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Extraction optimization of gallic acid, (+)-catechin, procyanidin-B2, (−)-epicatechin, (−)-epigallocatechin gallate, and (−)-epicatechin gallate: their simultaneous identification and quantification in *Saraca asoca*☆

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ABSTRACT

The objective of the present investigation was to optimize extraction conditions for maximum recovery of bioactive phenolics from different parts of *Saraca asoca*. Extraction recovery was optimized using a mixture of methanol and water in different proportions. For identification and quantification of six analytes, a rapid reversed phase ultra-performance liquid chromatography (UPLC) photo diode array detection method was developed. UPLC separation was achieved in a gradient elution mode on a C_{18} column with acetonitrile and aqueous phosphoric acid (0.1%, pH = 2.5). Extraction solvent for maximum recovery of analytes varied depending on the nature of matrices. The developed UPLC method was validated in accordance with International Council for Harmonisation (ICH) guidelines. Wide linearity range, sensitivity, accuracy, short retention time, and simple mobile phase composition implied that the method could be suitable for routine analysis of all six analytes with high precision and accuracy. The uniqueness of this study is the determination of the distribution of these compounds in the various parts of *S. asoca*.

1. Introduction

Saraca asoca (Roxb.) De Wilde (Syn. *S. indica* Linn.), which belongs to the family Caesalpiniaceae, is a medium sized evergreen tree distributed throughout India particularly in humid areas. *S. asoca* is considered as a sacred tree of Hindus and Buddhists. *S. asoca* has been traditionally used in Indian systems of medicine from time immemorial for treatment of uterine, genital, and other reproductive disorders in females [1]. The earliest chronicled mention of this tree is in the Ayurvedic treatise and later in Charaka Samhita (100 AD) where

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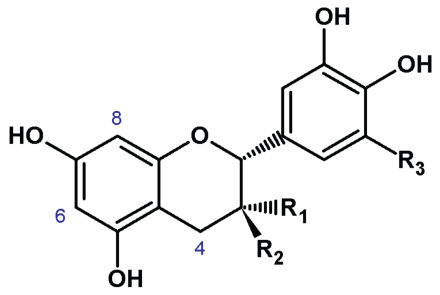
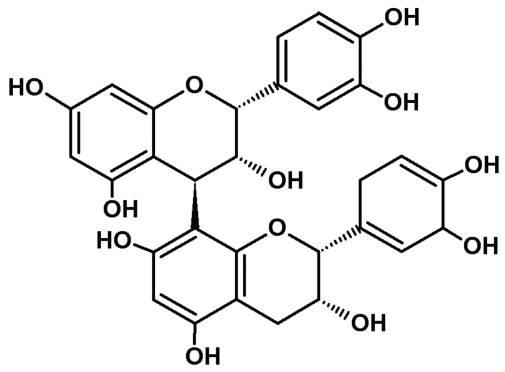
it was recommended as anodynes in formulations for the management of gynecological disorders [2–5]. Bark of *S. asoca* is reported to have stimulating effects on the endometrium, ovarian tissue, and is useful in menorrhagia during uterine fibroids [6]. A number of herbal formulations containing bark (Ashokarishta, Ashokaghrita, etc.) are available in the market. Ashokarishta, a well-known Ayurvedic formulation, is used for the treatment of menstrual disorders. Flowers are also used for the treatment of bleeding piles, cervical adenitis, biliousness, syphilis, hyperdipsia, and hemorrhagic skin diseases.

Phenolic compounds are important for dietary applications and include phenolic acids, polyphenols and flavonoids [7]. Flavonoids are a group of more than 4000 polyphenolics. Beneficial health effects of flavonoids are implicated because of their antioxidant properties and inhibitory role in the processes of carcinogenesis [8]. Catechins and anthocyanins, the glycosides of anthocyanidins contribute to a sizable proportion of total flavonoid consumption by humans. Catechins and anthocyanidins are biogenetically derived from a common C-15 tetrahydroxychalcone precursor, naringenin [9]. Catechins are well known flavonoids used for the symptomatic treatment (relieve the symptoms without addressing the basic cause of the disease) of several gastrointestinal, respiratory, and vascular diseases [10]. *S. asoca* contains significant amounts of phenolic compounds that are considered to be biologically active. A number of compounds including (+)-catechin (CA), (−)-epicatechin (EPC), and (−)-epigallocatechin were reported from *S. asoca*.

