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Research Article

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Quantification of camptothecin and some flavonoids from *Ixora javanica* (Blume) DC by HPLC

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ABSTRACT

Ixora javanica is a medicinally important plant reported to show anticancer, anti-inflammatory, antioxidant and hepatoprotective activities. The present study investigates four medicinally important phytoconstituents present in various parts of *I. javanica*. HPLC technique is used to detect the presence and quantities of three flavonoids rutin, kaempferol and quercetin in the leaves and flowers and alkaloid camptothecin in the bark and roots of *I. javanica*. Rutin was best extracted (43.92%) using 90% ethanol whereas quercetin (0.27%) was best extracted using 85% methanol both from the flowers of the plant. Kaempferol was best extracted from leaves (1.15%) using petroleum ether as a solvent. Alkaloid camptothecin was found to be present in bark (7.34%) as well as root extracts (3.52%) of *I. javanica*. However, higher camptothecin content was present in the bark as compared to the roots of the same plant. Both 60% methanol or 60% ethanol were found to be equally good solvents for camptothecin extraction from bark and root samples. This is the first report of HPLC quantification of alkaloid camptothecin from the bark and roots of *I. javanica*.

KEYWORDS: *Ixora javanica*, Flavonoids, Alkaloids, Camptothecin, HPLC

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INTRODUCTION

Ixora javanica (Blume) DC is native to Java, as its specific name indicates. It is an evergreen spreading shrub with flowers on terminal corymb inflorescence (Hooker, 1882). *I. javanica* is traditionally used to treat several ailments. The plant is reported for several therapeutic uses such as anticancer, anti-inflammatory, antioxidant and hepatoprotective activities (PayÁ *et al.*, 1993; Dontha *et al.*, 2015; Yerragunta *et al.*, 2016). *I. javanica* flowers were reported to exhibit a broad antitumor activity in mice. The mode of action was by inhibiting the formation and growth of tumors or by stopping further growth of already formed tumors (Dontha *et al.*, 2015). *I. javanica* flowers were reported for the presence of flavonoids, tannins, steroids, glycosides, carbohydrates, alkaloids, saponins, and terpenoids (Yerragunta *et al.*, 2016; Ghoshal *et al.*, 2022b). Although flowers are well studied, many other parts of *I. javanica* are also used to treat several ailments. However, there is little information available on the phytoconstituents present in other parts of *I. javanica*. Flavonoids, the largest class of naturally occurring plant phenolics (Hussein & El-Anssary, 2019) are known for their antiallergic, antithrombotic and vasoprotective properties and inhibition of tumor promotion (Richetti *et al.*, 2011; Javed *et al.*, 2012). Flavonoid Rutin also called vitamin P is a potent antioxidant and exhibits neuroprotective, cardioprotective and anticarcinogenic activities (La Casa *et al.*, 2000; Janbaz *et al.*, 2002; Schwedhelm *et al.*, 2003; Mellou *et al.*, 2006; Trumbeckaite *et al.*, 2006; Nassiri-Asl *et al.*, 2010; Lin *et al.*, 2012). Kaempferol is a natural polyphenolic flavonoid that inhibits MCP-1 gene expression at the transcription level and thus shows anti-inflammatory potential (Park *et al.*, 2006; Yoshida *et al.*, 2008). It exerts cytotoxic effects in many types of cancer cells yet not known for any toxicity towards the human body. Although extracted and isolated from flowers, leaves or seeds of plants, it is often present only in small amounts and thus too expensive to produce as a commercial product (He *et al.*, 2008). Kaempferol has a large demand in the pharmaceutical world (Fischer *et al.*, 1997). More plants thus need to be investigated to detect kaempferol which usually exists as glycosides in plants and influence their pharmacokinetic properties (Ross & Kasum, 2002). Quercetin belongs to the flavonol class of flavonoids (Hollman *et al.*, 1999). According to IUPAC- nomenclature quercetin is 3, 31, 41, 5, 7-pentahydroxyflavone. A glycosyl or sugar residue such as glucose, rhamnose, or rutinose is attached to quercetin to form quercetin glycoside during which one of the OH groups (commonly at position 3) is replaced. Depending on

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the type of glycosyl group attached, unique quercetin glycosides may be present in different plants. Thus, such molecules may have differing solubilities, absorption, and in vivo effects (Aguirre *et al.*, 2011; Li *et al.*, 2016). The term quercetin should be used to describe the aglycone moiety only; however, all quercetin-type molecules, including its glycosides are termed quercetin in research and industry (Davis *et al.*, 2009). The unique pharmacological properties of quercetin include its ability to inhibit platelet aggregation and capillary permeability, lipid peroxidation, and to stimulate mitochondrial biogenesis (Tadeusz, 2015).

