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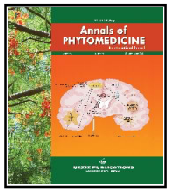

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GC-MS analysis and isolation of few bioactive phytoconstituents from *Ixora parviflora* Lam.

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Abstract

Ixora parviflora Lam. is a small garden tree which is very well known for its medicinal properties and health benefits as mentioned in Ayurveda and Siddha system of medicine. In spite of many uses of the plant, there is no much research done and only a few compounds were isolated from the plant. This article main aim is to present the extensive research work carried out on this plant for characterization and isolation of phytoconstituents. Hexane, ethyl acetate and methanol extracts were analysed by GC-MS to investigate the active components in the plant. After successive extraction of the leaves, the crude methanolic extract of the leaves was subjected to column chromatography for the isolation of compounds using various solvent systems and they were characterized using spectroscopic techniques like NMR, FTIR and mass for elucidation of the structures. Seven compounds were isolated and some of them are being reported for the first time in the plant such as oleanolic acid, betulinic acid, caffeic acid and gallic acid. Apigenin and chlorogenic acid are being reported first time in leaf. They were earlier reported in flowers of this plant. Betulin which was reported earlier was also isolated. The isolation of phytoconstituents is overriding to make the treatment of diseases or disorders easy and is a foremost step in new drug development process.

1. Introduction

Plants are the best resources for new prudent, guarded and renewable drugs. On explore of scientific explanations of medicinal plants for the therapeutic uses by primitive man, a well regulated investigation should explore plants as medicinal tools (Perumal, 2000; Mohd. Kashif Husain, 2021). Natural products are requisite sources of new pharmaceuticals, furthermore, the development of powerful analytical tools and other 21st century technologies are considerably facilitate identification and characterization of natural products (Linh, 2013; Yue Li, 2023). Unremitting discovery has been cleared the way by expansion of new methods (Duraipandiyan, 2003). The bioactive compounds are mostly plant metabolites naturally occurring compounds which have become dietary supplements, plant based medicines and other useful commercial products. These lead candidates can be promoted to modify the biological profiles of the compounds (Hideji Itokawa *et al.,* 2008; Sohaimy, 2012). Although, the use of modern medicines imply multiple of challenges, looking over severe side effects and drug resistance to antibiotics or even anticancer medications requires the development of novel inventions or discoveries (Lee *et al.,* 2008; Nabih *et al.*, 2023).

Regardless of the great diversity derived from the evolution of high yield screening methods over the past years, natural products continue to be extremely important elements of literatures (Newman, 2012; Gongora Castillo, 2013). Ahead, natural products and their associated structures are fairly to become important for development of advanced medicines, due to the diverse variety of functionally relevant secondary metabolites of plant species whose chemical and genetic diversity are being revealed by related genomics tools (Li, 2009; Walsh, 2010). Many bioactive compounds have been discovered from natural sources using bioactivity directed fractionation and isolation (Balunas and Kinghorn, 2005). Gas chromatography mass spectroscopy (GC-MS) is an analytical method that combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. GC-MS plays pivotal role in phytochemical components evaluation and chemotaxonomic studies regarding pharmacologically active components of medicinal plants (Hethelyi *et al.,* 1987; Uma and Balasubramaniam, 2012). This method analyses plant extracts that makes an interesting tool for testing the amount of few active principles in herbs used in drugs, pharmaceutical or food industry, cosmetic, environmental and forensic applications (Uma *et al.,* 2009).

Ixora parviflora Lam. (Syn. *Ixora pavetta* Andr.), is a small flowering tree found in gardens. It bears white aromatic flowers in bunches. The local names include Nevaari, Nepali, Navmalika and Vaasanti in Ayurveda and Shulundukora, Korivi in Siddha system of medicine. In Ayurveda, Nevaari is described as light, cooling, and bitter. Due to cooling property, it is given in case of bleeding disorders. It balances Vata, Pitta and Kapha. The plant mainly grows at dry deciduous forests distributed at areas of Western Bengal, Bihar, Western, Central and South India. Tribes of Nellore district, Andhra Pradesh, used root bark infusion as ethnic practice to cures jaundice and burning micturition (Srinivas, 2011). The flowers of *I. parviflora,* pounded

