Annals of Phytomedicine 11(2): 643-653, 2022

DOI: http://dx.doi.org/10.54085/ap.2022.11.2.79

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885

Original Article : Open Access

Phytochemical evaluation and physiochemical analysis of methanolic extract of *Thevetia peruviana* (Pers.) Schum. for future application in drug therapy

Pragyandip P. Dash*,,◆, Sushil Kumar***, Anuradha Mishra* and Sajal Srivastava***

**Amity Institute of Pharmacy, Amity University, Lucknow-226028, Uttar Pradesh, India*
***Faculty of Pharmacy, I.F.T.M University, Moradabad-244102, Uttar Pradesh, India*
****School of Pharmaceutical Sciences, I.F.T.M University, Moradabad-244102, Uttar Pradesh, India*

Article Info

Article history
Received 10 August 2022
Revised 27 September 2022
Accepted 29 September 2022
Published Online 30 December-2022

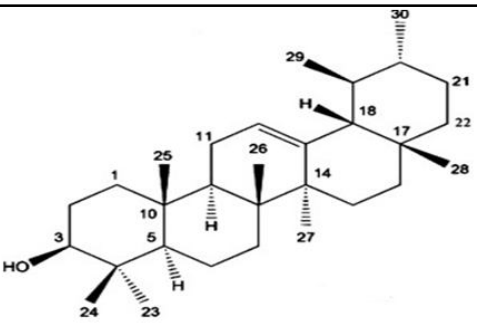
Keywords
Thevetia peruviana (Pers.) Schum.
Amyrin
Calotropin
LC-MS
FTIR and multidrug-resistant

Abstract

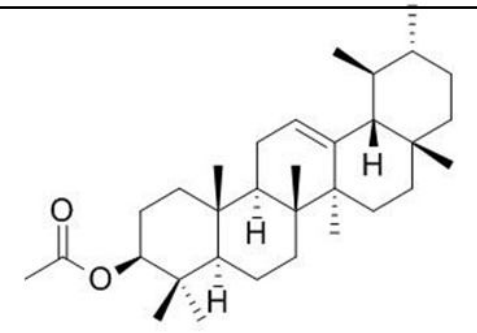
The current study was intended to analyze important phytoconstituents present in the methanolic extract of *Thevetia peruviana* (Pers) Schum. leaves. The preliminary study confirmed the presence of flavonoids, alkaloids, phenols, saponins, anthraquinone, phytosterols, coumarins, cardiac glycosides and anthocyanins, which was confirmed by TLC analysis. Physiochemical analysis showed acidic pH with foreign matters 0.19%.Total ash and water soluble ash are found 3.5% and 4.2%, respectively. The FT-IR spectrum showed the presence of alkyl, methyl, ether, amino, alkene, alcohol, carboxyl and carbonyl groups. UV spectral reports indicated the presence of unsaturated groups and heteroatoms such as S, N, and O. The absorption spectrum for *T. peruviana* extract showed some identifying peaks and confirmed about organic chromophores and a variety of functional groups. LC-MS analysis showed the existence of amyrin, amyrin acetate, calotropin, ursolic acids and beta-sitosterol. These results might be considered for potential biological activity and might largely be contributed to discovery of new drugs.

1. Introduction

Nowadays, herbal formulations have explored their areas in cosmetics, diagnosis or to treat the diseases of human beings. The majority of populations are still using herbal formulations due to their lesser side effect action as well as their pocket friendly nature. Herbal drugs have taken the market over synthetic ones and also provide their effectiveness to the populations (Ghavri and Adhav, 2018). *T. peruviana* is commonly known as yellow oleander or captain cook tree. It is cultivated in central and South America as well as in tropical Africa. The existence of several secondary metabolites makes the *T. peruviana* highly interesting for further study. It is a small evergreen herb that belongs to the family Apocynaceae (Ahamad *et al*., 2017). It is also seen in south Asian countries, especially India and Srilanka. Whole parts of the plants are toxic and contain cardiac glycosides (Bandara *et al*., 2009). It is cultivated as an ornamental plant and generally planted as large flowering shrubs or small ornamental trees (Bhavya *et al*., 2019). *T. peruviana* contributes to pharmacological action due to the occurrence of various important chemical constituents like alkaloids, flavonoids, tannins, cardiac glycosides and many more (Gezahegn *et al*., 2015). It has been used against toothpain, deafness and skin conditions, and it is ingested to reduce weight or deworming agent. Further used in chronic and obstinate skin diseases and alopecia. This study was intended to evaluate it is potential as a replacement for chemotherapy for microbial ailments (Gupta *et al*., 2011).



Alfa Amyrin



Amyrin acetate

Corresponding author: Mr. Pragyandip P. Dash
Amity Institute of Pharmacy, Amity University, Lucknow-226028, Uttar Pradesh, India
E-mail: parthasarathi27@gmail.com
Tel.: +91-9956927429

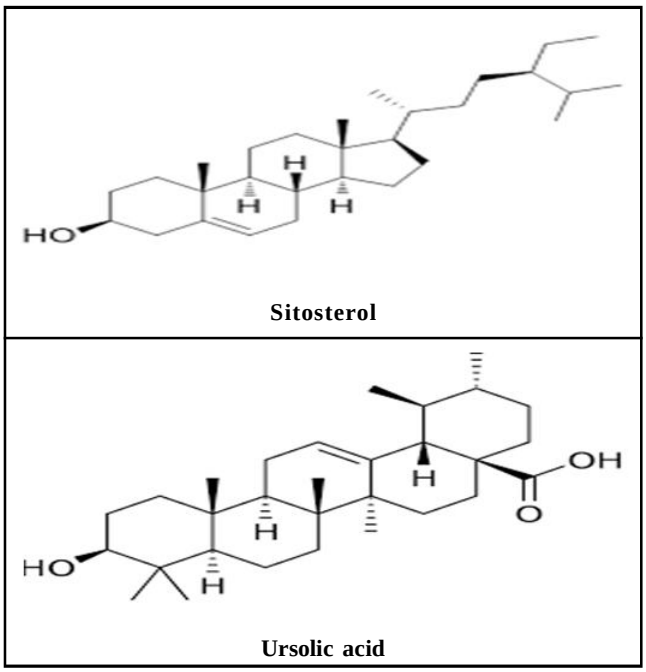


Figure 1: Important phytoconstituents of *T. peruviana*.

Thevetia peruviana (Pers.) Schum. is a small evergreen tree bearing a height of 5-6 meters. The leaves appear green in color, simple linear lanceolate, 10-12 cm in length, 1-2 cm wide, glaucous, glabrous having a subacute apex, short petiole and entire margin with few notches (Desmukh, 2014). The waxy coating of leaves prevents loss of water. Flowers are yellow or orange yellow and have odorless characteristics. The arrangement of flowers forms a funnel shape which enhances its beauty and appearance. Petals are spirally twisted with dark green sepals generally 5-7 cm long and 2-3 cm wide with a soft smooth touch (Mondal *et al*., 2016). The color of the fruits looks red or black in appearance having seeds within it. It is also assumed that it looks similar to a Chinese plant known as the "lucky nut". The seed has a flattened structure which has a small wing. Inflorescence includes sub-terminal, cymose and some few-flowered. Sepals show an acute and spreading pattern. The arrangement of stamens is completely inward and anthers are attached apically. Ovaries are attached with two carpels (Naz *et al*., 2013).

