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Research Article

Identification and Quantification of Phenolic Acids by HPLC, in Three Wild Edible Plants *Viz. Viburnum foetidum, Houttuynia cordata* and *Perilla ocimoides* Collected from North-Eastern Region in India

Tapan Seal*, Basundhara Pillai, Kausik Chaudhuri

Plant Chemistry Department, Botanical Survey of India, A. J. C. Bose Indian Botanic Garden, Shibpur, Howrah, INDIA

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method using photodiode array detector with gradient elution has been developed and validated for the estimation of free phenolic acids (gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, *p*-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, salicylic acid and ellagic acid), in two different solvent extracts of *Viburnum foetidum*, *Houttuynia cordata* and *Perilla ocimoides* collected from North-eastern region in India . The chromatographic separation of phenolic acids was carried out on Acclaim C 18 column (5 µm particle size, 250 x 4.6 mm), Dionex Ultimate 3000 liquid chromatograph and detection was carried out at three different wave lengths (272, 280 and 300 nm) using a mobile phase of methanol and 1% aqueous acetic acid solution with gradient elution. The experimental results showed a very high amount of protocatechuic acid in the methanol extract of *V. foetidum* (65.08±0.04 mg/gm dry extract) of the plant material. The high percentage of recovery (98-99%), 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 all phenolic acids in the three plants under investigation.

Keywords: Phenolic acids; Different solvent extracts; *V. foetidum*; *H. cordata*; *P. ocimoides*; Gradient HPLC

INTRODUCTION

Phenolic compounds are ubiquitous in plants and these are secondary metabolites which shield the plants against UV-radiation or resist the pathogenic aggression. The phenolic acids with common bio-genetic 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 depsides. These groups are well-known for their analgesic, antipyretic, cholagogic, sedative and anti-biotic properties[1]. The commonest is hydroxycinnamic acid which consists mainly of ferulic, *p*-coumaric, caffeic, sinapic acid etc. These acids occur chiefly in the form of ester of quinic acid or glucose *e.g*. chlorogenic acid. The derivatives of hydroxy 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 oxidative damaged diseases through consumption of fruits and vegetables. The amazing antioxidant cum nutraceutical 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]. *Viburnum foetidum* Wall, known as Soh lang in Meghalaya state, belongs to the family Caprifoliaceae. It is common in Khasi Hills of Meghalaya and in Assam of India. The plant is astringent, emmenagogue, juice of the leaves used internally in menorrhagia and in post-partum haemorrhage. It yields essential oil and crystalline alkaloid. The fruits of this plant are edible[3]. *Houttuynia cordata* known as 'Jamyr-doh' in Meghalaya state belongs to the family Saururaceae. The whole plant is eaten raw. The leaf juice is taken for the treatment of cholera, dysentery, curing of blood deficiency and purification of blood. The tender young shoots and leaves are eaten raw or cooked as a pot-herb. A decoction of this plant is used internally in the management of many ailments including cancer, coughs, dysentery, enteritis and fever. Externally, it is used in the treatment of snake bites and skin disorders The leaves and stems are harvested during the growing season and used fresh in decoctions The leaf juice is antidote and astringent[4]. *Perilla ocimoides* Linn., known as Nei in Meghalaya state, belongs to the family Labiatae. The leaves, stems and seeds of this plant considered as diaphoretic and cephalic in China and Indo-China. In Meghalaya state the seeds are roasted, crushed or pounded with salt and eaten as chutney. The seed contains a fixed oil similar in taste, odour and drying qualities to our common linseed oil. In Manchuria, this oil is used for edible purposes[4]. The nutritive value and the antioxidant activities of these plants have already been studied in our laboratory. The seeds of *P. ocimoides* and roots *H. cordata* are characterized by high protein content (23.85 % and 12.22 %) and substantial mineral content (Na, K, Ca etc.).

**Author for Correspondence: kaktapan65@yahoo.co.in*

The methanol extract of the fruits of *V. foetidum*, seeds of *P. ocimoides* and roots of *H. cordata* are reported to show high phenolic content (54.28, 20.72 and 24.60 mg GAE/gm of dry extract (DE) respectively) and strong DPPH radical scavenging activities[5,6]. Thus, the presences of an appreciable number of phenolic compounds in these plants 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 nutraceutical role provide unequivocal testimony to the fact. The extraction of polyphenolic compounds from plant is highly depending on the polarity of the solvent because 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[7]. Therefore this study was aimed to investigate the antioxidant potential and quantitative estimation of free phenolic acids such as gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, *p*-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, salicylic acid and ellagic acid in two different solvent extracts (methanol, 80% aq. ethanol) of three wild edible plants of *viz. Viburnum foetidum, Houttuynia cordata* and *Perilla ocimoides* collected from Meghalaya state of India (North-eastern region in India) using reversed phase HPLC with diode array detection.