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in milk treating for whooping cough. The decoction of the flowers is given for hemoptysis, catarrhal bronchitis and dysmenorrhea. The decoction of the roots was given for dysentery and as a sedative for hiccoughs, nausea, loss of appetite, fever and gonorrhea. The root is pulverized and mixed with ginger and rice water to treat dropsy (Srinivas and Baboo, 2011). The plant contains flavonoids, glycosides, saponins and tannins, phenols and triterpinoids produced a significant dose dependent increase in the enzymatic antioxidants of liver like superoxide dismutase, catalase and glutathione levels. The hepatoprotective and antioxidant activity produced by whole plant of methanolic extract may be due to the presence of flavonoids, glycosides, saponins, tannins, phenols and triterpinoids (Suneeta and Tirupathi, 2020). Butenolic fraction of the plant has antiobesity activity in high fat diet induced obesity in wistar rat model (Kanhere *et al.,* 2014). Major compounds like α-sitosterol, α-sitosterol-α-D-glucoside, kaempferol-7-O-methyl ether and kaempferol were isolated (Bachheti *et al.,* 2011).

There is no previous literature about the spectral studies and isolated compounds of *I.parviflora*. The main aim of the present study to characterize the phytoconstituents present in various extracts of *I. parviflora* by GC-MS chromatographic studies and isolate few bioactive compounds by column chromatography structurally confirmed by various spectroscopical studies. The images of flower, leaves, fruits and bark of *I. parviflora* are given in the Figure1.

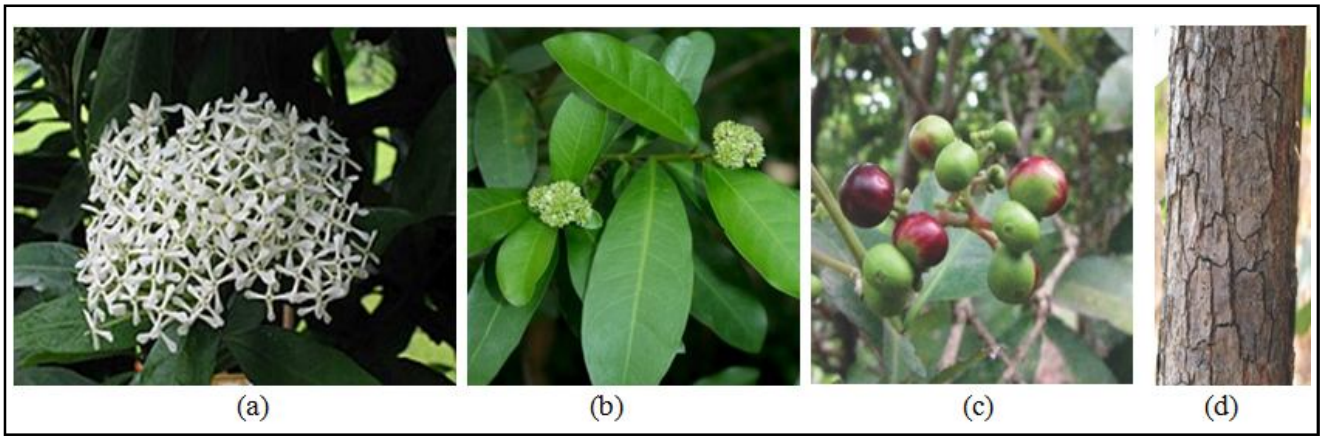


Figure 1: Various parts of *I. parviflora* a) flower, b) leaves, c) fruits, d) bark.