Alkaloids are nitrogen-containing organic compounds divided into different classes based on their chemical structure (Govindachari & Viswanathan, 1972). Camptothecin (CPT) is a quinoline alkaloid first extracted and isolated from the bark and stem of *Camptotheca acuminate* (*Camptotheca*, Happy tree), which is a tree native to China and is used to treat cancer as per Traditional Chinese Medicine (Efferth *et al.*, 2007; Kacprzak, 2013). It has so far been detected to be present in only a few plants which include *Chonemorpha fragrans* (Isah & Umar, 2018) and *Ixora coccinea*. Insufficient natural supply of camptothecin led to significant improvement of camptothecin total synthesis as well as to attempt its biotechnological production. Thus, search for plant sources having medicinally important alkaloid CPT is always on the forefront.

In the present study, the sophisticated and precise HPLC technique was used to investigate the presence and quantities of three medicinally important flavonoids namely rutin, quercetin and kaempferol in the leaves and flowers of *I. javanica*. As camptothecin was reported to be present in *I. coccinea* (Ghoshal *et al.*, 2022a), the presence and amount of this important alkaloid CPT in the bark and roots of *I. javanica* was also investigated.

MATERIALS AND METHODS

Collection and Preparation of the *Ixora Javanica* Plant Extracts

The fresh and healthy leaves, flowers, bark and roots of the experimental plant *Ixora javanica* [DG-03] were collected from the campus of Acharya Jagadish Chandra Bose Indian Botanical Garden, Botanical Survey of India, Central National Herbarium, Kolkata, India. The plant was taxonomically authenticated with Blatter Herbarium at the Centre itself. Plant parts of *I. javanica* were rinsed with water and shade dried. The dried material was ground into a coarse powder and stored in clean amber coloured glass bottles at room temperature for further analysis.

Extraction of Phytochemicals from Dried Powdered Leaf and Flower Samples by Successive Solvent Soxhlet Method

The extraction of phytochemicals from leaves and flower samples of *I. javanica* was carried out by the Successive solvent soxhlet method described by Ghoshal *et al.* (2022b). Three different successive HPLC grade solvents: Petroleum ether (100%), Methanol (85%) and Ethanol (90%) were used for the Soxhlet extraction process. The method in brief is as follows- 25 gm of each powdered leaf/flower of *I. javanica* was extracted in 250 mL of petroleum ether in a Soxhlet extractor for 4 hrs. The concentrated petroleum ether extract was filtered and concentrated in a rotary evaporator to obtain a semisolid mass which was used as petroleum ether extract. The residue obtained was dried overnight and further subjected to Soxhlet extraction with 250 mL of 85% methanol for 8 hrs. The concentrated 85% methanolic extract was filtered and concentrated in a rotary evaporator to obtain a semisolid mass which was used as 85% methanolic extract. The residue obtained after methanolic extraction was dried overnight and again subjected to Soxhlet extraction with 250 mL of 90% ethanol for 8 hrs. The concentrated 90% ethanolic extract was filtered and concentrated in a rotary evaporator to obtain a semisolid mass which was used as 90% ethanolic extract. 20 mg of dried leaf and flower semisolid mass obtained by the above method was each dissolved in 2 mL of methanol (HPLC grade). These stock solutions were further diluted to obtain 100 ppm stock solutions of each sample and then subjected to HPLC analysis to detect and determine the flavonoid rutin, quercetin and kaempferol content in various plant parts of *I. javanica*.

Extraction of Phytochemicals from Bark and Root Samples using Two Individual Solvents by the Shaker Extraction Method

The extraction of phytochemicals from bark and root samples of *I. javanica* was carried out by using two individual solvents by the Shaker extraction method described by Ghoshal *et al.* (2022a). The method followed is briefly described. 1 gm of dried powder of bark and roots of *I. javanica* was each extracted in 10 mL of 60% methanol and in 10 mL of 60% ethanol (HPLC grade) for 8 hrs in a shaker. Extracts were filtered and centrifuged at 10000 rpm for 15 min. The supernatant was then concentrated in a water bath to obtain a semi solid mass. 10 mg of each semi solid mass obtained from bark and root samples of *I. javanica* were then dissolved in 10 mL of methanol (HPLC grade). These were further diluted to obtain 50 ppm stock solutions and then each of these was subjected to HPLC analysis to determine alkaloid camptothecin content.

HPLC Analysis

The HPLC method of analysis was standardized and developed to detect the presence of 3 flavonoids and one alkaloid in *I. javanica*. Alkaloid analysis was carried out using the Agilent HPLC 1220 Infinity equipment system at Central Instrumentation Facility at Jai Hind College and analysis of flavonoids rutin, quercetin and kaempferol was carried out at "Chromatography world" situated at Sion. The method was developed and standardized by following the usual standardization protocol. The standard compounds rutin, quercetin, kaempferol were procured from Sigma & Merck Company and camptothecin from Sigma & Aldrich Company. HPLC grade Petroleum ether (40-60 °C),

Methanol, Ethanol, Acetonitrile as well as water were used for the present HPLC analysis.

