Future Journal of Pharmaceutical Sciences

RESEARCH

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RP-HPLC-based phytochemical screening of different polyphenolic compounds from floral extract of four species of *Saraca* L. (Leguminosae)

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Abstract

Background: *Saraca* L. is one of the treasures throve of medicinally important plants in Indian subcontinent with its four species among which two are naturally distributed. It is one of the important resources of highly active phytochemicals due to which it proclaims a legendary position from ancient medicinal practices to modern ages. The crude extracts of flowers of four species were prepared using Soxhlet apparatus in petroleum benzene, ethyl acetate and 90% methanol sequentially. RP-HPLC (reverse-phase high-performance liquid chromatography)-based analysis of the presence of different phytochemicals using 15 different polyphenolic phytochemical standards was done to assess and quantify different phytochemicals.

Results: RP-HPLC-based evaluation revealed the presence different polyphenolic compounds like catechins, chlorogenic acid, vanillic acid, caffeic acid, gallic acid, t-cinnamic acid, anthrol, p-coumaric acid, biochanin A, etc., in a considerable amount which is very crucial for the phytomedicinal field. The correlations of the presence of known phytochemicals give a basis of phytochemical correlation among the four species. The RP-HPLC chromatographic data were applied to develop the complete phytochemical coding according to the complete set of chemicals found among the species to evaluate phytochemical correlation among them in a different way. This application also produces strong evidence of distinguishing features of *S. indica* and *S. asoca* that were considered as same species by some traditional taxonomy.

Conclusion: Extracts of *S. thaipingensis* contain highest amount of polyphenolic compounds, and the lowest amount was found in *S. declinata*. The phytochemical relations among *S. asoca* and *S. declinata* are high, and *S. indica* also has close relations with them, but *S. thaipingensis* has distinct divergence.

Keywords: Phytochemical coding, Phytochemical screening, Polyphenolic compounds, RP-HPLC, *Saraca* L.

Background

Saraca, the marvel of herbal medicines, belongs to the family Leguminosae [1], sub-family Caesalpinioideae [2] and the tribe Deteriae [3], distributed through the rainforests of south and south-east Asia. *Saraca* along with its 11 species are known for its remarkable medicinal importance [4, 5], and the legacy has been going from the ancient period of time. The remarkable medicinal importance and the aesthetic beauty acclaimed its predominant position in the arts, sculptures, literature and ancient medicinal practices [6]. Four species of *Saraca* are available in India, and among them *S. asoca* (Roxb.) de wilde and *S. indica* L. were found naturally in the different tropical and sub-tropical rainforests,

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but *S. declinata* (Jack) miq. and *S. thaipingensis* Cantley ex Prain can only be found in artificial captivities [7]. Due to the intense medicinal importance, unscientific rearing is very common and in addition to that increasing urbanization is diminishing the natural populations of *Saraca* which made it as globally vulnerable species [8]. Extracts of flower of *Saraca* were reported to have remarkable therapeutic potentialities like antidiabetic [9, 10], anti-ulceric [11, 12], anti-microbial [13–15], anti-cancerous [16], anti-mutagenic and antigenotoxic [17]. Extract of flower of *S. asoca* and *S. indica* is reported to contain alkaloids, flavonoids, cardiac glycosides, steroids and tannins [18], anthraquinone glycosides [19]. Flower extracts of *Saraca* were reported to contain various kinds of highly important phytochemicals which are to be more critically evaluated. RP-HPLC (reverse-phase high-performance liquid chromatography)-based characterization and evaluation of different phytochemicals is more scientific and convenient technique than chemical tests and spectroscopic analysis. UPLC (ultra-performance liquid chromatography)-based analysis of ethanolic and aqueous extract of flowers of *S. asoca* was reported to contain gallic acid, (+)-catechin, procyanidin-B_2, (−)-epicatechin, (−)-epigallocatechin gallate and (−)-epicatechin gallate [20]. The phytochemical evaluation of the presence of catechins and gallic acid within the bark extracts of *S. asoca* confirmed their seasonal variability [21]. Methanolic extract of bark was reported to contain tannic acid, (−)-gallocatechin, (+)-catechin, (−)-epigallocatechin, (−)-epigallocatechin gallate, (+)-3'-deoxycatechin-3-O-α-L-rhamnopyranoside, (−)-3'-deoxiepicatechin-3-o-β-D-glucopyranoside, epicatechin, gallocatechin gallate, lyoniside, etc. [22]. HPTLC (high-performance thin-layer chromatography) of the extracts of the flower of *Saraca* showed the presence of gallic acid [17]. HPLC (high-performance liquid chromatography)-based phytochemical analysis is essential to be made for the characterization of the flower extract of *Saraca*.

Methods

Collection of samples and drying

Flowering inflorescences (20 days old) (Fig. 1) were collected from living plants at A.J.C Bose Indian Botanic Garden, West Bengal, India, during the flowering season (December to April) of 2015–2017, and voucher of the preserved samples (Table 1) was submitted and accessioned from BURD [23].

Mature flowers and flower buds were separated from the inflorescence and placed on dry water soaking papers for drying under open, airy and shady place for 6 days. Shade dried flowers were kept at 30 °C for 5 h within hot air oven. Dried samples were grinded coarsely and kept at 6 °C for future use.

