IJPSR (2023), Volume 14, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 November 2022; received in revised form, 11 January 2023; accepted, 01 May 2023; published 01 July 2023

HPLC FINGERPRINTING OF ETHNOMEDICINAL ANTITUBERCULOSIS PLANTS

Khushboo Vaghela [*1], Dhara Bhatt [2], Bhavita Dhru [1] and Maitreyi Zaveri [1]

K. B. Institute of Pharmaceutical Education and Research [1], Gandhinagar - 382023, Gujarat, India.
Medisynth Chemicals Pvt. Ltd. [2], Navi Mumbai - 400705, Maharashtra, India.

Keywords:

Catechin, Rutin, Quercetin, Kaempferol, *L. reticulate*, *C. hirsutus*

Correspondence to Author:
Dr. Khushboo Vaghela

K. B. Institute of Pharmaceutical Education and Research, Gandhinagar - 382023, Gujarat, India.

E-mail: Khushboovaghela19@gmail.com

ABSTRACT: Modern phytochemical analysis in terms of fingerprinting of different bioactive phytoconstituents in the roots of *Leptadenia reticulata* and whole herb of *Cocculus hirsutu* belonging to the family *Ascelpidiaceae* and *Menispermaceae* respectively. High-performance liquid chromatography (HPLC) analysis of roots of *Leptadenia reticulata* and whole herb of *Cocculus hirsutus* were carried out to develop the fingerprint and verify the presence of standards such as Gallic acid, Catechin, Rutin, Quercetin and Kaempferol in the plant extract. RP-HPLC was performed on an Agilent 1260 Infinity equipped with Inertsil ODS 3V (250 mm x 4.6 mm), 5 µm as a stationary phase, by injecting 20 µL of the sample (1µg/mL) at the flow rate 1.5 mL/min and temperature 30ºC using mixture of Mobile Phase A consist of 1 % Ortho phosphoric acid in water and mobile phase B consist of acetonitrile (20:80) with 60 min run time and the spectra were measured at 364nm using UV detector. The retention time of standard Gallic acid, Catechin, Rutin, Quercetin and Kaempferol were 6.4 min, 10.8 min, 18.2 min, 24.3 min and 29.1 min respectively. Retention time of roots of *Leptadenia reticulata* and whole herb of *Cocculus hirsutus extracts* were similar to that the standard retention time. HPLC fingerprinting shows the overlay between the four different markers such as catechin, Quercetion, Kaemferol and Rutin with aqueous extract of *L. reticulata* and *C. hirsutus.* In *L. reticulata* all the four markers are present and in *C. hirsutus* contains all three markers except Catechin in given quantity of sample.

INTRODUCTION: Plants are the major source of secondary metabolites that is the main interest to man since long. In current years' secondary metabolites are used directly as precursors or as main leading molecule, in the field of pharmaceutical industry [1]. *L. reticulata* is a restorative and stimulant. The leaves and roots are used in wounds, skin infections, asthma, and ear disorders. According to Ayurveda, it is act to give general strength of body.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(7).3539-43

This article can be accessed online on www.ijpsr.com

DOI link: http://doi.org/10.13040/IJPSR.0975-8232.14(7).3539-43

It also cures tuberculosis, hematemesis, emaciation, cough, dyspnoea, eye diseases, fever, and in burning sensation [2-4]. According to Unani system of medicine, *C. hirsutus* is used as tonic, antipyretic, good for fractures, lessens thirsty and useful in tubercular glands related problems. It relieves vata and kaphadoshas. It has potency as a detoxifier. It is has aphrodisiac properties.

Root is used as laxative, in joint pains, tonic, diuretic, demulcent, antiperiodic in fever, in malaria, kidney problems and in treatment of skin diseases. Mucilage is used externally for cooling medicine in eye problems in eczema, prurigo, dyspepsia and impetigo [5-6]. Roots of *Leptadenia reticulata* and whole herb of *Cocculus hirsutus* indicates the presence of Flavonoids, saponins,

steroids, tannins, terpenes, anthraquinone and alkaloids. These phytochemicals are responsible for anti-tubercular activity. Similar observations have been made in plants employed for traditional medicines, which were known to contain the said mentioned bioactive components [7]. The antimycobacterial activity of the selected plant extracts were investigated against the *M. smegmatis* strain of *Mycobacteria*, as the assays were performed in a biosafety containment level 2 setting. The results obtained indicate that *Leptadenia reticulata* and *Cocculus hirsutus* shows efficient antituberculosis activity [8-9].

