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Original article

Phytochemical studies of the endangered tree, *Saraca asoca* (Roxb.) De Wilde.

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Abstract

Saraca asoca (Roxb.) De Wilde. is an endangered medicinal tree that has immense medicinal importance. Six accessions of *S. asoca* were analyzed for phytochemicals to identify the accessions with the highest content of medicinal compounds and to characterize two pharmaceutically important compounds. The chloroform and ethanol extracts of bark and the ethanol and chloroform extracts of flowers of the accessions were qualitatively tested for secondary metabolites. Total phenolics and flavonoids were also estimated to select the accessions with the highest content, which was further investigated by a sensitive and reliable high performance liquid chromatographic (HPLC) method for the pharmacologically important quercetin and catechin. The accession, Sa-O, identified based on the results of phytochemical analysis incidentally is the fifty-year-old ornamental tree on the University campus, Osmania University, Hyderabad, India.

Key words: *Saraca asoca* (Roxb.) De Wilde., flavonoids, phenols, quercetin, catechin, micro-propagation

1. Introduction

The Ashoka (*Saraca asoca* (Roxb.) De Wilde), family Caesalpiniaceae, is a beautiful rain-forest tree, grown in gardens as an ornamental. Its original distribution is in the central areas of the Deccan plateau of India as well as the middle section of the Western Ghats in the western coastal zone of the Indian subcontinent. Ashoka also grows in countries such as Pakistan, Sri Lanka, Bangladesh, Mayanmar and Malaysia. The Ashoka is valued for its beautiful foliage and fragrant flowers. It is a small, erect evergreen tree, with thick green leaves, growing in dense clusters. Its flowering season is from February to April. The Ashoka flowers come in lush bunches and are bright orange-yellow in color, turning red before wilting. *S. asoca* is a threatened and vulnerable (IUCN 2.3) species which has low population number and is in considerable danger of becoming extinct due to its overharvesting (due to its medicinal value) along with high deforestation rates.

The bark of *S. asoca* tree is used for its medicinal properties such as uterine bleeding and menorrhagia due to uterine fibroids, leucorrhoea, internal bleeding and to treat depression in women (Sharma *et al.*, 2001). The flowers are medicinally important and used in the treatment of diabetes, cancer, hemorrhagic dysentery, piles, menorrhagia and other types of uterine disorders.

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The propagation is through nursery rising from seeds and stem grafting. However, the plant is difficult to grow and the success rate is less than 20%, necessitating the development of alternate methods of propagation (Puspangadan *et al.*, 2004). Hence, micropropagation of *S. asoca* would be highly useful for its conservation (Puspangadan *et al.*, 2004).

Plant phenolics include flavonoids and other compounds and they have many biological activities like antimicrobial, antiulcer, antiarthritic, anticancer, *etc*. Some of the most common flavonoids include quercetin (flavonol abundant in onion, broccoli, and apple) and catechin (flavanol found in tea and several fruits). *S. asoca* contains significant amounts of phenolic compounds and a comprehensive analysis of the phenolic components of the medicinal plant will be helpful in understanding the medicinal importance of the bark and flowers of the plant.

The present study investigates the phytochemical characteristics of the accessions of *S. asoca* with a view to identifying the accessions with the highest content of total phenolics and flavonoids and to quantify quercetin and catechin by HPLC.

2. Materials and Methods

Six accessions of *Saraca asoca* were studied presently. While one accession Sa-O,was the 50-year-old tree, growing in the Botanical garden of Osmania University, Hyderabad, India; four accessions (6-year old plants) were collected from different locations of Hyderabad (Sa-A from Herbal Garden, Rajendranagar; Sa-B from Medicinal Plants Board,Chilkur; Sa-C from Karthikavanam, Dhulapally; Sa-D from Hyderabad urban forestry, Erragadda) and one 6-year-old accession Sa-E, collected from Herbal Garden,

Rajahmundry, Andhra Pradesh. All the plants were authenticated by the Head, Department of Botany, Osmania University, Hyderabad and planted in the Botanical garden, Osmania University, Hyderabad, Telangana State, India. Phytochemical analysis (qualitative and quantitative) of the bark and flowers of all the six accessions of *S. asoca* was carried out for secondary metabolites, with a view to select accessions with a high content of desirable medicinally important compounds.