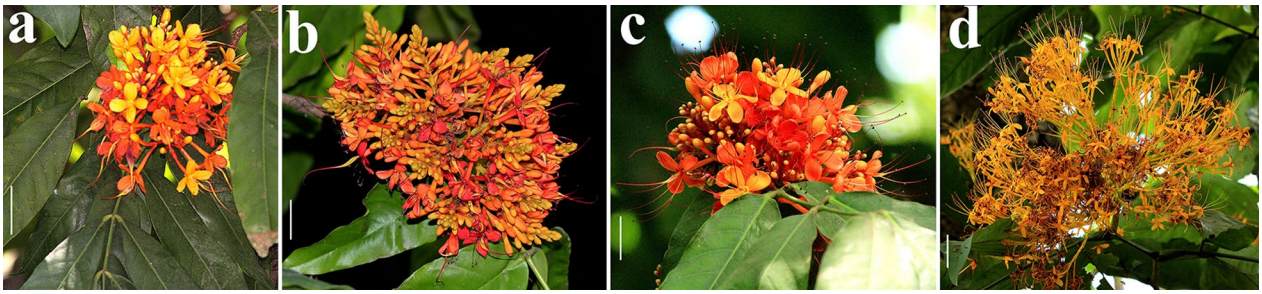

Fig. 1 Flowering twigs of four species of *Saraca* L. **a** *S. asoca*, **b** *S. declinata*, **c** *S. indica* and **c** *S. thaipingensis* (1 cm scale)

Table 1 Details of plant material applied for experiment

No	Name of the species	Date of collection	Place of collection	Geological position	Accession
1	*S. asoca*	12/04/2016	AJC Bose Indian Botanic Garden	88.3117°E, 22.572°N	BURD 1211/a/2018
2	*S. declinata*	29/01/2015	AJC Bose Indian Botanic Garden	88.3117°E, 22.572°N	BURD 12007/a/2018
3	*S. indica*	17/06/2017	AJC Bose Indian Botanic Garden	88.3117°E, 22.572°N	BURD 12004/a/2017
4	*S. thaipingensis*	26/01/2016	AJC Bose Indian Botanic Garden	88.3117°E, 22.572°N	BURD 12006/a/2017

Extraction

Mature flowers and flower buds were separated from the inflorescence and placed on dry water soaking papers for drying under open, airy and shady place for 6 days. Shade dried flowers were kept at 30 °C for 5 h within hot air woven. Dried flowers were grinded coarsely, and 20gm of samples was taken within cellulose thimble (80X20mm) and placed within the Soxhlet apparatus. 250 ml of petroleum benzene was poured within the Soxhlet, and the temperature was set at 30 °C. The Soxhlet was run for 72 cycles of extraction for each sample, and the extracts were collected separately in glass beakers. The thimble with the same sample was taken out and dried for 4 h at 30 °C and placed properly within cleaned Soxhlet setup, and the extraction was made with the help of ethyl acetate. Another sequence of extraction was followed from the same sample with 90% methanol. Extracts were kept within glass beakers covered with cotton cloth at 6–10 °C during which the extract became crystal dried. Dried extracts were weighed and kept at − 4 °C within separate glass micro-centrifuge tubes.

Standard used

Fifteen standards of different polyphenolic compounds were dissolved in methanol to get 1 mg/ml solution. 400 µl from each of the solution were taken into a single glass centrifuge tube.

RP-HPLC setup

RP-HPLC was established using Shimadzu chromatography system fitted with C-18 column. The system was equipped with degasser (DGU-2As), liquid chromatograph (LC-20AT), autosampler (SIL-20A), column oven (CTO-ASvp), fluorescent detector (RF-10AxL), diode array detector (SPD-M20-A), bus module (CMB-20) and fraction collector (FRC-10A).

Solvent and program setup

The reverse-phase HPLC was performed using 0.1% solution of acetic acid dissolved in Milli-Q® water (solvent A) and acetonitrile (solvent B), and the flow rate was maintained at 0.8 µl/min. The elution began with 10% of solvent B maintained from 0.01 to 2 min. It was changed from 10 to 30% from 2 to 27 min and followed by the change of concentration of solvent B from 30 to 90% from 27 to 50 min. From 50 to 52 min, the elution was increased from 90 to 100% and it was maintained to 53 min and then it was reduced to 10% till 63 min to stop. The chromatographic detection was done at 270 nm wavelength.

Preparation of samples

One milliliter of methanol was added to 2 mg of each of the dried extract of experimental samples and mixed thoroughly.

Method validation

The determination of linearity, precision and accuracy was done maintaining the ICH guidelines. The linearity was determined using aliquots of different concentrations (0.25, 0.5, 0.75, 1 µg/mL) of stock solutions for each of the standards. The calibration was done for each of the standards separately. The analytical method was run uninterruptedly in same day without changing stock or mobile phases, and the each of the sample was run at least six times to increase precision of the method. The precision was also determined by intra-day running of standard solution of four different concentrations. Recovery study was performed using addition of known amount standard with sample and following the run maintaining the same RP-HPLC method. % of recovery and % of RSD (relative standard deviation) were evaluated after the triplicate repetition of the study.

Run of sample

Ten microliters of each of the sample solution was placed consecutively for the analysis, and each of the report was developed with the specific software (AutoRun) equipped with the chromatographic system.

Statistical analysis

The comparison among the RP-HPLC data of flower extracts of four species of *Saraca* was done in the following way.