Due to variation in the concentration of secondary metabolites, various parts of *S. asoca* have different therapeutic values. Several studies are reported for the determination of individual compounds in different sources and formulations of *S. asoca* by high performance thin layer chromatography (HPTLC) [11–13], high performance liquid chromatography-diode array detection (HPLC-DAD) [14], ultra performance liquid chromatography-quadrupole-time-of-flight mass spectrometer (UPLC-QTOFMS) [15], high performance liquid chromatography-quadrupole-time-of-flight mass spectrometer (HPLC-QTOFMS) [8,16] but there is no report on simultaneous identification and quantification of gallic acid (GA), CA, procyanidin-B2 (PB2), EPC, (−)-epigallocatechin gallate (EGCG), and (−)-epicatechin gallate (EG) (Figure 1) in different parts (barks, flowers, leaves, stems, pods, seeds, and roots) of *S. asoca*. Earlier, Ketkar et al [17] reported an RP-HPLC-DAD method for analysis of GA, CA, and EPC in bark samples of *S. asoca*. However, the reported RP-HPLC-DAD method was not validated. Therefore, it was of paramount interest to study the distribution of polyphenols in different parts of *S. asoca*. In continuation to our earlier work for extraction optimization and profiling of main bioactive constituents of Indian medicinal plants, the principal objectives of the present studies were: (1) to optimize the extraction solvent for maximum recovery of main phenolics; and (2) to develop a simple, selective, precise, and reproducible ultra-performance liquid chromatographic (UPLC) method with a wide linear range and good sensitivity using photo diode array detection for identification and quantification of GA, CA, PB2, EPC, EGCG, and EG in different parts of *S. asoca*.



R_1=OH, R_2, R_3=H; Epicatechin
R_1, R_3=H, R_2=OH, Catechin
R_1=gallic acid ester, R_2, R_3=H; Epicatechin gallate
R_1=gallic acid ester, R_2=H, R_3=OH; Epigallocatechin gallate



Procyanidin B2

Figure 1 – Structure of catechins.

2. Experimental

2.1. Plant material

Different parts of *S. asoca* (barks, flowers, leaves, stems, pods, seeds, and roots) were collected from mature trees in the year 2013–2014. The collected plant materials were authenticated by a taxonomist. The specimen samples were deposited in a herbarium. All plant materials were air dried in shade for 1 week. Fine powder of dried samples was made using an electric grinder.

2.2. Reference compounds and chemicals

Reference compounds of the highest grade (purity > 99.0 %) namely CA {$[\alpha]_D^{20} = +26 \pm 2$, c = 1, water} was purchased from Natural Remedies (Bangalore, India), while GA, PB2{$[\alpha]_D = 29.2$}, EPC {$[\alpha]_D^{20} = -54$, c = 1, acetone:water (1:1)}, EGCG {$[\alpha]_D^{20} = -188$, c = 1, methanol}, and EG {$[\alpha]_D^{20} = -175.5$, c = 1, ethanol} were purchased from Sigma-Aldrich (Mumbai, India). Methanol, acetonitrile, and phosphoric acid (Merck, Mumbai, India) were HPLC grade. Milli Q grade water used throughout the experiment was prepared using a Millipore purification system (Millipore, Milli Q gradient A10, Molsheim, France).

2.3. Preparation of standard and sample solution

Plant samples of *S. asoca* (5 g each) were extracted overnight with methanol, water, and also with a mixture of water-methanol (1:1 and 4:1) at room temperature for 3 consecutive days in order to ensure the maximum possible recovery. Sample to solvent ratio was 1:20 [18]. Supernatants obtained were pooled together and concentrated under reduced pressure at 50–55°C using a vacuum rotary evaporator (Heizbed Hei-VAP, Heidolph, Schwabach, Germany). Stock solutions of different extracts were prepared by dissolving extract in water-methanol (1:1, 1.0 mg/mL) and filtered through a 0.45-μm membrane filter. Stock solution of GA, CA, PB2, EPC, EGCG, and EG were prepared in HPLC grade methanol (1.0 mg/mL, each). Working solutions of lower concentration were prepared by appropriate dilution of the stock solutions. Solutions of extract and standards were stored at 4 ± 1°C and before use brought to room temperature.