2.1 Phytochemical analysis

The bark and flower extracts were prepared from respective samples, collected from the plants. The samples were washed with water and air-dried at room temperature for 7 days and oven-dried at 40°C for 1 h to remove the residual moisture. They were pulverized and stored in air-tight containers at 4°C for future use. The powdered material was defatted with petroleum ether (60-80°C) before use. An equivalent amount of air-dried and powdered crude bark powder and flower samples of *S. asoca* were extracted in a Soxhlet extractor for 36 h with ethanol and chloroform, respectively and concentrated to dryness under reduced pressure at 40-50°C, using rotary evaporator and the percentage extract yield was calculated.

2.1.1 Qualitative analysis

The dried extracts of bark and flower were diluted with distilled water to the desired concentration (10 g/ml) and stored at 4°C until further use. The presence of alkaloids, steroids, terpenoids, tannins, glycosides, flavonoids, carbohydrates and saponins was assayed in the chloroform and ethanol extracts of bark and flowers using standard procedures (Parekh *et al.*,2005; Evans, 2009; Mukhopadhyay and Nath, 2011; Gupta *et al.*, 2013).

Test for alkaloids : The extract (5 ml) was dissolved in 5 ml dilute HCl solution and filtered. The filtrate was tested with Dragendroff's and Mayer's reagent. The treated solution was observed for yellow precipitation.

Test for steroids and terpenoids : Presence of steroids and terpenoids in the extract was tested by a mixture of acetic anhydride and chloroform (5 ml each) in presence of concentrated sulphuric acid (2 ml). Appearance of a brown-red ring at the interface between the two liquids indicated the steroids and a blue-green ring indicated the terpenoids.

Test for tannins : To 100 ml of the extract, 10% ferric chloride solution was added and was observed for a change in color to bluish black.

Test for glycosides : Presence of glycosides was tested by adding 5 ml dilute sulphuric acid to 10 ml extract. It was boiled for 5 min, filtered and cooled. Equal volume of chloroform was added and shaken well to separate into two layers. The lower chloroform layer was collected and half volume of ammonia solution added to it. The solution turns pink due to the presence of glycosides.

Test for flavonoids : Five ml ethyl acetate was added to 10 ml of extract, the mixture was shaken and allowed to settle. Production of greenish yellow color is taken as positive for flavonoids.

Test for carbohydrates: Presence of carbohydrate was indicated when 5 ml of the extract was slowly mixed with 5 ml Molisch reagent, and later, a small amount of concentrated sulphuric acid was added slowly, leading to the formation of purple ring at the interface.

Test for saponins : The bark/flower powder (0.5 g) was ground with 100 ml of distilled water and transferred to a test tube. The test tube was shaken vigorously for about 30 sec and allowed to stand in vertical position and observed for 30 min. If a honey comb froth above the surface of the liquid persists after 30 min, it indicates the presence of saponins.

2.1.2 Quantitative analysis

Quantitative analysis of the ethanolic bark and flower extracts (that were prepared for the qualitative tests) of *S. asoca* was carried out (0.5 g of bark/flower extract in 100 ml of water). Quantitative analysis was carried out to estimate total phenolic and total flavonoids contents by using appropriate reference standards. The standards of quercetin and gallic acid were procured from Alta Vista Phytochemicals, Hyderabad and the standard catechin was procured from Sigma Aldrich, Hyderabad.

Estimation of total phenolic content: Total phenolics were estimated with Folin-Ciocalteau reagent with a slight modification of the procedure described by Singleton *et al.* (1999). About 0.5 ml of sample (extract) was mixed with 1.5 ml Folin-Ciocalteau reagent (1:10 v/v diluted with distilled water) and incubated for 5 min at 22°C, after which, 2 ml of sodium carbonate (7% Na_2CO_3, w/v) was added and incubated for 90 min in the dark with intermittent shaking. The absorbance of the blue color was recorded at 725 nm using a spectrophotometer (Elico Ltd.). The standard graph was prepared by using gallic acid. The concentration of the total phenolic compounds in bark and flower extracts were deduced from the standard graph and expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) of extract.

Estimation of flavonoids: Flavonoids were estimated by the aluminum chloride colorimetric method (Chang *et al.*,2002) with some modifications. To 1.0 ml of plant extract, 1.0 ml methanol, 0.5 ml aluminium trichloride (1.2%) and 0.5 ml potassium acetate (0.1176%) were added and incubated for 30 min at room temperature. Later, the absorbance of the pink color was measured at 415 nm using a spectrophotometer (Elico Ltd.). Quercetin and catechin was used as standards for the bark and flower samples, respectively and the standard graphs were prepared. Flavonoid content was deduced from the respective standard graph and presented as quercetin equivalents (mg/g of bark extract) and catechin equivalents (mg/g of flower extract), respectively.