Preparation of Standard Stock Solutions of Selected Flavonoids Rutin, Quercetin and Kaempferol and Selected Alkaloid Camptothecin for Quantitative Analysis using HPLC

Stock solutions of each standard flavonoid rutin, quercetin and kaempferol and standard alkaloid camptothecin (1000 ppm each) were prepared. These were further diluted to get varying concentrations (100 ppm to 500 ppm) of each standard compound. From each of the diluted flavonoid and alkaloid standard stock solutions (100 ppm to 500 ppm), 10 µL of each flavonoid and 20 µL of alkaloid camptothecin were used for analysis on HPLC (Saravanan & Boopalan, 2011).

Instrument and Chromatographic Conditions for HPLC Analysis of Rutin, Quercetin and Kaempferol

The High-Performance Liquid Chromatography (HPLC) was performed using the Agilent HPLC 1260 equipment system including a 2-solvent delivery system, binary pump 0-2 mL/min, a UV detector, and a 2-chamber in-line degasser. The analysis was performed on an Agilent ZORBAX Eclipse XDB-C18 column (4.6×150 mm i.d., 5 µm particle size) at ambient room temperature. The standardization of the method was carried out using the HPLC grade Acetonitrile: Methanol (solvent A): Water (solvent B) as mobile phase in 40:15:45 v/v ratio with 1% Acetic acid (Zu *et al.*, 2006; Saeed *et al.*, 2020). The injection volume for each standard flavonoid was 10 µL with a flow rate of 0.8 mL/min and at 35 °C temperature of the column. Each run was followed by an equilibration time of 4 min. Detection was carried out at 254 nm wavelength. Each of these standard compounds such as rutin, quercetin and kaempferol as well as the *I. javanica* plant samples were analyzed using the same HPLC conditions and furthermore, the calibration of the detector response was done. The calibration curve for quantitative estimation of the standard compounds rutin, quercetin and kaempferol were plotted.

Instrument and Chromatographic Conditions for HPLC Analysis of Camptothecin

The High-Performance Liquid Chromatography (HPLC) analyzed on the Agilent HPLC 1220 Infinity equipment system including a 2-solvent delivery system, binary pump 0-2 mL/min, a UV detector, and a 2-chamber in-line degasser. The analysis was performed on an Agilent ZORBAX Eclipse XDB-C18 column (4.6×150 mm i.d., 5 µm particle size) at ambient room temperature. The standardization of the method was carried out using the HPLC grade Acetonitrile (solvent A) and Water (solvent B) as mobile phase in 40:60 v/v ratio with 20 µL injection volume of standard alkaloid camptothecin with a flow rate of 1.2 mL/min (Saeed *et al.*, 2020). Each run was followed by an equilibration time of 4 min. Detection was carried out at 254 nm wavelength. Open Lab CDS Version A.04.06 chromatographic software was used for data acquisition. The calibration curve for quantitative estimation of the standard compound camptothecin was plotted.

Validation Method for Studying Flavonoids Rutin, Quercetin and Kaempferol and Alkaloid Camptothecin

The validation methods used for the standard stock of three flavonoids rutin, quercetin and kaempferol and standard alkaloid camptothecin were initially standardized with the help of calibration parameters as specified below. The HPLC method was validated in terms of specificity, accuracy, precision and sensitivity (LOD and LOQ) as shown in Table 1.

Specificity

The specificity of the method was determined by analyzing the bands of each standard compound. The bands of the compound in the sample solution were confirmed by comparing the Rf values and UV spectra with the reference standards. The peak purity of the compound was assessed by comparing the spectra at three different levels, i.e., peak start, peak apex and peak end position.

Table 1: Method validation parameters for the quantification of all studied standard compounds by using HPLC method

S. No.	Standard Compound	Method used	Parameter	Results
1	Rutin	HPLC	R_T	2.1 min
			R^2	0.99
			Linearity range	100-500 ppm
			Precision (≤ 2%)	
			Intraday	0.818%
			Interday	0.666%
			Sensitivity	
			LOD	109 ppm
			LOQ	330 ppm
2	Quercetin	HPLC	R_T	3.8 min
			R^2	0.99
			Linearity range	100-500 ppm
			Precision (≤ 2%)	
			Intraday	0.674%
			Interday	0.761%
			Sensitivity	
			LOD	97 ppm
			LOQ	295 ppm
3	Kaempferol	HPLC	R_T	5.5 min
			R^2	0.99
			Linearity range	100-500 ppm
			Precision (≤ 2%)	
			Intraday	0.770%
			Interday	0.796%
			Sensitivity	
			LOD	68 ppm
			LOQ	205 ppm
4	Camptothecin	HPLC	R_T	2.3 min
			R_2	0.99
			Linearity range	100-500 ppm
			Precision (≤ 2%)	
			Intraday	0.740%
			Interday	0.742%
			Sensitivity	
			LOD	66 ppm
			LOQ	200 ppm

Accuracy

The accuracy of the method was determined by analyzing the percent recovery of the compound in samples. The analysis was done in three sets of different concentrations (100, 200 and 400 ppm). The linearity of the required flavonoid compounds rutin, quercetin and kaempferol as well as alkaloid camptothecin was validated by linear regression and correlation coefficient. The five-point calibration curve was found to be linear in the range of 100-500 ppm and is described in the result section.