Comparative analysis based on known standards

The comparison of RP-HPLC data based on the presence and quantitative estimation of known phytochemicals was done with the help of 'PAST 3' software. These data were applied in principal component analysis (PCA) and similarity clustering.

Comparative analysis based on complete phytochemical data

The better resolution of chemotaxonomic relations among different species of *Saraca* was established applying the RP-HPLC data of complete phytochemical sets. The retention times were regarded as independent characters, and on the basis of the presence and absence of such characters, a binary data matrix was obtained. The

binary data were applied in 'PAST 3' software for PCA and similarity clustering.

Quantification of phytochemicals

The phytochemicals present within each of the sample on the basis of known standards were quantified according to the following formula [24].

$$\text{The percentage of compound} = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \frac{\text{Concentration of standard}}{\text{Concentration of sample}} \times \text{purity of standard}$$

Results

The percent relative standard deviation (% RSD) in linearity test was observed below 2, and the mean relative coefficient is 0.9997. Intra-day repeatability had provided the range of value of % RSD from 0.31 to 0.48 (n = 4). The recovery data states range from 99.48 to 99.86% for different standards with the % RSD value ranging from 0.28 to 0.39. The extractive yields of each of the fractions of four species of *Saraca* are given in Table 2. The mixture of 15 standards gives the chromatogram represented in Fig. 2.

Table 2 Total quantity of phytochemicals (mg/kg of plant materials)

Standards	*S. asoca*	*S. declinata*	*S. indica*	*S. thaipingensis*
Gallic acid	92192.99	209956.4	192296.3	329939.8
Protocatechuic acid	4123.283	13850.58	1585.371	14265.88
Catechin	0	393.5961	15162.18	66.16143
Hydroxybenzoic acid	0	0	0	0
Chlorogenic acid	0	111.6441	3956.238	1155.707
Vanillic acid	5353.813	3004.12	10107.05	782.4114
Syringic acid	16025.7	9766.306	1954.577	1739.703
Caffeic acid	0	0	37362.29	68881.72
m-hydroxybenzoic acid	15866.93	3902.247	45396.04	0
p-coumaric acid	26749.27	0	0	119106.8
Sinapic acid	5017.516	4042.664	25320.18	42608.17
t-ferulic acid	10164.78	0	32696.02	405,934.9
t-cinnamic acid	12828.86	18303.68	25440.64	7908.605
Biochanin A	33274.22	9764.952	21400.51	6801.211
Anthrol	2628.686	0	0	4655.386

Specification of samples

Petroleum benzene (PB) extract of the flowers of *S. indica* showed the presence of 90 different phytochemicals among which m-hydroxybenzoic acid was identified. In the petroleum benzene extract of flower of *S. declinata,* gallic acid was found to be present. The extract of flower of *S. thaipingensis* contained gallic acid and chlorogenic acid as important chemicals, and the extract of flower of *S. asoca* contained m-hydroxybenzoic acid and biochanin A as major phytochemicals. Petroleum benzene extract of flower of *S. declinata*, *S. thaipingensis* and *S. asoca* was found to contain total 97, 58 and 97 types of compounds, respectively (Fig. 3).

Ethyl acetate (EA) extracts of flowers of *S. indica*, *S. declinata*, *S. thaipingensis* and *S. asoca* comprised a total of 121, 110, 111 and 121 types of phytochemicals, respectively (Fig. 4). The extract of *S. indica* contained gallic