In the present study, the selected accession of *S. asoca* that recorded the highest quantity of flavonoids was chosen and analyzed further by HPLC for quantification of quercetin and catechin in the bark and flowers, respectively.

2.1.3 HPLC analysis

HPLC analysis of the bark and flower ethanol extracts of *S. asoca* was carried out for the estimation of quercetin and catechin, respectively. The reference compounds were used as external standards to set up and calculate appropriate calibration curves. The experimental conditions were performed using Shimadzu HPLC (LC-2010 model) (dual wavelength) and LC solutions software.

Preparation of reference standards: Five mg quercetin was dissolved and made upto 10 ml with methanol, and 10 mg catechin was dissolved and made upto 20 ml with methanol.

Sample preparation : An accurate amount of 42 mg dried extract of the bark was weighed and dissolved in methanol in a 50 ml volumetric flask and the final volume made up to the mark. One mg of dried extract of the flowers was weighed accurately and dissolved in methanol in a 250 ml volumetric flask and the final volume was made up to the mark.

HPLC operating conditions : The operating conditions for quercetin consisted of Phenomenex Luna C-18 column (150 × 4.6 mm) 3 µm, 0.1% (v/v) Ortho phosphoric acid in water (50:50) and Acetonitrile-water (50:50) (0.1-100) as Eluents-A and B, Flow rate of 0.6 ml/minute, Diluent-Methanol, λ max -254 nm (for quercetin) and 210 nm (for catechin), 20 µl injection (for quercetin) and 40 µl (for catechin), with accurate weight of standard and sample of 5 mg in 10 ml and 10 mg in 10 ml methanol, respectively with acolumn temperature of 20ºC to 30ºC.

Identification of compounds : Compounds were identified by comparing the peak of the specific compound in the chromatogram with that of the standard peak. The quantity of quercetin and catechin was determined from the respective standard curves. The percentage of compounds present in crude bark and flower extract of *S. asoca* was calculated using the following formula:

$$\frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \frac{\text{concentration of standard}}{\text{concentration of sample}} \times \text{purity of standard}$$

The retention time and peak area of the corresponding chromatogram were taken into consideration for quantification of quercetin and catechin.

2.2 Statistical analysis

All the experiments of phytochemical analysis were set up in a completely randomized design with three replications per assay to verify the reproducibility of results and expressed as mean ± SE.

3. Results and Discussion

The results obtained in the study concerning phytochemical characteristics of six accessions of *S. asoca,* identification of accessions with the highest content of total phenolics and flavonoids including HPLC analysis are discussed.

3.1 Phytochemical analysis

3.1.1 Qualitative analysis

Several workers have advocated the medicinal importance of phytochemicals in *S. asoca* (Pradhan *et al.,* 2009; Bhadauria *et al.,*2012; Mishra *et al.,* 2013; Singh *et al.,* 2015). Brownish mass of bark extract weighing 160 g of ethanolic and 155 g of chloroform extract was obtained which gave a yield of 16% and 15.5%, respectively w/w on dried bark powder. Reddish brown mass of flower extract weighing 90 g of ethanolic and 60 g of aqueous extract was obtained with a yield of 9% and 6%, respectively w/w on dried flower powder.

The qualitative phytochemical analysis was carried out on the bark and flower extracts of six accessions of *S. asoca* and all of them have shown the presence of most of the secondary metabolites (Table1). Terpenoids were absent in the bark samples of some accessions and saponins were completely absent in the bark samples of all the accessions. Saponins and terpenoids were absent in the flower samples of some accessions but, alkaloids were completely absent in the flower samples of all the accessions. However, the phenolics, especially the flavonoids were consistently present in the bark and flower samples of all the accessions. Hence, quantitative estimations of the same were taken up for intensive study. The results are in agreement with the reports of Sadhu *et al.* (2007), Panchawat and Sisodia (2010), Pradhan *et al*. (2010), Mukhopadhyay and Nath (2011) and Manohar *et al.* (2012) in *S. asoca*. Toxicity of some secondary metabolites of various plant parts was tested by Mukhopadhyay and Nath (2011) while the antioxidant properties of bark and flower extracts were reported by Sadhu *et al*. (2007), Panchawat and Sisodia (2010), Pandey *et al.* (2011) and Pal *et al.* (2014). Singh *et al.* (2012) reported the anti-acne property of the bark extract.

