Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 13 (2), 429-435

Qualitative and quantitative screening of phytoconstituents and characterization of various extracts of *Wedelia trilobata* leaf extracts

Research Article

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Abstract

The birth control pills (contraceptive) are safe, in some cases the combination of the contraceptive pill can increase your risk of health. The side effects include heart attack, stroke, blood clots, and liver tumors. In very rare cases, they can lead to death. The risk of health problems and side effects were minimal or no side effects recorded while using the herbal contraceptives. The present investigation was focused on the preliminary phytochemical analysis, UV-VIS spectrum and Fourier Transform Infrared Spectral analysis and HPLC analysis of *Wedelia trilobata* L. The leaves were extracted using soxhlet extractor with seven different solvent in the order of relative polarity (aqueous, methanol, ethanol, acetone, petroleum ether, benzene and hexane) the leaves of the plant of *Wedelia trilobata* L. were tested for the availability of alkaloids, glycosides, flavonoids, phenols, saponins, steroids, amino acids, tannins, terpenoids, quinones, anthraquinones etc. And the phenol, flavonoid and terpenoids were analysed quantitatively. The UV-VIS spectrum showed the peaks with the absorption for the seven solvents respectively. The FT-IR spectrum showed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, aromatics, nitro compounds and amines. With the HPLC results we confirm that the compound caryophyllene which was used as contraceptive now a days was present in the two extracts (aqueous and ethanol) The results confirm the fact that this plant possesses important bioactive constituents useful for our health, so further scientific investigation is needed to investigate the biological activities of this plant.

Key Words: *Wedelia trilobata L.,* Phytochemicals, UV-VIS, FTIR, HPLC, Caryophyllene.

Introduction

Contraception also known as Birth control and fertility control, is a method or device used to prevent pregnancy. Birth control has been used since ancient times, but effective and safe methods of birth control only became available in the 20th century. (WHO) Even though the contraceptives were used since 20th century there will be a side effects of taking oral contraceptive pills. Most side effects of Oral Contraceptive Pill's are mild and disappear with continued use or switching to another pill formulation. The most common adverse effect of combined oral contraceptive pills is break through bleeding. Women will also complain of nausea, headaches, abdominal cramping, breast tenderness, and an increase in vaginal discharge or decreased libido(1). To overcome this problem from the synthetic medicines, conventional medicines were used which having very low or no side effects. Herbal based contraceptive medicine were promisingly improve the physical and mental health of the woman.

The herbal plant Wedelia, known officially by the scientific name, *Sphagneticola trilobata* (L.) Pruski, but still commonly referred to by its former name, *Wedelia trilobata* (L.) Native of this plant were West Indies, Hawaii, India, Burma, China, Japan, Ceylon, especially at low elevation (2). *W. trilobata* has antioxidant activity, anti bacterial activity, analgesic activity, and antimicrobial activities, these pharmacological activities may depend on its phytoconstituents such as tannin, saponins, flavonoids, phenol and terpenoids. *W. trilobata* is employed to treat backache, muscle cramps, rheumatism, stubborn wounds, sores and swellings, and arthritic painful joints. Anticonceptive activity was described for some extracts and the isolated compounds, kaurenoic acid and luteolin, from S. trilobata (3). The present study was designed to develop the plant based anti-conceptive medicine using *W. trilobata* leaves extract.

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Materials and methods

Plant Collection and extraction

The *W. trilobata* leaves were collected from Thanjavur nursing garden. Then leaves were washed with tap water and distilled water before initiating the experiment. 100g of W. trilobata (L.) leaves were weighed and extracted in soxhlet's apparatus using

distilled water, methanol, ethanol, acetone, petroleum ether, benzene, hexane as a solvent. Then the extract was filtered using what man No. 1 filter paper and stored at 4 °C. The solvents were removed using rotary vacuum evaporator and stored in desiccator for further use(4).

Qualitative analysis of phytochemicals

Test for Alkaloids (Wagner's test)

2-3 ml of plant extracts was mixed with few drops of Wagner's reagent and kept undisturbed for 3-4 minutes. The reddish-brown precipitate indicates the presence of alkaloid (5).