2.4. Apparatus and chromatographic conditions

Chromatographic separation was achieved using an UPLC system consisting of quaternary pumps, an in-line vacuum degasser, an auto sampler, a column heater, and a photodiode array detector, PDA (Acquity UPLC H-class, Waters, Milford, USA). Injection volume was 1 μL. Column oven temperature was set at 37°C in order to minimize the viscosity of the mobile phase. The instrumentation was controlled by using Empower 3.0 software (Waters). Compounds were separated on an RP-18 column (50 × 2.1 mm internal diameter, 1.8 μm, pore diameter 100 A°, Acquity UPLC HSS T3, Waters) in a gradient elution mode. The mobile phase was a mixture of aqueous phosphoric acid (0.1%, v/v) (Solvent A) and acetonitrile (Solvent B) at a flow rate 0.75 mL/min. Gradient programming was set as follows: 0 minutes 5% B, 0.5 minutes 5% B, 6 minutes 20% B, 7 minutes 80 % B, 9 minutes 80 % B, then initial conditions were restored at 9.10 min. Total run time was extended up to 11 minutes in order to ensure any late eluting peak. The peaks obtained in the chromatogram were monitored in the range of 200–350 nm using a photodiode array detector. Wavelength selected for quantitative analysis of GA, CA, PB2, EPC, EGCG, and EG in different extracts of *S. asoca* was 210 nm. At this wavelength, the best base line separation with maximum absorbance was achieved as compared to chromatograms recorded at 230 nm or 280 nm (data not shown).

2.5. Method development and validation

Catechins are unstable in alkaline medium, therefore, presence of acid in the mobile phase was essential for both complete resolution and elimination of peak tailing. Acidified mobile phases comprising methanol-water and acetonitrile-water in different proportions, both in isocratic and gradient elution mode, were tried in the present study. Chen et al [19] reported an HPLC method for identification and quantification of catechins, EPC and (–)-epigallocatechin together with their gallate derivatives EG and EGCG in oolong tea using a mobile phase comprised of water containing 0.5% acetic acid and acetonitrile in a gradient elution mode.

Validation of the developed method was performed as per International Council for Harmonisation (ICH) guidelines [20] for validation of analytical procedures. A series of assays including accuracy, precision, detection and quantification limit, linearity, recovery, selectivity, and robustness were carried out. All the data were evaluated using MS Excel 2010 (Microsoft Corporation, Redmond, USA) and SAS 9.2 (SAS, Mumbai, India).

3. Results

Gradient elution was carried out with phosphoric acid in water (0.1%) along with acetonitrile as an organic phase. This mobile phase provided sharp peaks and better resolution. The UV absorption maxima of all analytes were evaluated using a PDA detector. The elution of analytes was in the following order: GA (0.45 minutes), CA (2.44 minutes), PB2 (3.23 minutes), EPC (3.52 minutes), EGCG (3.70 minutes), and EG (5.19 minutes) (Table 1). As no peaks were detected after the peak of EG (5.19 minutes), the chromatograms of the extracts were shown up to 6 minutes only. The elution order of the present study was in a similar pattern as reported by earlier researchers [21,22].

Chromatographic peaks in the extract samples were identified by matching their retention time and UV absorption spectra with the peaks in the chromatogram of mixed standards. An external standard calibration method was used for quantification of all analytes in the extract samples. The linear equation between the concentration of the standard injected and the peak area were expressed as $y = mx + c$, where y is the peak area, x is the concentration of the standard, and m and c are constants. Using the equation for

Table 1 – Linear relationships between peak area and concentration of individual analytes.

Analyte	Retention time mean (%RSD)	Regression equation ($y = ax + b$)	Correlation coefficient r^2	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
GA	0.45 (0.13)	12400x + 8350	0.9994	0.1–40.0	0.005	0.017
CA	2.44 (0.15)	12200x + 7990	0.9995	0.1–40.0	0.011	0.038
PB2	3.23 (0.13)	12900x + 8460	0.9994	0.1–40.0	0.011	0.038
EPC	3.52 (0.11)	13300x + 8960	0.9994	0.1– 40.0	0.010	0.033
EGCG	3.70 (0.09)	19200x + 7060	0.9998	0.1–40.0	0.010	0.029
EG	5.19 (0.06)	13100x + 8400	0.9995	0.1–40.0	0.012	0.040

CA = (+)-catechin; EG = (–)-epicatechin gallate; EGCG = (–)-epigallocatechin gallate; EPC = (–)-epicatechin; GA = gallic acid; LOD = limit of detection; LOQ = limit of quantification; PB2 = procyanidin-B2; RSD = relative standard deviation.

individual analytes, concentrations (x) of respective analytes in the extract samples were calculated by putting the value of integrated peak area (y) of the individual analytes in the calibration equation prepared by that of corresponding standards. Santagati et al [21] reported simultaneous determination of catechins, rutin, and GA in extracts of the *Cistus* species by HPLC with diode array detection. Catechins [CA, EPC, (−)-gallocatechin, (−)-epigallocatechin, EGCG], rutin, and GA were eluted with acetonitrile-phosphate buffer (50 mM, pH 2.5) in a runtime of 35 minutes. In the present study, the total runtime was 11 minutes.