MATERIALS AND METHODS

Plant material

The fruits of *Viburnum foetidum,* roots *of Houttuynia cordata* and seeds of *Perilla ocimoides* 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 2 and BSITS 3 and BSITS 6 for future reference. The plant part was shed-dried, made coarse powder and stored in an air-tight container for extraction.

Chemicals

The standards chemicals like ascorbic acid, phenolic acids *viz*. gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, *p*-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, salicylic acid and ellagic acid 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 20 μl loop and Chromeleon 6.8 system manager as data processor. The separation was achieved by a reversed-phase Acclaim^{TM} 120 C18 column (5 μm particle size, i.d. 4.6 x 250 mm).

Preparation of standard solutions

The stock solution of concentration 1mg / ml was prepared by dissolving 1 mg gallic acid in 0.5 ml HPLC-grade methanol followed by sonication for 10 minutes and the resulting volume was made up to 1 ml with methanol. The same method was followed to prepare the standard stock solutions of the phenolic acids. The working solutions of the sample under investigation were prepared by further dilution of the standard solution with methanol. 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 two solvents of different polarity

One gm of each coarsely powdered plant samples were extracted using 5 ml methanol with constant stirring for 24 hours 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 process was followed for the preparation of plant extract in 80% aq. ethanol.

Chromatographic analysis of phenolic compounds

The chromatographic analysis was carried out following the method as described by Violeta Nour et al[8] with minor modification. The mobile phase contains methanol (Solvent A) and 1% aq. acetic acid solution (Solvent B), the column was thermostatically controlled at 28^0 C and the injection volume was kept at 20 µl. A gradient elution was performed by varying the proportion of solvent A to solvent B. The gradient elusion was 10 % A and 90 % B with flow rate 1ml/min to 0.7 ml/min in 27 min, from 10 % to 40% A with flow rate 0.7ml/min for 28 min, 40 % A, with flow rate 0.7 to 0.6 ml/min for 5 min, from 40 to 44 % A with flow rate 0.6 to 0.3 ml/min in 5 min, 44 % A with flow rate 0.3 to 0.6 ml/min in 5 min. The mobile phase composition back to initial condition (solvent A: solvent B: 10: 90) in 73 min and allowed to run for another 8 min, before the injection of another sample. Total analysis time per sample was 81 min. HPLC Chromatograms were detected using a photo diode array UV detector at three different wavelengths (272, 280 and 300 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 concentration of the respective standard sample. The data were reported as means ± standard error means of three independent analyses.

Validation of the method

According to the USP and ICH guidelines, there are various parameters to validate the reproducibility of the

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

Name of the Standard phenolic acids	Detected at wavelength λ nm	Retention time	RSD (%) of the retention time	RSD (%) of the peak area at conc 40 µg/ml	RSD (%) of the peak area at conc 60 µg/ml	Regression Coefficient R^2	LOD µg/ml	LOQ µg/ml	Percentage of recovery (%)
Gallic acid	280	7.11	0.956	0.138	0.149	99.88	0.186	0.565	98.76
Protocatechuic acid	280	15.32	0.706	0.206	0.171	99.83	0.277	0.839	98.50
Gentisic acid	280	27.50	0.712	0.799	0.382	99.91	1.062	3.219	98.15
p-OH benzoic acid	280	31.91	0.830	0.173	0.103	99.89	0.233	0.705	98.33
Chlorogenic acid	280	42.88	0.475	0.220	0.227	99.10	0.309	0.935	99.20
Vanillic acid	280	44.54	0.462	0.025	0.032	99.73	0.034	0.103	98.24
Caffeic acid	280	46.49	0.453	0.114	0.144	99.68	0.156	0.472	98.25
Syringic acid	280	49.60	0.787	0.118	0.047	99.58	0.163	0.492	98.13
p-Coumaric acid	280	56.61	0.796	0.061	0.027	99.93	0.083	0.250	98.78
Ferulic acid	280	59.71	0.621	0.176	0.199	99.98	0.230	0.698	98.14
Sinapic acid	280	60.35	0.546	0.450	0.292	99.96	0.584	1.770	98.30
Salicylic acid	280	69.02	0.383	0.335	0.509	99.52	0.414	1.255	98.13
Ellagic acid	280	74.25	0.747	0.333	0.325	93.61	0.448	1.357	98.33