Test for Flavonoids (Sodium hydroxide test):

0.2 g of the plant extract was dissolved in a cold dilute solution of NaOH and diluted hydrogen chloride. The Appearance of the yellow-colored solution that turns colorless indicates the presence of flavonoids (5).

Tests for Carbohydrates (Molish's test)

2-3 ml of plant extracts was added with few drops of α-napthol solution and shake well then add few drops of concentrated sulphuric acid along the sides of the test tubes. The formation of violet ring at the junction indicates the presence of carbohydrates (5).

Tests for Protein (Biuret's test)

2-3 ml of plant extracts was added with a few drops of 4 % sodium hydroxide and 1% Copper sulphate solutions. Appearance of Violet or pink color indicates the presence of proteins(5).

Test for Terpenes (Copper acetate test)

3 ml of plant extracts was added with 8-10 drops of copper acetate solution and the formation of emerald green color indicates the presence of terpenes (5).

Test for Tannin (Lead acetate test)

2-3 ml of plant extracts was added with 3 ml of lead acetate solution and the occurrence of white precipitates indicates the presence of tannins and phenols (5).

Test for Saponins (Foam test)

Few ml of the plant extract was added with small amount of water and shaked vigorously for about 10 min and the presence of stable foam indicates the presence of saponins (5).

Test for Anthraquinone glycosides (Borntrager's test)

Few ml of plant extracts was boiled with dilute sulphuric acid, filtered and then filtrate is added with chloroform is added and shaken well. The organic layer is separated to which ammonia is added slowly. The ammoniacal layer shows pink to red color due to presences of anthraquinone glycosides (5).

Test for Cardiac glycosides (KellarKillani's test)

Few ml of plant extracts was dissolved in water with Glacial acetic acid and ferric chloride and concentrated sulphuric acid. The formation of brown ring at the junction indicates the presence of cardiac glycosides (5).

Test for Phenols and Tannins (Ferric chloride test)

0.5 ml of plant extract mixed with 5 ml of D. H_2O and boiled for 10 min then 2 ml of collected filtrate was added with a few drops of 10% ferric chloride solution. Appearance of greenish blue or violet color indicates the presence of a phenolic hydroxyl group (5).

Test for Steroids (Salkowski Tests)

2-3 ml of plant extracts mixed with 2 ml of chloroform & 2 ml of concentrated sulphuric acid and shaken well. The chloroform layer appear red and acid layer shows greenish yellow fluorescence shows the presence of steroids (5).

Test for Fat and Oils (Saponification test)

2-3 ml of plant extracts was mixed with 0.5 N alcoholic potassium hydroxide solution along with 2 drops of phenolphthalein and heated for about 2 hrs. The formation of soap indicates the presence of fixed oils and fats (5).

Test for sterols (Lieberman test)

The plant extracts were evaporated and the residues were extracted with petroleum ether and acetone. The insoluble residues left after extraction were dissolved in chloroform and few drops of acetic anhydride were added along with a few drops of concentrated sulphuric acid from the side of the tube. The appearance of blue to blood red color indicates the presence of sterols in the extracts (5).

UV analysis

The light of ultra-violet region (200-400 nm) is absorbed by the molecule which is used for qualitative and quantitative characterization of sample compounds .The sample is diluted to 1:10 with the same solvent. The extract was scanned at wave length ranging from 200 to 400 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. The peak values of the UV-VIS were recorded (6).

FTIR analysis

Fourier transform infrared spectrophotometer (FTIR) is perhaps the most powerful tools for identifying the types of chemical bonds (functional groups) present in compounds. Dried powders of different solvent extracts of plant extract were used for FTIR analysis. 10mg of the dried W. trilobata powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc. The powdered sample of the extract was loaded in FTIR spectroscope (Shimadzu, Japan), with a Scan range from 400 to 4000 cm-1 with a resolution of 4 cm-1 (7).