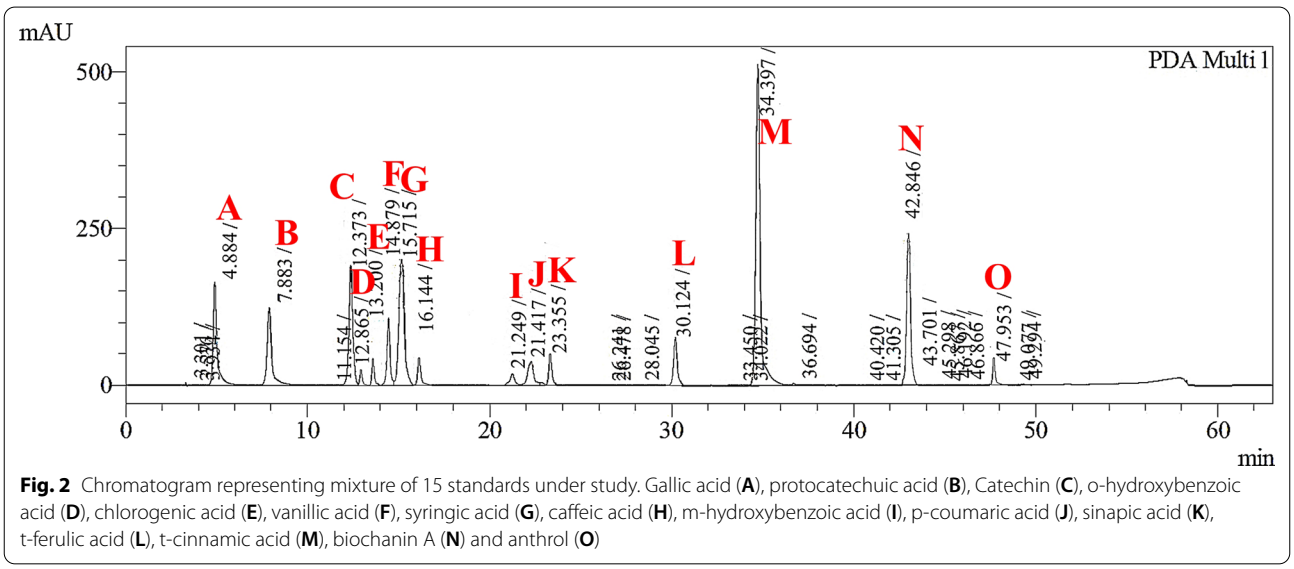

Fig. 2 Chromatogram representing mixture of 15 standards under study. Gallic acid (**A**), protocatechuic acid (**B**), Catechin (**C**), o-hydroxybenzoic acid (**D**), chlorogenic acid (**E**), vanillic acid (**F**), syringic acid (**G**), caffeic acid (**H**), m-hydroxybenzoic acid (**I**), p-coumaric acid (**J**), sinapic acid (**K**), t-ferulic acid (**L**), t-cinnamic acid (**M**), biochanin A (**N**) and anthrol (**O**)

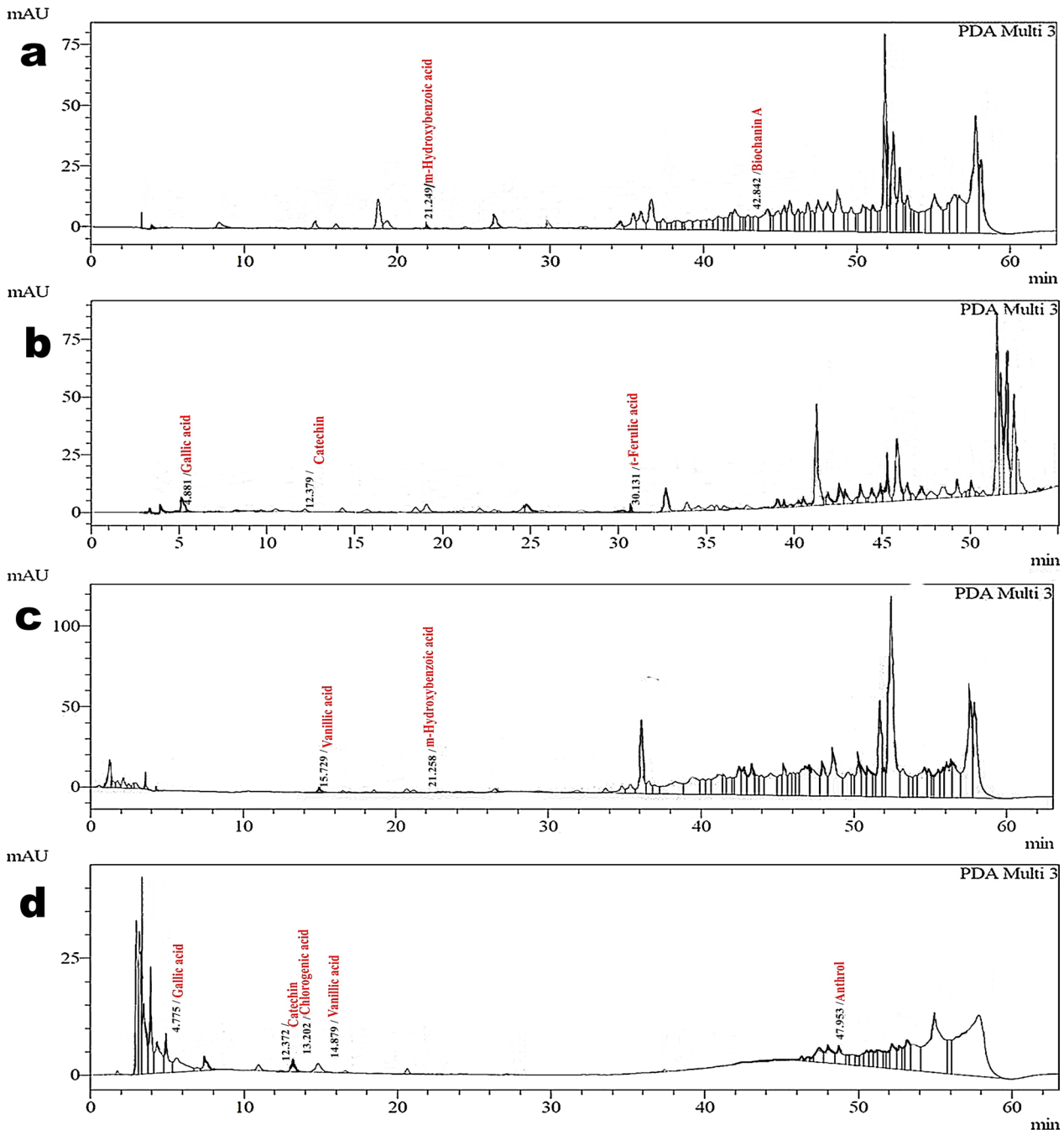

Fig. 3 Chromatograms of petroleum benzene extracts of four species of *Saraca* **a** *S. asoca*, **b** *S. declinata*, **c** *S. indica* and **d** *S. thaipingensis*

acid, catechins, chlorogenic acid, syringic acid, caffeic acid, sinapic acid, t-ferulic acid, t-cinnamic acid and biochanin A. The extract of *S. declinata* was found to contain gallic acid, catechins, chlorogenic acid, m-hydroxybenzoic acid, t-ferulic acid, t-cinnamic acid, biochanin A, anthrol. *S. thaipingensis* contained gallic acid, syringic acid, caffeic acid, sinapic acid, t-ferulic acid, t-cinnamic acid, biochanin A. *S. asoca* contained gallic acid, syringic acid, t-ferulic acid, t-cinnamic acid, biochanin A and anthrol.