2. Materials and Methods

2.1 Preparation of extract

Fresh leaves of *I. parviflora* were collected by G. Ravi, Taxonomist from Manuguru forest, Telangana and authenticated by Dr. P. Laxman, Associate Professor, Govt. Degree College, Kukatpally, Telangana. The leaves were shade dried for 15 days and made into powder. A voucher specimen with number GDC/IPL/5626 was stored. The shade dried leaf powder of *I. parviflora* each 500 g was extracted by hot percolation with hexane, ethyl acetate and methanol (1000 ml) using Soxhlet apparatus. The residue solvents were vacuum evaporated and the crude hexane extract, ethyl acetate extract and methanol extracts were kept in desiccators for further use. Percentage yield of the extracts were calculated using formula:

Percentage yield (%w/w) = Practical yield/ Theoritical yield × 100

2.2 Gas chromatography mass spectrum (GC-MS) analysis and identification of compounds

Hexane extract, ethyl acetate extract and methanol extract were separately analysed for the various constituents present by GC-MS. Instrument used for analysis was GC-MS-QP2010 plus (Shimadzu, Kyoto, Japan) with headspace sampler (AOC-20s) and auto injector (AOC-20i). The system was equipped with mass selective detector with an ion source having temperature 250°C and interface temperature 300°C. The column used was capillary column with 30 m × 0.25 mm (length × diameter) and 0.25 ìm of film thickness. The temperature of the injector was adjusted to 250°C, possessing a split injection mode. The initial temperature applied was 50°C (3 min), which was further programmed to increase to 280°C at a ramp rate of 10°C /min. Helium (99.99%) was used as carrier gas with 35.3 cm/sec of linear velocity. The total flow programmed was 8.7 ml/min, with column flow of 0.95 ml/min. Relative quantity of the chemical compounds present in each of the extracts was expressed as percentage based on peak area produced in the chromatogram. Identification of compounds achieved based on retention indices and interpretation of mass spectrum using the database library of National Institute of Standards and Technology (NSIT).

2.3 Fractionation and isolation of phytoconstituents from *I. parviflora* methanol extract

The methanol extract was chosen for the isolation of compounds as it showed more activity for antimitotic assay compared to other extracts which was reported in the previous study (Srivani and Krishna Mohan, 2022). 90 g of methanolic extract of *I. parviflora* was used for packing of column. The methanol extract was adsorbed on silica powder for dry loading of the sample. Initially the column was packed with silica gel (60-120 mesh silica gel) using an eluent system of increasing polarity of ethyl acetate: n-hexane (40:60) up to 40 major fractions. Later, the column elution was done using methanol: chloroform starting with 10:90 thereby increasing the concentration of methanol: chloroform to 50:50 to collect 50 more fractions. All the fractions were developed TLC and the similar spots identified were pooled up. These fractions were again subjected to column (100-200 mesh silica gel) with increasing polarity of the solvent system starting with chloroform: n-hexane (10:90) and ended up with methanol: chloroform (30:70). Fractions (F) 1-10 gave a fatty waxy residue which was kept aside, F11-16 in chloroform:

hexane (20:80) yielded compound 1, F17- 23 in chloroform: hexane (30:70) yielded compound 2, F24 to F32 does not contain any active compound. F33- 39 in methanol: chloroform (2:98) gave compound 3, F40-48 in methanol: chloroform (8:92) yielded compound 4. F49 to F60 gave a brown sticky matter. F61-68 in methanol: chloroform (10:90) yielded compound 5, F69- 76 in methanol: chloroform (20:80) yielded compound 6, F77-84 in methanol: chloroform (30:70) yielded compound 7. All the compounds were purified by solvent washing or subjecting to flash chromatography. They were submitted to various analytical techniques for the elucidation of the structures.

3. Results

3.1 Percentage yield and physical status of extracts

Dried hexane, ethyl acetate and methanol extract were calculated for their percentage yield (%w/w) and the physical status of the extracts was presented in the following Table 1.

Table 1: Percentage yield of *I. parviflora* extracts

Extract	Percentage yield (%w/w)	Physical status of extract
Hexane extract	10.4	Light green and dry
Ethyl acetate extract	17.6	Green and wet
Methanol extract	21.2	Dark green and sticky

3.2 Gas chromatography mass spectrum (GC-MS) analysis of the extracts

Hexane, ethyl acetate and methanol extracts of *I. parviflora* were characterized for the various phytoconstituents using GC-MS. It revealed that hexane extract consists of seventy compounds, ethyl acetate extract sixty six compounds and methanol extract has thirty three compounds. The GC-MS chromatogram of hexane, ethyl acetate and methanol extracts of *I. parviflora* were presented in the Figures 1, 2 and 3, respectively. In all the extracts some important constituents are screened from the obtained data and presented in the Tables 2, 3 and 4.

