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Original Article

Comparative Extraction and Quantification of Scutellarein from Leaves of *Triumfetta rhomboidea* Using RP-HPLC

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ABSTRACT

Aim: The purpose of this paper was to develop a sensitive, reproducible Reverse Phase High Performance Liquid Chromatography (RP-HPLC) for extraction and quantitative estimation of Scutellarein, major flavone glycoside from leaves of *Triumfetta rhomboidea*. **Materials and Methods:** To optimize best solvent system and ideal extraction methodology, various leaves extract was prepared by using different solvent systems such as ethanol, ethanol: water (50:50) and water through different extraction methodologies includes, maceration, Soxhlet assisted extraction (SAE), Ultrasound Assisted Extraction (UAE) and Accelerated Solvent Extraction (ASE). Chromatographic separation of Scutellarein was performed on C_{18} column. **Results:** The results showed that the highest concentration of Scutellarein was found to be 1.547 ng in ethanolic extract. This study states about, existence of flavone glucocidic content in leaves extract of *Triumfetta rhomboidea* which can be extracted using ethanol as best solvent system and ASE as optimized extraction methodology. **Conclusion:** Extracts from *T. rhomboidea* obtained by maceration, SAE, UAE and ASE methods were analysed by HPLC-ESI-MS/MS to quantify flavonoid scutellarein contents. Ethanol was found to be a promising solvent system and Accelerated Solvent Extraction methodology was found to be the promising methodology for the extraction of Scutellarein from leaves of *Triumfetta rhomboidea*.

Keywords: *Triumfetta rhomboidea*, Scutellarein, Accelerated Solvent extraction, Ultrasound Assisted Extraction, RP-HPLC.

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INTRODUCTION

Extraction is an important step in studies involving the discovery and isolation of active compounds of plant materials. The preparation of crude extracts from plants is the starting point for the isolation and purification of chemical constituents present in vegetable tissues. Usually, the traditional techniques require long extraction time and have low efficiency. Moreover, many natural products are thermally unstable and may degrade during thermal extraction hence use of modern extraction techniques have been developed for the extraction of phytoconstituents from plants in order to shorten the extraction time, decrease the solvent consumption, increase the extraction yield and enhance the quality of extracts. The plant *Triumfetta rhomboidea* is an under shrub belonging to the family Tiliaceae, widely distributed in tropical and subtropical India, Ceylon, Malay peninsula, Chine, Africa and in America.[1]

Various extraction methodology most commonly used mainly for the extraction of active compound from the medicinal plant material includes conventional extraction (CEM) and non-conventional extraction methodology (N-CEM). The conventional extraction methodology is simple and mostly includes Maceration, Percolation, Infusion, Decoction etc. on the other hand; the non-conventional methodology includes Ultrasound Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), Accelerated Solvent Extraction (ASE) and Supercritical Fluid Extraction (SFE). Recently, this N-CEM has gained increasing interest and has been rapidly spread globally for the isolation of therapeutically important phytochemical from the medicinal plants as this N-CEM has an advantage over the CEM. The N-CEM is fast and environmentally friendly in terms of solvents and energy consumptions and several parameters can be controlled at a time. Furthermore, improvement in the extraction efficiency, efficacy, batch to batch variation, consistency and selectivity are also the benefits of the N-CEM.[2-4] After the extraction of medicinal plants by CEM and N-CEM, the estimation of bioactive can be done by RP-HPLC. Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is an important analytical tool for quantification of bioactive compound in herbal extract. It is commonly used chromatographic technique for the estimation of secondary metabolites in the plants. It has wide applications in different

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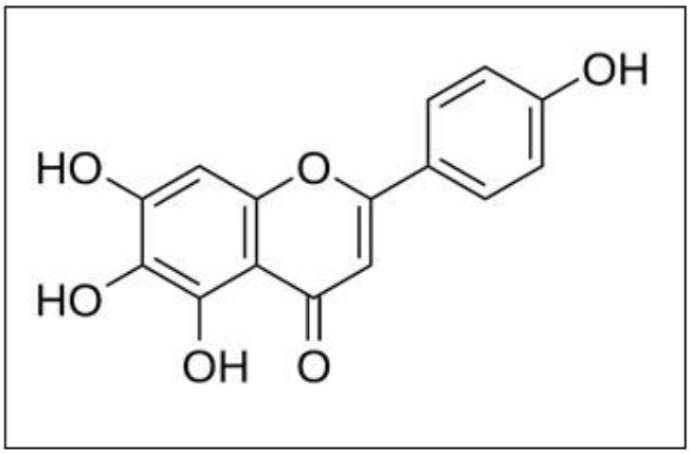