HPLC analysis

The modified protocol for HPLC analysis were used the acetone, acetonitrile (ACN) and water HPLC

grade were purchased from Finar Chemicals (Ahmedabad, India), Beta-caryophyllene standard (>98.5%) were Purchased from Sigma Aldrich (St. Louis, USA). The extracts used for HPLC analysis were passed through a 0.45-µm filter (Milli-pore, MSI, West boro, MA) and then the filtrates were passed into the column flow rate of 1.0 ml/min (Waters, C18 silicon column, reverse phase, Australia) and the mobile phase set as isocratic elution mode (50:50 proportion of acetonitrile:water) Then, the hits were detected at 200 to 400 nm(8).

Result and Discussion

Qualitative analysis

The qualitative phytochemical analysis of seven solvents (Polar to non-polar) of W. trilobata revealed that presence of various Phyto constituents. Anthraquinones were absent in all the extracts. Terpenoids were only present in the aqueous and ethanolic extract. The least number of phytoconstituents were seen in acetone extract. Aqueous extract and ethanolic extract showed the presence of all phytochemicals except anthraquinones.

Table 1: Qualitative analysis of various extracts of W. trilobata

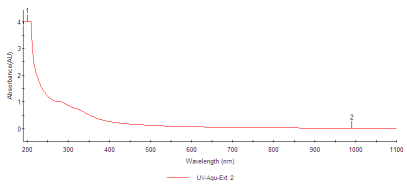
Phytochemical	Hexane	Benzene	Petroleum	Acetone	Ethanol	Methanol	Aqueous
Alkaloids	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+
Protein	-	+	+	-	+	+	+
Terpenoids	-	-	-	-	+	-	+
Tannins	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-	-
Cardiac glycosides	-	+	-	-	+	+	+
Phenol	+	+	+	+	+	+	+
Steroids	+	-	+	_	+	+	+
Fixed oil and fats	+	+	+	+	+	+	+
Sterol	-	+	-	-	+	+	+

UV Analysis

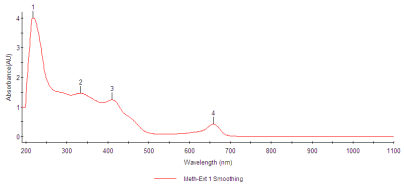
Table 2 indicates the qualitative UV-VIS profile of seven extracts form polar to non-polar such as aqueous methanol, ethanol, acetone, petroleum ether, benzene, hexane extracts of W. trilobata and the wavelength taken at 200 nm to 400 nm due to the sharpness of the peaks and proper baseline. The presence of an absorbance band at a particular wavelength is a good indicator for the presence of a chromophore and results in the excitation of the electrons from the ground state to higher energy state. The profile showed the peaks at the absorbance of 4.00 and 0.02 with the wavelength of 200.2 and 908.5. Acetone, benzene and hexane shows higher number of peaks. Acetone extract shows the lowest absorbance of 0.01 in the wavelength 970.8. conversely hexane extract shows the highest absorbance of 4.20 with the wavelength 235.4. Ethanol and Acetone having three peaks between the UV wave length which shows the maximum absorption of UV rays.

Figure 1 shows the absorption spectrum of seven extracts of W. trilobata and these are almost transparent in the wavelength region of 200-400 nm. Absorption bands observed pertaining to aqueous extract of *W. trilobata* were displayed.

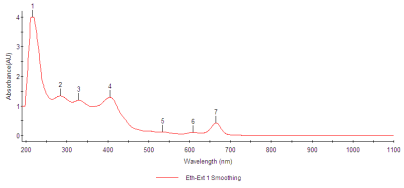
Figure 1: UV spectrum of various extract of *W.trilobata*