Precision

For the instrumental precision, intraday variations for standard flavonoids rutin, quercetin and kaempferol and standard alkaloid camptothecin, each standard compound with a concentration of 100 ppm was loaded six times on the same day and each was repeatedly scanned. The % RSD in the replicate analysis is used as a measure of the precision of the results. The interday precision variation was carried out using the repeated concentrations of 100 ppm of each standard compound for 3 days and expressed as % RSD. The precision for the proposed HPLC method was found to be precise due to the low values of % RSD. The result is shown in Table 1.

Sensitivity

The sensitivity of the method was determined with values of limit of detection (LOD) and limit of quantification (LOQ). For the sensitivity of the method, aliquots of standard solution of all the standards in different solvents were analyzed. The results were calculated as per the standard ICH guidelines which indicate that -LOD=3.3 σ/S and LOQ=10 σ/S. Here 'σ' is the standard deviation of the blank and 'S' is the slope of the calibration curve. The result is shown in Table 1.

After standardizing and developing the method for quantification of all standard flavonoids rutin, quercetin and kaempferol, all the plant samples of *I. javanica* were further analyzed for flavonoid estimation by using the above standardized method. However, while running the bark and root samples for alkaloid camptothecin estimation by above mentioned method, the plant constituents were not getting separated into discrete and separate peaks. Thus, we altered some parameters for estimating alkaloid camptothecin content in bark and root samples to detect and obtain good separation of plant metabolites. For this separation, the mobile phase of HPLC grade Acetonitrile (solvent A) and Water (solvent B) in 40:60 v/v ratio was changed to 50:50 v/v and a flow rate of 1.2 mL/min. was changed to 0.8 mL/min for alkaloid camptothecin estimation. Further, the run was carried out at a controlled temperature of 35 °C. These parameters were then again used for quantification of standard alkaloid camptothecin as well as the quantification of camptothecin in bark and root samples of *I. javanica*.

Preparation of *I. javanica* Plant Part Extracts for HPLC Analysis

Semi-solid mass of leaf and flower extracts of *I. javanica* extracted in petroleum ether, 85% methanol and 90% ethanol were each weighed accurately and dissolved in HPLC grade respective mother solvents and sonicated for 10 min to make a 10000 ppm solution. This was further diluted to make a 100 ppm solution for each plant sample and 10 µL of each of plant sample was injected into the HPLC column along with 10 µL of 10 ppm of each standard flavonoid after filtering through a 0.45 µm pore size filter and all samples analyzed under the same HPLC conditions (Bressolle *et al.*, 1996).

Semi-solid mass of bark and root extracts of *I. javanica* extracted in 60% methanol and 60% ethanol were each weighed accurately and dissolved in HPLC grade respective mother solvents and sonicated for 10 min to make a 1000 ppm solution. This was further diluted to make a 50 ppm solution for each plant sample. 20 µL of each 50 ppm plant sample thus prepared was loaded on the column along with 20 µL of 10 ppm standard alkaloid camptothecin after filtering through a 0.45 µm pore size filter and all samples analyzed under the same HPLC conditions (Bressolle *et al.*, 1996). Quantification of all the standards was obtained by calculating peak area *vs* the retention time of injected standards with the plant samples. This helped to determine the percentage of the above flavonoids and alkaloids present in the plant samples in terms of percentage.

Statistical Analysis

The observation data obtained on different parameters as studied in the present work were subjected to statistical analysis using Microsoft Excel and GraphPad prism-5 software. The analysis was performed in triplicate mode and the data was represented as mean ± standard deviation. The computed arithmetic mean values for each of the plant extracts and the data obtained were tested for regression analysis, standard deviation, and standard error.

RESULT AND DISCUSSION

Standardization and Quantification of Flavonoids Rutin, Quercetin and Kaempferol and Alkaloid Camptothecin Content

The calibration curve of all standard flavonoids namely rutin, quercetin and kaempferol and standard alkaloid camptothecin in concentration *vs* area obtained was plotted and the method for estimation of these compounds by HPLC was standardized. The five-point calibration curve was found to be linear in the range of 100-500 ppm for all standard samples. The regression equation and correlation coefficient were Y=348084x and R^2=0.9989 for standard rutin, Y=635754x and R^2=0.9982 for quercetin, Y=528781x and R^2=0.9995

for kaempferol and Y=131171x and R^2=0.9995 for standard alkaloid camptothecin. The retention time and retention area of standard rutin was found to be 1.87 min and area 721.8 (Figure 1), standard quercetin was found to be 2.51 min and area 1592.6 (Figure 2) and standard kaempferol was found to be 3.46 min and area 562.4 (Figure 3). The retention time and retention area of standard camptothecin was found to be 1.42 min and area 27592859 (Figure 4). These standardized methods were developed to authenticate the quantity of 3 flavonoids rutin, quercetin and kaempferol in flower and leaf extracts and alkaloid camptothecin in the bark and root extracts of *I. javanica*.