3.1.2 Quantitative analysis

The quantitative phytochemical analysis was carried out in the six accessions of *S. asoca* for the estimation of total phenols and flavonoids from bark and flowers. The highest amount of total phenols recorded were: 1.734 ± 0.09 and 4.045 ± 0.04 mg GAE/g in the bark and flower extracts, respectively of the Sa-O accession (Tables 2 and 3). Similarly, the highest amount of flavonoids were also recorded (1.068 ± 0.02 and 1.403 ± 0.06 mg/g in the bark and flower extracts, respectively) in the Sa-O accession, which is a 50 year-old tree, growing in the Botanical garden of Department of Botany, Osmania University (Tables 2 and 3). Therefore, from among all the accessions estimated, the Sa-O accession was found to possess the highest quantities of phenols and flavonoids.

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites that possess an aromatic ring bearing one or more hydroxyl constituents (Sadhu *et al.,* 2007). The values obtained presently were much higher than earlier reports by Sadhu *et al*. (2007), Pradhan *et al.* (2010), Pandey *et al.* (2011) and Ghatak *et al.* (2015). Therefore, the Sa-O accession was chosen for the HPLC analysis of quercetin and catechin.

3.1.3 HPLC for estimation of quercetin and catechin

Secondary metabolites present in medicinal plants are essential components of drugs in herbal medicine and pharmaceutical industry. The bark of *S. asoca* is extensively available as a component in mixtures with other drugs in the market. It has, however, been little investigated as a single drug. Presently, the bark and flower extracts of accession Sa-O of *S. asoca* were analyzed for the estimation of quercetin and catechin by high performance liquid chromatography (HPLC) and the compounds were quantified by comparing the peak of the compound in the chromatogram, its retention time and peak area with that of the respective standard. Among all the tested stationary phases, symmetry shield Phenomenex Luna C-18 (150 × 4.6 mm) was found to be most suitable for quantification of quercetin and catechin, with two mobile phases (mobile phase A-0.1% $^{v}/_{v}$ Ortho Phosphoric acid in water, B-Acetonitrile-water; (0.1-100), methanol). Throughout the run, the flow rate was maintained at 1ml/1 min. The column effluent was monitored at 210 nm, 243 nm, 254 nm with 25ºC -30ºC temperature.

The retention time for standard quercetin was recorded with three runs in HPLC as 20.192 min at 254 nm while the quercetin of the bark extract of *S. asoca* was recorded with a retention time of 21.011 min at 254 nm (Figures 1 and 2). Since the quercetin in case of the standard and the bark extract showed different retention times, both the samples (standard and bark extract) (50 + 50%) were run twice, and by spiking the highest peak in the chromatogram, the compound was identified as quercetin with a concentration of 0. 344 mg /g.

The retention time for standard catechin was recorded with three runs in HPLC as 6.948 min at 280 nm while that of the catechin in the flower extract of *S. asoca* was recorded with the retention time of 6.622 min at 280 nm (Figures 3 and 4). Since different retention times were recorded for the catechin standard and that of the bark extract, both the samples (standard and flower extract) (50% + 50%) were run twice, and by spiking the highest peak in the chromatogram, the compound was identified as catechin with a concentration of 0.994 mg /g.

Table 1: Qualitative analysis of chloroform and ethanol extracts of bark and flowers of *S. asoca*

Phytochemicals	Accessions						Accessions					
	Sa-O	Sa-A	Sa-B	Sa-C	Sa-D	Sa-E	Sa-O	Sa-A	Sa-B	Sa-C	Sa-D	Sa-E
	Chloroform extract of bark						**Ethanol extract of bark**					
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	–
Steroids	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	–	+	–	+	+	+	–	+	–
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids												
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	–	–	–	–	–	–	–	–	–	–	–	–
	Chloroform extract of flowers						**Ethanol extract of flowers**					
Alkaloids	–	–	–	–	–	–	–	–	–	–	–	–
Steroids	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	–	+	–	+	+	+	–	+	–
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	–	–	+	–

\+ Presence of the compound
– Absence of the compound

Table 2: Total phenolic content in bark and flower extracts of six accessions of *S. asoca*

S.No.	Accessions	Phenolic content in bark extract (mg GAE/g*) (Mean ± SE**)	Phenolic content in flower extract (mg GAE/g*) (Mean ±SE**)
1	Sa-O	1.734±0.09	4.045±0.04
2	Sa-A	0.453±0.08	3.463±0.05
3	Sa–B	0.896±0.06	1.634±0.06
4	Sa–C	1.146±0.05	2.154±0.02
5	Sa–D	0.768±0.04	1.021±0.01
6	Sa–E	0.634±0.09	2.345±0.06

* Milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) of extract

** The analysis was carried out in triplicate and expressed as mean ± SE

Table 3: Total flavonoid content in bark and flower extracts of six accessions of *S. asoca*

S.No.	Accessions	Flavonoids in bark extract (mg/g*) (Mean ± SE**)	Flavonoids in flower extract (mg/g*) (Mean ± SE**)
1	Sa-O	1.068±0.02	1.403±0.06
2	Sa-A	0.303±0.01	0.136±0.05
3	Sa–B	0.466±0.01	0.658±0.09
4	Sa–C	0.032±0.07	1.045±0.07
5	Sa–D	0.136±0.03	1.021±0.01
6	Sa–E	0.564±0.06	0.364±0.05

* Flavonoid content in terms of quercetin equivalent (mg/g of bark extract) / catechin equivalent (mg/g of flower extract)

** The analysis was carried out in triplicate and expressed as mean ± SE

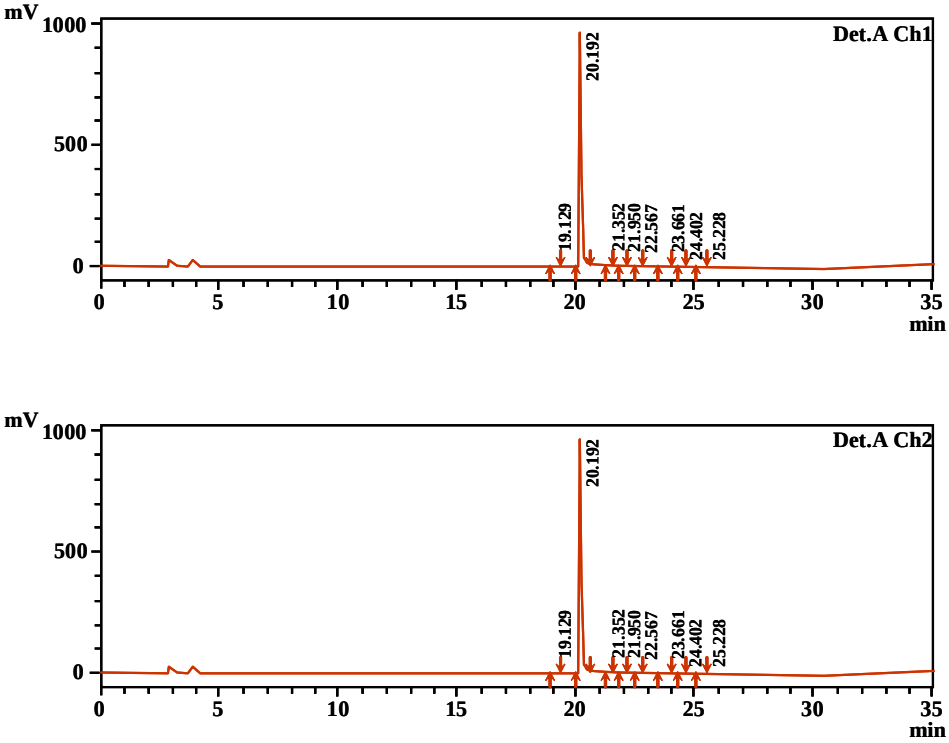

Figure 1: HPLC graph of standard quercetin

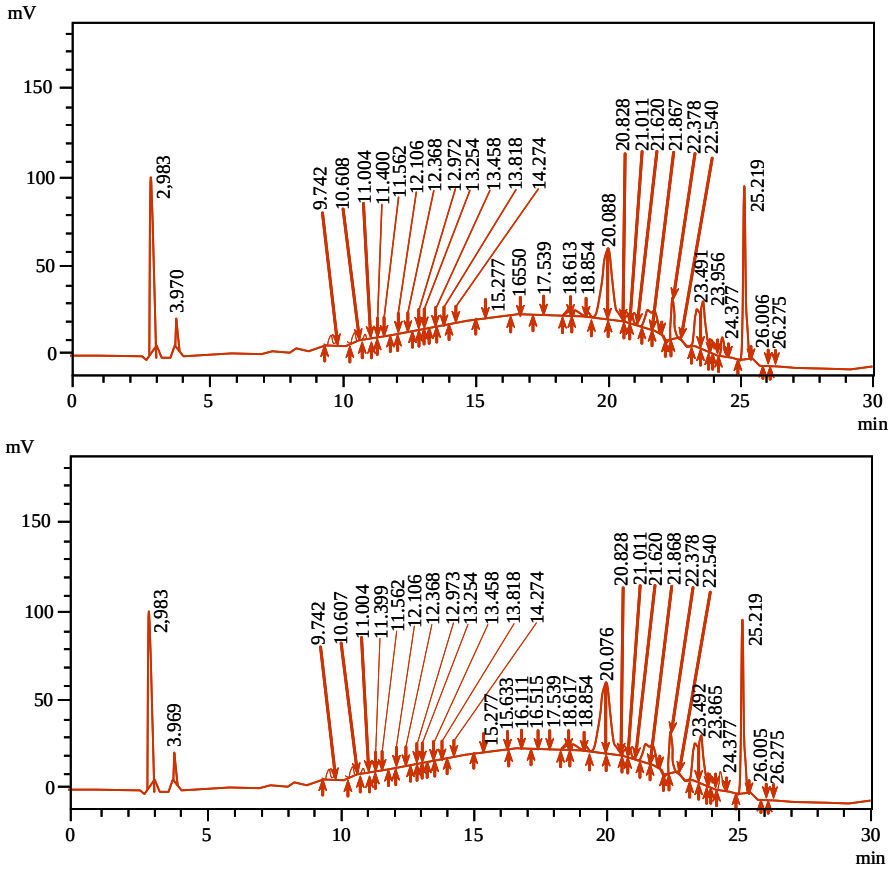

Figure 2: HPLC graph of bark extract of *S. asoca* for quercetin

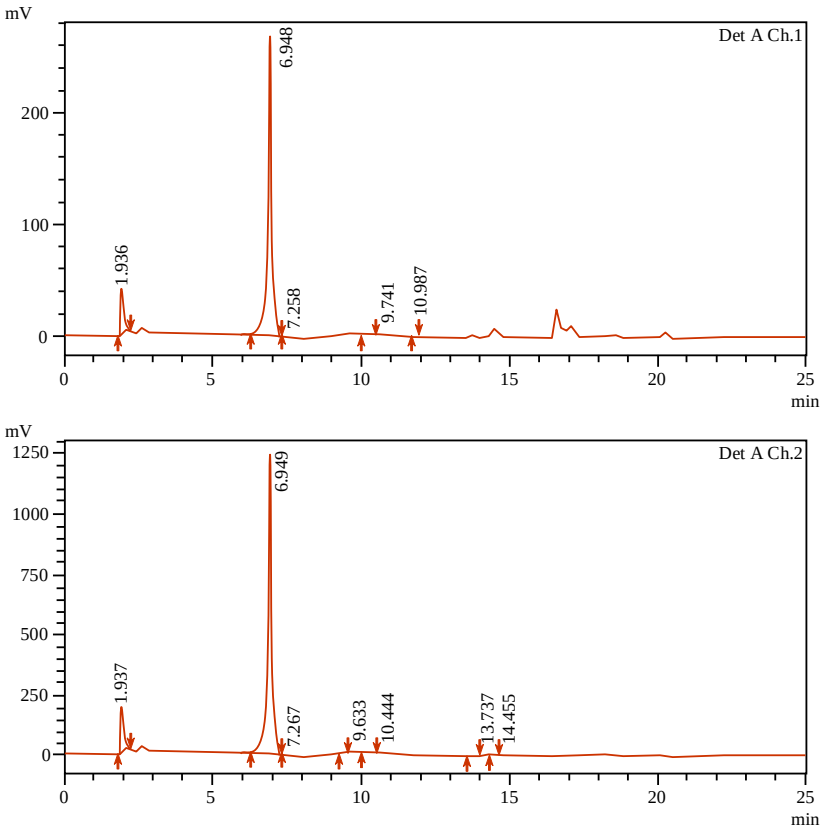

Figure 3: HPLC graph of the standard Catechin.