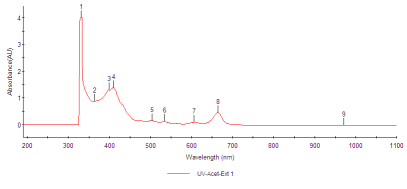
a) Aqueous extract



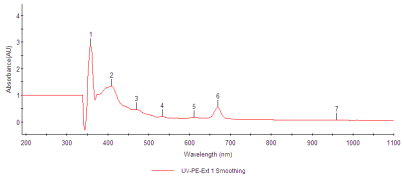
b) Methanol extract



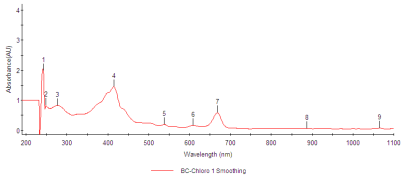
c) Ethanol extract



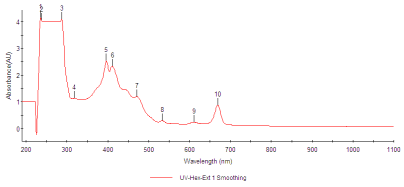
d) Acetone extract



e) Petroleum ether extract



f) Benzene extract



g) Hexane extract

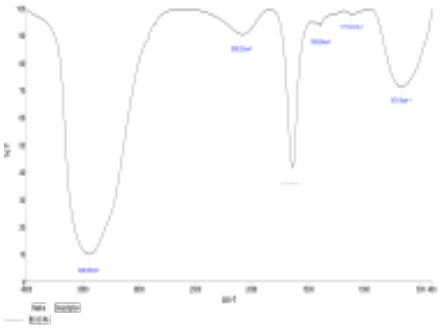
Table 2: UV analysis of various extract of *W. trilobata*

Hexane		Benzene		Pt. ether		Acetone		EtOH		MeOH		Aqueous	
W	Ab	W	Ab	W	Ab	W	Ab	W	Ab	W	Ab	W	Ab
235.4	4.20	241.5	2.05	357.9	2.88	331.3	4.00	215.6	4.00	217.5	4.00	200.2	4.00
237.8	4.06	247.7	0.84	408.7	1.33	364.2	0.87	283.6	1.33	333.2	1.46	989.5	0.02
287.3	4.09	276.2	0.82	469.7	0.46	398.8	1.30	329.4	1.19	410	1.23		
318.3	1.12	415.0	1.44	533.0	0.20	410.0	1.38	405.0	1.29	658.2	0.41		
396.3	2.53	538.0	0.19	611.2	0.17	504.3	0.14	534.2	0.12				
411.2	2.32	608.7	0.15	669.4	0.54	534.2	0.11	608.7	0.10				
470.9	1.19	668.2	0.58	959.6	0.06	606.2	0.08	664.5	0.42				
533.0	0.29	887.4	0.06			664.5	0.44						
611.2	0.23	1,065.4	0.07			970.8	0.01						
669.4	0.87												

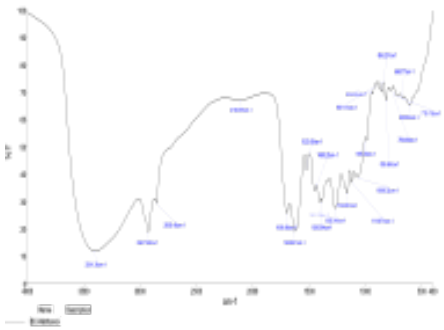
FTIR analysis

The FTIR spectrum was used to identify the functional groups of the active components present in plant extracts based on the peak values in the region of IR radiation. When the plant extract was analyzed into the FTIR, the functional groups of the compounds were appeared on different wave's length. The results of analysis of crude extract W. trilobata were given in tables 10-16 and figs. 8-14. These results showed that alkenes, esters, nitro compounds, alcohols, ethers, aromatics, carbonyls, aldehydes, alkyl halides were present in the extracts. FTIR spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular composition.

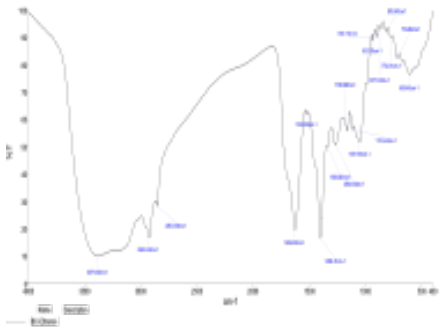
Figure 2: FTIR spectrum of various extract of *W.trilobata*