Figure 1: Structure of Scutellarein.

fields in term of isolation, qualitative and quantitative estimation of active molecules.[5-13]

Scutellarein (4,5,6,7-tetrahydroxy flavone) is one of the phytochemical, a type of flavonoid, originally obtained from *Scutellaria lateriflora* and other members of the genus Scutellaria, as well as the fern *Asplenium belangeri* (Structure of Scutellarein is shown in Figure 1). It is also reported in *Triumfetta rhomboidea* belonging to family Liliaceae. It is a commonly used herbal medicine in an Ayurvedic system. Roots, leaves, and stems of *T. rhomboidea* have been used as a single drug or as a component of certain compound drug preparations in the Indian Ayurvedic system of medicine for treatment of various disorders as well as tonic and rasayana drug. Various studies indicated that *T. rhomboidea* posseses anti-inflammatory, anticancer, antioxidant, hepatoprotective, antibacterial, antimicrobial, anti-diabetic, anti-tubercular and anthelmintic, chemopreventive and lactogenic activity.[14]

The isolation of flavone glycosidal derivative of Scutellarein, Triumboidin, from the leaves of *T. rhomboidea* was reported by Shrinivasan and Subramanian who wrongly characterized it as Scutellarein-7-O-L-rhamnosylarabinoside and also no evidence was provided for the presence of the disaccharide moiety.[15] Again further investigation was done on the same compound, Triumboidin, by Bernard Voirin and Jean Favre-bonvin using conventional extraction methodology i.e boiling with 80% of ethanol.[16]

Although, there are various CEM available for the Scutellarein, due to its exhaustive nature, time-consuming nature that directly affects the percent yield of the Scutellarein extract and concentration of the pure Scutellarein in the extract. Considering the importance of Scutellarein, there is a need to enhance the extraction efficiency and extraction yield like important parameters by using efficient N-CEM. Keeping in view to that, there is no such report or documentation available related to the comparative evaluation of extraction method, extraction percent yield, and concentration of pure Scutellarein, present research work was carried out.

The main objective of this study was to develop an optimized procedure of Soxhlet Assisted Extraction (SAE), Ultra-sound Assisted Extraction (UAE) and Accelerated Solvent Extraction (ASE) techniques for preparation of extracts from *T. rhomboidea* leaves and to compare these extraction techniques. Further, the quantification of bioactive compound Scutellarein was done using HPLC technique. It is anticipated that these data will help to find the optimal method of extraction of flavonoids from *Triumfetta rhomboidea* leaves.

MATERIALS AND METHODS

General

The authenticated dried leaves of *T. rhomboidea* were grofund to a powder using a mixer grinder (Havells India Ltd., Delhi, India). Leaf powder was sifted in a sieve shaker (CIP Machineries, Ahmedabad, GJ, India) to select uniform particle size, leaf powder was passed through sieves of different sizes (12, 24, 45, 85 and 120 mesh, Swastika Electric and Scientific Works, Ambala, HR, India) for a period of 20 min. The leaf powder passed through 120 mesh sieves was collected and used for further extraction experiments. The standard Scutellarein (purity 98% by HPLC) was purchased from Sigma–Aldrich (St-Louis, MO, USA). All solvents used for the extraction and the chromatographic purpose were of analytical grade (Finar Chemicals Ltd., Ahmedabad, GJ, India) and HPLC grade (MERCK Specialties Pvt. Ltd. Mumbai, MH, India) respectively. Ultrasonic bath (Model: USB 6.5L (H), power: 230VPCi Analytics, Thane, MH, India) and accelerated solvent extractor (E-914, Buchi India, Mumbai, MH, India) were used for the extraction purposes. The extracts were prepared freshly and stored in desiccators (Riviera Glass Pvt. Ltd., Mumbai, MH, India) under vacuum until the analysis.