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

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 phenolic acids. 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 methanol 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 extracts of *V. foetidum, H. cordata* and *P. ocimoides* were spiked with two known concentration of standard solutions (40 µg/ml and 60 µg/ml). The amounts of phenolic acids present in the investigated plants 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 98 – 99% 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 $(\sigma)/S$ and LOQ = 10 $(\sigma)/S$, where (σ) = standard deviation of response (peak area) and S = 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 (40 µg/ml and 60 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 280 nm is presented in fig. 1. As shown in the chromatogram, all investigated compounds had responses at 280 nm, where they were successfully separated. The constituents under investigation were also identified by the recorded absorption spectra, which were comparable both for plant extracts 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 280 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.*, 40 µg/ml and 60 µg/ml was found to be below one percent. The significantly high rate of recovery of the standard phenolics 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 estimation of phenolics in the two different solvent extracts of these three plants under investigation.

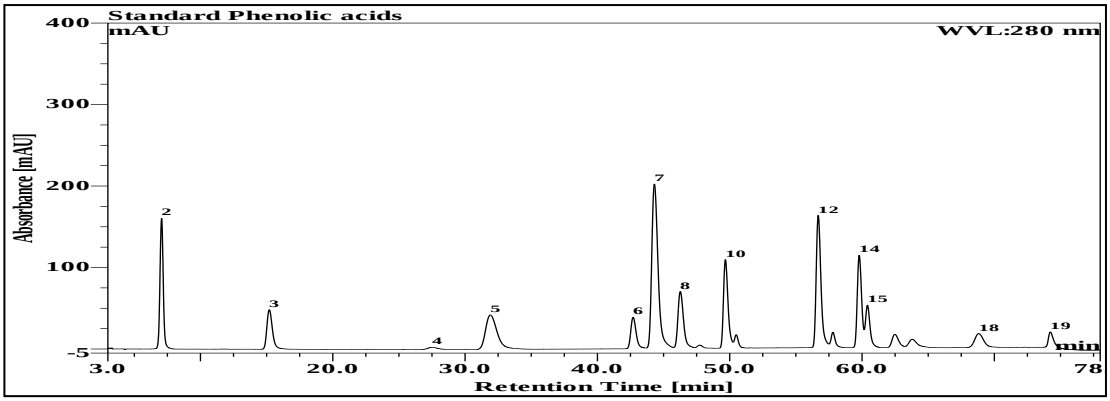

Figure 1: HPLC Chromatogram of standard phenolic acids

2. Gallic acid, 3. Protocatechuic acid 4. Gentisic acid 5. p-Hydroxy benzoic acid 6. Chlorogenic acid 7. Vanilic acid 8. Caffeic acid, 10. Syringic acid 12. p-Coumaric acid 14. Ferulic acid 15. Sinapic acid 18. Salicylic acid 19. Ellagic acid