METHODS:

Collection of Plant: Fresh plant material of two selected medicinal plants, such as roots of *Leptadenia reticulata* and whole herb of *Cocculus hirsutus* were collected from Dhandhiya village of Rajkot district, Gujarat, India during the flowering season in the month of January and were authenticated. The root part of *Leptadenia reticulata* and herb of *Cocculus hirsutus* were separated and dried under sunlight and used for the present work. Herbarium specimens of selected plant materials (PH/015/010-PH/015/011) were deposited at the Pharmacognosy department, K.B.I.P.E.R., Gandhinagar.

Chemicals and Reagents: HPLC grade, Ortho phosphoric acid, Acetonitrile and Methanol were procured from Thermo Fisher and Milli-Q water of HPLC grade was used and purchased from Merck Ltd. All the chemicals and reagents are of analytical grade and Standard Gallic acid, Catechin, Rutin, Quercetinand Kaempferol were purchased from Sigma Aldrich (St. Louis, MO, USA).

Preparation of Methanolic Extract of Roots of *Leptadenia reticulata* and Whole herb of *Cocculus hirsutus*: Dried powder of roots of *Leptadenia reticulata* and whole herb of *Cocculus hirsutus* was separately passed through the sieve of 60 mesh (#) size and stored in airtight containers. Shade dried powder was extracted with methanol. It was then refluxed for about 1 h with occasional shaking, consecutively 3 times and filtered. The filtrates were pooled and concentrated to dryness, percentage yield was calculated. The prepared extracts were labeled and stored in an airtight container for further investigation.

Preparation of Standard Solutions of Bioactive Phytoconstituents: Standard solutions of bioactive phytoconstituents Gallic acid, Catechin, Rutin, Quercetin and Kaempferol were prepared by dissolving appropriate weights in methanol, and diluting to the desired concentration. By using the stock solutions, a series of mixed working standard solutions were prepared with different concentration ranges, and all the solutions were stored under refrigeration.

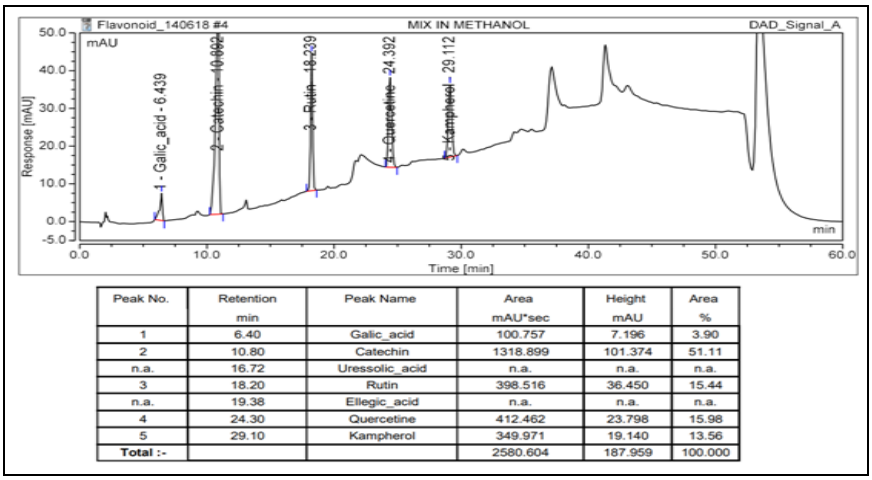
Development of HPLC Fingerprints: This experiment used the Agilent 1260 Infinity II HPLC system. The quantification of bioactive phytoconstituents in the methanolic extract was performed by the HPLC method on a base-deactivated RP phase. The phytoconstituents were separated on Inertsil ODS 3V (250 mm x 4.6 mm) 5µm column. It was furnished with an automated gradient controller, a UV-Visible detector. The column oven temperature was maintained 30°C and 20µL of sample was injected to develop the chromatogram. The method was run for 60 min. The phytoconstituents were detected at 364 nm (UV-VIS detector). The flow rate was maintained at 1.5mL min^{-1}. The mobile phase was optimized to give the best separation between the chromatographic peaks. Mobile phase A consist of 1% Ortho phosphoric acid in water and mobile phase B consists of acetonitrile (20: 80). The standard compounds at different concentrations used were Gallic acid, Catechin, Rutin, Quercetin and Kaempferolfor generating HPLC standard fingerprints. Membrane filters of 0.45 mm pore size (Millipore) were used for filtration of the mobile phase, and Whatman' s syringe filters (NYL 0.45 mm) were used for filtration of the sample. Both extracts' fingerprints were analyzed by interpolating data from the extract and standard bioactive phytoconstituents.