Maceration

Maceration is a commonly used conventional extraction technique widely used in the medicinal plant research. This method is the easiest and simple method of extraction. It involves the socking of the powdered plant material in a container containing solvent with proper stopper. The maceration extraction process (n=5) was done by taking 60 g of dried powdered leaves and subjected to macerate with Water, Ethanol: Water (50:50) and ethanol for 24 hr. After 24 hr the mixtures were further filtered concentrated using rotary vacuum evaporator and analyzed for Baicalein content by HPLC.[2]

High-Performance Liquid Chromatography (HPLC) analysis

All extracts of *T. rhomboidea* macerated with Water, Ethanol: Water (50:50) and ethanol were analyzed using high-performance liquid chromatography (HPLC) at 335 nm. The chromatographic

separation was performed on a C_{18}, 250 x 4.6 mm, 5um (X-Bridge, Waters) having oven temperature 40°C, with the mobile phase composed of acetonitrile: water (25:75, v/v), adjusted to pH 2.4 with 1M phosphoric acid at a flow rate of 1.0 mL/min with total run time of 20 min.[5]

Soxhlet Assisted Extraction technique (SAE)

SAE was used for the maximum recovery of Scutellarein from the *T. rhomboidea* leaves. 60 g of powdered leaves were placed in porous bag or thimble made by using filter paper, which is placed in thimble chamber (Borosil, Mumbai, MH, India), which was inserted into a Soxhlet apparatus and extracted (n=5) with 180 ml Water, Ethanol: Water (50:50) and ethanol as an extraction solvent. The extraction solvent is heated in the round bottom flask and vaporizes into the thimble chamber and lastly condensed into the condenser. When the condense vapors reach to the chilled siphon tube arm it gets converted into the liquid and drip back into the round bottom flask again and the process was performed continued for 5 hr. After the extraction process completion, the sample present in the round bottom flask was collected and concentrated using vacuum rotary evaporator and analyzed for Scutellarein content by using RP-HPLC.[2]

Ultra-sonic Assisted Extraction technique (UAE)

UAE involves the application of high intensity, high-frequency sound waves and their interaction with materials. UAE works by using the ultrasounds ranging from the 20 kHz to 2000 kHz. 60 g of dried powder was mixed with 200 ml of ethanol in a beaker. The extraction of *T. rhomboidea* powder (n=5) was carried out by placing the beaker in an ultrasonic bath with the fixed power of 150W. The beaker was immersed in the ultrasonic bath and extracted for 30 min. The water in the ultrasonic bath was circulated at 25°C to avoid the overheating produce by the ultrasound waves. The sample was then collected and concentrated using rotary vacuum evaporator and analyzed for the content of Scutellarein by using RP-HPLC.[3]

Accelerated Solvent Extraction technique (ASE)

ASE is an efficient extraction method compare to maceration and SAE as the use of a minimum solvent. ASE based on parameters like temperature, time, pressure and hold time.5 gm of dry powder of *T. rhomboidea* (n=5) was mixed with 115 g of silica sand and placed into the stainless-steel extraction cell to prevent the sample from aggregating and block the system tubing. Packed ASE cell contains the layer of silica sand and powder mixture cellulose filter papers and sand layers. 97% Ethanol was used as an extraction solvent for the overall extraction process with a temperature range of 78°C and pressure 78 psi*. Two static cycles were carried out in the overall extraction process. After the extraction, the total solvent volume was collected in the vial and the extracts were concentrated using rotary vacuum evaporator and analyzed for the content of Scutellarein by using RP-HPLC.[2,3]

Identification and quantification of Scutellarein in extracts using HPLC

Ethanolic Extracts of different samples of *T. rhomboidea* by Accelerated solvent extraction, Ultrasound-assisted extraction, Soxhlet Extraction and Maceration were analyzed using high-performance liquid chromatography (HPLC) at 335nm. The chromatographic separation was performed on a C_{18}, 250 × 4.6 mm, 5um (X-Bridge, Waters) having oven temperature 40 degree Celsius, with the mobile phase composed of acetonitrile: water (25:75, v/v), adjusted to pH 2.4 with 1M phosphoric acid at a flow rate of 1.0 mL/min with total run time of 20 min.