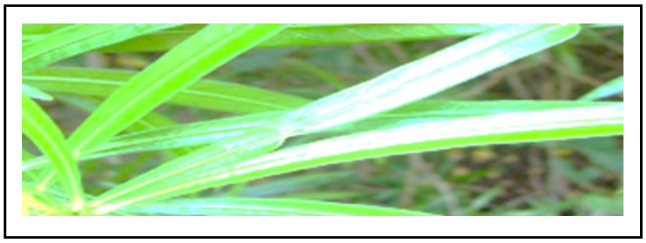


Figure 2: Morphological structure of *T. peruviana* leaves.

2. Materials and Methods

2.1 Collection, authentication of leaves of *T. peruviana*

Freely available leaves of *T. peruviana* (Apocynaceae) were collected from the local area of Lucknow, Uttar Pradesh India, and were authenticated taxonomically at Integral University, Lucknow, India (Voucher Ref. No.: IU/PHAR/HRB/21/15).The leaves collected were cut into small pieces, shade dried and coarsely pulverized for extraction.

2.2 Extract preparation

The powdered sample was extracted with methanol by soxhlation. The extracts were collected and distilled off a rotavapour and the last trace of any solvent was removed in a vacuum. The resulting extracts were kept in the refrigerator at 4°C for further use (Phuse and Khan, 2018).

2.3 Study of extractive value

The extraction of plant material is done with a variety of solvents for analysis of some number of secondary metabolites. These solvents include non-polar solvents like petroleum ether, chloroform and polar solvents like methanol, ethanol and water. Extracts obtained using alcohols were used for the determination of tannins, glycosides, resins, *etc*. Extracts obtained using ether are used for volatile and fat constituents, but when this extract is heated at 105°C, it contains resin, coloring matter and fixed oil as the volatile matters are lost on heating (Gomashe *et al*., 2021).

2.4 Total ash content

The estimation of total ash content is necessary to find out the presence of sand, soil, chalk powder, calcium oxalate and various inorganic contents. The optimum temperature employed is less than 450°C as alkali chlorides are thermolabile**.** For estimation of total ash, 2 g of the extract was taken in a crucible and ignited in a muffle furnace at a temperature not more than 450°C until a constant weight is obtained (Kokate *et al*., 2005).

2.5 Estimation of foreign matter

Any substance other than the parts of the active metabolite is termed foreign organic matter. These substances include parts of insects, moulds, bacteria and particles, animal excreta, *etc*. The separation of foreign matters is necessary to get an accurate analysis of the secondary metabolites and the active ingredients of the plant extract. For estimation, percentage of these matters is calculated.

2.6 Determination of pH

pH of 1% solution: The solution was prepared by dissolving 1 g of extract in 100 ml of methanol. The resulting solution was filtered and analyzed in a calibrated pH meter.

pH of 10% solution: The solution was prepared by dissolving 10 g extract in 100 ml of methanol. The resulting solution was filtered and was analyzed in a calibrated pH meter (Idris *et al*., 2021).

2.7 Preliminary phytochemical screening

Preliminary standard screening of methanolic extract of *T. peruviana* was carried out to find out various plant constituents. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins and anthraquinones using standard procedures (Kareru *et al*., 2010; Kohl *et al*., 2012; Das, and Gezici, 2018; Sivakumar *et al*., 2022).

Table 1: Phytochemical screening

Name of test	Procedure	Observation	Inference
Mayer's test	Sample added to potassium mercuric iodide solution.	Yellow colored precipitate	Presence of alkaloids.
Wagner's test	Sample added to iodine in potassium iodide	Reddish brown precipitate.	Presence of alkaloids.
Dragendroffs test	Sample added to the solution of potassium bismuth iodide.	Red colored precipitate	Presence of alkaloids.
Hager's test	Sample added to the saturated picric acid solution.	Yellow colored precipitate	Presence of alkaloids.
Ferric chloride test	The sample was added to 3 drops of 1% $FeCl_3$ and potassium ferrocyanide	Bluish-green color.	Presence of phenol.
Alkaline reagent test	Sample added to a few drops of sodium hydroxide solution.	Yellow ppt. turns colorless with dil. HCl.	Presence of flavonoids.
Lead acetate test	Sample added to lead acetate solution.	Yellow color precipitate.	Presence of flavonoids.
Borntrager's test	Sample was shaken vigorously with 10 ml benzene and then filtered. 5 ml of 10% ammonia solution was added to the filtrate and mixture was shaken.	Red/pink/violet color present in the ammonia layer.	Presence of anthraquinone.
Modified Borntrager's test	Sample added to $FeCl_3$ solution and immersed in boiling water, cooled and extracted with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution.	Rose-pink color formed in the ammonia layer.	Presence of anthraquinone.
Salkowski's test	Sample dissolved in 2 ml chloroform; Con. H_2SO_4 was added to the wall of the test tube to form a lower layer.	Reddish brown color at the interface.	Presence of steroid.
Liebermann Burchard's test	The sample was added with chloroform and filtered. A few drops of acetic anhydride were added to the filtrate, boiled and cooled. con. sulphuric acid was added.	Brown ring formed at the junction.	Presence of phytosterols.
Test for terpenoids	Sample was treated with 2 ml acetic anhydride and con. sulphuric acid.	Blue, green rings formed.	Presence of terpenoids.
Ferric chloride test	The sample was dissolved in water and filtered. 10% ferric chloride solution added	Yellow colored precipitate	Presence of tannin.
Lead acetate test	Sample dissolved in water and 10% lead acetate solution was added.	Yellow precipitate formed.	Presence of tannin
Potassium dichromate test	The sample was dissolved in water and a strong potassium dichromate solution was added.	Yellow color precipitate.	Presence of tannin and phenolic.
Fehling's test	The sample was hydrolyzed first with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions.	Red precipitate formed.	Presence reducing sugars.
Keller-Kiliani test	Sample solution dissolved in 2 ml chloroform. $H_2SO_4$was added to form a layer at the interphase.	Brown ring at the interphase.	Presence cardiac glycosides.
Test for fatty acid	Sample was mixed with 5 ml of ether evaporated on filter paper and dried.	Filter paper appears transparent.	Presence of fatty acids.
Froth test	Sample diluted with distilled water up to 20 ml.	1 cm layer of honey comb froth.	Presence of saponin.
Test for anthocyanins	Sample mixed with 2 ml of 2N HCl and ammonia color.	Pinkred color to blue-violet	Presence of anthocyanins.
Test for leucon-thocyanin	Sample was added to 5 ml of isoamyl alcohol. colour.	Upper layer appears red in colour.	Presence of l-anthocyanins
Test for coumarins	Sample was mixed with 3 ml of 10% NaOH.	Yellow color confirms.	Presence of coumarins.
Test for emodin	Sample mixed with 2 ml of NH_4OH and 3 ml of benzene.	Red color.	Presence of emodins.

2.8 Physicochemical parameter analysis

The different parameters of physicochemical analysis like the extractive values of, total ash, foreign matter and moisture content of *T. peruviana* extract were conducted as per the standard procedure. pH of 1% and 10% solution of methanolic extract was checked by a calibrated pH meter (Kokate *et al*., 2005).

2.8.1 Thin layer chromatography

Commercial TLC sheets were used for the analysis and were cut to the required size and baseline was drawn above 1 cm from one end. The mobile phase was selected by testing out the samples in various solvents. A small quantity of methanolic extract was applied to the plate by a thin capillary tube on the baseline. Plates are placed inside the TLC chamber where the spotted sides were kept down into the chamber for development. The chromatographic plates were held inside the chamber until the mobile phase travelled up to ¾th of the distance of the plate. The plate is removed and allowed to dry, and then the spots were observed using chromatogenic reagents (Kumari *et al*., 2017).