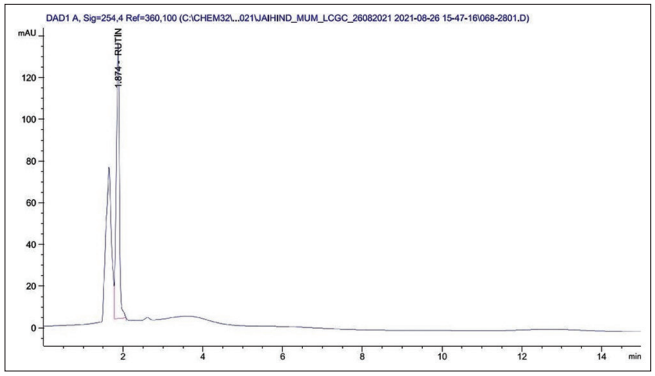

Figure 1: HPLC chromatogram for the standard Rutin

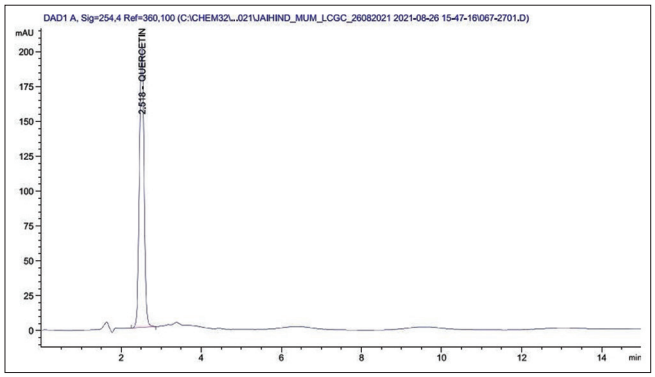

Figure 2: HPLC chromatogram for the standard Quercetin

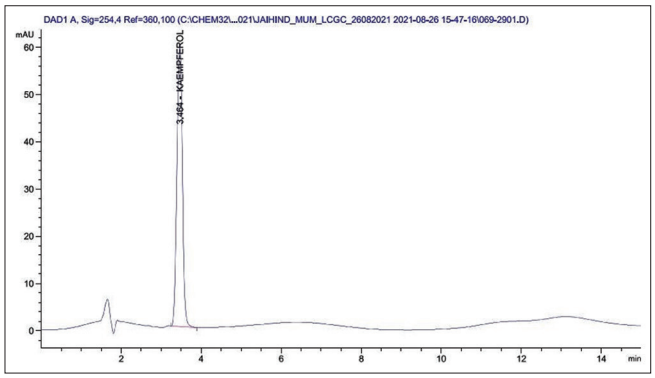

Figure 3: HPLC chromatogram for the standard Kaempferol

Analysis of Chromatograms of Petroleum Ether, 85% Methanol and 90% Ethanolic Extracts of Leaf and Fower Samples of *I. javanica* for Flavonoids Rutin, Quercetin and Kaempferol Content

Quantification of rutin, quercetin and kaempferol was done by calculating peak area *vs*. the retention time of injected standards corresponding to the peak area *vs* the retention time of injected plant samples i.e., flowers and leaf samples of *I. javanica* extracted in petroleum ether, 85% methanol and 90% ethanol extracts. The chromatographic profiles of the petroleum ether extract of the *I. javanica* leaf show a peak at a retention time of 2.87 min and 3.70 min homologous to the standard quercetin and standard kaempferol, respectively. No presence of rutin was detected in leaf petroleum ether extracts (Figure 5). Similarly, the petroleum ether extract of *I. javanica* flower did not show any presence of any of the standard flavonoids - rutin, quercetin or kaempferol.

The 85% methanolic extract of *I. javanica* leaf and flower shows a peak at a retention time of 1.88 min (Figure 6) and 1.98 min (Figure 7) respectively homologous to the standard rutin and indicating its presence in both flower and leaf methanolic extracts. Further, the 85% methanolic flower extracts additionally showed a peak at a retention time of 2.82 min (Figure 7) homologous to the standard quercetin and indicated its presence in the flower extract. Flavonoids quercetin and kaempferol were both absent in methanolic leaf extracts and only flavonoid kaempferol was absent in methanolic flower extracts.

The 90% ethanolic extract of *I. javanica* leaf and flower show a peak at a retention time of 1.87 min (Figure 8) and 1.90 min (Figure 9) respectively homologous to the standard rutin. Flavonoids quercetin and kaempferol were absent in ethanolic extracts of both leaves as well as flowers.