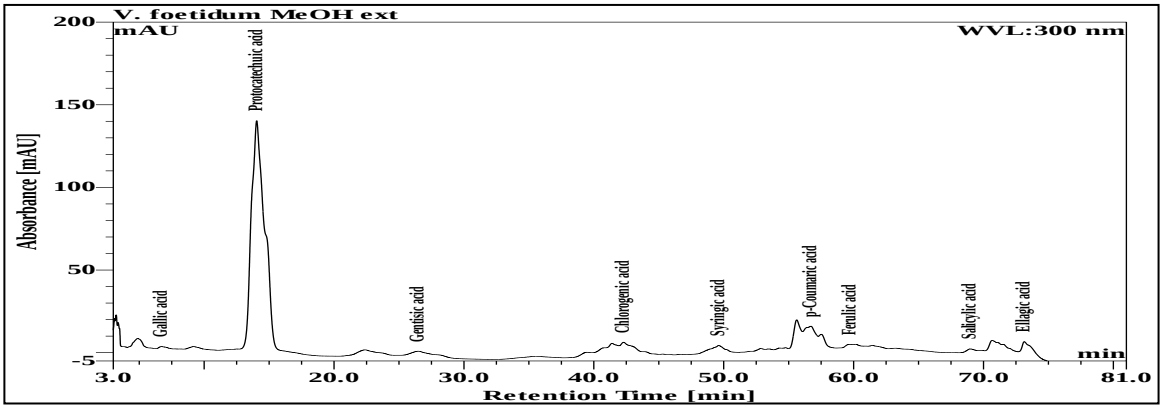

Figure 2: HPLC chromatogram of the methanol extract of V. *foetidum*

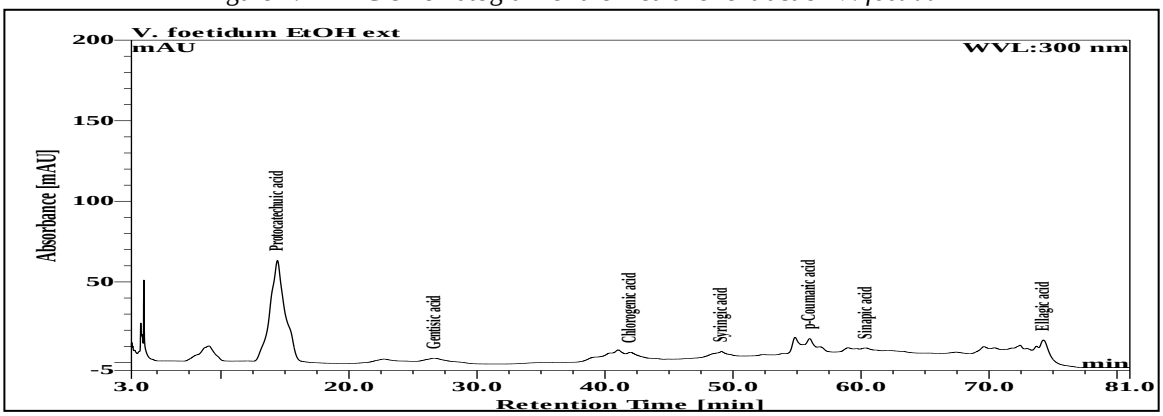

Figure 3: HPLC chromatogram of the 80 % aq. ethanol extract of *V. foetidum*

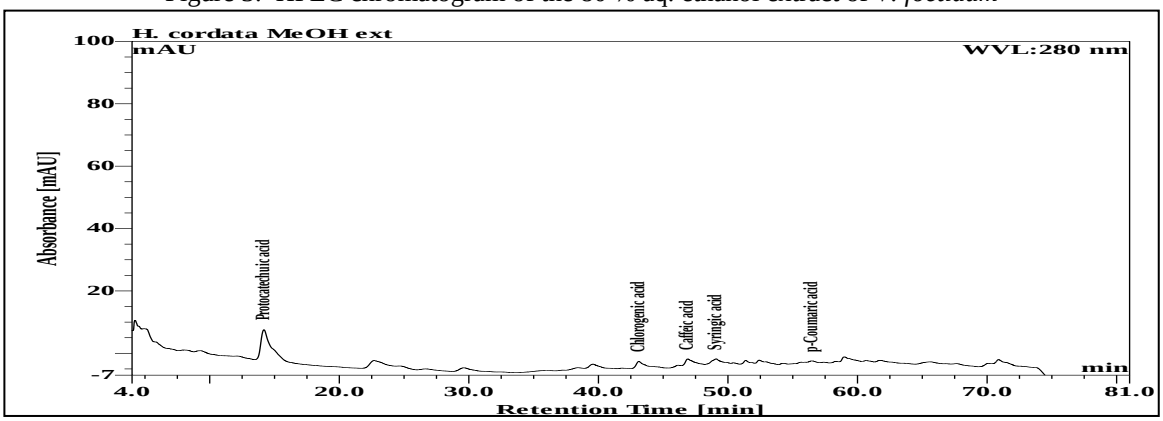

Figure 4: HPLC chromatogram of the methanol extract of *H. cordata*

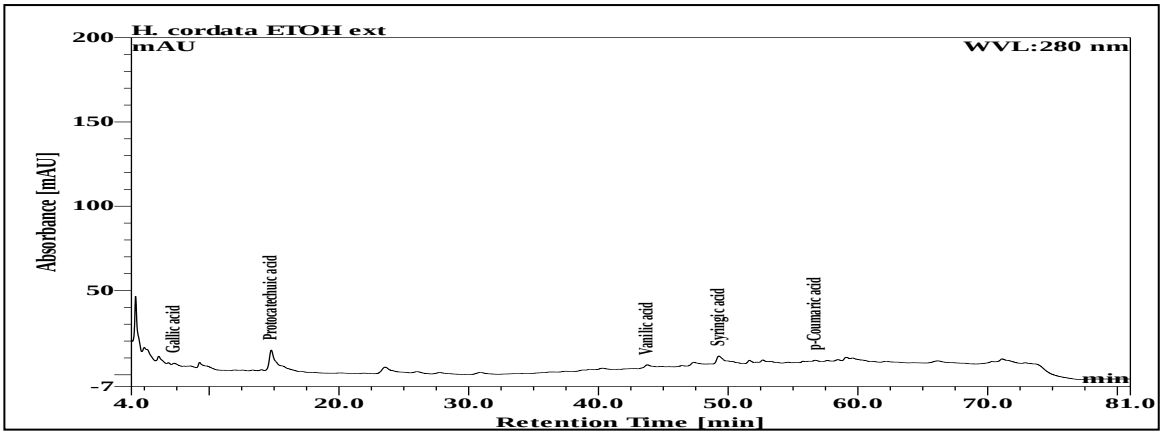

Figure 5: HPLC chromatogram of the 80 % aq. ethanol extract of *H. cordata*