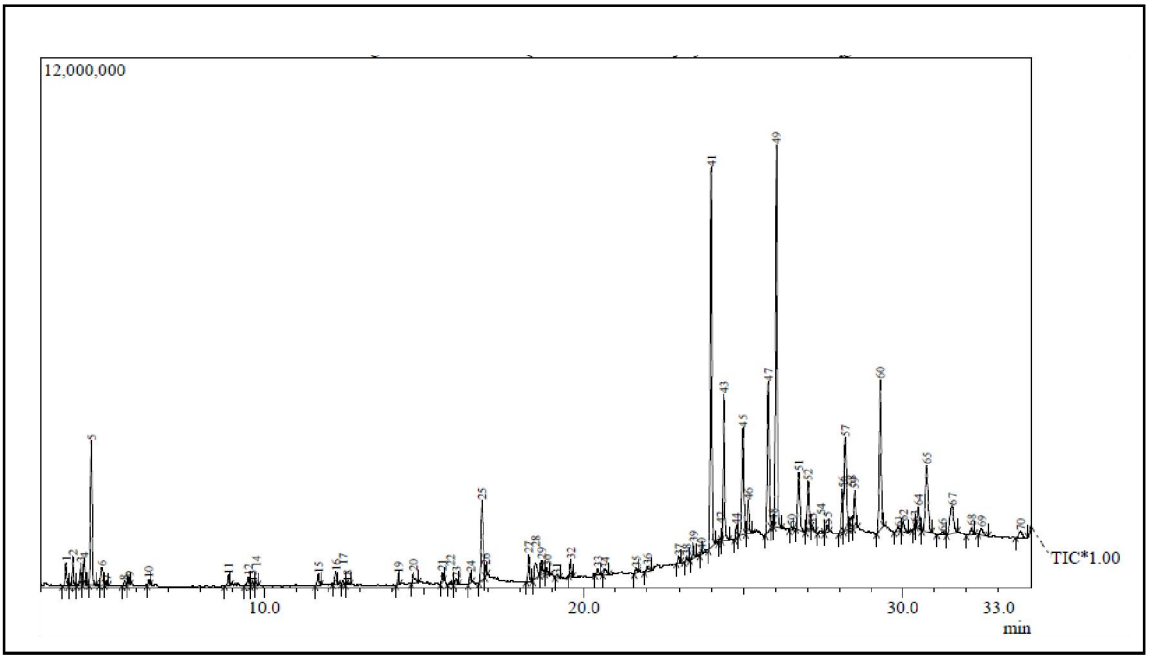
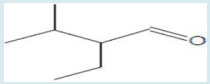
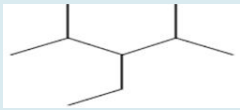
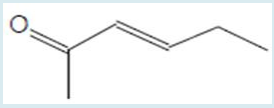
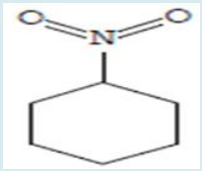
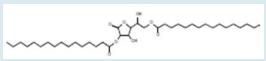
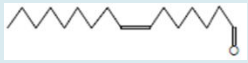
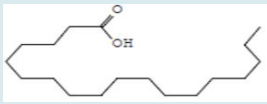
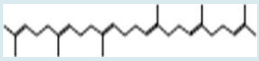
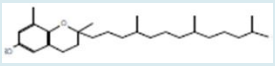
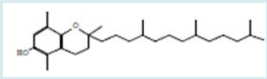
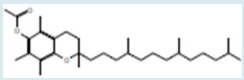
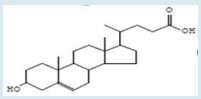
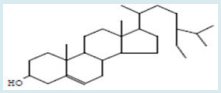
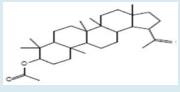


Figure 2: GC-MS chromatogram of hexane extract of *I. parviflora*.