3.1. System suitability test

The system suitability test of a standard mixture of individual analytes was performed using Empower 3.0 software. System suitability parameters of individual analytes such as repeatability, plate counts, tailing factor, capacity factor, and resolution in standard mixture were determined by injecting six replications. Repeatability of the method in terms of RSD values of retention time is shown in Table 1. Tailing factor, resolution, capacity factor, and plate count values are described in Table 2. It was verified from the results that all the values were within the range of recommended limits and the system was adequate for the analysis of all six analytes.

Table 2 – System suitability data of all analytes.

Parameter	GA	CA	PB2	EPC	EGCG	EG
Plate count	3469	23106	37041	53664	53196	88278
Tailing	1.06	0.97	0.98	0.99	1.00	0.99
Capacity factor	3.52	23.38	31.28	34.18	35.96	50.88
Resolution	–	41.18	11.82	4.45	2.81	21.87
Selectivity	–	6.63	1.34	1.09	1.05	1.41

CA = (+)-catechin; EG = (−)-epicatechin gallate; EGCG = (−)-epigallocatechin gallate; EPC = (−)-epicatechin; GA = gallic acid; PB2 = procyanidin-B2.

3.2. Linearity and sensitivity

The solutions of different concentrations of six analytes were used for construction of calibration curves. Peak areas were recorded for each concentration of analytes and plotted against concentrations. Different concentrations ranging from 0.1 μg/mL to 40 μg/mL were used for establishing the linearity between peak area and concentration of all six analytes. The data for peak area versus analytes concentration were treated by the lack of fit test. Looking at the F-value and its corresponding *p* value (Pro > F), it was observed that values for all analytes are < 0.05, which indicated fitness of the model (Table 3). Limit of detection (LOD) and limit of quantification (LOQ) were defined as the lowest concentration of analytes that can be detected and quantified in a sample. Under the chromatographic conditions employed in the present study, signal-to-noise ratios of 3 and 10 were considered as LOD and LOQ, respectively. The detailed description of calibration curves and limit of sensitivity are depicted in Table 1.

3.3. Accuracy and precision

Repeatability is the degree of agreement of results when experimental conditions are maintained as constant as possible [22]. Repeatability of the developed UPLC method was evaluated at three different concentrations of GA, CA, PB2, EPC, EGCG, and EG. The developed method was precise as the RSD values for intraday and interday precision were in the range of 0.12–1.74% and 0.14–3.27%, respectively. Analytical recovery was performed by analyzing the analytes by spiking with the six standards in blank extracts. The recovery percentage was calculated by using the following formula:

Table 3 – Lack of fit test for linearity.

Analyte	Residual	Degree of freedom	Sum of squares	Mean square	F Value	Pr > F
GA	Lack of fit	3	3.0912	1.0304	71.27	< 0.0001
	Pure error	30	0.4337	0.0144		
	Total error	33	3.5249	0.1068		
CA	Lack of fit	3	3.7934	1.2644	121.96	< 0.0001
	Pure error	30	0.3110	0.0103		
	Total error	33	4.1044	0.1243		
PB2	Lack of fit	3	0.1367	0.0455	10.75	< 0.0001
	Pure error	30	0.1271	0.0042		
	Total error	33	0.2639	0.0079		
EPC	Lack of fit	4	1.6382	0.5460	39.91	< 0.0001
	Pure error	35	0.4105	0.0136		
	Total error	39	2.0488	0.0620		
EGCG	Lack of fit	3	1.9472	0.6490	23.54	< 0.0001
	Pure error	30	0.8271	0.0275		
	Total error	33	2.7743	0.0840		
EG	Lack of fit	4	2.6421	0.8807	175.70	< 0.0001
	Pure error	35	0.1503	0.0050		
	Total error	39	2.7925	0.0846		

CA = (+)-catechin; EG = (−)-epicatechin gallate; EGCG = (−)-epigallocatechin gallate; EPC = (−)-epicatechin; GA = gallic acid; PB2 = procyanidin-B2.

Table 4 – Precision and recovery study of phenolics.

Analyte	Concentration (μg/mL)	(%RSD)		Recovery (%)
		Interday	Intraday	
GA	0.1	2.58	1.08	102.4
	10	2.31	0.74	100.9
	40	0.20	0.10	99.3
CA	0.1	2.00	0.81	98.5
	10	1.67	0.44	99.3
	40	0.14	0.12	99.0
PB2	0.1	3.27	0.60	98.2
	10	0.83	0.54	101.0
	40	0.19	0.06	100.8
EPC	0.1	1.88	1.09	98.9
	10	0.21	0.88	99.0
	40	0.42	0.23	99.1
EGCG	0.1	0.40	1.74	100.4
	10	0.25	0.12	101.0
	40	0.30	0.28	99.8
EG	0.1	2.32	1.36	101.3
	10	0.55	0.19	99.7
	40	0.14	0.18	99.7

CA = (+)-catechin; EG = (–)-epicatechin gallate; EGCG = (–)-epigallocatechin gallate; EPC = (–)-epicatechin; GA = gallic acid; PB2 = procyanidin-B2.

recovery (%) = [(amount found- original amount)/spiked amount] × 100.