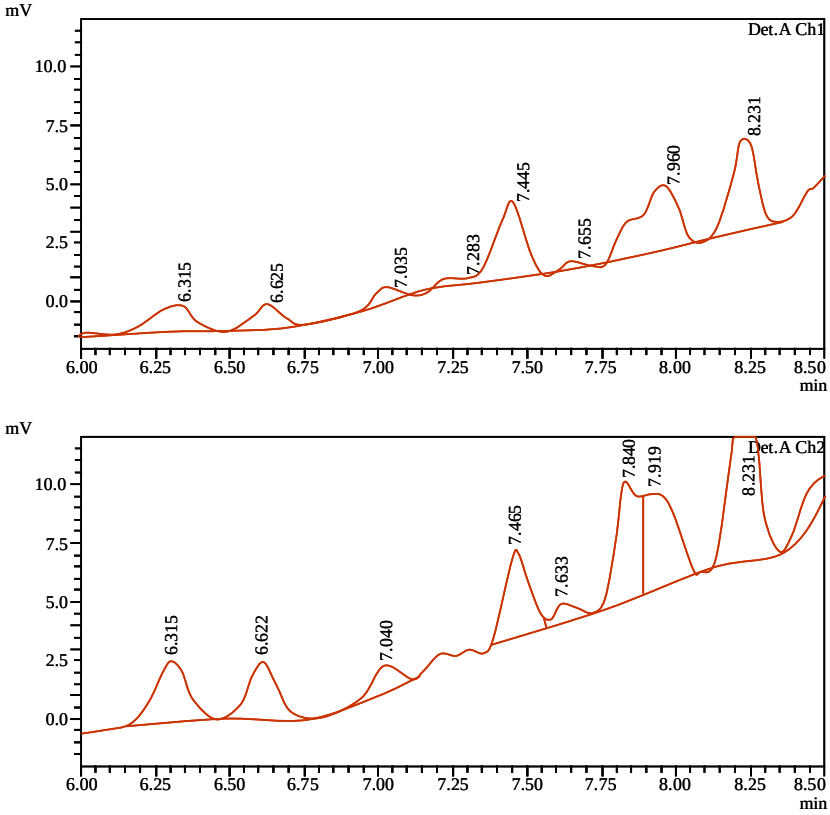

Figure 4: HPLC graph of flower extract *S. asoca* for catechin.

The bark and flowers contain numerous polyphenolic compounds and flavonoids. The unusual flavonoid compound quercetin is responsible for the fragrance in bark organs of *S. asoca* (Saha *et al.*,2012). Catechin is a well-known flavonoid known for antioxidant activity and is also used for the symptomatic treatment of several gastrointestinal respiratory and vascular diseases and has also been reported to induce cancer preventive activity mediated through a chaperone like property (Pradhan *et al.*,2009; Shirolkar *et al.*,2013).

Therefore, the concentration of quercetin obtained presently was 0.344 mg/g with 21.011 min retention time at 254 nm and that of catechin was 0.994 mg/g with 6.622 min retention time at 280 nm. These results are comparable and higher contents recorded presently than those of Rathee *et al.* (2010), Saha *et al.* (2012 and 2013), Shirolkar *et al.* (2013), Ghatak *et al.* (2015) and Ketkar *et al.* (2015). This indicates that the content of the compounds can vary with the accession. The fact that the accession Sa-O is a fifty-year old tree also indicates the possibility of higher contents of quercetin and catechin in older trees (compared to other accessions, which were six years old).

4. Conclusion

The current endeavor has accomplished an investigation of the phytochemical constituents both qualitatively and quantitatively with the HPLC analysis of specific and important medicinal compounds, quercetin and catechin. One accession, Sa-O has been identified to possess a very high content of the valuable compounds.

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Conflict of interest

We declare that we have no conflict of interest.

References

Bhadauria, P.; Arora, B.; Sharma, A. and Singh, V. (2012). A review on *Saraca indica* plant. Int. Res. J. Pharm., **3**:80-84.

Chang, C.C.; Yang. M.H.; Wen, H.M. and Chern, J.C. (2002). Estimation of total flavonoid content in Propolis by two complementary colorimetric methods. J. Food Drug Analysis, **10**:178-182.

Evans, W.C. (2009). Trease and Evans' Pharmacognosy, (16th Ed.).W. B. Saunders Ltd., ISBN Number 9780702029332.

Ghatak, A.; Nair, S.; Vajpayee, A.; Chaturvedi, P.; Samant, S.; Soley, K.; Kudale, S. and Desai, N. (2015). Evaluation of antioxidant activity, total phenolic content, total flavonoids, and LC-MS characterization of *Saraca asoca* (Roxb.) De.Wilde. Int. J. Adv. Res., **3**:318-327.

Gupta, M.; Thakur, S.; Sharma, A. and Gupta, S. (2013). Qualitative and quantitative analysis of phytochemicals and pharmacological value of some dye yielding medicinal plants. Oriental J. Chem., **2**:475-481.

Ketkar, P.M.; Nayak, S.U.; Pai, S.R. and Joshi, R.K.(2015). Monitoring seasonal variation of epicatechin and gallic acid in the bark of *Saraca asoca* using reverse phase high performance liquid chromatography (RP-HPLC) method. J. Ayurveda Integr. Med., **6**:29-34.

Manohar, V.R.; Chandrashekar, R. and Rao, S.N. (2012). Phytochemical analysis of *Saraca asoca* bark extracts. Int. J. Pharm. Sci. Rev. Res., **17**:101-103.