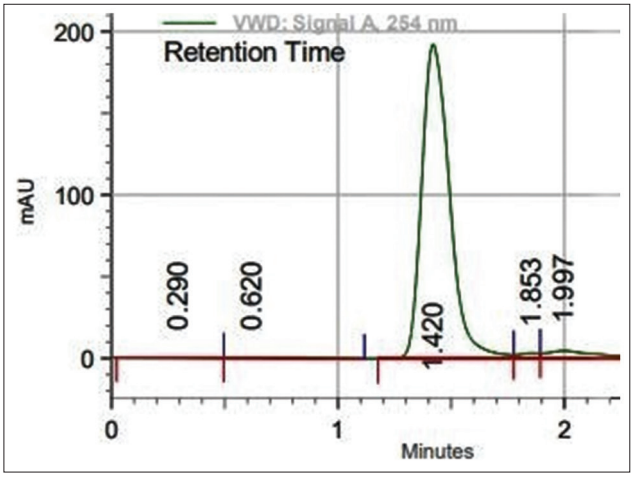

Figure 4: HPLC chromatogram for the standard Camptothecin

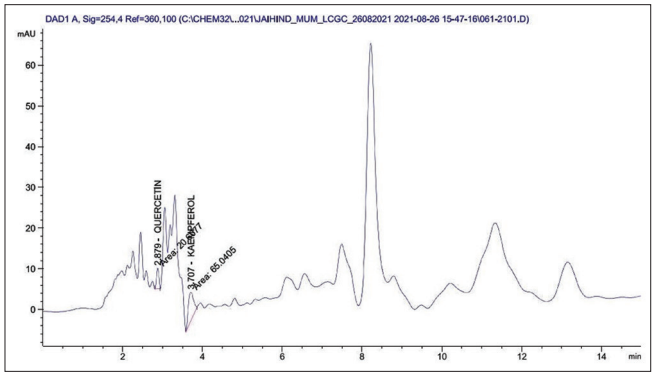

Figure 5: HPLC chromatogram of *Ixora javanica* leaf in petroleum ether extract

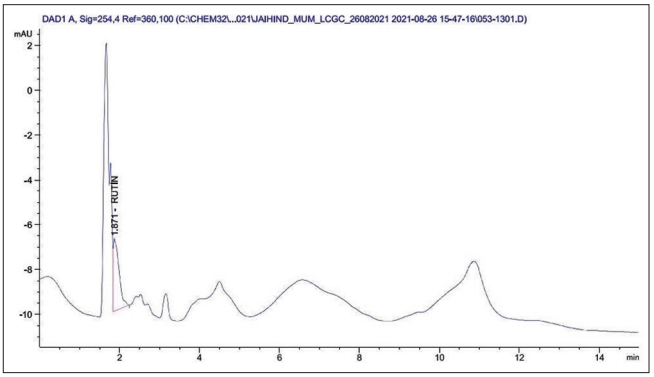

Figure 8: HPLC chromatogram of *Ixora javanica* leaf in 90% ethanolic extract

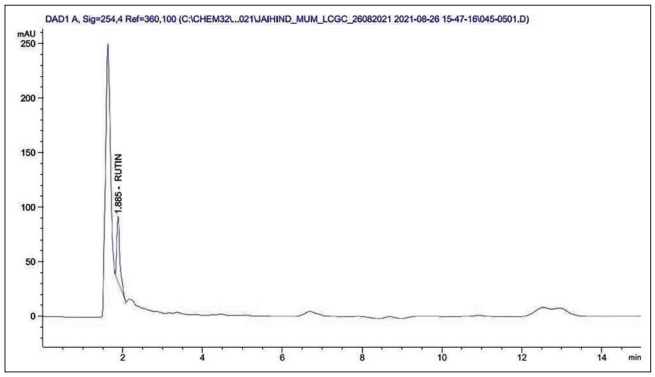

Figure 6: HPLC chromatogram of *Ixora javanica* leaf in 85% methanolic extract

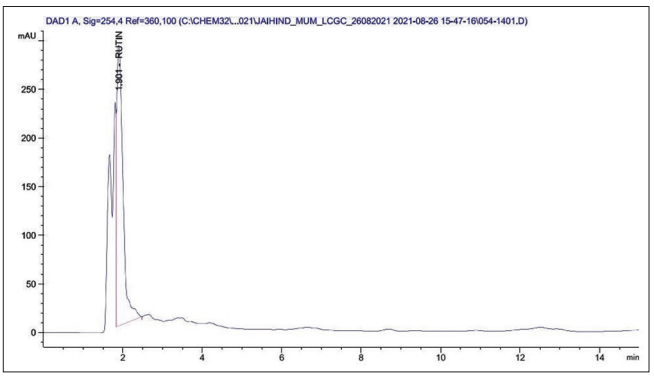

Figure 9: HPLC chromatogram of *Ixora javanica* flower in 90% ethanolic extract

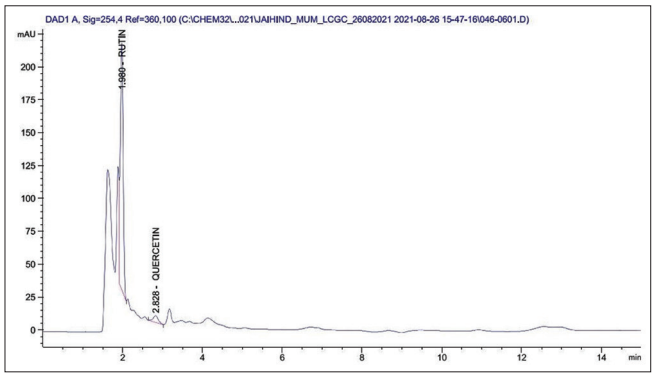

Figure 7: HPLC chromatogram of *Ixora javanica* flower in 85% methanolic extract

Quantification of Rutin, Quercetin and Kaempferol Content in *I. javanica* Flower and Leaf Extracts by HPLC

A summary of flavonoids rutin, quercetin and kaempferol content in leaf and flower samples of *I. javanica* is mentioned in Table 2. *I. javanica* flower showed the presence of very high rutin content in 90% ethanolic solvent when compared to *I. javanica* leaf. Interestingly, it was observed that petroleum ether solvent extracts did not show any presence of rutin in both flower and leaf extracts of *I. javanica* as per HPLC analysis. Thus, from the above result, we conclude that 90% ethanol was the best solvent for extraction of rutin from flowers compared to 85% methanol. Further, we also conclude that the flowers are the best source for rutin extraction when compared to leaves at least in *I. javanica* although leaves may also be used for extraction but by using 85% methanol as the solvent. *I. javanica* thus contains very high amount of rutin similar to the well-studied and reported *I. coccinea* plant (PayÁ *et al.*, 1993; Baliga & Kurian, 2012; Chen *et al.*, 2016; Raju *et al.*, 2021). Literature survey points towards the traditional use of the aerial parts of *I. javanica* for several diseases or ailments (PayÁ *et al.