Methanolic (90%) extracts of flowers of four species of *Saraca* contained 88, 82, 67 and 90 types of compounds, respectively (Fig. 5). *S. indica* contained gallic acid, catechins, vanillic acid. *S. declinata* contained gallic acid

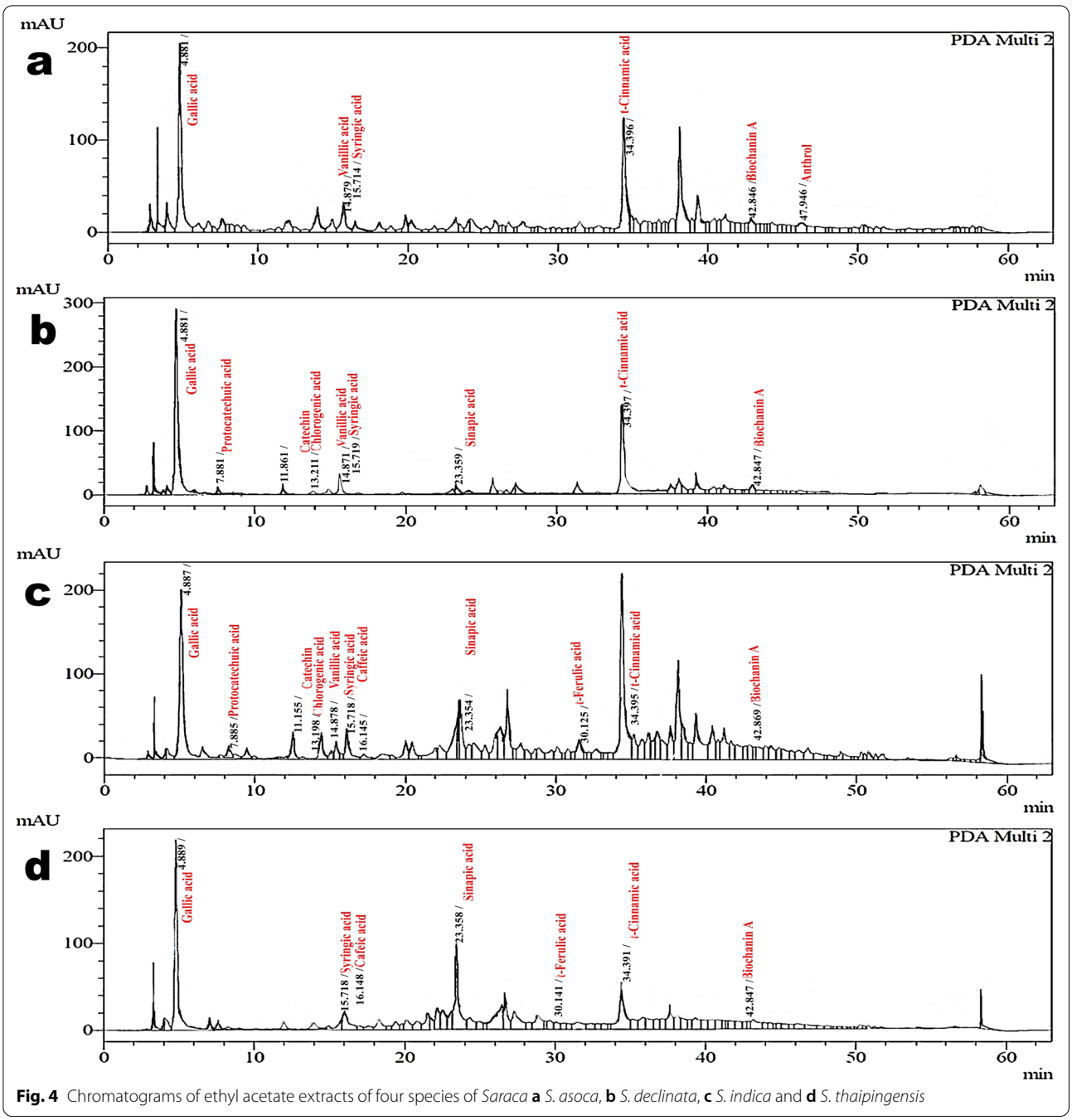

Fig. 4 Chromatograms of ethyl acetate extracts of four species of *Saraca* **a** *S. asoca*, **b** *S. declinata*, **c** *S. indica* and **d** *S. thaipingensis*

and protocatechuic acid. *S. thaipingensis* contained gallic acid, protocatechuic acid, caffeic acid, coumaric acid, t-ferulic acid. *S. asoca* contained gallic acid, protocatechuic acid, coumaric acid, sinapic acid and biochanin A.