The overall recovery percentages were in the range of 98.2–102.4%. The developed method was specific for determination of GA, CA, PB2, EPC, EGCG and EG as their peak purity values established that peaks were pure and had no coeluting peaks. Results of the intraday and interday precision experiments, as well as recovery data, are described in Table 4.

3.4. *Robustness*

To test the robustness of the developed UPLC method, chromatographic conditions which could affect the performance of the method, were deliberately changed. In the present study, phosphoric acid content in the mobile phase (0.05%, 0.08%, and 0.1%), flow rate (0.65 mL/min, 0.75 mL/min, and 0.85 mL/min) and wavelength of detection (± 5 nm) were changed. The relative standard deviation of retention time and peak area of all analytes were calculated for change in each parameter. The results demonstrated that the developed UPLC method was insensitive to minor changes (%RSD < 2).

4. Discussion

Traditional medicines, despite being in existence and continued use over centuries, have not been officially recognized in many countries due to lack of proper quality control. Selection of suitable solvent is a crucial step for the extraction of phytochemicals from the plant. Catechins are among the top 10 ingredients with health claim/index compounds for quality control in permitted health foods of Taiwan [23]. The major catechins in tea are EGCG, (–)-epigallocatechin, and EG [24]. Decoction process is used for preparation of most of the

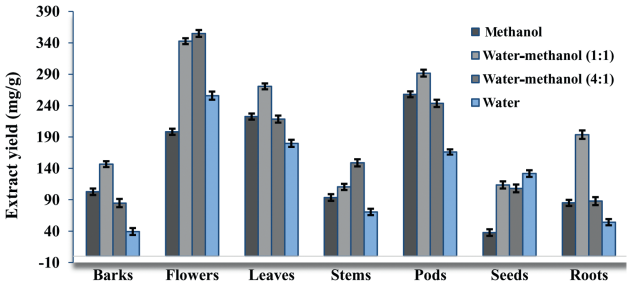


Figure 2 – Extract yield using different solvent systems.

herbal formulations. Water extract of *S. asoca* is used to prepare various Ayurvedic and herbal drugs which are rich sources of CA, EC, EG, their polymers, and glucosides. To ensure the maximum recovery of GA, CA, PB2, EPC, EGCG, and EG from different parts of *S. asoca*, extraction was carried out using solvents of four different polarities. The extract yield was expressed as mg/g (Figure 2). Also, the representative chromatograms corresponding to maximum recovery (sum of total polyphenols) from different plant parts are shown in Figure 3.

Methanol was reported as the most effective solvent for extraction of CA, EPC, and EG from grape seed. However, ethanol (75%) provided the maximum extraction of GA [25]. Also in the present study, methanol was more effective for extraction of all analytes from flowers, stems, and roots. Maximum extraction recovery of polyphenols was obtained using water from bark, leaf, and pod samples. However, in the case of seed, water-methanol (1:1) was able to extract the maximum polyphenols content (Figure 4).

The developed UPLC method was applied for quantification of GA, CA, PB2, EPC, EGCG, and EG in different extracts of *S. asoca* (Table 5). Although baseline separation was achieved for extract samples (Figures 3B–D, 3F, and 3G), however, baseline was drifted in chromatograms of Figures 3E and 3H. This could be due to the matrix effect of these samples. GA was not quantified in bark and root extracts due to trace amount. However, it was identified and quantified in all extracts of flowers, leaves, stems, pods, and seeds. Its concentration was significantly higher in water extract of pods than extracts of other plant parts. CA was quantified in all plant parts except seed and flowers, where smaller quantities were detected. Methanol extract of stems had maximum concentration of PB2. PB2 was detected in all extracts but it could not be quantified in water, water-methanol (1:1 and 4:1) extracts of flowers, and also in methanol extract of seeds. Similar results were obtained for EPC. Its maximum concentration was present in water-methanol (1:1) extract of leaves. EGCG was not detected in bark extract. Also, it could not be quantified in water, water-methanol (4:1) extracts of flowers and water extract of leaves. Further, it could not be quantified in water as well as water-methanol (4:1) extracts of pods and in all four extracts of seeds. The presence of EG was confirmed in all parts of *S. asoca*. However, it could not be quantified in water extract of flowers and methanol extract of seeds. Its maximum concentration was in barks (water extract). It is pertinent to mention that in *S. asoca* seed methanol extract,