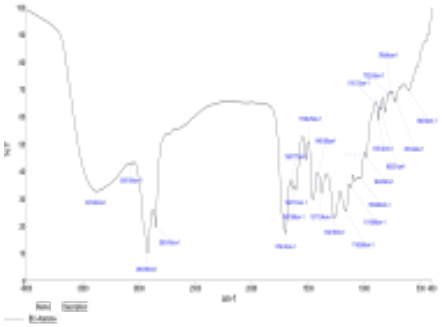
a) Aqueous extract



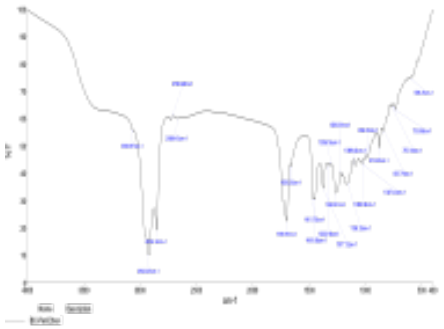
b) Methanol extract



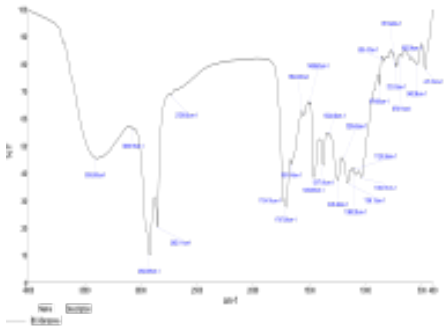
c) Ethanol extract



d) Acetone extract



e) Petroleum ether extract



f) Benzene extract

g) Hexane extract

Table 3: FTIR analysis of various extract of *W. trilobata*

Functional group	HE	BE	PE	A	E	M	Aq
Alcohols and phenols	-	3395.93	-	-	-	-	3438.49
1˚,2˚ amines, amides	-	1499.63,	-	3376.02, 1518.25, 1607.51,	-	3391.36, 1629.21	-
Alkanes	2922.08, 2852.45, 1377.06, 1451.29	2922.09, 2852.11, 731.62	2922.25	2853.06	2926.23, 1402.41, 1340.82, 719.49	2927.95, 2855.44, 1450.23, 1385.04, 719.13	1385.24
Carboxylic acids	2578.06	1657.44	-	3067.07, 2925.06	-	934.55	-
Aldehydes	2727.98	2729.83, 1734.74	2862.32, 2728.48, 2668.45	-	2853.39	-	-
α, β-unsaturated aldehydes, ketones	-	-	1668.75	-	-	-	-
Alkenes	3064.39, 1657.62,	1552.27, 1324.40	984.63	1657.77, 984.02, 720.34,	1629.35, 900.16, 933.78, 871.72, 772.01, 818.47	985.54, 901.18	672.16
Aromatics	874.47, 767.56	1377.81, 874.60, 720.33	3064.97, 1451.75, 874.20	1377.94, 873.82, 855.31	1523.08	856.27	-
Nitro compounds	-	1462.66	1481.88, 1328.18, 1306.16	1461.65	-	1523.83, 1492.05	-
Aromatic amines	1326.52	1245.26, 1205.65	-	-	-	1285.41	-
Alkyl halides	1261.26, 837.0, 600.28	835.47, 679.11, 504.08	1206.61, 1326.16, 1377.12, 1158.33, 837.79, 767.97, 733.98, 599.25	816.12, 732.60, 768.64, 604.64	1051.50, 1116.62	1163.01, 1187.71, 816.84, 768.88.	-
Aliphatic amines	1207.11, 1176.86, 1089.34, 1028.37	1090.03, 1158.15, 1129.56, 1034.73	1089.40, 1027.40	1118.80, 1034.84	-	1069.32	-
Alcohols, carboxylic acids, esters, ethers	1306.57, 1007.17	-	-	1162,68	1163.20	-	1117.21

Note: HE – Hexane, BE – Benzene, PE – Petroleum ether, A – Acetone, E – Ethanol, M – Methanol, Aq – Aqueous