RESULTS AND DISCUSSION

High-Performance Liquid Chromatography (HPLC) analysis

HPLC fingerprinting of ethanolic extract of *T. rhomboidea* obtained from the Maceration, SAE, UAE, and ASE techniques was performed. Different calibration standards viz. 1, 2, 3, 4, 6, 8 and 10 ng/ml of Scutellarein were prepared and seven points standard calibration curve for scutellarein was developed using pre-calibrated HPLC method. The regression equation for linearity was found to be y= 20161x + 269.37 with R^2 value 0.999 which is nearer to 1 indicates the graph is linear. The curve of Area vs. Amount was plotted as shown in Figure 2. After the development of HPLC method standard solution of scutellarein, the solution was prepared like that, the final concentration lies in the range of calibration standards. The scutellarein sample was injected and the method was run for 20 min. The standard peak of scutellarein was obtained at the retention time of 7.324 as shown in Figure 3.

Maceration

It is a basic technique used for the extraction process; it softens and breaks the plant cell wall to release the phytochemicals into the extraction solvent. It is CME and involves the transfer of heat

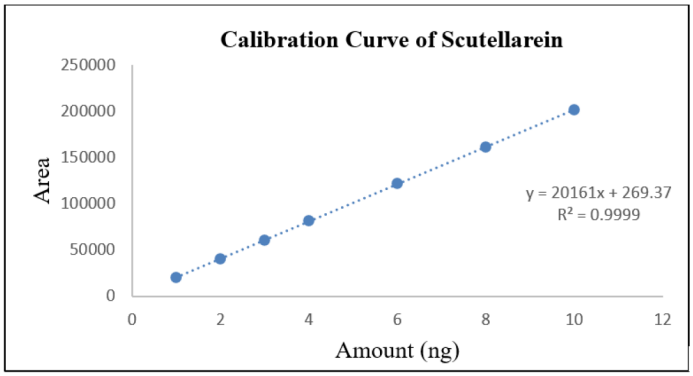

Figure 2: Calibration curve for Scutellarein.

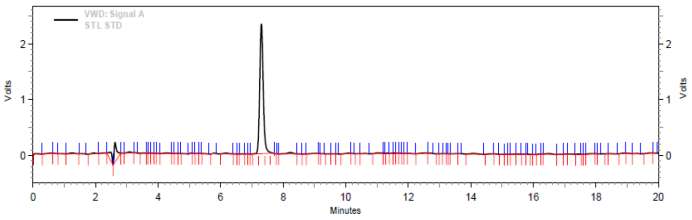

Figure 3: HPLC fingerprinting of standard Scutellarein.

Table 1: Percent yield of maceration extraction of *T. rhomboidea* using different solvents.

Sl. No.	Solvents used for Extraction (ml)	Mean Percent yield (%)
1	Water	3.21 +- 0.037
2	Ethanol: water (50:50)	4.82 +- 0.029
3	Ethanol	5.20 +- 0.041

*The extraction was performed separately in terms of batch (n=5)

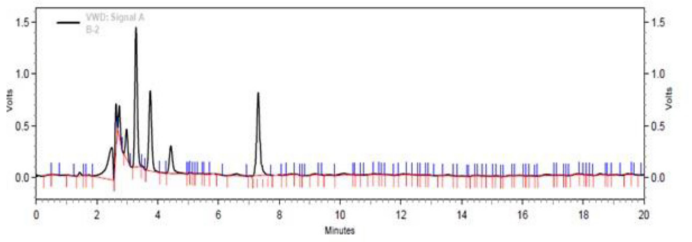

Figure 4: HPLC fingerprinting of Scutellarein using Maceration.

through the mechanism of convection and conduction and also the choice of solvents determines the type of compound extracted from the plant material. In this research work, Maceration extraction process was performed by using three different extraction solvents viz. water, ethanol: water (50:50), and ethanol. The extraction of *T. rhomboidea* in ethanol as extracting solvent shows maximum yield. On the basis of a percent extraction yield, ethanol was optimized as the better extraction solvent. The percentage yield in each solvent was determined and showed in Table 1. The HPLC estimation of Scutellarein in *T. rhomboidea* by using ethanol as extracting solvent was shown the higher concentration of Scutellarein as compare to the other extracting solvents. For the further extraction methods which include Soxhlet assisted extraction (SAE), Ultrasound extraction (UAE) and accelerated solvent extraction (ASE) ethanol were selected as extracting solvent.