2.8.2 UV spectrophotometry

The UV analysis was performed to identify the phytoconstituents present in the methanolic extract of *T. peruviana*. The UV spectra were performed to identify the compounds containing s-bonds, p-bonds and lone pair of electrons, chromospheres and aromatic rings. The qualitative UV profile of methanol extract of *T. peruviana* taken at the wavelength of 200-400 nm and maximum wavelength of different active metabolites are obtained (Save *et al*., 2015).

2.8.3 FT-IR analysis

The leaves were powdered and mixed with potassium bromide with the help of a mortar and pestle and transformed into a slender pellet. Infrared spectra were recorded as potassium bromide and sample pellets on an IR21E28SEP19 transmission, between 4000-400 cm^{-1} (Rahaman *et al*., 2014).

2.8.4 LC-MS analysis

The LC-MS spectrums interpretation was performed using a spectrum database for organic compounds in SDBS application. The results of spectrum interpretation on methanol extracts of *T. peruviana* leaves were obtained (Sangodare *et al*., 2012).

3. Results

The results of physicochemical parameters like extractive values, water soluble ash, total ash and foreign matter are tabulated in Table 2.

Table 2: Physiochemical analysis of *T. peruviana* leaves

Parameter	Findings
Methanolic extractive value	11
Water-soluble ash	3.5
Total ash	4.2
Foreign matter	0.19
pH of 1 % extract	4.9
pH of 10 % extract	5.1

3.1 Phytochemical screening reports

The preliminary phytochemical investigation of methanolic extract of *T. peruviana* leaves showed the presence of flavonoids, alkaloids, saponins, phenols, cardiac glycosides, phytosterols, anthraquinones, coumarins, anthracyanins, leucoanthocyanins, tannin, fatty acids and emodins shown in Table 3.

Table 3: Phytochemical analysis results of methanolic extract of *T. peruviana*

Bioactive components	Methanol extract
Alkaloids	+
Phenol	+
Flavonoids	+
Anthraquinone	+
Phytosterols	+
Tannin	+
Cardiac glycosides	+
Fatty acid	+
Saponin	+
Anthocyanins	+
Leucothocyanins	+
Coumarins	+
Emodin	+

(*+ indicates present)

3.2 Thin layer chromatography

Rf values obtained from the analysis range from 0.19 to 0.91with the different solvent systems. These values indicated compounds like flavonoids, phenols, saponins, anthraquinones, phytosterols, coumarins, anthocyanins, leucoanthocyanin, emodins, fatty acids, cardiac glycosides and alkaloids. Mobile phase composition and Rf values are given in Table 4.

Table 4: TLC analysis of *T. peruviana* leaves extract

S.No.	Mobile phase composition	Ratio	Rf value
1	Toluene: Ethyl acetate	8:2	0.91
2	Toluene: Ethyl acetate :Glacial acetic acid	5:4:1	0.75
3	Petroleum ether:Chloroform	6.5:3.5	0.28
4	Ethyl acetate:Methanol	1:1	0.64
5	Hexane:Dichloromethane	1:1.5	0.19
6	Ethyl acetate + Methanol	2:1	0.84

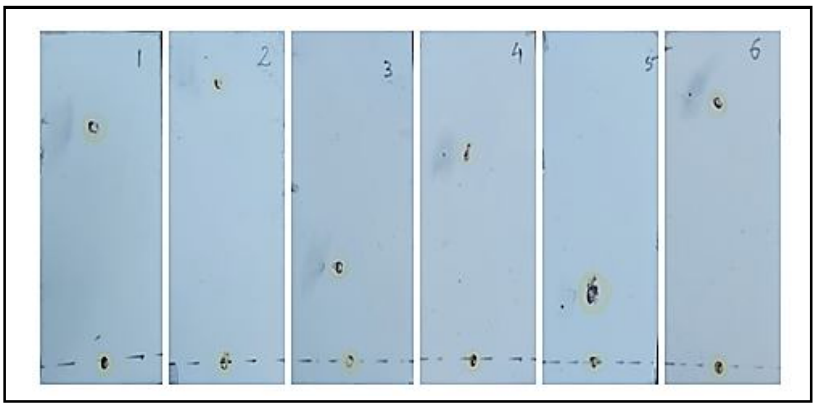


Figure 3: TLC plate chromatograms of methanolic extract of *T. peruviana*.

3.3 UV spectroscopy results

The profile showed the peaks at 268 nm, 212 nm and 228 nm with the absorption 1.342, 1.118 and 1.482, respectively.The band at 268 nm reveals the presence of flavones and chalcones type of flavonoids and reserpine type of indole alkaloid. Where the wavelength at 228 nm indicates the presence of halides and OH group in conjugation with each other and also indicates the presence of glycosides. The wavelength 212 nm indicates the presence of steroidal nucleus. Figure 4 shows standard graphs of some identified compounds from methanolic extracts of *T. peruviana* leaves.

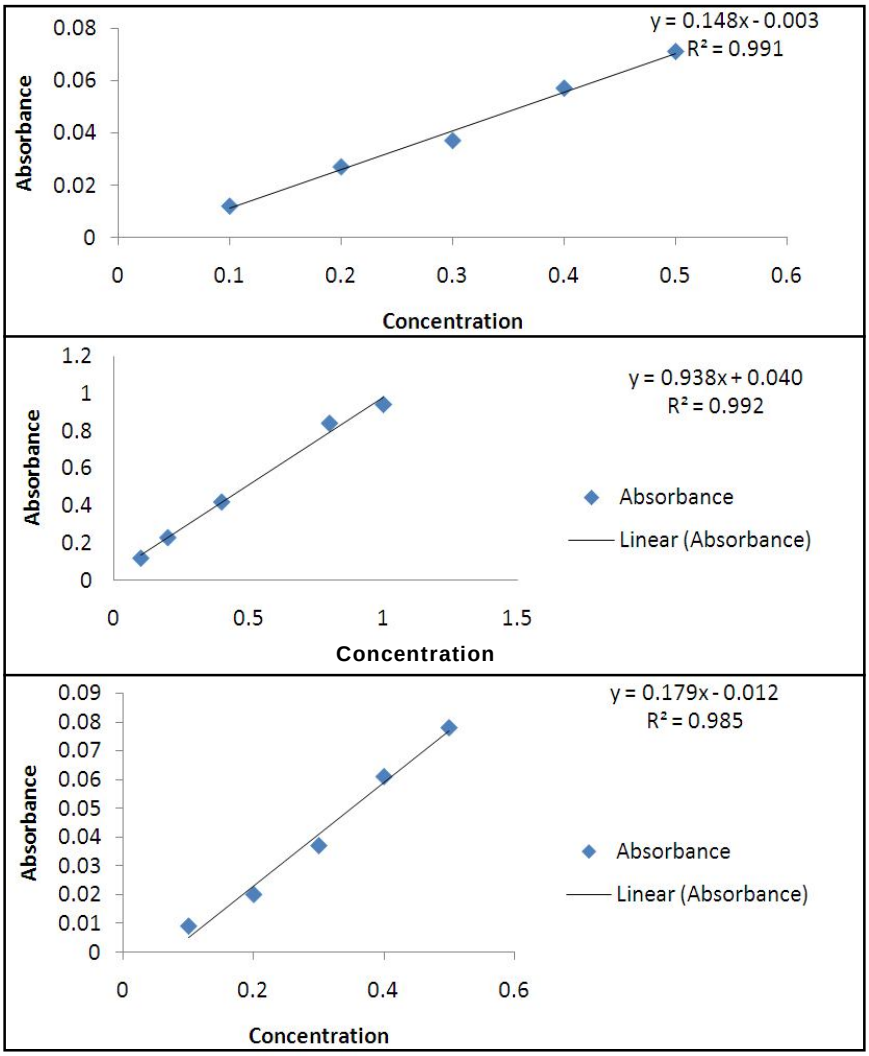


Figure 4: Standard graph of compounds (A) a-amyrin, (B) Chlorendic acid (C) Sitosterol.