Table 2: Characterization of phytoconstituents of hexane extract of *I. parviflora*

Peak	Retention time	Area%	Name	Formula	Structure
2	4.019	1.01	2-ethyl-3-methyl- butanal	$C_7H_{14}O$	
4	4.374	0.84	3-ethyl-2,4-dimethyl- pentane	C_9H_{20}	

5	4.594	4.68	3-Hexen-2-one	$C_6H_{10}O$	
8	5.632	0.15	Nitrocyclohexane	$C_6H_{11}NO_2$	
16	12.241	0.49	Heneicosane	$C_{21}H_{44}$	
25	16.819	3.67	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	
28	18.489	1.06	7-Hexadecenal	$C_{16}H_{30}O$	
29	18.691	0.60	Octadecanoic acid	$C_{18}H_{36}O_2$	
41	23.997	13.26	2,6,10,14,18,22-Tetracosahexaene	$C_{30}H_{50}$	
45	24.988	4.56	2H-1-Benzopyran-6-ol	$C_{27}H_{46}O_2$	
46	25.159	1.18	Tetrapentacontane	$C_{54}H_{110}$	
47	25.778	5.70	beta.-Tocopherol	$C_{28}H_{48}O_2$	
50	26.505	0.12	Vitamin E acetate	$C_{31}H_{52}O_3$	
56	28.103	1.79	3Beta-hydroxy-5-cholen-24-oic acid	$C_{24}H_{38}O_3$	
60	29.288	7.41	Gamma-sitosterol	$C_{29}H_{50}O$	
65	30.736	4.47	Lup-20(29)-en-3-ol, acetate, (3.beta.)	$C_{32}H_{52}O_2$	

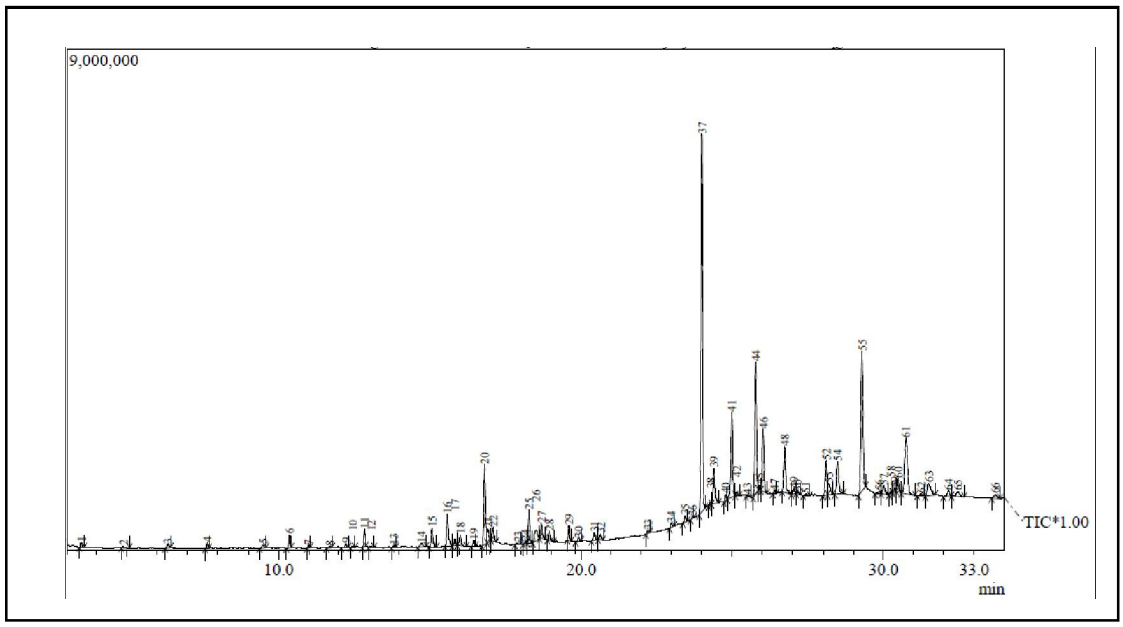
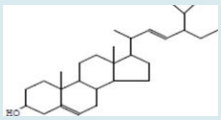
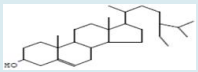
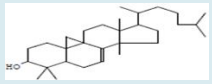
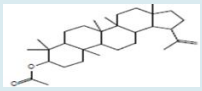
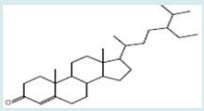
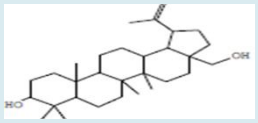
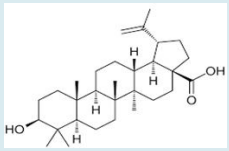
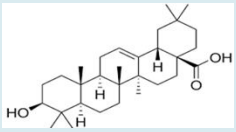