Phytochemical coding

Two types of phytochemical codes were used for the analysis of RP-HPLC data of the respective extracts. The phytochemical codes based on the known phytochemicals were prepared according to the quantitative data (Table 2) of different phytochemicals present in each of the species. The complete phytochemical codes were obtained from the complete set of phytochemicals present as per the chromatograms. The sum of all of the phytochemicals was estimated with the help of compilation of the chromatograph data of three fractions for

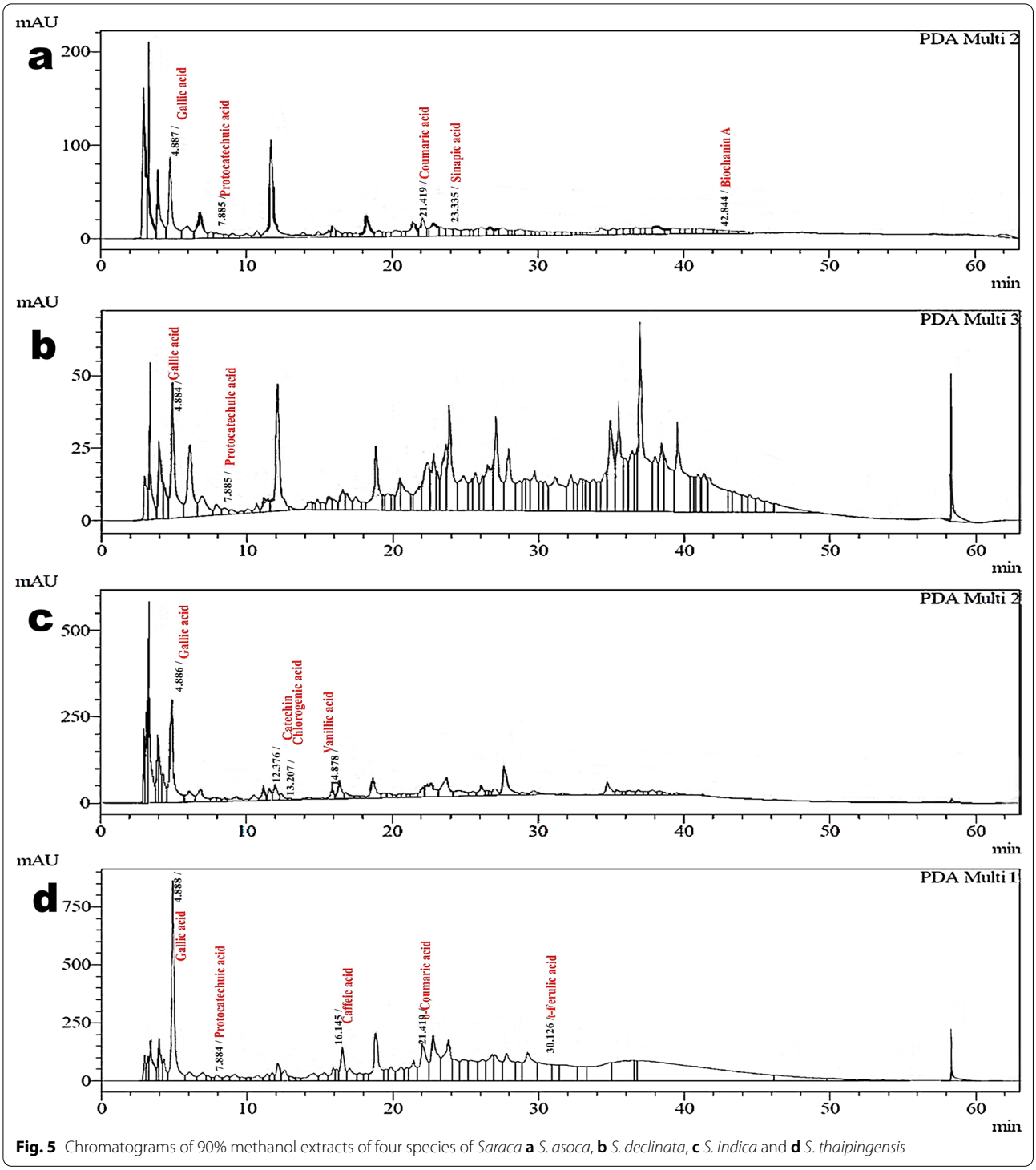

Fig. 5 Chromatograms of 90% methanol extracts of four species of *Saraca* **a** *S. asoca*, **b** *S. declinata*, **c** *S. indica* and **d** *S. thaipingensis*

each species. The comparative account of phytochemical codes was represented as binary code presented in Additional file 1: Table 5.

Quantitative evaluation

The HPLC data of the mixed standards of known concentrations (Additional file 1: Table 1 and Fig. 1) represented

a standard chromatograms profile (Figs. 3, 4, 5) which was compared with the chromatograms of similar compounds present in different solvent extracts of the flowers (Additional file 1: Tables 2, 3 and 4) to prepare the complete quantitative estimation of phytochemicals present in each species (Table 2).

Statistical application of quantitative and qualitative data

The quantitative data of each of the phytochemicals gave both quantitative and qualitative expression of floral extracts of each species which was applied for statistical analysis. Figure 6a shows the comparative account of phytochemicals present within each of the species. Principal component analysis of the same data showed the chemotaxonomic distinctness of each of the species (Fig. 6c) and revealed that *S. declinata* and *S. indica* are closely related and *S. thaipingensis* and *S. asoca* are chemotaxonomically distantly related. The loading plot (Fig. 6e) showed that *S. asoca* is most distantly correlated with the other species of *Saraca*. PCA analysis of complete phytochemical data (6c) showed the close chemotaxonomic similarity between *S. asoca*, *S. Indica* and *S. declinata* is equally correlated with *S. thaipingensis*. The loading plot (Fig. 6d) showed the distinctness of *S. asoca* from other three species.