The aqueous extract of W. trilobata showed characteristic absorption bands between 3438.49 – 1637.93 with the functional group of alcohols, alkanes, acids, nitro compounds. Benzene extract shows the higher number of peaks followed by the hexane extract and petroleum ether extract. The least number of peaks were found in the aqueous extract. Alkyl halide and alkanes were the functional group present in all the extracts in higher number. Alcohols and phenols were only present in the benzene and aqueous extracts. Primary and secondary amines were present in Benzene, acetone, and methanolic extracts (1499.63), (3376.02, 1518.25, 1607.51), (3391.36, 1629.21) respectively. Ethanolic extract contains alkanes, alkenes, aromatics, aldehydes, alkyl halides and alcohols (2926.23, 1402.41, 1340.82, 719.49), (1629.35, 900.16, 933.78, 871.72, 772.01, 818.47), (1523.08), (2853.39), (1051.50, 1116.62), (1163.20) in the respective peaks. Aldehydes and ketones were only present in the Petroleum ether extract. The result revealed that ethanol and aqueous extract having alkenes, aromatics and alcohols which was compared with the functional groups of Beta-caryophyllene compound. From the above result it was confirmed that Beta Caryophyllene was present in the aqueous and ethanolic extract.

Further the HPLC analysis of aqueous and ethanolic extract was performed for the Conformation of presence Beta-caryophyllene of the compound.

HPLC analysis

Aqueous extract

Figure 3: HPLC chromatogram of aqueous extract of *W. trilobata*

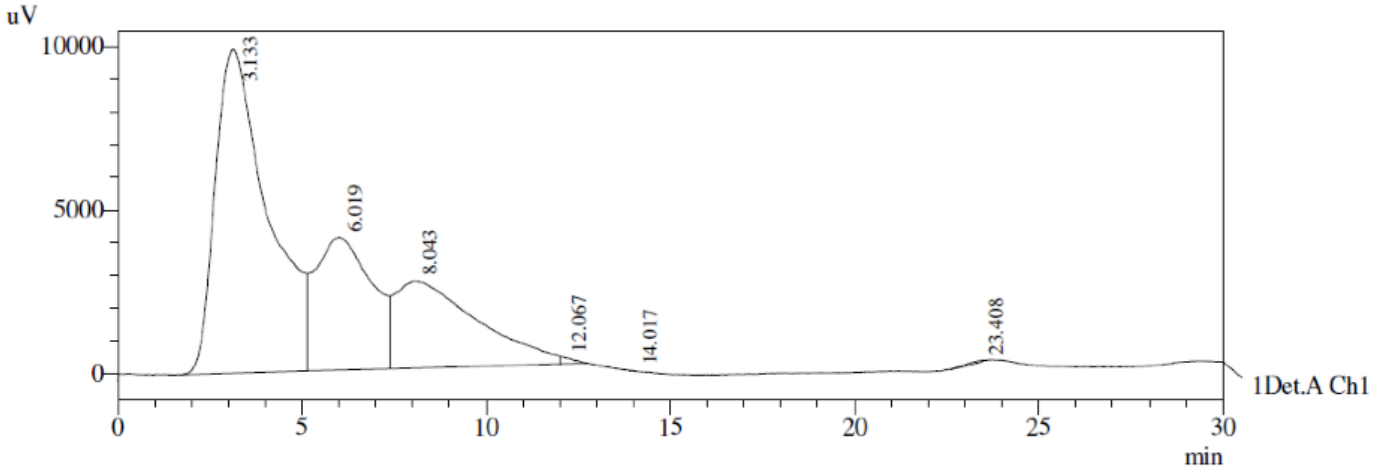


Table 4: HPLC peak report of aqueous extract of *W. trilobata*

Peak	Ret. time	Area	Height	Area%	Height %
1	3.133	980564	9886	53.580	58.640
2	6.019	431321	4056	23.568	24.058
3	8.043	412096	2654	22.518	15.742
4	12.067	4500	213	0.246	1.262
5	14.017	62	8	0.003	0.048
6	23.408	1565	42	0.085	0.250
Total		1830108	16860	100.00	100.00

Results of HPLC analysis of *W. trilobata* aqueous extract, shows presence of various constituents as evidenced by the chromatogram obtained at various retention times (3.133, 6.019, 8.043, 12.067, 14.017, 23.408) are the Phyto constituents found in *W. trilobata* leaves.