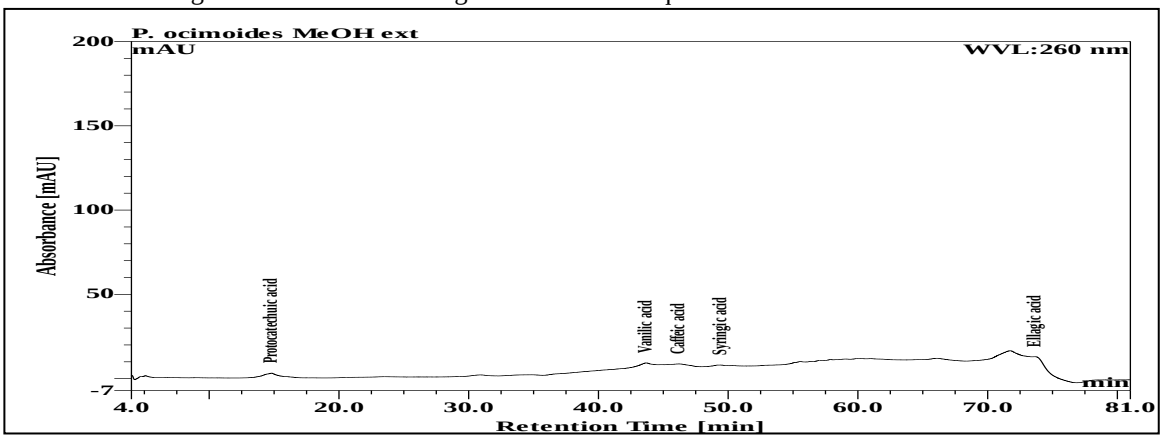

Figure 6: HPLC chromatogram of the methanol extract of *P. ocimoides*

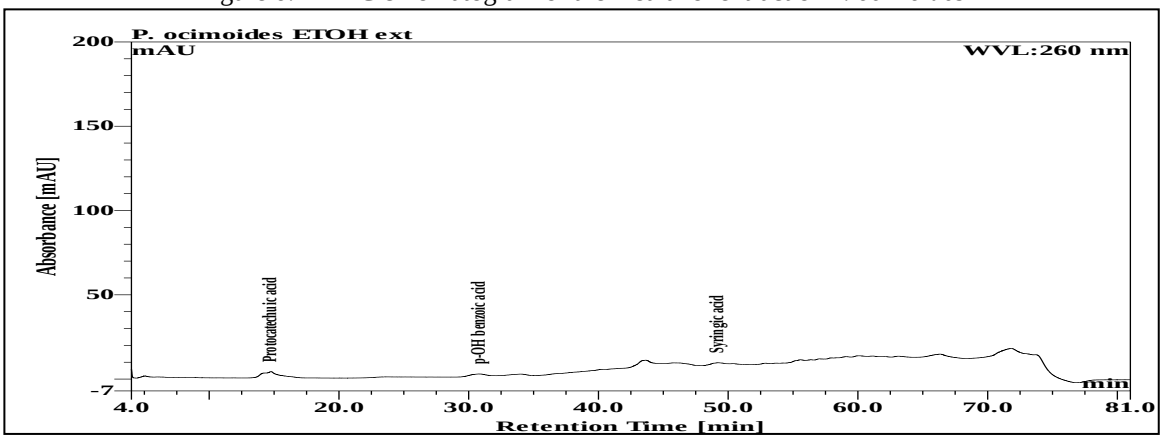

Figure 7: HPLC chromatogram of the 80 % aq. ethanol extract of *P. ocimoides*

Identification and quantification of different phenolic acids in the methanol and 80% aq. ethanol extracts of *V. foetidum*, *H. cordata* and *P. ocimoides*