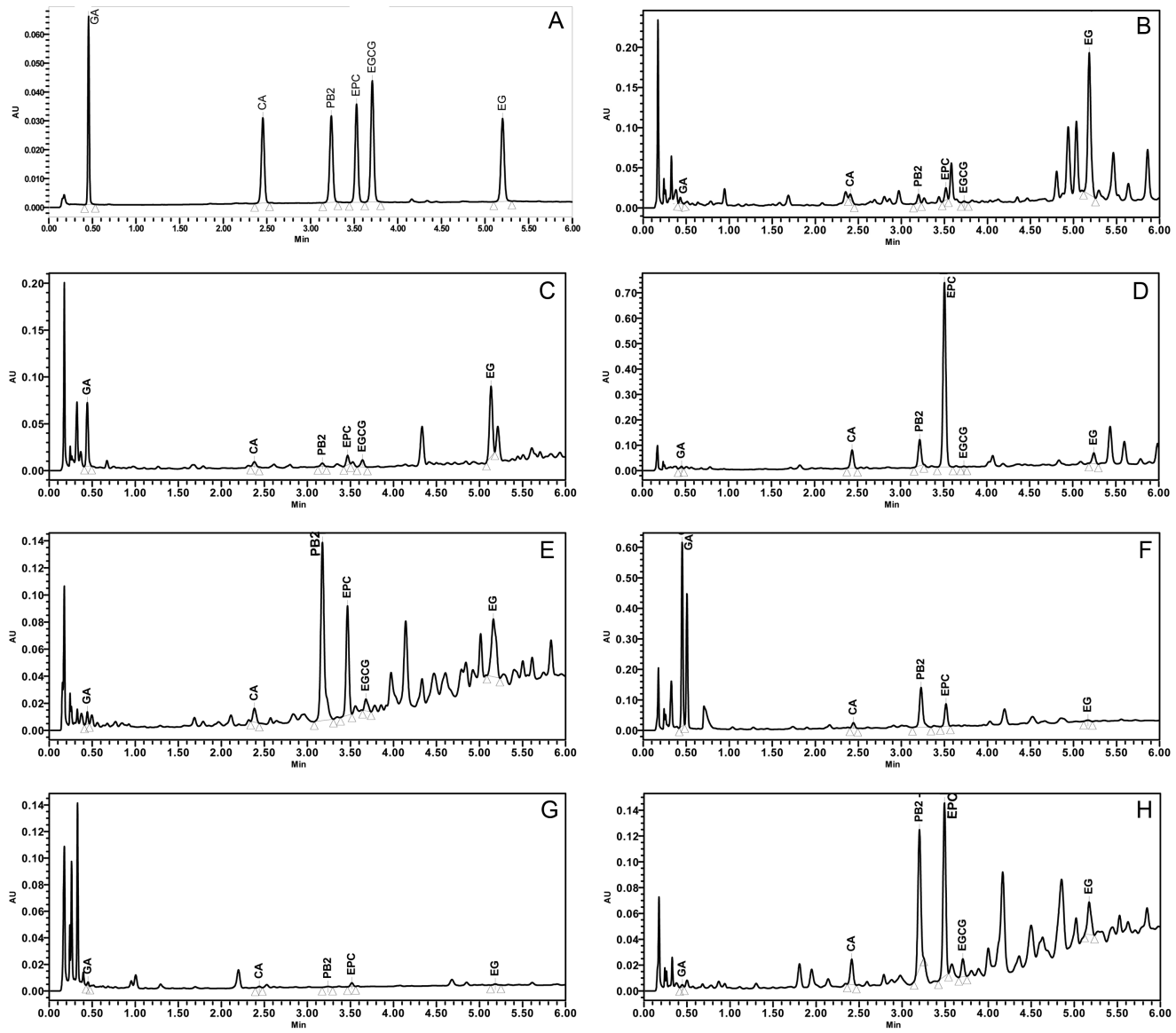


Figure 3 – Ultra-performance liquid chromatography (UPLC) chromatograms of (A) standard mixture of gallic acid (GA), (+)-catechin (CA), procyanidin-B2 (PB2), (–)-epicatechin (EPC), (–)-epigallocatechin gallate (EGCG), and (–)-epicatechin gallate (EG); (B) water extract of barks; (C) methanol extract of flowers; (D) water extract of leaves; (E) methanol extract of stems; (F) water extract of pods; (G) water-methanol extract of seeds; and (H) methanol extract of roots of *Saraca asoca*.

