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Research Article

SEPARATION OF FLAVONOIDS FROM ALCOHOLIC EXTRACT OF *SALVADORA PERSICA* BY HPLC

MANU ARORA[1*], ANEES A SIDDIQUI[2], SARVESH PALIWAL[3]

[*][1,3]Department of Pharmacy, Banasthali University, P.O. Banasthali Vidyapith, Distt. Tonk, Rajasthan, 304022, [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi, 110062 India. Email: manu_cognosy@yahoo.co.in.

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ABSTRACT

Objective: *Salvadora persica* is commonly known as toothbrush tree which contains numbers of flavonoids. The objective of the research work was separation and identification of flavonoids. Methods: These flavonoids were analyzed by the HPLC method from the alcoholic extract of stems of *Salvadora persica*. Mobile phase was composed of acetonitrile: water. Isolation of these flavonoids was done by using RP-18 column with UV variable wavelength detector (set at 254 nm). Chromatogram of standards was compared with the chemical compounds present in sample. Results: It revealed the presence of rutin, kaempferol, quercetin and quercetrin in the crude extract. Among all the flavonoids rutin was present in higher concentration. Quercetrin was one of the flavonoid which was detected first time in alcoholic extract of stem of the plant.

Keywords: Tooth brush tree, Phytochemistry, *Salvadora persica*, Kharajal.

INTRODUCTION

Khara jal is one of the traditional remedy used as anti-fungal[1], hypoglycaemic[2], anti-microbial[3], anti-bacterial[4], anti-plasmodial[5], anti-spasmodial[6], anti-mycotic[7], oral hygiene tool[8] and also used in hepatic disorders[9]. These therapeutic activities of kharajal are due to the presence of different secondary plant metabolites like amino acids[10], volatile oil[11] and benzylamides[12]. It also contains steroids and steroidal glycoside[13].

Salvadora persica also possess the flavonoids and flavonoid glycosides like Kaempferol, Quercetin, Kaempferol 3-*α*-L-rhamnosyl-7-*β*-xylopyranoside, iso rhamnetin-3-O-robinobioside, kaempferol-3-O-robinobioside, narcissin, kaempferol-3-O-rutinoside, isorhamnetin-3-O-*β*-galactoside,astragalin,isorhamnetin-3-O-*β*-D-glucoside,isorhamnetin-3-(2,6-dirhamnopyranosyl-galactopyranoside), Mauritanian, isorhamnetin-3-O-(2-Glc-rhamnosylrutinoside) and kaempferol 3-O-(2-Glc-rhamnosylrutinoside)[15]

MATERIALS AND METHODS

Plant material

The fresh stems were collected in the month of July-August 2007 from Agricultural University Campus, Bikaner and authenticated by Dr. Shekhar Bhargava, Head of the Botany Department, Rajasthan University. After the collection, stems dried in shade at temperature 25-30 °C for 10 days and were crushed to obtain coarse powder which could pass through sieve number 40.

Preparation of extract

100 g of the air dried coarse powdered defatted plant material was percolated with alcohol (95%) for 16 hrs. and repeated three times until exhaustion. The alcoholic extract was evaporated by Rotatory evaporator at about 50 °C.

Standards and chemicals

HPLC-gradient grade methanol and other chemicals (acetonitrile, trifluoroacetic acid) of analytical – reagent grade were purchased from Merck. The authentic standards of the studied flavonoids were taken from Indian Institute of Integrative Medicine and Research, Jammu.

Conditions

HPLC analysis was carried out on injection valve with a 25 µl, a UV variable wavelength detector (set at 254 nm) sensitivity was 0.001, 5 µm RP-18 column (30°C). Mobile phase consisted of acetonitrile: water (containing 5% TFA each). The analytes were eluted gradientally at a flow rate of 1.0 ml/min. Chromatograms were generated on software. The HPLC instrument was operated at room temperature (23 ± 2°C). Each diluted extract 10 µl was injected in to the HPLC three times and the average peak area was reported and used for quantification.