*, 1993; Yerragunta *et al.*, 2016; Oktaviyanti *et al.*, 2020). Rutin is reported to be an anticancer compound (Satari *et al.*, 2021) and the presence of rutin in *I. javanica* makes this plant an ideal candidate for medicinal use as well as for extraction of rutin for industrial purposes. This plant should be further investigated for its pharmacological properties.

Flowers of *I. javanica* showed the presence of quercetin only in 85% methanolic solvent and none in the petroleum ether extract or 90% ethanol extract. Leaves of *I. javanica* showed the presence of quercetin only in petroleum ether extract and none in 85% methanol or 90% ethanol extracts. Thus, no quercetin could be detected in the ethanol extracts of both leaves as well as flowers of *I. javanica*. There was no quercetin detected in the petroleum ether flower extracts and methanol leaf extracts. Thus, it was concluded that quercetin can be extracted from flowers using 85% methanol and from leaves using petroleum ether. Kaempferol was detected to be present only in the leaf

Table 2: Total Rutin, Quercetin and Kaempferol content in leaf and flower extracts of *Ixora javanica* and total Camptothecin content in bark and root extracts of *Ixora javanica* as determined from HPLC analysis

Secondary metabolites	Rutin % (Flavonoid)			Quercetin % (Flavonoid)			Kaempferol % (Flavonoid)			Camptothecin % (Alkaloid)	
Plant part used	100% Pet.E	85% M.E	90% E.E	100% Pet.E	85% M.E	90% E.E	100% Pet.E	85% M.E	90% E.E	60% E.E	60% M.E
Leaf	-	4.60%	0.43%	0.12%	-	-	1.15%	-	-	-	-
Flower	-	12.65%	43.92%	-	0.27%	-	-	-	-	-	-
Bark	-	-	-	-	-	-	-	-	-	7.34%	6.84%
Root	-	-	-	-	-	-	-	-	-	3.52%	3.74%

Pet.E-Petroleum ether extract; 85% M.E-85% Methanolic extract; 90% E.E-90% Ethanolic extract; 60% E.E-60% Ethanolic extract; 60% M.E-60% Methanolic extract.

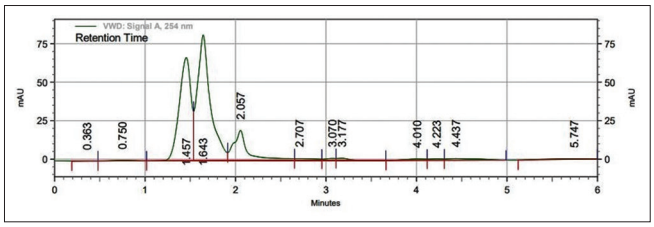

Figure 10: HPLC chromatogram for Camptothecin in the bark of *Ixora javanica* in 60% ethanolic extract