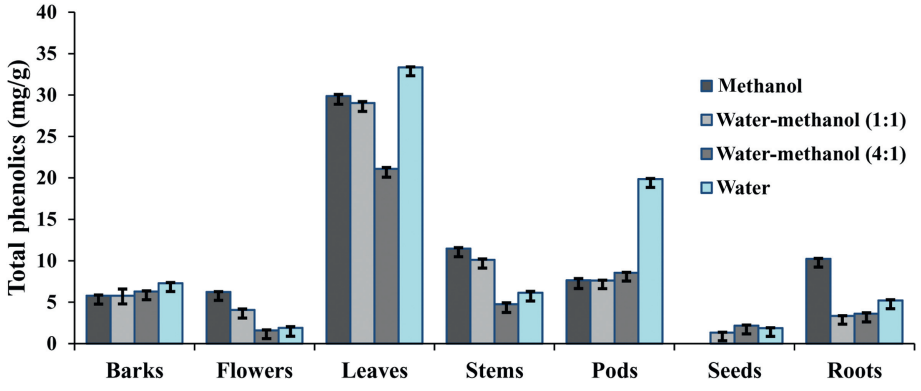


Figure 4 – Distribution of phenolics in different parts of *Saraca asoca*.

Table 5 – Distribution of phenolics in different parts of *Saraca asoca*.

	GA (mg/g ± sd)	CA (mg/g ± sd)	PB2 (mg/g ± sd)	EPC (mg/g ± sd)	EGCG (mg/g ± sd)	EG (mg/g ± sd)	Total (mg/g ± sd)
Barks							
Methanol	t	0.28 ± 0.01	0.84 ± 0.01	1.19 ± 0.02	nd	3.45 ± 0.06	5.76 ± 0.09
Water:methanol (1:1)	t	0.27 ± 0.02	0.84 ± 0.02	1.21 ± 0.02	nd	3.47 ± 0.08	5.79 ± 0.07
Water:methanol (4:1)	t	0.17 ± 0.02	0.48 ± 0.03	0.88 ± 0.03	nd	4.76 ± 0.03	6.29 ± 0.08
Water	t	0.12 ± 0.02	0.20 ± 0.01	0.31 ± 0.02	nd	6.66 ± 0.07	7.29 ± 0.07
Flowers							
Methanol	1.60 ± 0.01	0.09 ± 0.01	0.01 ± 0.00	0.20 ± 0.02	0.14 ± 0.01	2.66 ± 0.03	4.70 ± 0.05
Water:methanol (1:1)	3.55 ± 0.09	0.01 ± 0.01	t	t	0.09 ± 0.01	0.44 ± 0.01	4.09 ± 0.10
Water:methanol (4:1)	1.36 ± 0.03	t	t	t	nd	0.24 ± 0.02	1.60 ± 0.05
Water	1.89 ± 0.15	t	t	t	nd	t	1.89 ± 0.15
Leaves							
Methanol	1.68 ± 0.04	0.85 ± 0.02	4.45 ± 0.06	21.88 ± 0.08	0.02 ± 0.00	1.00 ± 0.01	29.88 ± 0.18
Water:methanol (1:1)	1.72 ± 0.08	0.85 ± 0.00	4.32 ± 0.06	21.94 ± 0.03	0.04 ± 0.01	0.16 ± 0.00	29.03 ± 0.18
Water:methanol (4:1)	1.13 ± 0.11	0.38 ± 0.08	3.44 ± 0.44	15.52 ± 0.40	0.02 ± 0.01	0.55 ± 0.04	21.04 ± 0.16
Water	0.11 ± 0.01	2.77 ± 0.02	3.66 ± 0.07	25.30 ± 0.02	t	1.50 ± 0.03	33.34 ± 0.06
Stems							
Methanol	0.08 ± 0.02	0.31 ± 0.00	5.80 ± 0.05	2.69 ± 0.01	0.19 ± 0.01	2.41 ± 0.05	11.48 ± 0.11
Water:methanol (1:1)	0.09 ± 0.02	0.32 ± 0.02	0.46 ± 0.07	2.66 ± 0.02	0.22 ± 0.01	2.38 ± 0.04	6.13 ± 0.10
Water: methanol (4:1)	0.05 ± 0.03	0.55 ± 0.04	1.07 ± 0.09	0.88 ± 0.03	0.16 ± 0.03	2.05 ± 0.08	4.76 ± 0.15
Water	0.04 ± 0.02	0.61 ± 0.02	1.67 ± 0.13	0.95 ± 0.00	0.01 ± 0.00	2.87 ± 0.04	6.15 ± 0.16
Pods							
Methanol	2.62 ± 0.13	0.51 ± 0.01	0.14 ± 0.01	0.39 ± 0.02	0.07 ± 0.01	3.92 ± 0.06	7.65 ± 0.21
Water:methanol (1:1)	2.71 ± 0.06	0.54 ± 0.02	0.35 ± 0.02	0.58 ± 0.01	t	3.45 ± 0.06	7.63 ± 0.03
Water: methanol (4:1)	4.21 ± 0.09	0.45 ± 0.02	2.44 ± 0.05	0.28 ± 0.04	0.04 ± 0.03	1.13 ± 0.05	8.55 ± 0.06
Water	11.43 ± 0.04	0.51 ± 0.03	5.43 ± 0.03	2.40 ± 0.03	nd	0.07 ± 0.01	19.84 ± 0.08
Seeds							
Methanol	t	t	t	t	t	t	t
Water:methanol (1:1)	0.20 ± 0.01	t	0.50 ± 0.01	0.46 ± 0.02	t	0.18 ± 0.01	1.34 ± 0.03
Water:methanol (4:1)	0.09 ± 0.11	t	0.22 ± 0.04	0.41 ± 0.05	t	0.11 ± 0.03	0.83 ± 0.07
Water	0.04 ± 0.08	t	0.19 ± 0.05	0.22 ± 0.04	t	0.04 ± 0.03	0.49 ± 0.04
Roots							
Methanol	t	0.66 ± 0.01	3.83 ± 0.02	4.43 ± 0.02	0.27 ± 0.01	1.04 ± 0.02	10.23 ± 0.06
Water:methanol (1:1)	t	0.20 ± 0.01	1.12 ± 0.01	1.48 ± 0.00	t	0.56 ± 0.01	3.36 ± 0.02
Water:methanol (4:1)	t	0.27 ± 0.04	1.48 ± 0.06	1.34 ± 0.05	t	0.54 ± 0.02	3.63 ± 0.10
Water	t	0.53 ± 0.02	1.28 ± 0.01	1.76 ± 0.06	t	1.64 ± 0.05	5.21 ± 0.08