The HPLC chromatogram of methanol extract of the fruits of *V. foetidum* showed the presence of gallic acid (0.38±0.007 mg/gm dry extract DE), gentisic acid (5.09±0.03 mg/gm DE), chlorogenic acid (0.83±0.0004 mg/gm DE), syringic acid (3.19±0.008 mg/gm DE), *p*-coumaric acid (0.29±0.003 mg/gm DE), ferulic acid (0.27±0.001 mg/gm DE), salicylic acid (0.21±0.006 mg/gm DE) and ellagic acid (12.83±0.004 mg/gm DE) whereas the 80 % aq. ethanol extract of this plant contained gentisic acid (3.27±0.011 mg/gm DE), chlorogenic acid (0.294±0.001 mg/gm DE), syringic acid (2.21±0.02 mg/gm DE), *p*-coumaric acid (0.172±0.004 mg/gm DE), sinapic acid (0.137±0.001 mg/gm DE) and ellagic acid (6.84±0.04 mg/gm DE) as presented in fig. 2 and fig.3. The methanol extract of *V. foetidum* contained a very high amount of protocatechuic acid (65.08±0.04 mg/gm DE), as compared to the 80 % aq. ethanol extract (30.45±0.07 mg/gm DE) of the plant. Both the methanol and 80 % aq. ethanol extracts of the roots of *H. cordata* revealed the presence of a good amount of protocatechuic acid, syringic acid and *p*-coumaric acid. HPLC analysis also showed the presence of chlorogenic acid and caffeic acid in the methanol extract of the plant and these

phenolics were not found in the 80 % aq. ethanol extract of the plant, whereas gallic acid and vanillic acids were detected only in the 80 % aq. ethanol extract of same plant as depicted in the HPLC chromatogram in fig. 4 and fig. 5. The 80 % aq. ethanol extract of *P. ocimoides* showed the presence of good amount of protocatechuic acid (2.18±0.012 mg/gm DE) and syringic acid (1.98 ± 0.013mg/gm DE) as compared to the methanol extract of the plant. The methanol extract of the plant contained a good amount of caffeic acid, vanillic acid and ellagic acid which were not found in the 80% aq. ethanol extract of the plant as shown in fig. 6 and fig. 7. 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 found (Table 2) in methanol extract of *V. foetidum* (0.38±0.007 mg/gm DE**)** and 80 % aq. ethanol extract *H. cordata* (0.052 ± 0.002 mg/gm DE) are well compared to that in fruits such as, chilli pepper (3.33 mg/gm), lemon (2.03 mg/gm), spinach (1.82 mg/gm), onion bulb (1.55 mg/gm), cabbage (0.49 mg/gm) etc.[9]. Protocatechuic acid is a type of widely distributed naturally occurring phenolic acid. It has structural similarity with gallic acid, caffeic acid, vanillic acid, and syringic acid which are well-known antioxidant compounds. It is widely distributed and present in most edible plants used in folk medicine. It is also a very common compound present in human diet, present in bran and grain brown rice and also found in many fruits, such as plums gooseberries, grapes and nuts. This phenolic acid showed various pharmacological activity and these effects are due to their antioxidant activities, along with other possible mechanisms, such as anti-inflammatory properties and interaction with several enzymes[10]. A very high amount of protocatechuic acid present in the methanol (65.08±0.04 mg/gm DE) and 80 % aq. ethanol (30.45±0.07 mg/gm DE) extract of *V. foetidum*, which might be responsible for the strong antioxidant properties of the plant and thus help in prevention and therapy of various oxidative stress related diseases such as neurodegenerative and hepatic diseases[10].