3.4 FT-IR analysis

The FTIR analysis of methanolic extract of *T. peruviana* showed the following characteristic absorption peaks as shown in Table 4 and Figure 5. From the FT-IR spectral data, C-O strong stretching (1271.33 cm^{-1} and 1071.33cm^{-1}), C-H stretching (2926.19 cm^{-1}), C-H bending (1384.65 cm^{-1}) (C=C stretching (1619.58 cm^{-1}),C=C strong bending (886.31 cm^{-1}) C-H strong bending(718.16 cm^{-1}), C-CHO, N-H stretching (3393.53 cm^{-1}), CH_3, N=C=S stretching (2113.17 cm^{-1}), and C-X strong stretching (609.50 cm^{-1}) were identified.

Table 4: IR spectroscopic data

S.No.	Group stretching	Frequency (cm^{-1})	Group
1.	N-H stretching	3393.53	Aliphatic primary amine
2.	C-H stretching medium	2926.19	Alkane
3.	N=C=S stretching	2113.17	Thiocyanate
4.	C=C stretching	1619.58	conjugated diene
5.	C-H medium bending	1384.65	Aldehyde
6.	C-O strong stretching	1271.33	Aromatic ester
7.	C-O strong stretching	1071.31	Primary alcohol
8.	C=C strong bending	886.31	Alkene
9.	C-H strong bending	718.16	1,3 di-substituted
10.	C-X strong stretching	609.50	Halo compounds

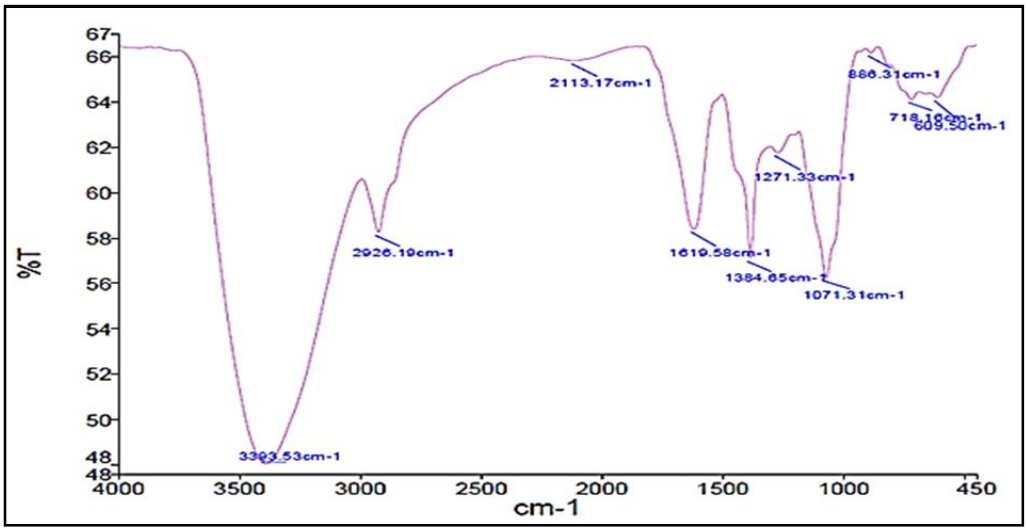


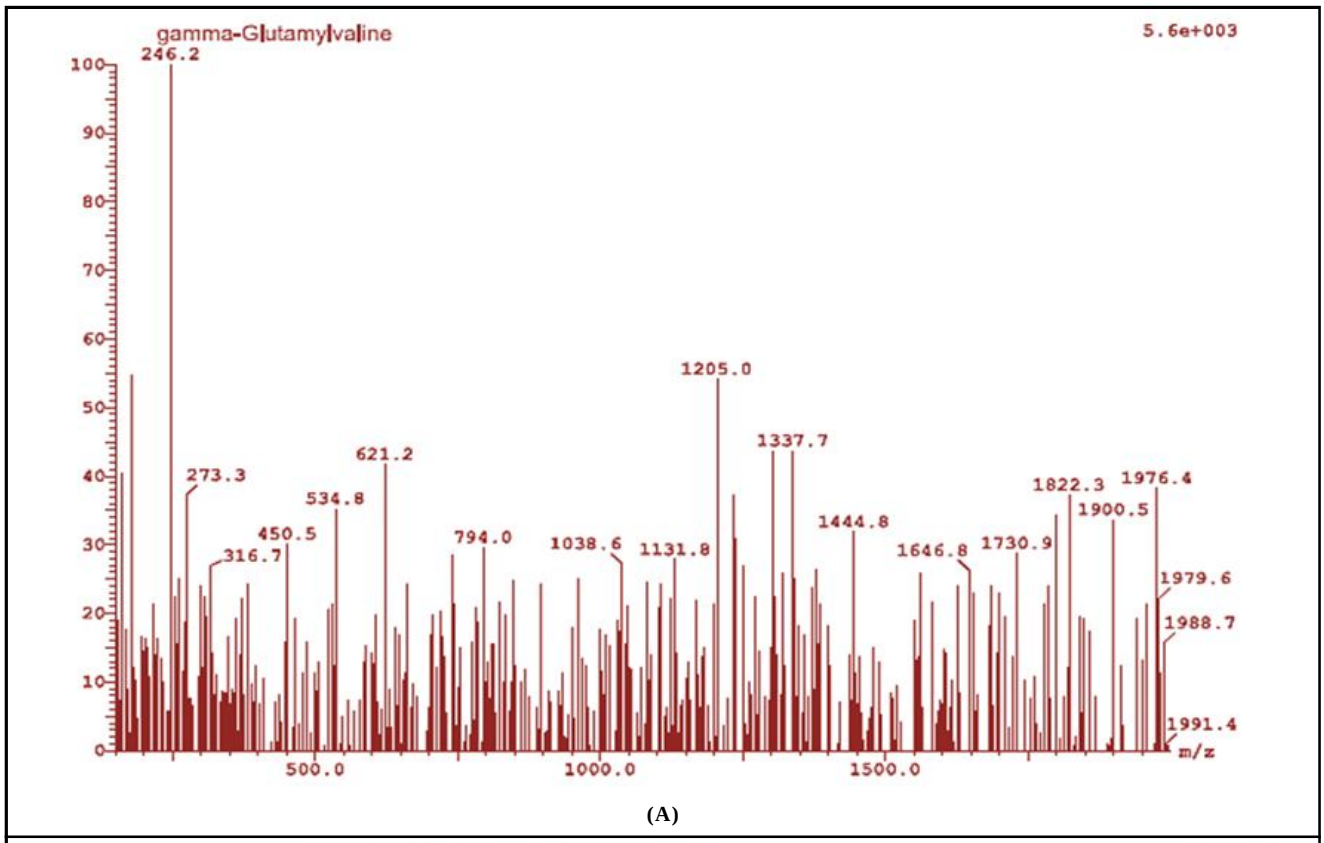
Figure 5: FTIR spectra of *T. peruviana* extract.