RESULTS:

Standardization of Fingerprint: The objective of the works was to establish the best way to have appropriate fingerprints for all bioactive phytoconstituents present in the samples. The relationship between the fingerprints was notified. For the traditional quality control system, gallic acid, catechin, rutin, quercetin and kaempferol are used as the marker substances to evaluate the methanolic extract of roots of *Leptadenia reticulata*

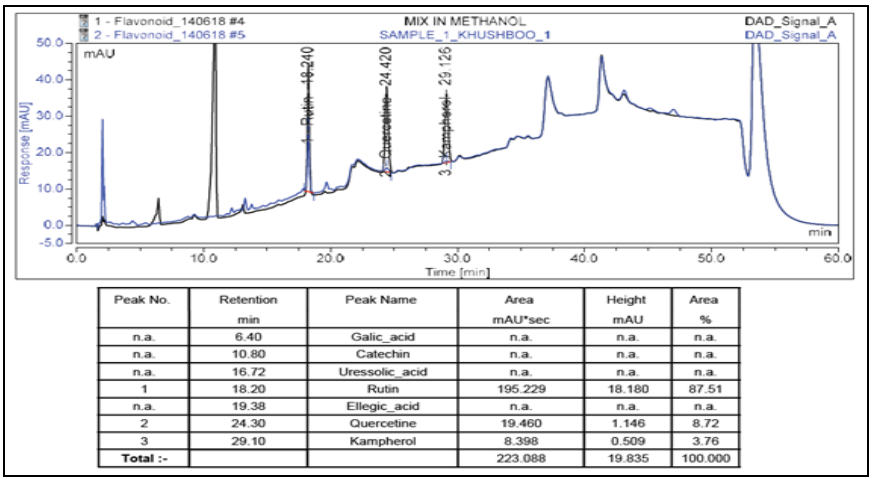
and whole herb of *Cocculus hirsutus*. The contents of these compounds present in the sample were determined by linear regression analysis. A series of standard solutions at different concentrations were tested to determine the linearity equations and scope for the analysts. All results indicated that the conditions for the fingerprint analysis were satisfactory.

Sample Analysis by HPLC: HPLC analysis was performed using the different bioactive phytoconstituents dissolved in methanol at different concentrations. HPLC chromatograms of the bioactive phytoconstituents such as Gallic acid, Catechin, Rutin, Quercetin and Kaempferol were shown in **Fig. 1.** HPLC fingerprint analysis was performed based on the relative retention time. HPLC chromatograms overlay of methanolic extract of roots of *Leptadenia reticulata* and whole herb of *Cocculus hirsutus* over the standards gallic acid, catechin, rutin, quercetin and kaempferol were shown in **Fig. 2** and **3** respectively. The retention time of gallic acid 6.4 min, catechin 10.8 min, Rutin 18.2 min, Quercetin 24.3 min, and Kaempferol 29.1 min. The peaks were observed at a similar retention time in the methanolic extract of roots of *Leptadenia reticulata* and the whole herb of *Cocculus hirsutus.* This confirms the presence of bioactive phytoconstituents such as Catechin (0.0 and 12.00%), Rutin (87.51 and 67.56%), quercetin (8.72 and 13.57%) and Kaempferol (3.76 and 6.87%) in the methanolic extract of roots of *Leptadenia reticulata* and whole herb of *Cocculus hirsutus* respectively. Hence, based on our findings it was concluded that the Methanolic extract of both the plant, such as roots of *Leptadenia reticulata* contained all four markers while *C. hirsutus* contains all three markers except Catechin in a given quantity of sample.