Preparation of standards

1.2 mg of each standard (Rutin, Kaempferol, Quercetin and Quercetrin) was taken in 5ml of methanol (HPLC grade). From which 5, 10, 15, 20, 25µL were injected in HPLC system for making standard curve.

Preparation of sample

10 mg of dry 50% alcoholic extract was dissolved in 10 ml extraction solvent (HPLC grade) to get 1 mg/ml solution, filtered through 0.45 µm Millipore and injected to Waters HPLC system.

Calibration plots

Calibration plots were prepared in order to find out the range of marker concentration, which shows a linear relation with respect to response of analytical technique. Once this range of response was established, concentrations of all test samples were so adjusted as to give the established linear range.

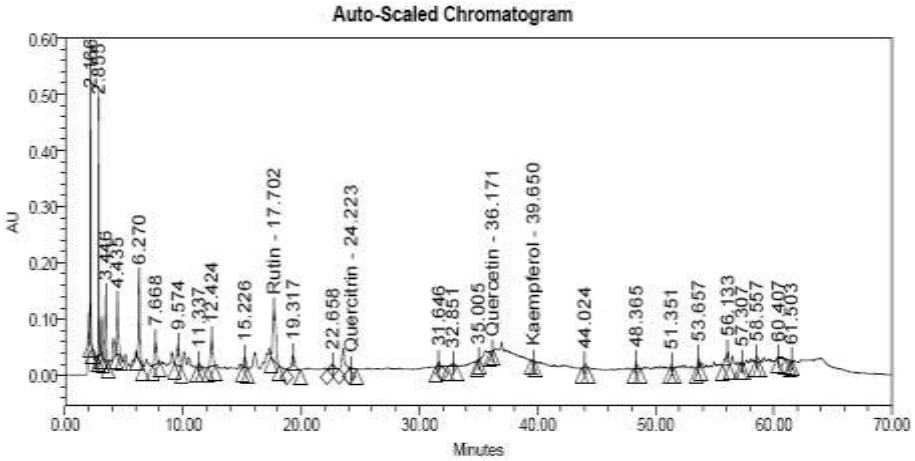
Quantification

The compounds exhibited linear responses in the calibration curves, which were prepared by using the multipoint calibration method. Samples and standards as such and after mixing were injected in different amounts. Calibration curves were plotted for Rutin, Quercetin, Kaempferol, and Quercetrin.

RESULTS AND DISCUSSION

The RP-HPLC behavior of the flavonoids on the reversed phase column in kharajal was tested sequentially varying the proportion of the Acetonitrile-water elution mixture. The separation was achieved by modifying the mobile phase with a small amount of trifluoroacetic acid. HPLC fingerprints of alcoholic extract of *Salvadora persica* given in graph 1 and their retention times & amounts were calculated as shown in Table 1.

Results of HPLC analysis of *Salvadora persica* alcoholic extract (Figure-1), at 254 nm, had shown presence of various constituents as evidenced by the chromatogram obtained at various retention times (17.702, 24.223, 36.171, 39.650) are the constituents found in *Salvadora persica*. In which the compound having retention time 24.223 was the new constituent in alcoholic stem extract. Kaempferol had shown the highest retention time (39.650) among all.



Graph 1: Shown the retention time of compounds present in the sample extract.

Table 1: Retention time & amount of compounds present in sample extract

Peak name	Retention time	Area (µv*sec)	% age Area	Height	Amount (ng)
RUTIN	17.702	1668430	13.11	91835	1390.814
QUERCETRIN	24.223	69941	0.55	3569	13.981
QUERCETIN	36.171	4697	0.04	-497	0.939
KAEMPFEROL	39.650	4247	0.03	251	1.969

These constituents detected were present in (1390.814, 13.981, 0.939 and 1.969) ng amounts. Out of these constituents rutin was present in higher amount. Although quercetrin was present in very less amount but that was not reported earlier. The highest peak of rutin was shown on 91835 in the chromatogram of alcoholic extract of *Salvadora persica* stem. Other flavonoids namely quercetrin, quercetin and kaempferol showed the height of peak at 3569, -497, 251 respectively.