3.5 LC-MS analysis result

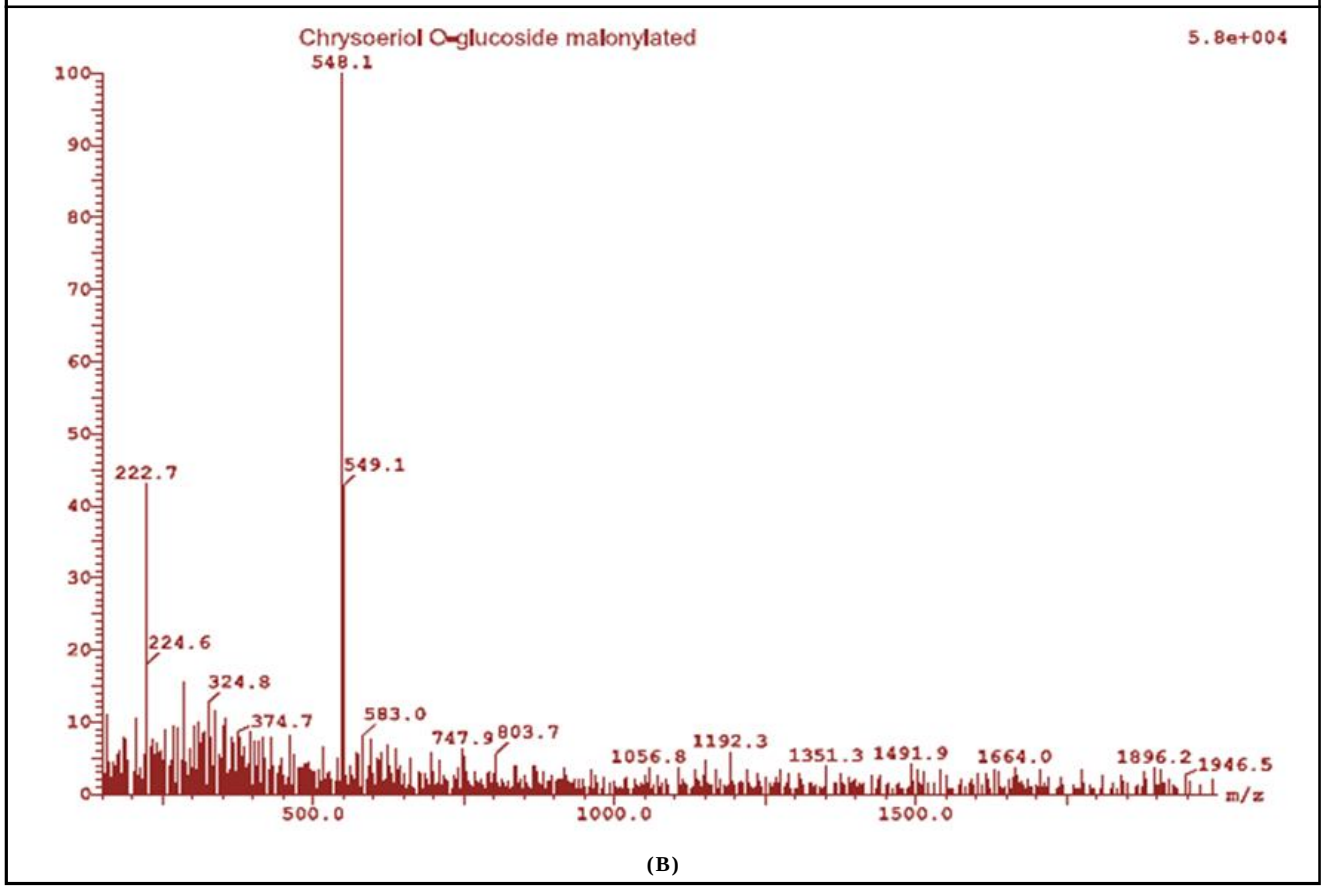
The result of LC-MS data interpretation indicated that there are the substance of chrysoeriol O-glucoside-malonylated with a retention time 14.35 min with the candidate mass 548.1 m/z, chlorendic acid with a retention time 25.13 min and a candidate mass 385.82 m/z, fenarimol with a retention time 27.97 min and a candidate mass 329.9 m/z , eriocitrin with a retention time 35.13 min and a candidate mass 596.8 m/z and gamma-glutamyl valine with a retention time 37.53 min and a candidate mass 246.2 m/z. The values are shown in the Table 5 and the results were confirmed by each fragmentation pattern in Figure 6 (A, B, C, D and E).

Table 5: LCMS findings with m/s and retention time of identified bioactive

Peak No.	RT in min	m/z (Candidate mass)	Proposed compound	Molecular formula
1.	14.35	548.1	Chrysoeriol O-glucoside	$C_{25}H_24O_{14}$
2.	25.13	385.82	Chlorendic acid	$C_9H_4Cl_6O_4$
3.	27.97	329.9	Fenarimol	$C_{17}H_{12}Cl_2N_2O$
4.	35.13	596.8	Eriocitrin	$C_27H_{32}O_{15}$
5.	37.53	246.2	Gamma-glutamylvaline	$C_{12}H_{21}N_3O_6$