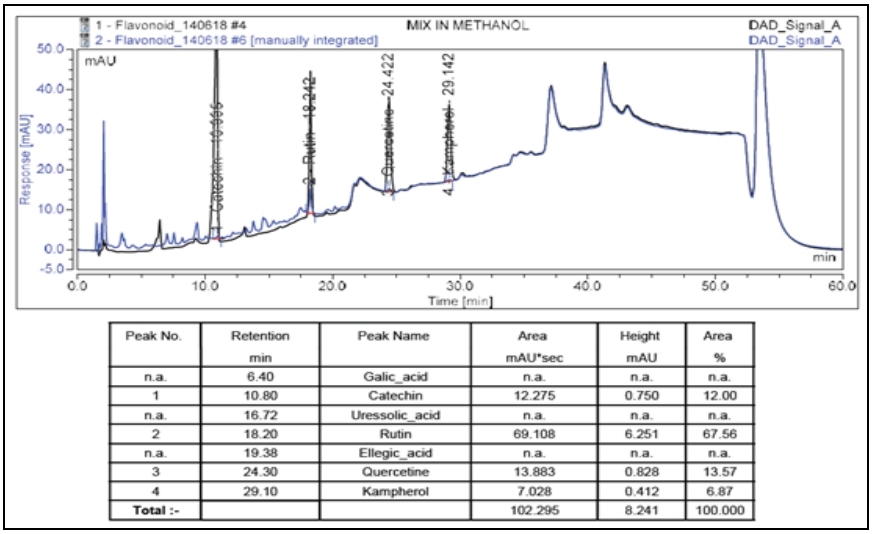
Peak No.	Retention min	Peak Name	Area mAU*sec	Height mAU	Area %
1	6.40	Galic_acid	100.757	7.196	3.90
2	10.80	Catechin	1318.899	101.374	51.11
n.a.	16.72	Uressolic_acid	n.a.	n.a.	n.a.
3	18.20	Rutin	398.516	36.450	15.44
n.a.	19.38	Ellegic_acid	n.a.	n.a.	n.a.
4	24.30	Quercetine	412.462	23.798	15.98
5	29.10	Kampherol	349.971	19.140	13.56
Total :-			2580.604	187.959	100.000

FIG. 1: HPLC CHROMATOGRAMS OF THE BIOACTIVE PHYTOCONSTITUENTS SUCH AS GALLIC ACID, CATECHIN, RUTIN, QUERCETIN AND KAEMPFEROL



Peak No.	Retention min	Peak Name	Area mAU*sec	Height mAU	Area %
n.a.	6.40	Galic_acid	n.a.	n.a.	n.a.
n.a.	10.80	Catechin	n.a.	n.a.	n.a.
n.a.	16.72	Uressolic_acid	n.a.	n.a.	n.a.
1	18.20	Rutin	195.229	18.180	87.51
n.a.	19.38	Ellegic_acid	n.a.	n.a.	n.a.
2	24.30	Quercetine	19.460	1.146	8.72
3	29.10	Kampherol	8.398	0.509	3.76
Total :-			223.088	19.835	100.000

FIG. 2: HPLC CHROMATOGRAMS OVERLAY OF METHANOLIC EXTRACT OF ROOTS OF *LEPTADENIA RETICULATA* AND THE BIOACTIVE PHYTOCONSTITUENTS SUCH AS GALLIC ACID, CATECHIN, RUTIN, QUERCETIN AND KAEMPFEROL



Peak No.	Retention min	Peak Name	Area mAU*sec	Height mAU	Area %
n.a.	6.40	Galic_acid	n.a.	n.a.	n.a.
1	10.80	Catechin	12.275	0.750	12.00
n.a.	16.72	Uressolic_acid	n.a.	n.a.	n.a.
2	18.20	Rutin	69.108	6.251	67.56
n.a.	19.38	Ellegic_acid	n.a.	n.a.	n.a.
3	24.30	Quercetine	13.883	0.828	13.57
4	29.10	Kampherol	7.028	0.412	6.87
Total :-			102.295	8.241	100.000

FIG. 3: HPLC CHROMATOGRAMS OVERLAY OF METHANOLIC EXTRACT OF WHOLE HERB OF *COCCULUS HIRSUTUS* AND THE BIOACTIVE PHYTOCONSTITUENTS SUCH AS GALLIC ACID, CATECHIN, RUTIN, QUERCETIN AND KAEMPFEROL