The retention time of standards rutin, quercetrin, quercetin and kaempferol appeared at 17.644, 24.471, 36.193, 39.533 as shown in Graph 2 and amount of these standards were calculated in ng and tabulated in table 2.

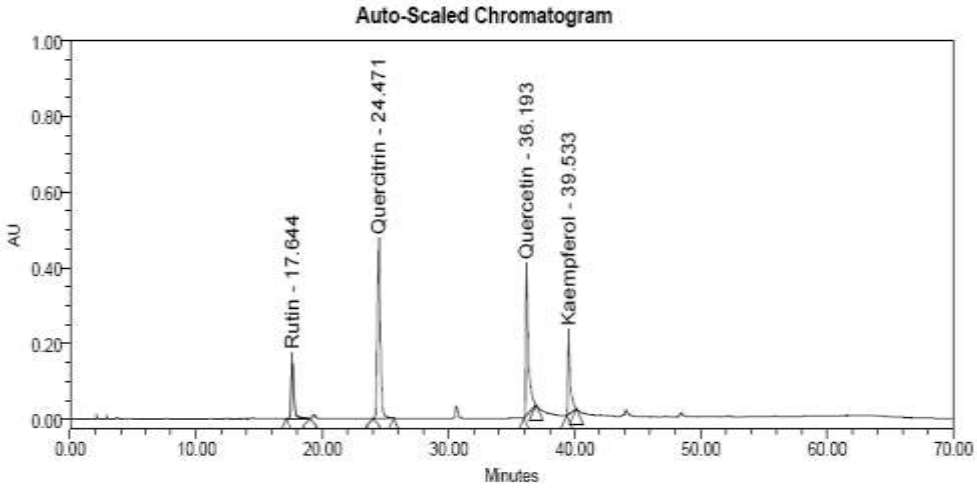
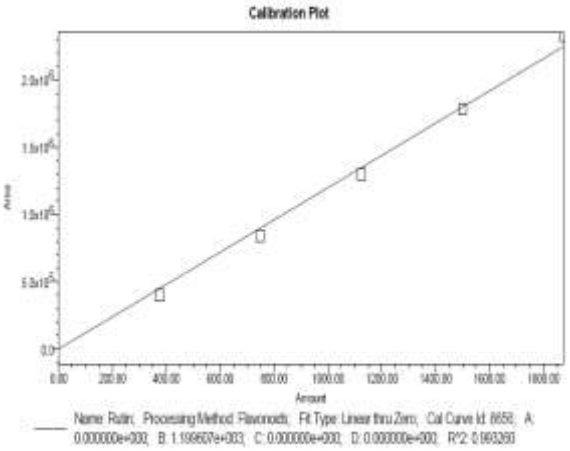


Fig. 2: Shown the retention time of standards

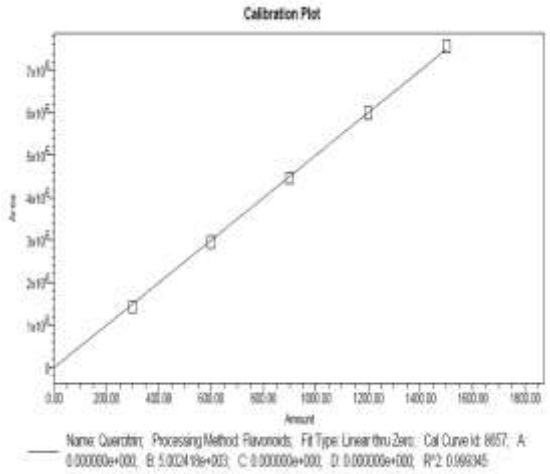
Table 2: Retention Time & amount calculated of all standards