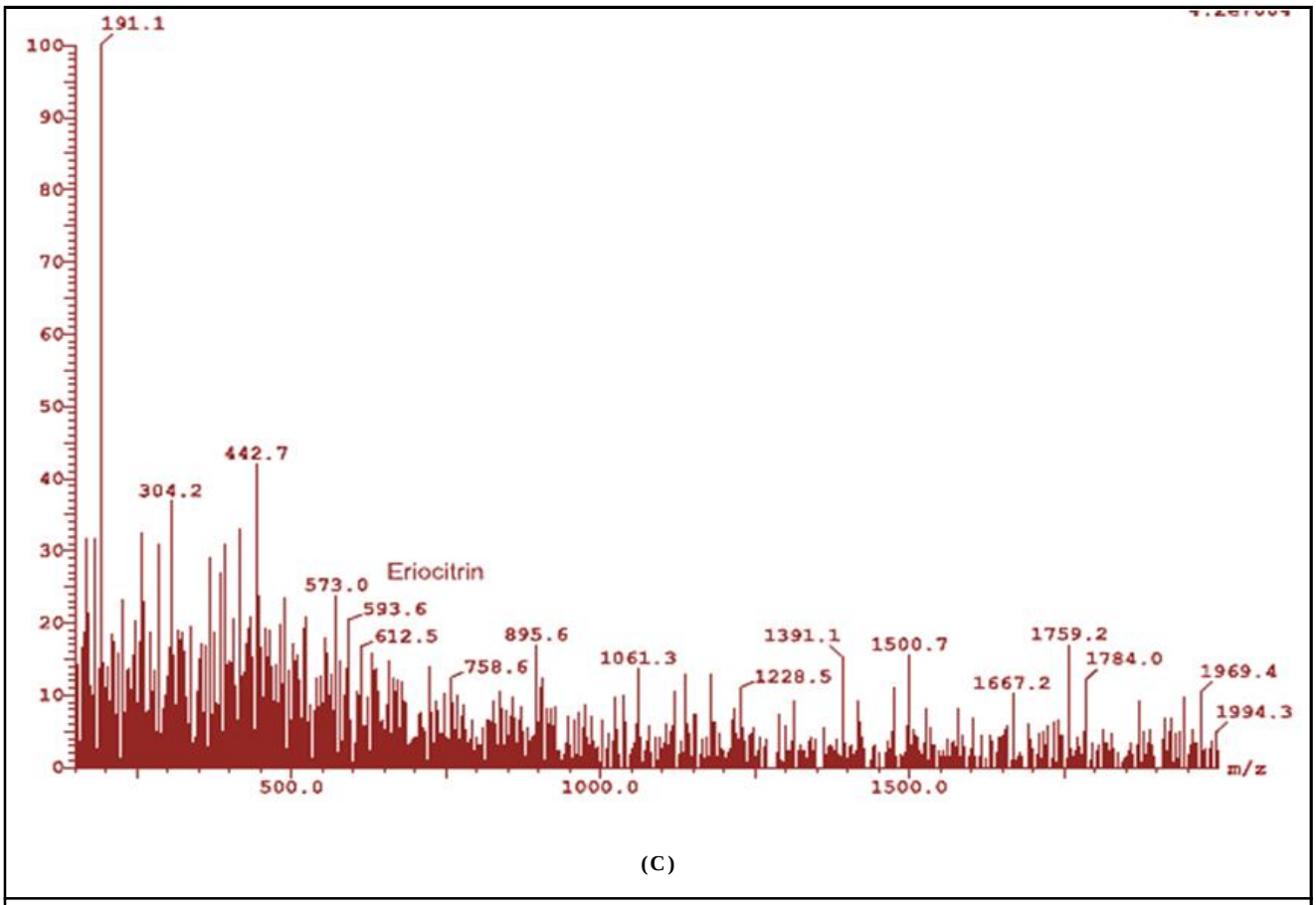

gamma-Glutamylvaline
5.6e+003
246.2
273.3
316.7
450.5
534.8
621.2
794.0
1038.6
1131.8
1205.0
1337.7
1444.8
1646.8
1730.9
1822.3
1900.5
1976.4
1979.6
1988.7
1991.4
m/z
500.0
1000.0
1500.0

(A)

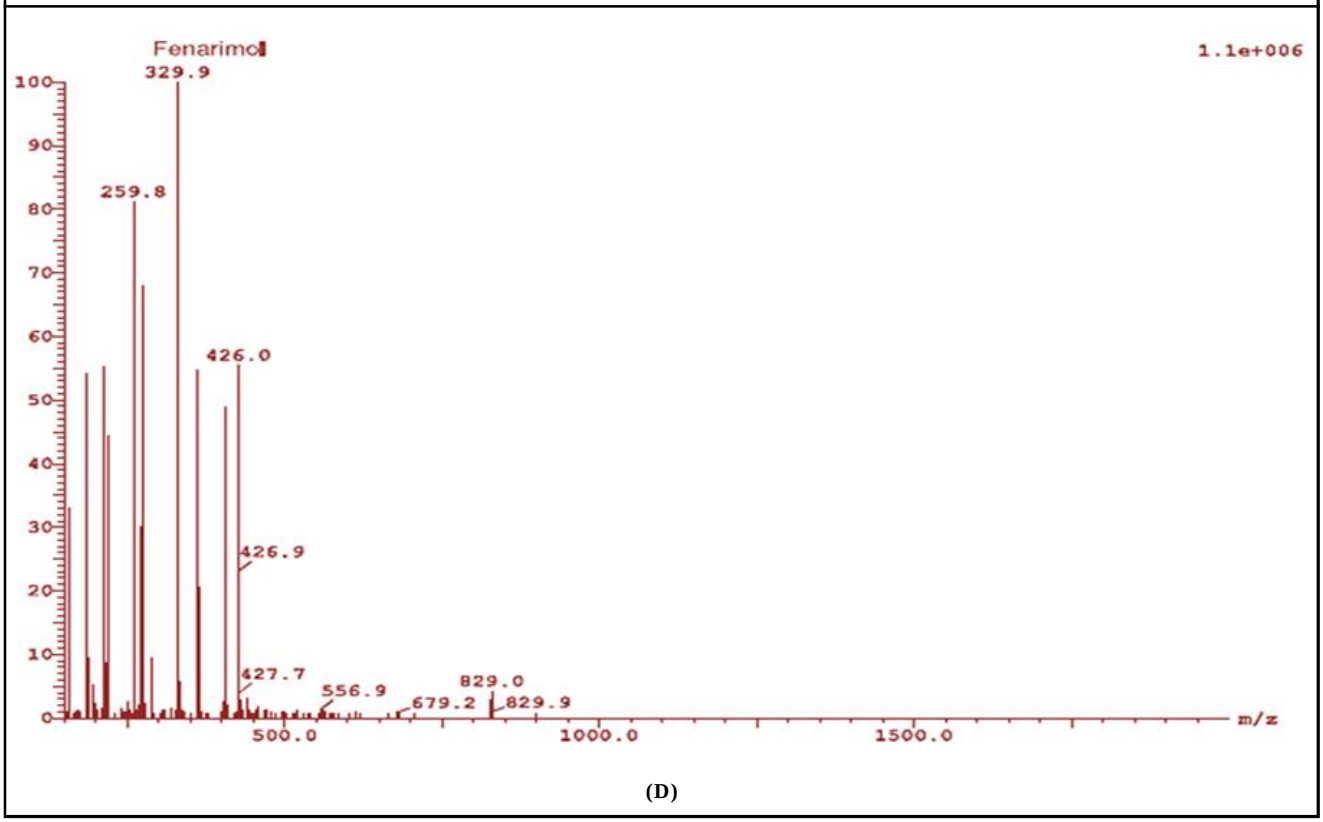

Chrysoeriol C-glucoside malonylated
5.8e+004
548.1
549.1
222.7
224.6
324.8
374.7
583.0
747.9
803.7
1056.8
1192.3
1351.3
1491.9
1664.0
1896.2
1946.5
m/z
500.0
1000.0
1500.0

(B)

650


191.1
100
90
80
70
60
50
40
30
20
10
0
442.7
304.2
Eriocitrin
573.0
593.6
612.5
895.6
758.6
1061.3
1391.1
1500.7
1228.5
1667.2
1759.2
1784.0
1969.4
1994.3
500.0
1000.0
1500.0
m/z

(C)


Fenarimol
1.1e+006
329.9
100
90
80
70
60
50
40
30
20
10
0
259.8
426.0
426.9
427.7
556.9
679.2
829.0
829.9
500.0
1000.0
1500.0
m/z

(D)