Dendrograms

Dendrograms were obtained by the application of both quantitative and qualitative data obtained from RP-HPLC analyses. The dendrogram (Fig. 6f) based on known phytochemical data (Table 3) showed the relative similarity between *S. declinata* and *S. thaipingensis*, whereas in *S. asoca* and *S. Indica* were different from each other in terms of chemotaxonomic features. The dendrogram obtained with the help of quantitative and qualitative phytochemical data (Fig. 6g) showed two distinct clusters in which *S. thaipingensis* is out grouped and *S. declinata* and *S. indica* are more closely related as opposed to *S. asoca* within the same cluster.

Discussion

Saraca L. is the genus with potential therapeutic importance, and both *S. indica* and *S. asoca* are used to cure many diseases in India from ancient ages. RP-HPLC-based phytochemical analysis of bark samples of *S. asoca* and *S. indica* revealed the presence of catechins and gallic acid [22, 24]. RP-HPLC-based analysis of extracts of flower is relatively new but essential since flowers of *Saraca* spp. had been reported as therapeutically potent [6, 14, 16, 25, 26] and have several traditional uses. Extracts of flowers were reported to contain several medicinal properties, but phytochemical evidence behind those potentialities was not recognized. Present study based on RP-HPLC analysis of floral extract comparing with 15 phytochemical standards approaches to confer the reason behind the therapeutic potentialities of flower of *Saraca*. Present analysis confirms the presence of protocatechuic acid, chlorogenic acid, caffeic acid, coumaric

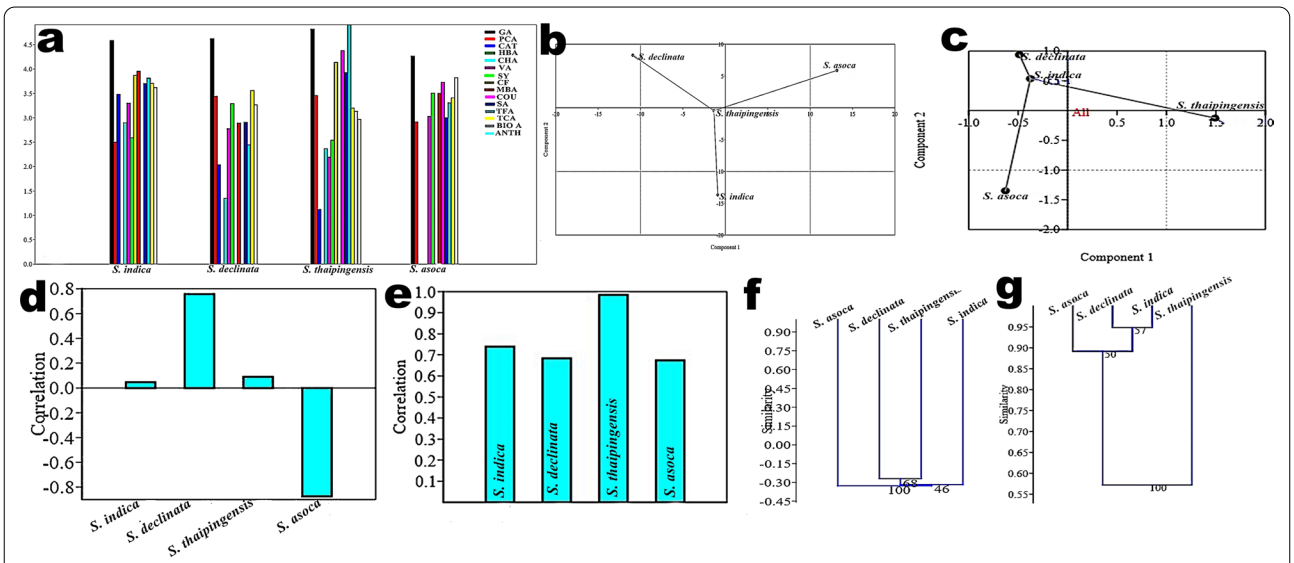

Fig. 6 Statistical analysis of biochemical data among four species of *Saraca* **a** Bar graph showing quantitative and qualitative phytochemical differences, **b** PCA scatter plot obtained from complete phytochemical data, **c** PCA scatter plot obtained by applying phytochemical data based on specific studied standards, **d** PCA loading plot obtained by using complete phytochemical data, **e** PCA loading plot obtained by using phytochemical data based on known standards, **f** Dendrogram obtained with UPGMA analysis applying complete phytochemical data and **g** Dendrogram obtained applying known phytochemical data based on known standards

Table 3 Binary codes representing the presence and absence of traces of standards in different species