Ethanol Extract:

Figure 4: HPLC chromatogram of ethanolic extract of *W. trilobata*

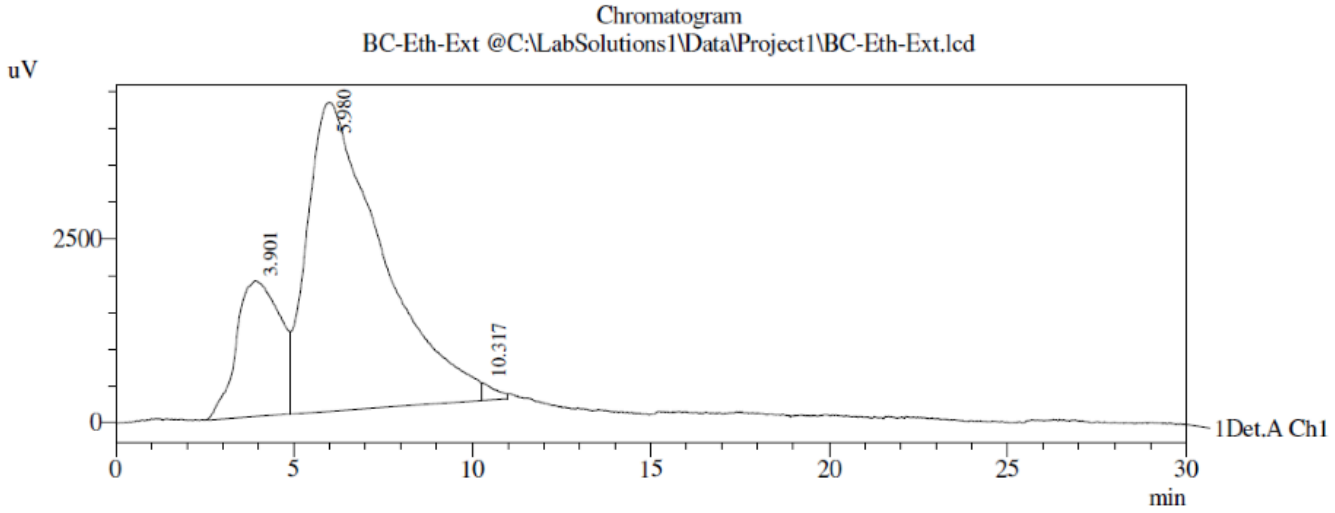


Table 5: HPLC peak report of ethanolic extract of *W. trilobata*

Peak	Ret. time	Area	Height	Area%	Height%
1	3.901	157675	1840	20.210	29.360
2	5.908	616487	4191	79.020	66.865
3	10.317	6008	237	0.770	3.774
Total		780170	6268	100.00	100.00

Results of HPLC analysis (Figure-4) of *W. trilobata* ethanolic extract, shows presence of various constituents as evidenced by the chromatogram obtained at various retention times (3.901, 5.980, 10.317)

The above HPLC analysis revealed that the compound beta caryophyllene which was used as contraceptive were present in the above two extracts aqueous and ethanol was revealed. From the above results these two extracts of W. trilobata was taken for the further studies for the herbal contraception of the females.

Conclusion

In the present study analysis of the plant extract of W. trilobata sample under FTIR and UV-VIS spectroscopic technique and HPLC analysis showed that the presence of Flavonoid phenol and terpenoid compound. The presence of these bioactive compounds in W. trilobata plants lends credence to its use by the human community. It is revealed that the terpene compound Beta caryophyllene which was present in the two extracts of the W. trilobata leaves such as aqueous and ethanol. It also holds for the production of Herbal contraceptive. It could be concluded that W. trilobata contains various bioactive compounds. So, it is recommended as a plant of phytopharmaceutical importance. However, further studies will need to be undertaken to determine fully its biological activity, toxicity profile for herbal contraceptive medicine.

Conflict of interest

The authors declare that they have no conflict of interest for this study.

Funding support

The authors declare that they have no funding support for this study.

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