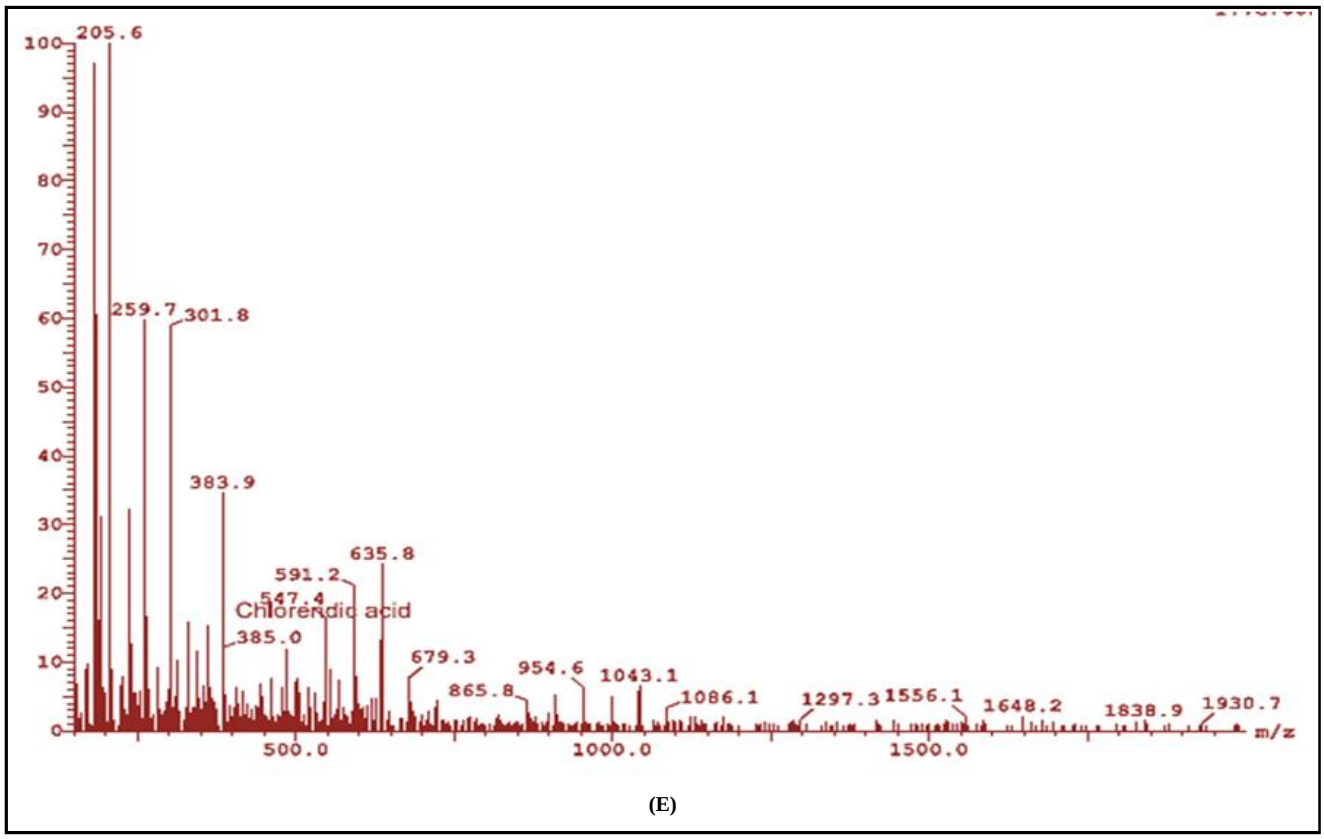


Figure 6: (A, B, C, D and E): LCMS fragmentation pattern of *T. peruviana* methanolic extract.

4. Discussion

From ancient times, it is a well-established fact that herbal drugs are very important for mankind. Plant and their material are a major source of secondary metabolites which not only protects the plants from microbes, viruses and other competing plants, but also attracts insects and other animals for pollination. Phytochemical estimation of the plant was a major step to understanding and ensuring the presence of active metabolites which can be utilized as a potential drug candidate. The preliminary phytochemical analysis of our research reveals the presence of a variety of active secondary metabolites like flavonoids, alkaloids, saponins, phenols, cardiac glycosides, phytosterols, anthraquinones, coumarins, anthocyanins, leucoanthocyanins, tannin, fatty acids and emodins in methanolic extract of *T. peruviana*. These results showed that the plant extract possesses maximum medicinal value which can be useful for pharmaceutical purposes. As we know from the previous literature data, flavonoids are useful in antioxidant and anticancer activities. Alkaloids have cardioprotective, anaesthetic and anti-inflammatory properties. Saponins have great potential in reducing blood glucose and lipid level. Phenols are used as antibacterial and antiseptic effects. Cardiac glycosides were used as life saving drugs in heart failure and other heart related problems. Phytosterols were used for controlling blood cholesterol levels and cardiovascular diseases. Anthraquinone has laxatives and antimicrobial properties. Coumarins play important role in the treatment of prostate and renal cancer. Anthocyanins and leucoanthocyanins have antidiabetic, anticancer, anti-inflammatory, antimicrobial and antiobesity properties. Tannins are responsible for wound healing and the protection of mucous membranes. Emodins were used in asthma, osteoarthritis, diabetes, atherosclerosis and hepatic diseases (Kokate *et al.*, 2005).

Thin layer chromatography was performed to confirm the result found in the preliminary phytochemical screening. Analytical grade TLC plates were used with different mobile phases to find the isolated active metabolites. Array of solvent systems are used to develop the chromatogram were Toluene: ethyl acetate (8:2), toluene: ethyl acetate : glacial acetic acid (5:4:1), petroleum ether: chloroform (6.5:3.5), ethyl acetate: methanol (1:1), hexane: dichloromethane (1:1.5) and ethyl acetate + methanol (2:1).The Rf values obtained were 0.091,0.075,0.028,0.064,0.019,0.084, respectively, which confirms the presence of all the secondary metabolites.

The UV spectrometry analysis showed three λ_{max} with 268 nm, 228 nm and 212 nm with the absorbance of 1.342, 1.118 and 1.482, respectively. Standard graphs were drawn taking different concentration v/s absorbance at λ_{max} 268 nm, 228 nm and 212 nm. The value confirmed the presence of three important compounds such as a-amyrin at 268 nm, chlorendic acid at 228 nm and sitosterol at 212 nm.

Fourier transform infrared spectrophotometer analysis is done to find out the band of the spectrum present in the sample. The result of our study showed different peaks such as 1271.33 cm^{-1} and 1071.33 cm^{-1} which accounted for C-O strong stretching indicated the presence of aromatic ester and primary alcohol, peak at 2926.19

accounted for C-H bending indicated the presence of saturated hydrocarbon chain, band at 3393.53 cm^{-1} accounted for N-H stretching indicated the presence of aliphatic primary amine group, peak at 1619.58 cm^{-1} accounted for C=C stretching indicated presence of conjugated dienes, 1384.65 cm^{-1} peak accounted for C-H medium bending indicated presence of Aldehyde functional group, 2113.17 cm^{-1} band accounted for N=C=S stretching indicated presence of thiocyanate group, 886.31cm^{-1} band accounted for C=C strong bending indicated presence of unsaturated hydrocarbons such as alkenes, 718.16 cm^{-1} band accounted for C-H strong bending indicated 1,3 disubstituted alkenes and peak at 609.5 cm^{-1} accounted for C-X strong stretching indicated presence of halo compounds.

The most important part of our study was liquid chromatography mass spectroscopy which confirms the presence of some new findings. As per the previous literature *T. peruviana* seed extract contains some glycosides like thevetin A (895.39 m/z), thevetin B (876.47 m/z), thevetin C (892.45 m/z), acetyl thevetin A (932.45 m/z), acetyl thevetin B (918.47 m/z), acetyl thevetin C (934.46) which is determined by HR-MS measurement ($Q\text{-}T_0F$) (Kohls *et al*., 2012). Flavanone glucosides, (2*R*) and (2*S*)-5-*O*-b-D-glucopyranosyl-7,49-dihydroxy-39,59-dimethoxyflavan-one[pervianoside I, peruvianoside II] and a new flavonol glycoside, quercetin 3-*O*-{b-D-glucopyranosyl-(1®2)-[a-L-rhamnop-yranosyl-(1®6)]-b-D-galactopyranoside} (peruvianoside III,) were isolated from the leaves of *T. peruviana*.

The LC-MS analysis of *T. peruviana* leaf extract showed five retention times 14.35 min, 25.13 min, 27.97 min, 35.13 min and 37.53 min. Each peak was fragmented, resulting 5 fragmentation spectrum with candidates mass (m/z) 548.1, 358.82, 329.9, 596.8 and 246.2 (Table 5 and Figure 6). From the fragmentation pattern and the candidate mass value the compounds are identified as chrysoeriol O-glucoside malonylated with molecular formula $C_{25}H_{24}O_{14,}$ chlorendic acid with molecular formula $C_9H_4Cl_6O_{4,}$ fenarimol with molecular formula $C_{17}H_{12}Cl_2N_2O$, eriocitrin with molecular formula $C_{27}H_{32}O_{15}$, gamma-glutamyl valine with molecular formula $C_{12}H_{21}N_3O_6$. From the results, the retention times of findings are matching with the standard compounds which confirms the presence of these chemical entities which can be used for pharmacological screenings and might be potential candidates for drugs.