S. asoca	*S. declinata*	*S. indica*	*S. thaipingensis*
0	1	0	1
0	0	0	0
0	1	0	1
0	0	0	0
0	0	0	1
0	0	1	1
0	0	0	0
0	0	0	0
1	0	1	0
0	0	0	0
0	0	0	0
0	0	0	0
0	1	0	0
1	0	0	0
0	0	0	1
1	1	1	1
0	1	1	0
0	1	1	0
0	0	0	0
0	1	1	0
1	1	1	0
1	1	1	1
0	0	1	1
0	1	0	0
0	0	0	0
0	1	1	1
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1	1	1	1
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1	0	1	0
1	1	1	1
1	1	0	1
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0	0	0	0
0	0	0	1
0	0	0	0
1	0	0	1
1	0	0	0
0	0	0	1
0	0	0	0
1	0	0	0
0	0	0	0

acid, ferulic acid, cinnamic acid, biochanin A in different extracts of flower. The presence of gallic acid may be the responsible for the anti-fungal [27], anti-cancerous [28, 29], anti-rheumatoid arthritis [30] activities of the flower of *Saraca*. Protocatechuic acid is an antioxidant and is also an anti-inflammatory agent with hepatoprotective [31], anti-cytotoxic [32], anti-tumorigenic [33, 34], anti-apoptotic to neural stem cell [35], and above therapeutic potentialities are reported to be found in extracts of *S. indica* and *S. asoca*. Caffeic acid has the properties like anti-cancerous [36] potentialities. Chlorogenic acid causes the activities like anti-hypersensitiveness [37, 38] and anti-inflammatory [39]. Ferulic acid possesses dermato-protective activity [40]. P-coumaric acid is anti-tumorigenic [41]. Cinnamic acid is the precursor of many other phenolic compounds as coumaric acids, sinapic acid, ferulic acid and has perfumery and antioxidant utilities. These therapeutic activities of each phytochemical may have cumulative influence to produce the remarkable therapeutic potentialities of *Saraca*.

RP-HPLC can effectively detect and quantify phytoestrogenic compounds [42], and it had been proven to be present in legumes in high amount [43, 44]. Based on the previous reports of being uterogenic therapeutant, both *S. indica* [6] and *S. asoca* [10] could be presumed to be the source of phytoestrogens. Biochanin A, the isoflavone, being a potent phytoestrogenic and anti-bacterial compound [45] was targeted within the floral extracts of all of the four species of *Saraca*. The amount of the compound in the ethyl acetate extract of flowers of *S. asoca* and *S. indica* is considerably high.

The antioxidant potentiality of flower extracts of *Saraca* is reported by many authors [24, 46, 47] which is due to the presence of considerable number of polyphenolic compounds with higher quantity. It proves that the flowers of *Saraca* are a great commercial resource of therapeutic components.

DNA marker-based barcoding is reported to be effective in identification and to estimate the diversity of *S. asoca* and *S. indica* [24, 48]. On the other hand, palynological data are also reported [7] to distinguish these four species along with many other morphological data [49]. However, the phytochemical-based correlation among them is also very essential due to their relation with highly medicinal properties. The presence of catechins, chlorogenic acid, vanillic acid, caffeic acid and absence of protocatechuic acid in *S. indica* differ it from *S. asoca*. Gallic acid, t-cinnamic acid and biochanin A are common compound of these four species. The presence of vanillic acid and anthrol is the distinguishing chemotaxonomic evidence of *S.*

indica and *S. thaipingensis*, respectively. Though p-coumaric acid is the essential intermediate for the biochemical cycle of other polyphenols, only *S. thaipingensis* and *S. asoca* showed its presence and the reason may either be the degradation of it during extraction or the quick conversion of it into other phytochemicals in other species.

The statistical analysis is based on the complete set of phytochemicals, and considering each of the retention time equivalent to distinct character gives an innovative and effective idea for the chemotaxonomic characterization to resolve more complex taxonomic problems. Characterization of medicinally important plants based on chemotaxonomic traits gives the exposure to the quantitative evaluation of certain phytochemicals in different species and also in different ecological variations.

Conclusions

The method designed was properly validated and found best suitable for the phytochemical evaluation and quantification of different polyphenolic compounds in floral extracts of plant parts. The phytochemical analysis reveals the presence of valuable phytochemicals in different extracts of flowers of four different species of *Saraca*, and the quantitative evaluation gives the approximate value of specific phytochemicals. The comparative basis preparation of quantitative and qualitative phytochemical coding helps to evaluate the phytochemical relations among them. The qualitative coding including all of the known and unknown data set of phytochemicals gives more comprehensive expression of relation among four species of *Saraca,* which properly correlates with that evaluated previously through pollen morphological data [49].

Abbreviations

RP-HPLC: Reverse-phase high-performance liquid chromatography; UPLC: Ultra-performance liquid chromatography; PCA: Principal component analysis; PB: Petroleum benzene; EA: Ethyl acetate; RSD: Relative standard deviation; UPGMA: Unweighted pair group method with arithmetic mean.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-022-00430-8.

Additional file 1: RP-HPLC based phytochemical data of four species of *Saraca* L. *Sil et al.*

Acknowledgements

Authors are thankful to Director(s) of Botanical Survey of India for their permission and cooperation for the collection of specimens and CAL herbarium consultation. Authors thankfully acknowledge the Director of Central Instrument Facility of Bose Institute for instrument facilities.

Author contributions

SS, KKD and AG had equal contributions in the hypothesis, experimental work, manuscript preparation and finalization of manuscript. All the authors read and approved the manuscript for final communication.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Availability of data and materials

Data and materials have been provided with the manuscript as tables and figures. Supplementary tables and figures have also been provided as additional data set.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have approved the publication of this study.

Competing interests

All authors hereby declare that there is no conflict of interest.

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Received: 3 June 2022 Accepted: 26 September 2022
Published online: 12 October 2022

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