Gentisic acid is widely found in various plants like citrus fruits, *Vitis vinifera*, *Helianthus tuberosus*, *Sesamum indicum*, *Gentiana* spp, *Pterocarpus santalinus*, *Eucalyptus grandis*, olive *Olea europaea* etc. Gentisic acid has an effective role in the anti-carcinogenetic activity of China-rose hibiscus (*Hibiscus rosa-sinensis*) extract and recently it has been established as Fibroblast Growth Factor (FGF) inhibitor. Gentisic acid is reported to be used as analgesic, anti-inflammatory, anti-rheumatic, anti-arthritic, and cytostatic agent and it inhibits low-density lipoprotein oxidation in human plasma[11]. The present study showed the presence of a reasonable amount of gentisic acid in both the methanol (5.09±0.03 mg/gm DE) and 80% aq. ethanol (3.27 ±0.011) extract of *V. foetidum* where as it was not detected in *H. cordata* and *P.ocimoides.* *p*-Hydroxybenzoic acid, reported to possesses antifungal, anti-mutagenic, anti-sickling, estrogenic, and anti-microbial activities has been detected only in the 80 % aq.ethanol extract of *P. ocimoides* (2.18± 0.012) mg/gm DE) among three plants taken for investigation. It has been isolated from many sources *viz. Daucus carota*, oil palm, grapes and numerous other species including east African satinwood, yellow-leaf tree, *Paratecoma peroba*, *Tabebuia impetiginosa*, red sandalwood, southern catalpa, *Vitex negundo*, betel palm, *Roystonea regia* and *Mespilus germanica*[11]. Chlorogenic acids are a phytochemical found in coffee and coffee beans and also found in higher plants. It has been touted as being able to reduce blood sugar levels and potentially exert an anti-diabetic effect. The methanol extracts of *V. foetidum* and *H. cordata* are found to contain a moderate amount of chlorogenic acids. The consumption of the plant containing chlorogenic acids are associated with a lower risk of a variety of liver diseases, including liver cirrhosis and liver cancer[12]. Vanillic acid is a benzoic acid derivative used as a flavouring agent. The highest quantity of vanillic acid in plants has been reported in the roots of *Angelica sinensis* and also isolated from numerous plants like *Panax ginseng, Pterocarpus santalinus, Picrorhiza kurrooa* etc. Various studies showed that vanillic acid has a hepatoprotective effect through its suppressive action on immune-mediated liver inflammation in concanavalin A-induced liver injury[13]. In our study vanillic acids were found in the methanol extract of *P. ocimoides* and 80 % aq. ethanol extract of *H. cordata* whereas it was not detected in any extracts of *V. foetidum*. Caffeic 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 present study showed that the methanol extracts of *H. cordata* and *P. ocimoides* were found to contain a very good amount of caffeic acid ranging from 0.203±0.001 to 0.34±0.003 mg/gm DE and these were compatible with the same in cauliflower (0.058 mg/gm), carrot (0.09 mg/gm), lettuce (1.57 mg/gm) and potato (2.80 mg/gm)[14]. Syringic acid with hydroxy benzoic acid skeleton is found in fruits and is well known for its anti-cancer, anti-proliferative, sedative, decongestant and hepato-protective actions[15]. Our investigation with these three plants showed that a very good amount of syringic acids were found in methanol and 80% aq. ethanol extracts of *V. foetidum* and a reasonable amount were also found in the both extracts of *H. cordata* and *P. ocimoides* in different concentrations. The content of the acid in the different extracts of plants under investigation were higher than that reported for common leafy vegetables, such as, cauliflower (0.0113 mg/gm)[16], *Salvia officinalis* (0.0335 mg/gm), *Origanum vulgare* (0.0375 mg/gm)[17]. One of the important phenolics, ferulic acid which is only detected in the methanol extract of *V. foetidum* in our study, 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

Table 2: Quantification of phenolic acids in the two different solvent extracts of *V. foetidum, H. cordarta* and *P.ocimoides*