Figure 3: GC-MS chromatogram of ethyl acetate extract of *I. parviflora.*

Table 3: Characterization of phytoconstituents of ethyl acetate extract of *I. parviflora*

Peak	Retention time	Area%	Name	Formula	Structure
1	3.482	0.27	1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	
11	12.843	0.86	9-Eicosene, (E)	$C_{20}H_{40}$	
13	13.807	0.11	8-Pentadecanone	$C_{15}H_{30}O$	
16	15.581	2.22	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	
20	16.815	5.41	Pentadecanoic acid	$C_{15}H_{30}O_2$	
25	18.283	1.82	Phytol	$C_{20}H_{40}O$	
27	18.691	1.02	Octadecanoic acid	$C_{18}H_{36}O_2$	
29	19.602	0.77	Hexadecyl Oxirane	$C_{18}H_{36}O$	
36	23.686	0.11	Pentatriacontane	$C_{35}H_{72}$	
37	23.996	21.50	2,6,10,14,18,22-Tetracosahexaene	$C_{30}H_{50}$	
41	24.989	6.30	3,4-dihydro-2,8-dimethyl- 2H-1-Benzopyran-6-ol	$C_{27}H_{46}O_2$	
48	26.733	3.44	Vitamin E	$C_{29}H_{50}O_2$	
52	28.100	2.84	Beta-hydroxy-5-cholen-24-oic acid	$C_{24}H_{38}O_3$	

54	28.488	2.49	Stigmasterol	$C_{29}H_{48}O$	
55	29.289	11.18	gamma.-Sitosterol	$C_{29}H_{50}O$	
60	30.484	0.92	9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	$C_{30}H_{50}O$	
61	30.739	6.24	Lup-20(29)-en-3-ol, acetate, (3.beta.)	$C_{32}H_{52}O_2$	
63	31.496	1.50	Stigmast-4-en-3-one	$C_{29}H_{48}O$	
64	32.148	0.48	Betulin	$C_{30}H_{50}O_2$	
65	32.457	0.60	Betulinic acid	$C_{30}H_{48}O_3$	
66	33.697	0.44	Oleolonic acid	$C_{30}H_{48}O_3$	