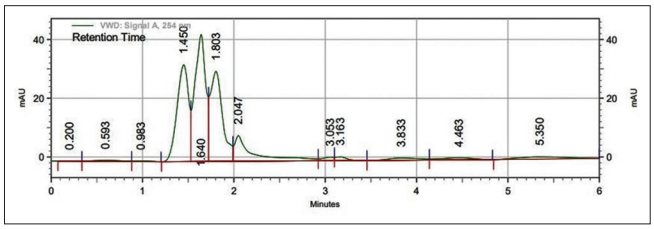

Figure 11: HPLC chromatogram for Camptothecin in the root of *Ixora javanica* in 60% ethanolic extract

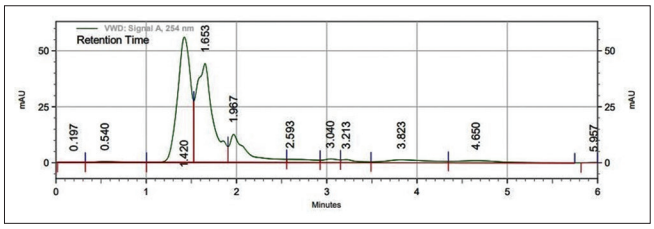

Figure 12: HPLC chromatogram for Camptothecin in the bark of *Ixora javanica* in 60% methanolic extract

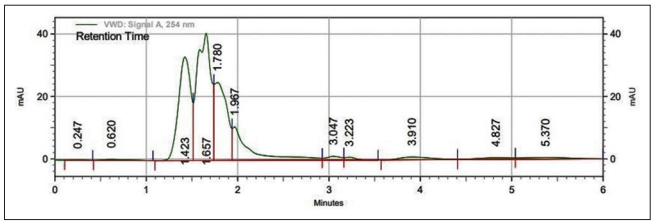

Figure 13: HPLC chromatogram for Camptothecin in the root of *Ixora javanica* in 60% methanolic extract

petroleum ether extracts of *I. javanica* and absent in flower petroleum ether extracts. Thus, as per HPLC results, we conclude petroleum ether to be the best solvent for extraction of kaempferol from leaves of *I. javanica*.

Analysis of Chromatograms of 60% Ethanolic and 60% Methanolic Extracts of Bark and Root Samples of *I. javanica* by HPLC for Alkaloid Camptothecin Content

Quantification of alkaloid camptothecin was done by calculating peak area *vs* the retention time of injected standards corresponding to the peak area *vs* the retention time of injected plant samples i.e., bark and root extracts of *I. javanica* and thus compared with standard alkaloid camptothecin. The chromatographic profiles of the 60% ethanolic extract of the *I. javanica* bark and root shows a peak retention time of 1.45 min (Figures 10 & 11) which was homologous to the standard camptothecin. The chromatographic profiles of bark and root samples of *I. javanica* in 60% methanolic extracts shows a peak retention time of 1.42 min (Figures 12 & 13) which was also homologous to standard camptothecin.

Quantification of Camptothecin Content in *I. javanica* Bark and Root Extracts by HPLC

A summary of alkaloid camptothecin content in bark and root samples of *I. javanica* is mentioned in Table 2. Camptothecin was present in both bark and root samples of *I. javanica*. Thus, both the bark and roots of this plant can be used for the extraction of alkaloid camptothecin. However, as a higher amount of camptothecin is present in the bark of *I. javanica* it is preferred to the root of the same plant for alkaloid extraction. Both 60% methanol or 60% ethanol can be used as potential candidates for the extraction of camptothecin as there is not much difference in the amounts of camptothecin extracted using any one of these two solvents.

CONCLUSION

In the present study, HPLC analysis is used to investigate and report the presence and quantities of three selected medicinally important flavonoids rutin, kaempferol and quercetin in the leaf and flowers of *I. javanica*. Similarly, the bark and root of the same plant are investigated to detect the presence and quantities of alkaloid camptothecin. From the results obtained, it can be concluded that flowers are a better source for rutin extraction when compared to leaves at least in *I. javanica* and 90% ethanol proved to be the best solvent for rutin extraction. Further, rutin could also be used for extraction from leaves of the same plant but by using 85% methanol as the extraction solvent. Quercetin

can be best extracted from flowers of *I. javanica* using 85% methanol and from leaves using petroleum ether.

We further conclude leaves of *I. javanica* are the best source for kaempferol extraction with petroleum ether as the preferred solvent. Results of quantification of camptothecin content in *I. javanica* concluded both bark and root to be the good sources of alkaloid camptothecin. However, a higher amount of camptothecin is present in the bark of *I. javanica* and therefore bark is preferred to the root for camptothecin extraction and both 60% methanol or 60% ethanol gave similar extractive values for camptothecin extraction. The presence of flavonoids rutin, kaempferol and quercetin in *I. javanica* as well as the presence of an important alkaloid camptothecin in the same plant makes this plant an ideal candidate for medicinal use as well as for extraction of rutin or camptothecin for industrial purposes. The bark and the root samples of this plant must specifically be analyzed to study pharmacological activities possibly arising due to its higher alkaloid content. This is the first report of HPLC quantification of anticancer alkaloid camptothecin from the bark and roots of *I. javanica*.

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