DISCUSSION: The proposed RP-HPLC conditions ensure sufficient resolution and the use of reference standards guarantees the presence of the phytoconstituents. An HPLC method was successfully developed for fingerprint analysis of the methanolic extract of roots of *Leptadenia reticulata* and whole herb of *Cocculus hirsutus*. The HPLC fingerprint of bioactive phytoconstituents such as Gallic acid, Catechin, Rutin, Quercetin and Kaempferol represent the characteristic markers of this herb's constituents for the first time.

HPLC chromatogram of the standards Gallic acid, Catechin, Rutin, Quercetin and Kaempferol were taken using the same mobile phase with the retention time of Gallic acid 6.4 min, Catechin 10.8 mins, Rutin 18.2 min, Quercetin 24.3 min, and Kaempferol 29.1 min. The peaks were observed at a similar retention time in the methanolic extract of roots of *Leptadenia reticulata* and whole herb of *Cocculus hirsutus.*

This confirms the presence of bioactive phytoconstituents such as Catechin (0.0 and 12.00%), Rutin (87.51 and 67.56%), quercetin (8.72 and 13.57%) and Kaempferol (3.76 and 6.87%) in the methanolic extract roots of *Leptadenia reticulata* and whole herb of *Cocculus hirsutus* respectively.

CONCLUSIONS: Based on our developed HPLC fingerprinting study, we concluded that the presence of bioactive phytoconstituents such as Catechin, Rutin, Quercetin, and Kaempferol in the methanolic extract ofin *L. reticulata* are present and in *C. hirsutus* contains all three markers such as Rutin, Quercetin, and Kaempferol except Catechin in a given quantity of sample. These phytochemicals may be responsible for anti-tubercular activity.

ACKNOWLEDGEMENTS: The authors thank the Department of Science and Technology for providing DST-Inspire Fellowship.

CONFLICTS OF INTEREST: None

REFERENCES:

1. Shokeen P, Bala M and Tandon V: Evaluation of the activity of 16 medicinal plants against *Neisseria gonorrhoeae*. Int J of Antimicro Agents 2009; 33: 86-91
2. Kirtikar KR and Basu BD: Leptadeniareticulata. Indian Medicinal Plants, 2nd ed., Lalit Mohan Basu Publication, Allahabad, 1933; I: 1786-1789.
3. Chopra RN, Chopra IC, Handa KL and Kapur LD: *Leptadenia reticulata*. Indigenous drugs of India, UN Dhur and sons Pvt. Ltd., Calcutta 1958; 289: 665-666.
4. Hakim RA: A preliminary report on the use of malkanguni with other indigenous drugs in the treatment of depression. Indian Journal of Psychiatric 1964; 6: 142-146.
5. Caius JE: The medicinal and poisonous plants of India, Jodhpur, Scientific Publishers 1986; 166-171.
6. Nandkarni KM: *Cocculus hirsutus*. Indian Materia Medica, Vol. I, Popular Prakashan, New Delhi 1976; 362-363.
7. Jethva K, Bhatt D and Zaveri M: Phytopharmacognostical study of some selected antituberculosis plants and study of its cytotoxicity screening using vero cell line, International Journal of Pharmaceutical Sciences and Research 2021; 12(3): 1685-1698.
8. Jethva KD, Bhatt DR and Zaveri MN: Antimycobacterial Screening Of Selected Medicinal Plants Using Mtt And The Microplate Resazurin Assay. Int J of Pharmaceutical Sciences and Research 2021; 12(3): 1537-1545.

9. Jethva KD, Bhatt DR and Zaveri MN: Antimycobacterial screening of selected medicinal plants against Mycobacterium tuberculosis H37Rv using agar dilution method and the microplate resazurin assay. International Journal of Mycobacteriology 2020; 9(2): 150.

How to cite this article:
Vaghela K, Bhatt D, Dhru B and Zaveri M: HPLC fingerprinting of ethnomedicinal antituberculosis plants. Int J Pharm Sci & Res 2023; 14(7): 3539-43. doi: 10.13040/IJPSR.0975-8232.14(7).3539-43.

All © 2023 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)