nd = not detected; t = trace.

peaks of all analytes were detected but could not be quantified due to their low concentration. However, as the water was added in methanol, these peaks were quantified (Table 5).

Total polyphenolics were expressed as the sum of the content of GA, CA, PB2, EPC, EGCG, and EG in different extracts. It was in the range of 5.76 ± 0.09–7.29 ± 0.07, 1.60 ± 0.05–4.70 ± 0.05, 21.04 ± 0.16–33.34 ± 0.06, 4.76 ± 0.15–11.48 ± 0.11, 7.63 ± 0.03–19.84 ± 0.08, 0.49 ± 0.04–1.34 ± 0.03, and 3.36 ± 0.02–10.23 ± 0.06 mg/g for bark, flower, leaf, stem, pod, seed, and root extracts, respectively. On the basis of the above findings, leaf was identified as the plant part of *S. asoca* having the maximum total polyphenolics content. Therefore, this part could be used as a renewable substitute for bark in different herbal formulations. This would prevent the destructive harvesting of the *S. asoca* plant, thereby, promoting its conservation. Liquid-liquid partitioning for further enrichment of fractions can be used by using a solvent of appropriate polarity such as ethyl acetate for plant parts in which polyphenolics could not be detected due to their low concentration. Preliminary work done by us in this direction has yielded satisfactory results.

5. Conclusions

The effects of different solvents on the extraction of six phenolics from different parts of *S. asoca* were investigated. These phenolic compounds are representative of bioactive phenolics found in the different parts of *S. asoca*. Methanol, water, and water-methanol (1:1 and 4:1) were compared for extraction efficiency of GA, CA, PB2, EPC, EGCG, and EG. Their concentrations were monitored by the developed and validated UPLC method. The developed UPLC method was able to provide an efficient and repeatable separation of GA, catechins, and PB2. Also, in order to overcome the detection limit of UPLC-PDA, selective ion monitoring or multiple reaction monitoring methods using LC-MS could be used for identification and quantification of those analytes present in concentrations which are below the quantification limit of the present developed method.

Worldwide medicinal properties of *S. asoca* are being explored commercially for treatment of many health disorders. The results of the present investigation may be utilized for exploring the possibilities of other renewable parts of *S.*

asoca in place of barks. Also, the developed UPLC method could be utilized for bioprospection and validation for use of species such as *Bauhinia variegate*, *Brownea ariza*, *Polyalthia longifolia*, *Shorea robusta*, and *Trema orientalis*. These species are known adulterants of *S. asoca*.

Conflicts of interest

The authors have no conflicts of interest.

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