in cereal grain is ferulic acid which constitutes 0.8 to 2 g/kg (DW) of wheat grain were polyphenolic account for 90%.

Another hydroxy cinnamic acid, *p*-coumaric acid, well-documented for its antioxidant behavior are widely distributed in foodstuffs, such as, barley, peanuts, navy beans, tomato, carrots, etc. and is believed to have antioxidant behaviour thereby reducing the formation of carcinogenic nitrosamines in the stomach [18].

Rutin is a phenolic compound with glycoside linkage and is used for the treatment of varicose veins, haemorrhoids, haemorrhagic stroke and mucositis. The rutin content in the extract of aq. 80% ethanol (0.307 mg/g) is comparable to that in the leaves of *Fagopyrum esculentum* (0.12 mg/g) and *Melisa officinalis* (0.30 mg/g) [19].

Quercetin is distributed in distributed in different parts of plants of the plant not only as aglycones but also as glycosides and is known to impart luxuriant color to the fruits, flowers, leafy parts, etc. It is reported to display anti-histamine, anti-cancer as also anti-inflammatory activities which mostly follow its antioxidant traits. The dietary sources of quercetin include citrus fruits, apples, onions, parsley, sage, tea and red wine. The famous "French Paradox" is related to the rich presence of this compound in red wine.

In fact, onion (*Allium cepa* L) is the richest source of this flavonol (2.60 mg/g) whereas the content of this compound in the leaves of the plant under study (0.289 mg/g in 1% aq. acetic acid) well exceeds the same in apple (0.021 mg/g), lettuce (0.011 mg/g) and tomato (0.055 mg/g) [20].

The dietary adjuncts, such as, fruits, vegetables, spices, herbs, etc. are the rich source of apigenin, a flavone, which is known to reduce the risk of cardiac ailments, neurological syndromes, mutagenesis. It is reported that raw parsley is abundant in this nutraceutical (3.02 mg/g) and the amount of this component in the leaves of wild edible *Z. acanthopodium* (0.035 mg/g) matches well with the harvested vegetables, such as, celery (0.046 mg/g), cabbage (0.0001 mg/g), sweet potato leaves (0.0012 mg/g), peppermint (0.087 mg/g) etc. [21].

Among natural poly-phenolics, kaempferol, a flavonol, is reported to possess potent pharmacological and nutraceutical activities thereby conferring innumerable health benefits in the form of reducing scourge of CVD, cancer, arteriosclerosis, etc. The antioxidant properties are known to be responsible for these health benefits [21].

It is worthwhile to mention that the polarity increases the quantification of highly polar phenolic acids and a few flavonoids like rutin, quercetin and apigenin are detected in low concentration in the least polar solvent (Chloroform extract). However, the extract in. 80% aq. ethanol is found to be the optimum solvent of choice as it contains the maximum variety and the extent of bio-active components.

CONCLUSION

The reversed-phase HPLC method with diode array detection was developed for the quantitative estimation of ascorbic acid, phenolic acids and flavonoids in the four different solvent extracts of *Z. acanthopodium.* The established HPLC assay showed a good separation of the compounds and also the developed method was linear, sensitive, accurate, meticulous and reproducible. Therefore, the method can be used for the simultaneous determination of phenolic acids and flavonoids in different formulations with 'shorter runtime' and 'high efficiency'. The presence of significant amount of respective bio-active components in the plant under study and variation of quantity determined based on the polarity of the solvent taken for the extraction process, ensures its unequivocal recommendation for the use in the pharmaceutical and nutraceutical sector.

ACKNOWLEDGEMENT

The Author owe deep gratitude to Dr. P. Singh, Director, Botanical Survey of India, Kolkata for extending necessary scientific facilities & express sincere indebtedness to Mr. R. Shanpru, Scientist, Botanical Survey of India, North Eastern Circle, Shillong, Meghalaya for identifying the plant specimens.