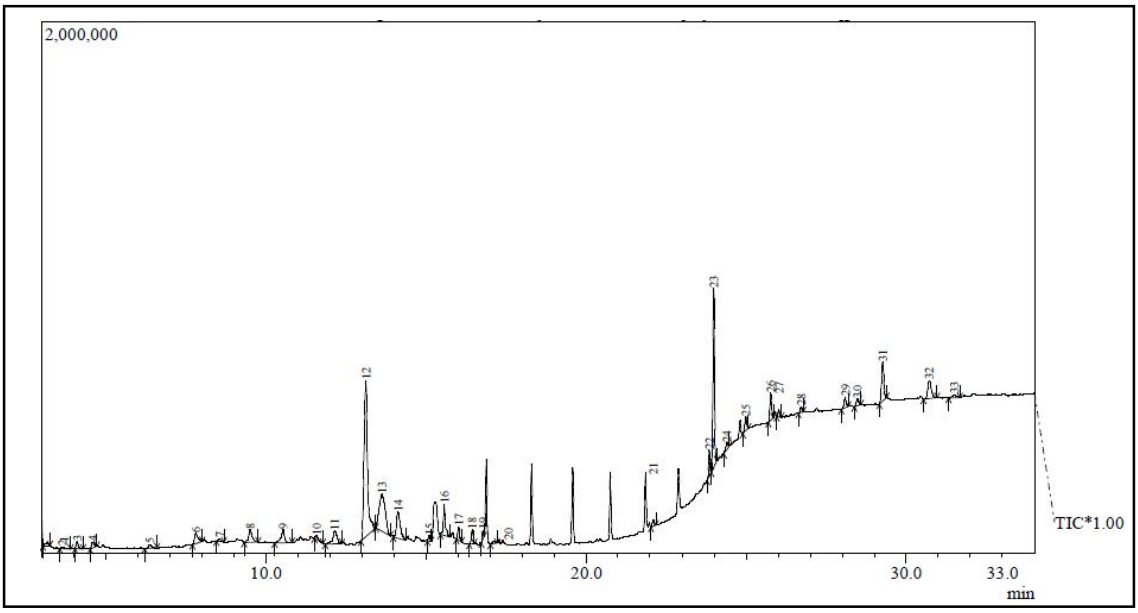
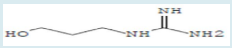
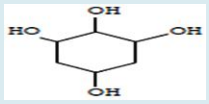
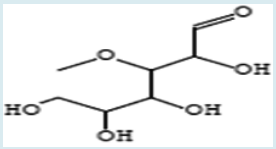
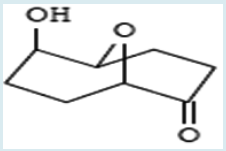
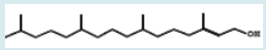
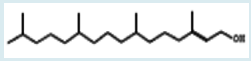
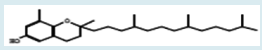
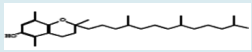
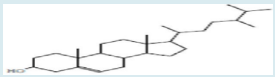
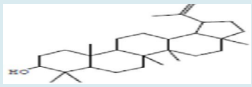


Figure 4: GC-MS chromatogram of methanol extract of *I. parviflora.*

Table 4: Characterization of phytoconstituents of methanol extract of *I. parviflora*

Peak	Retention time	Area%	Name	Formula	Structure
3	4.109	0.71	2-Cyclopenten-1-one, 2-hydroxy-	$C_5H_6O_2$	
6	7.811	1.96	1,2-Benzenediol	$C_6H_6O_2$	
8	9.508	2.41	3-Guanidino-1-propanol mononitrate	$C_4H_{11}N_3O$	
9	10.538	2.57	Silane, [(1,1-dimethyl-2-propenyl)oxy]dimethyl-	$C_7H_{16}OSi$	
11	12.152	2.96	1-Methyl-1-(3-methylbutyl)oxy-1-silacyclobutane	$C_9H_{20}OSi$	
12	13.124	27.31	1,2,3,5-Cyclohexanetetrol	$C_6H_{12}O_4$	
13	13.617	12.01	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	
14	14.127	5.09	9-Oxabicyclo 6-hydroxy- [3.3.1]nonan-2-one	$C_8H_{12}O_3$	
16	15.572	3.46	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	
17	16.014	1.38	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	
18	16.441	1.27	Heptacosanoic acid, methyl ester	$C_{28}H_{56}O_2$	
22	23.849	2.04	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	
25	24.985	1.61	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)	$C_{27}H_{46}O_2$	
26	25.765	2.43	Beta-Tocopherol	$C_{28}H_{48}O_2$	
29	28.085	1.33	Campesterol	$C_{28}H_{48}O$	
31	29.262	4.62	Cholest-5-en-3-ol (3.beta.)-	$C_{27}H_{46}O$	
32	30.713	3.32	Lupeol	$C_{30}H_{50}O$	

3.3 Fractionation and isolation of phytoconstituents from *I. parviflora* extract

The methanol extract (90 g) of *I. parviflora* after subjecting for repeated column chromatography resulted in the isolation of seven compounds. From the eluent chloroform: n-hexane (20:80) white powder was obtained, named as compound 1**,** chloroform: n-hexane (30:70) white amorphous powder was obtained that was compound 2, from methanol: chloroform (2:98) white solid compound 3 was obtained, from methanol: chloroform (8:92) yellow solid compound 4 was obtained, in methanol: chloroform (10:90) yellowish white solid powder compound 5 was obtained, methanol: chloroform (20:80) yellow solid compound 6 was obtained, in methanol: chloroform (30:70) cream color solid compound 7 was obtained.