Phenolic acids	Amount of phenolic acids (mg/gm dry extract DE) in the two different extracts of *V. foetidum, H. cordata* and *P. ocimoides*					
	V. foetidum		*H. cordata*		*P. ocimoides*	
	Methanol	80% Aq. ethanol	Methanol	80 % Aq. ethanol	Methanol	80 % Aq. ethanol
Gallic acid	0.38±0.007	ND	ND	0.052±0.002	ND	ND
Protocatechuic acid	65.08±0.04	30.45±0.07	2.91±0.02	2.41±0.001	0.214±0.001	2.18±0.012
Gentisic acid	5.09±0.03	3.27±0.011	ND	ND	ND	ND
p-Hydroxy benzoic acid	ND	ND	ND	ND	ND	0.296±0.003
Chlorogenic acid	0.83±0.0004	0.294±0.001	0.84±0.02	ND	ND	ND
Vanillic acid	ND	ND	ND	0.062±0.001	0.027±0.001	ND
Caffeic acid	ND	ND	0.34±0.003	ND	0.203±0.001	ND
Syringic acid	3.19±0.008	2.21±0.02	0.252±0.003	0.638±0.001	0.069±0.001	1.98±0.013
*p*_coumaric acid	0.29±0.003	0.172±0.004	0.026±0.001	0.023±0.001	ND	ND
Ferulic acid	0.27±0.001	ND	ND	ND	ND	ND
Sinapic acid	ND	0.137±0.001	ND	ND	ND	ND
Salicylic acid	0.21±0.006	ND	ND	ND	ND	ND
Ellagic acid	12.83±0.004	6.84±0.04	ND	ND	0.131±0.002	ND

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

Note: ND denote Not detected

viability[18]. It is the most widely distributed phenolics in cereal grain is ferulic acid which constitutes 0.8 to 2 gm/Kg (DW) of wheat grain where polyphenolics account for 90%. Another Hydroxycinnamic acid, *p*-coumaric acid, well-documented for its antioxidant behaviour, is widely distributed in food stuffs, such as, barley, peanuts, navy beans, tomato, carrots etc. and is believed to have antioxidant behavior thereby reducing the formation of carcinogenic nitrosamines in the stomach[19]. The HPLC analyses of the three plants under investigation revealed that both the methanol and 80% aq.ethanol extracts of *V. foetidum* contain very good amount of *p*-coumaric acid ranging from 0.172±0.004 to 0.29±0.003 mg/gm DE. The result of analysis also showed the presence of lesser amount of *p*-coumaric acid in *H. cordata* indicating its lower antioxidant activities than *V. foetidum*. Sinapic acid is a substance widely prevalent in the plant kingdom and has been identified in various fruits, vegetables, cereal grains, oilseed crops, some spices, and medicinal plants. Sinapic acid showed antioxidant, anti-microbial, anti-inflammatory, anticancer, and anti- anxiety activity. In present study the 80 % aq. ethanol extract of *V. foetidum* found to contain 0.137±0.001 mg/gm DE sinapic acid whereas this acid was not detected in two other plants. Ellagic acid is a natural phenol antioxidant found in numerous fruits and vegetables. The highest levels of ellagic acid are found in blackberries, cranberries, pecans, pomegranates, raspberries, strawberries, walnuts, wolfberries, and grapes. These molecules have a variety of benefits like anti-mutagenic, antimicrobial and antioxidant properties, and inhibitors of human immunodeficiency virus (HIV). Ellagic acid has been marketed as a dietary supplement with a range of claimed benefits against cancer, heart disease, and other medical problems[20]. A very good amount of ellagic acid was detected in the methanol extract (12.83±0.004 mg/gm DE) of *V. foetidum* followed by the 80 % aq. ethanol extract of the same plant. The methanol extract of *P. ocimoides* also was found to contain a sensible amount of ellagic acid. So the fruits of *V. foetidum* plant might be considered as a good source of ellagic acid and consumption of this plant would be useful for health promotion.

CONCLUSION

The reversed-phase HPLC method with diode array detection was developed for the quantitative estimation of phenolic acids in the two different solvent extracts of *V. foetidum, H. cordata* and *P. ocimoides.* 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 run time' and 'high efficiency'. RP-HPLC results showed the plants contained several phenolic compounds: gallic acid, protocatechuic acid, gentisic acid, p-hydroxy benzoic acid, caffeic acid, syringic acid, vanillic acid, *p*-coumaric acid, sinapic acid, chlorogenic acid ferulic acid and ellagic acid in varying amounts. So these wild plants are valuable in antioxidant components. The presence of significant amount of respective bio-active components in these plants under study and variation of quantity determined based on the polarity of the solvent taken for extraction process, ensures its unequivocal

recommendation for the use in the pharmaceutical and nutraceutical sector. More work should be done to evaluate phenolic compounds and their antioxidants activity for greater number of plants grown in the north –eastern region in India, in order to create nutritional and medicinal reference for these plants and to evaluate their health benefits.

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