Ethanolic Extracts of different samples of *T. rhomboidea* by Accelerated solvent extraction, Ultrasound assisted extraction, Soxhlet Extraction and Maceration were analyzed using high performance liquid chromatography at 335nm. The concentration of ethanolic extract of scutellarein obtained from maceration was found to be 0.129 ng and HPLC fingerprint of ethanol extract obtained was shown in Figure 4.

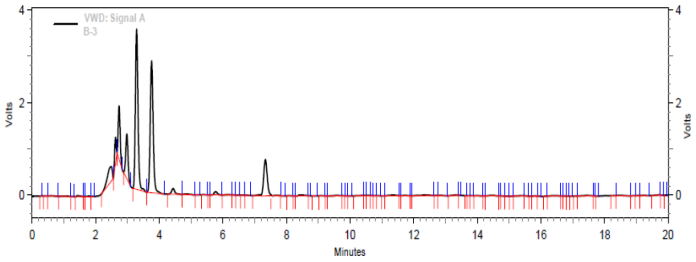

Figure 5: HPLC fingerprinting of Scutellarein using Soxhlet Assisted Extraction (SAE).

Soxhlet assisted extraction technique (SAE)

This CEM requires a small quantity of solvent compared to the maceration process. Various factors such as temperature, solvent-sample ratio etc. need to be considered for this method. Soxhlet assisted extraction of *T. rhomboidea* powder was performed by using ethanol as extracting solvent which was pre-optimized extraction solvent by maceration process. Total percent yield after ethanolic extraction was found to be 8.6%. The concentration of ethanolic extract of scutellarein obtained from SAE was found to be 1.027 ng and HPLC fingerprint of ethanol extract obtained was shown in Figure 5.

Ultra-sonic assisted extraction technique (UAE)

The UAE has received great interest to overcome the disadvantages of Maceration and SAE such as small extraction percent yield. UAE is work on the basis of formation of ultrasound waves which transferred throughout the extraction solvent to form a cavity in the form of bubbles. Due to the continuous circulation of extraction solvent from the bubble cavities, that improves the mass transfer rate of extraction solvent. Due to increase in the mass transfer rate, the fractures forms in the plant cell walls which will enhance the permeation rate and a large amount of solvent enters into the plant tissue to extract the therapeutically active phytochemicals. The choice of suitable solvents for the SAE is an important parameter because of the physical properties like polarity, viscosity, surface tension, and vapor pressure that enhances the cavitation mechanism in the UAE process. Other parameters like frequency and power also considered a major factor for the UAE process, because the power produces physical effects like turbulence. Considering the importance of these factors, ethanol was selected as an extraction solvent. Ultrasonic assisted extraction of *T. rhomboidea* powder was also performed by using ethanol as the pre-optimized extraction solvent. The total percent yield of ethanolic extract of *T. rhomboidea* by UAE was found to be 11.8% which were better than SAE. Parameters like irradiation time, exposure to ultrasonic waves plays a major role to get the high percent yield. The concentration of ethanolic extract of scutellarein obtained from UAE was found to be 1.358 ng and HPLC fingerprint of ethanol extract obtained was shown in Figure 6.

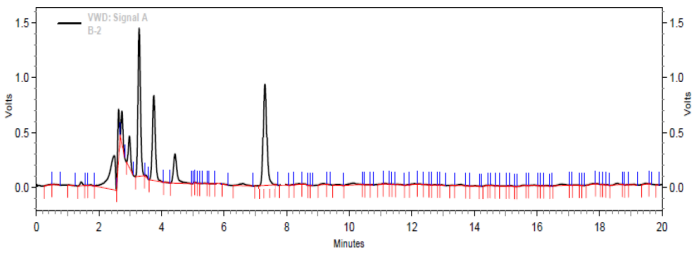

Figure 6: HPLC fingerprinting of Scutellarein using Ultrasound Assisted Extraction (UAE).