Peak name	Retention time	Area (µv*sec)	%age Area	Height	Amount (ng)
RUTIN	17.644	2329005	13.00	14.3366	1875
QUERCETRIN	24.471	7566862	42.25	445840	1500
QUERCETIN	36.193	5500590	30.71	374129	1500
KAEMPFEROL	39.533	2512380	14.03	192016	1500

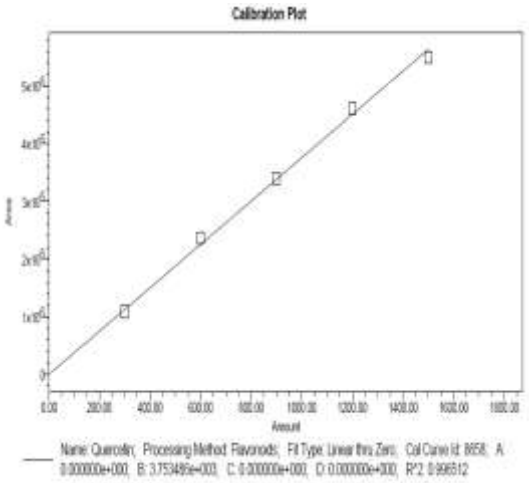
Standard rutin was present in higher concentration among all standards. Calibration curves of standards were plotted and compared with the sample.



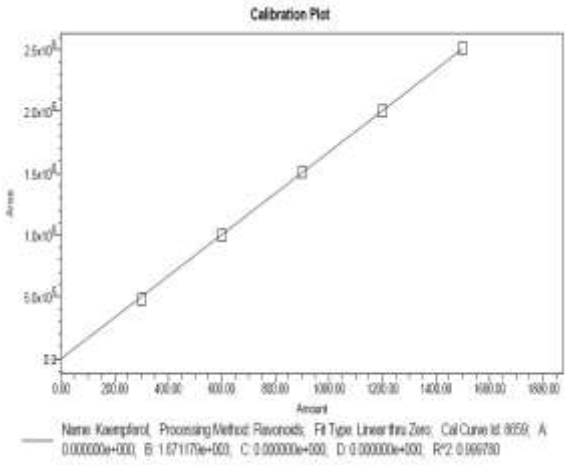
Graph 3a



Graph 3b



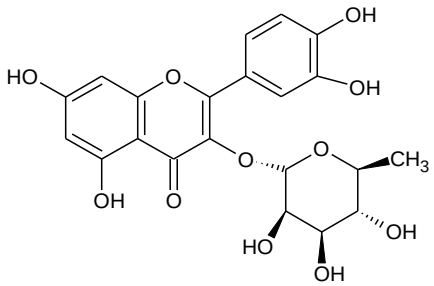
Graph 3c



Graph 3d

Fig. 3a-d: Calibration plots of standards (rutin, quercetrin, quercetin and kaempferol)

The results obtained by this method revealed the presence of four flavonoids Rutin, Quercetrin Quercetin and Kaempferol. Quercetrin was new identified compound.



Quercetrin

Fig. 1: New novel compound identified by high performance thin layer chromatography

In the present study, we described a method that was developed to profile alcohol soluble Phenols, which are mainly flavonoid glycosides and showed the intense absorption in UV region. This technique provided us chromatogram of different compounds present in alcoholic extract of plants. In the absence of authentic standards these UV spectra only allowed determination of compound class. Therefore, this method must be coupled to HPLC-MS and/or NMR analyses in order to identify completely the compounds detected and get some insight into their structure.

CONCLUSION

Based upon the HPLC fingerprints of alcoholic extract of *Salvadora persica*, it can be concluded that Quercetrin is the flavonoid which was not reported earlier. Rutin was present in higher concentration as compared to quercetrin. All these flavonoids are responsible for therapeutic activity of *Salvadora persica* which is commonly known as toothbrush tree. Further research can be done on isolation of all the flavonoids.

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