Mukhopadhyay, M.K. and Nath, D. (2011). Phytochemical screening and toxicity study of *Saraca asoca* bark methanolic extract. Int. J. Phytomed., **3**:498-505.

Mishra, A.; Kumar, A.; Rajbhar, N. and Kumar, A. (2013). Phytochemical and pharmacological importance of *Saraca indica*. Int. J. Pharm. Chem. Sci., **2**:1009-1013.

Mukhopadhyay, M.K. and Nath, D. (2011). Phytochemical screening and toxicity study of *Saraca asoca* bark methanolic extract. Int. J. Phytomed., **3**:498-505.

Pal, T.K.; Bhattacharya, S. and Dey, A. (2014). Evaluation of antioxidant activities of flower extract (fresh and dried) of *Saraca indica* grown in West Bengal. Int. J. Curr. Microbiol. App. Sci., **3**:251-259.

Panchawat, S. and Sisodia, S.S. (2010). *In vitro* antioxidant activity of *Saraca asoca* Roxb. De Wilde stem bark extracts from various extraction processes. Asian J. Pharm.Clin. Res., **3**:231-233.

Pandey, A.K.; Ojha, V.; Yadav, S. and Sahu, S.K. (2011). Phytochemical evaluation and radical scavenging activity of *Bauhinia variegata*, *Saraca asoca* and *Terminalia arjuna* barks. Res. J. Phytochem., **5**:89-97.

Parekh, J.; Jadeja, D. and Chanda, S. (2005). Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turk. J. Biol., **29**:203-210.

Pradhan, P.; Joseph, L.; Gupta, V.; Chulet, R.; Arya, H.; Verma, R. and Bajpai, A. (2009). *Saraca asoca* (Ashoka): A review. J. Chem. Pharm. Res., **1**:62-71.

Pradhan, P.; Joseph, L.; George, M.; Kaushik, N. and Chulet, R. (2010). Pharmacognostic, phytochemical and quantitative investigation of *Saraca asoca* leaves. J. Pharm. Res., **3**:776-780.

Puspangadan, P.; Mehrotra, S.; Rawat, A.K.S.; Tewari, S.K.; Sikar, R.L.S and Misra, N. (2004). Package of practices for organic cultivation of important medicinal plant (Part I). Economic Botany Information Service, National Botanical Research Institute, Lucknow, India, pp:85.

Rathee, P.; Rathee, S.; Rathee, D. and Rathee, D. (2010). Quantitative estimation of (+)-catechin in stem bark of *Saraca asoka* Linn using HPTLC. Der Pharma Chemica, **2**:306-314.

Sadhu, S.K.;Khatun, A.;Phattanawasin, P.; Ohtsuki, T. and Ishibashi M. (2007). Lignan glycosides and flavonoids from *Saraca asoca* with antioxidant activity. J. Nat. Med.,**61**:480-482.

Saha, J.; Mitra, T.; Gupta, K. and Mukherjee, S. (2012). Phytoconstituents and HPTLC analysis in *Saraca asoca* (Roxb.) Wilde. Int. J. Pharm. Sci., **4**(1):96-99.

Saha, J.;Mukherjee, S.; Gupta, K. and Gupta, B. (2013). High-performance thin-layer chromatographic analysis of antioxidants present in different parts of *Saraca asoca* (Roxb.) de Wilde. J. Pharm. Res., **7**(9):798-803.

Sharma, P.C.; Yelne, M.B. and Dennis, T.J. (2001). Database on medicinal plants used in Ayurveda, CCRAS, New Delhi, **3**:76.

Shirolkar, A.;Gahlaut, A.; Chhillar, A.K. and Dabur, R. (2013). Quantitative analysis of catechins in *Saraca asoca* and correlation with antimicrobial activity. Anal. J. Pharm., **3**:421-428.

Singh, D.; Yadav, P.; Nayak, S. and Hatwar, B. (2012). Antiacne, preliminary phytochemical and physicochemical investigation of *Saraca asoca* bark. J. Nat. Prod. Plant Resour., **2**:119-126.

Singh, S.; Krishna, T.H.A.;Kamalraj, S.;Kuriakose, G.C.; Valayil, J.M. and Jayabaskaran, C. (2015). Phytomedicinal importance of *Saraca asoca* (Ashoka): An exciting past, an emerging present and a promising future. Curr. Sci., **109**:1790-1801.

Singleton, V.L.; Orthofer, R. and Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. Methods Enzymol., **299**:152-178.