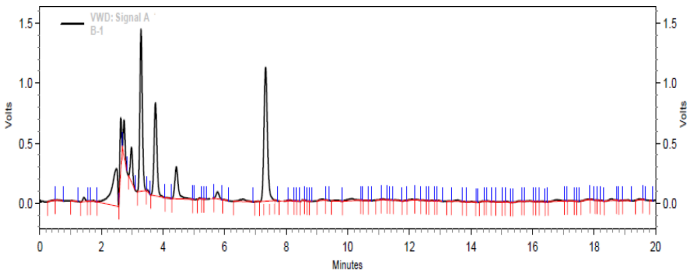

Figure 7: HPLC fingerprinting of Scutellarein using Accelerated Solvents Extraction (ASE).

Table 2: Percent yield of extraction of *T. rhomboidea* by various extraction techniques using ethanol.

Sl. No.	Extraction technique	Solvents used for extraction	Mean Percent yield (%)
1	SAE	Water	7.1 +- 0.028
2		Ethanol: water (50:50)	7.9 +- 0.034
3		Ethanol	8.6 +- 0.048
4	UAE	Water	8.1 +- 0.031
5		Ethanol: water (50:50)	9.5 +- 0.042
6		Ethanol	11.8 +- 0.059
7	ASE	Water	10.2 +- 0.026
8		Ethanol: water (50:50)	11.6 +- 0.039
9		Ethanol	13.4 +- 0.051

*The extraction was performed separately in terms of batch (n=5)

Accelerated solvent extraction technique (ASE)

ASE is an automated rapid extraction technique that utilizes common solvents at elevated temperature and pressure and thereby increases the efficiency of extraction of various compounds. ASE is an efficient form of liquid solvent extraction compared to maceration and Soxhlet extraction as the method using a minimal amount of solvent. In this technique, there is control of temperature and pressure for each individual sample and requires less than an hour for extraction. Accelerated solvent extraction of *T. rhomboidea* powder was performed by using pre-optimized ethanol solvent. The ASE shows higher percentage yield as compared to the other extraction methodologies. The total percent yield of ethanolic extract of *T. rhomboidea* was found to be 13.4%. Percent yield of extraction of *T. rhomboidea* by various extraction techniques using ethanol were shown in Table 2. The concentration of ethanolic extract of scutellarein obtained from ASE was found to be 1.547 ng and HPLC fingerprint of ethanol extract obtained was shown in Figure 7.

Table 3: Total concentration of scutellarein obtained from the various extraction techniques.

Samples	Area	Retention time	Mean Concentration of Scutellarein (ng)
Standard scutellarein	3,05186	7.324	3.000 +- 0.046
Maceration	9138	5.313	0.129 +- 0.061
Soxhlet assisted extraction (SAE)	102117	7.319	1.027 +- 0.071
Ultrasound assisted extraction (UAE)	1,37431	7.318	1.358 +- 0.084
Accelerated solvent extraction (ASE)	1,56558	7.315	1.547 +- 0.049

From the HPLC analysis, the total scutellarein concentration obtained using Maceration, UAE, SAE, and ASE was shown in Table 3 and it was observed that the extract obtained from the ASE technique shows the highest amount of scutellarein.

CONCLUSION

Soxhlet Assisted (SAE), Ultrasound Assisted (UAE) and accelerated solvent extraction (ASE) techniques were successfully optimized for the extraction of Scutellarein from leaves of *Triumfetta rhomboidea*. Extracts from *T. rhomboidea* obtained by maceration, SAE, UAE and ASE methods were analysed by HPLC-ESI-MS/MS to quantify flavonoid scutellarein contents. Concentration of standard Scutellarein was 3 ng, Maximum concentration of Scutellarein (1.547 ng) and extractive yield (13.4%) were found by using ethanol solvent system and ASE technique. Hence, Ethanol was found to be a promising solvent system and Accelerated Solvent Extraction methodology was found to be the promising methodology for the extraction of Scutellarein from leaves of *Triumfetta rhomboidea*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CEM: Conventional Extraction Methodology; **N-CEM:** Non-Conventional Extraction Methodology; **SAE:** Soxhlet Assisted Extraction; **UAE:** Ultrasound Assisted Extraction; **ASE:** Accelerated Solvent Extraction; **RP-HPLC:** Reverse Phase High Performance Liquid Chromatography.

SUMMARY

The proposed work explored the comparative extraction of Scutellarein from leaves of *Triumfetta rhomboidea* along with RP-HPLC study. The results obtained from given research work will be useful for further phytochemical analysis of *Triumfetta rhomboidea*.

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