5. Conclusion

Phytochemical screening isessential, for the estimation of important bioactive components in herbal resource. The present study was focused on pharmacognostic and phytochemical screening of methanolic leaves extract of *T. peruviana*. The LCMS result of current study showed the presence of chemical constituents like chrysoeriol O-glucoside malonylated,, fenarimol, eriocitrin and gamma-glutamylvaline, which might play a dynamic role in prevention and remedy for reported diseases like antimicrobial, antibacterial, fungicidal, insecticidal and anti-inflammatory properties. It can be concluded that methanolic extracts of *T. peruviana* leaves have great potential to be used as an alternative to biomedicine for antimicrobial diseases.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

Acknowledgements

The author wish to thank to Professor (Dr).Suneela Dhaneshwar, Director Amity Institute of Pharmacy, Amity University Uttar Pradesh, Lucknow campus for her support in conducting my research work in the laboratory.

References

Ahmad, T.; Hamid, A.; Sharma, A. and Bhardwaj, U. (2017). *Thevetia peruviana*: A multipurpose medicinal plant, a review. Int. J. Adv. Res., **5**(8):486-493.

Bandara, V.; Weinstein, S. A.; White, J. and Eddleston, M. (2009). A review of the natural history, toxinology, diagnosis and clinical management of *Nerium oleander* (common oleander) and *Thevetia peruviana* (yellow oleander) poisoning. Toxicon, **56**(3):273-281.

Bhavya, P.; Jhansi R. S.; Rithika, R.S.; Supriya, N.; Bakshi, V. and Boggula, N. (2019) Phytophamacological importance of *Thevetia peruviana*: A literature based review. Int. J. Pharmacol. Pharm. Res., **1**(1):33-37.

Das, K.; and Gezici, S. (2018). Secondary plant metabolites, their separation and identification, and role in human disease prevention. Ann. Phytomed., **7**:13-24.

Deshmukh, A.S. (2014). Pharmacognostic preliminary physiochemical investigations of plant *Thevetia peruviana* leaves and flowers. Reviews, **1**(10):650-653.

Gezahegn, Z.; Akhtar, M.S.; Woyessa, D. and Tariku, Y. (2015). Antibacterial potential of *Thevetia peruviana* leaf extracts against food associated bacterial pathogens. J. Coast. Life Med., **3**(2):150-157.

Ghavri, K. and Adhav, M. (2018). Comparative phytochemical screening of morphotypes of *Thevetia peruviana* (Pers.). J. Pharmacogn. Phytochem., **7**(5):539-541.

Ghavri, K. and Adhav, M. (2018). Phytochemical screening, ultra violet and infra-red spectroscopy of ethanolic leaf extract of *Thevetia peruviana* (Pers.) Thevetia yellow. J. Pharm. Innov., **7**:314-318.

Gomashe, S. S.; Ingle, K. P.; Sarap, Y. A.; Chand, D.andRajkumar, S. (2021). Safflower (Carthamustinctorius L.): An underutilized crop with potential medicinal values. Ann. Phytomed., **10** (1):242-248.

Gupta, R.; Kachhawa, J. B.; Gupta, R. S.; Sharma, A. K.; Sharma, M. C. and Dobhal, M.P. (2011). Phytochemical evaluation and anti-spermatogenic activity of *Thevetiaperuviana* methanol extract in male albino rats, Hum. Fertil., **14**(1):53-59.

Idris, S.; Mishra, A. and Khushtar M. (2021). Phytochemical estimation of germinated *Trigonellafoenum-graecum* L. seed extract for better application in phytotherapy, Ann. Phytomed., **10**(2):213-222.

Kareru, P. G.; Keriko, J. M.; Kenji, G. M. and Gachanja, A. N. (2010). Anti-termite and antimicrobial properties of paint made from *Thevetia peruviana* (Pers.) Schum. oil extract. Afr. J. Pharmacy Pharmacol., **4**(2):87-89.

Kohls, S.; Scholz, B.M.; Teske, J. and Rullkotter, J. (2010). Isolation and quantification of six cardiac glycosides from the seeds of *Thevetia peruviana* provide a basis for toxological survey. Ind. J.of Chem., **15**:1502-1510.

Kohls, S.; Scholz, B.M.; Teske, J.; Zark, P. and Rullkotter, J. (2012). Cardiac glycosides from yellow oleander (*Thevetia peruviana*) seeds. Phytochem., **75**:114-127.

Kokate, C.K.; Purohit, A.P. and Gokhale, S.B. (2005). A Textbook of Pharmacognosy. Nirali Prakashan, Pune. 2008.

Kumari, P.; Kumari, C. and Singh, P.S. (2017). Phytochemical screening of selected medicinal plants for secondary metabolites. Int. J. Life Sci. Res., **3**(4):1151-1157.

Mondal,K.; Chatterjee,A.; Bhattacharya, S.; Biswas, R. and Auddy, S. (2016). A review on various biological and pharmacological properties of *Thevetia peruviana*. Int. J. Adv. Res. Biol. Sci., **3**(9):178-182.

Naz, S.; Zafar, M.; Ahmad, M.; Memon, R. A.; Sultana, S.; Bahadur, S. and Shah, M. A. (2013). Palynological investigation of lactiferous flora (Apocynaceae) of district rawalpindi, pakistan, using light and scanning electron microscopy. Microsc. Res. Tech., **82**(9):1410-1418.

Phuse, S.S. and Khan, Z. H. (2018). Antioxidant and hemolytic quantification of Thevetia flowers in different extracts. Reviews **8**(2):110-116.

Rahman, N.; Mahmood, R.; Rahman, H. and Haris, M. (2014). Systematic screening for phytochemicals of various solvent extracts of *Thevetia peruviana*. Schum. leaves and fruit rind. Int. J. Pharm. Pharm. Sci., **6** (8):173-179.

Sangodare, R.S.A.; Agbaji, A. S.; Dakare, M. A.; Usman, Y. O.; Magomya, A.; Paul, E. D. and Shikeola, O. (2012). Investigation of the chemical constituent of extracts of *Thevetia peruviana* seed using GC-MS and FT-IR. Int. J. Food Nutr. Saf., **2**(1):27-36.

Save, S. A.; Padmanabhan, U.; Kothari, S. T.; Lokhande, R. S. and Chowdhary, A. S. (2015). Chemical and pharmacological study of the twigs of *Thevetia peruviana* plant and its bioactivity profiles. Int. J. Res. in Eng. App. Sci., **5**(6):123-38.

Save, S.A.; Lokhande, R.S. and Chowdhary, A.S. (2015). Determination of 1, 2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester from the twigs of *Thevetia peruviana* as a Colwell Biomarker. J. Innov. Pharm. Biol. Sci., **2**(3):349-362.

Sivakumar, P.; Monisha, S.; Selvaraj, K. V.; Chitra, M.; Prabha, T.; Santhakumar, M. and Velayutham, A. (2022). Nutritional value, phytochemistry, pharmacological and in vitro regeneration of turmeric (*Curcuma longa* L.): An updated review. Ann. Phytomed., **11**(1):236-246.

Citation **Pragyandip P. Dash, Sushil Kumar, Anuradha Mishra and Sajal Srivastava (2022). Phytochemical evaluation and physiochemical analysis of methanolic extract of *Thevetia peruviana* (Pers.) Schum. for future application in drug therapy. Ann. Phytomed., 11(2):643-653. http://dx.doi.org/10.54085/ap.2022.11.2.79.**