CONFLICT OF INTERESTS

Declare none

REFERENCES

1. Waksmundzka–Hajnos M, Oniszczuk A, Szewczyk K, Wianowska D. Effect of sample preparation methods on the HPLC quantitation of some phenolic acids in plant materials. Acta Chromatogr 2007;19:227-37.
2. Mattila P, Hellstrom J. Phenolic acids in potatoes, vegetables, and some of their products. J Food Compos Anal 2007;20:152-60.
3. Maheshkumar SK, Kirti SL. Determination of total flavonoids content and quantification of rutin in *Momordica tuberosa* (Roxb) Cogn. fruits by RP-HPLC. Asian J Tradit Med 2012;7:220-5.
4. Kayang H. Tribal knowledge on edible wild plants of Meghalaya, Northeast India. Indian J Traditional Knowledge 2007;6:177-81.
5. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. Seventh reprint; 2006. p. 170-241.
6. Wijaya CH, Hadiprodjoi TI, Apriyantono AJ. Identification of volatile compounds and key aroma compounds of aliman fruits (*Z. acanthopodium*). Food Sci Biotechnol 2002;11:680–3.
7. Nayar MP, Sastry ARK. Red Data Book of Indian plants. Vol. I. Botanical survey of India; 1987.
8. Seal Tapan. Determination of nutritive value, mineral contents and antioxidant activity of some edible wild plants from Meghalaya State. India Asian J Appl Sci 2011;4:238-46.
9. Nur Syukriah AR, Liza MS, Harisun Y, Fadzillah AAM. Effect of solvent extraction on antioxidant and antibacterial activities from *Quercus infectoria* (Manjakani). Int Food Res J 2014;21:1067-73.
10. Center for Drug Evaluation and Research (CDER). Reviewer Guidance', Validation of Chromatographic Methods; 1994. p. 7-28.
11. Zennie TM, Dwayne OC. Ascorbic acid and vitamin a content of edible wild plants of ohio and kentucky. Economic Bot 1977;31:76-9.
12. Romaric GB, Fatoumata AL, Oumou HK, Mamounata D, Imael HNB, Mamoudou HD. Phenolic compounds and antioxidant activities in some fruits and vegetables from burkina faso. Afr J Biotechnol 2011;10:13543-7.
13. Schmidtlein H, Herrmann K. On the phenolic acids of vegetables. IV Hydroxycinnamic acids and hydroxybenzoic acids of vegetables and potatoes (GeL). Z Lebensmittel Untersuch. Forsch 1975;159:255-63.
14. Vinayagam R. Preventive effect of syringic acid on hepatic marker enzymes and lipid profile against acetaminophen-induced hepatotoxicity rats. Int J Pharm Biol Arch 2010;1:393-8.
15. Ping L, Xu-Qing W, Huai-Zhou W, Yong-Ning W. High-performance liquid chromatographic determination of phenolic acids in fruits and vegetables. Biomed Environ Sci 1993;6:389-98.
16. Kivilompolo M, Oburka V, Hyotylainen T. Comparison of GC-MS and LC-MS methods for the analysis of antioxidant phenolic acids in herbs. Anal Bioanal Chem 2007;388:881-7.
17. Mussatto SI, Dragone G, Roberto IC. Ferulic and *p*-coumaric acids extraction by alkaline hydrolysis of brewer's spent grain. Ind Crops Prod 2007;25:231–7.
18. Karthikeyan R, Devadasu C, Srinivasa Babu P. Isolation, characterization, and RP-HPLC estimation of *p*-coumaric acid from methanolic extract of durva grass (*Cynodon dactylon*

(Linn.) Pers). Int J Anal Chem 2015;1-7. Doi:10.1155/ 2015/ 201386. [Epub 18 Feb 2015].

19. Moghaddasian B, Eradatmand AD, Alaghemand A. Simultaneous determination of rutin and quercetin in different parts of *Capparis spinosa.* Bull Environ Pharmacol Life Sci 2013;2:35-8.
20. Wach A, Pyrzynska K, Biesaga M. Quercetin content in some food and herbal samples. Food Chem 2007;100:699–704.
21. Mohammad A, Elham KK. Medicinal uses and chemistry of flavonoid contents of some common edible tropical plants. J Paramed Sci 2013;4:119-38.