3.4 Spectral details and identification of isolated compounds

All the compounds were analysed using NMR, FTIR and Mass spectroscopic studies and they were identified as follows by interpretation of obtained values. Basic concepts of NMR spectra were given in the Figures 5, 6.

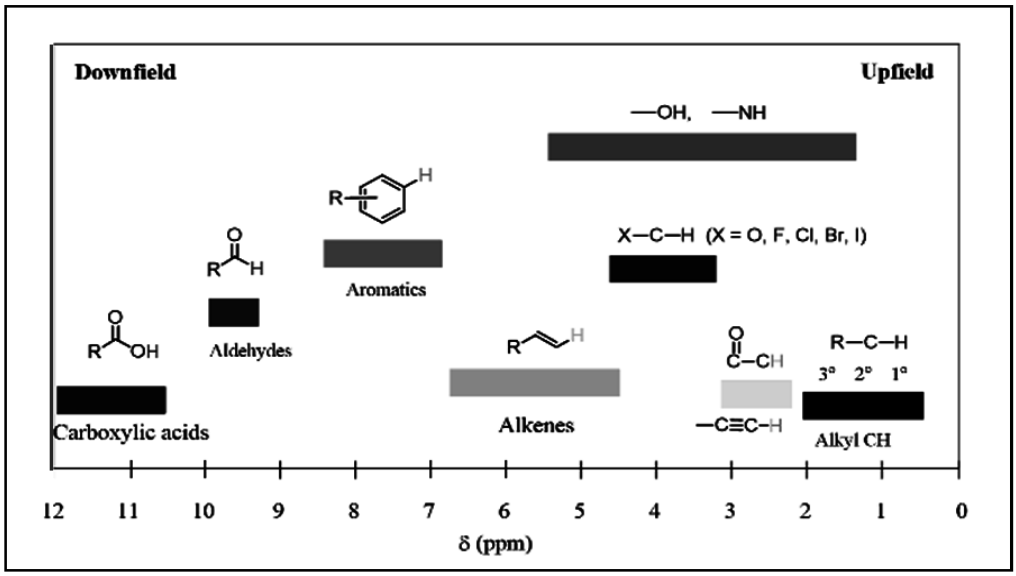


Figure 5: Chemical shift values of ^{1}H NMR spectra.

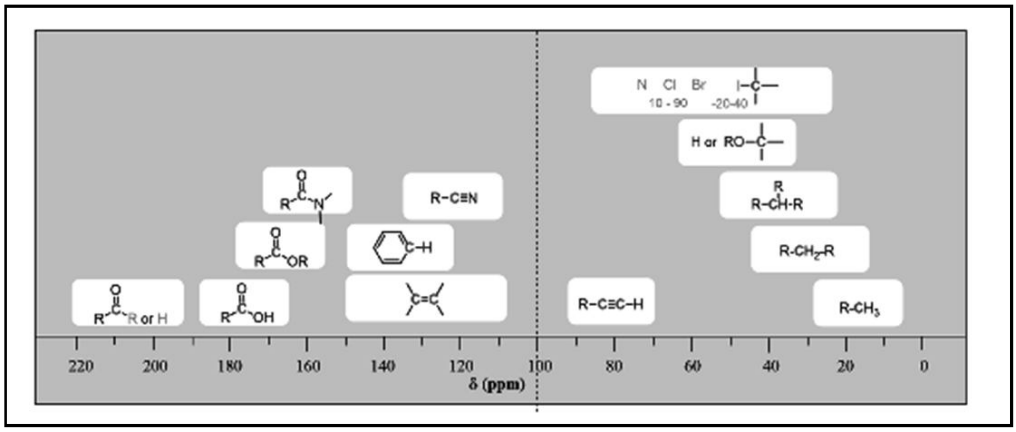


Figure 6: Chemical shift values of ^{13}C NMR spectra.

3.4.1 Characterization of compound 1 from methanol extract fraction F11-16

^{1}H-NMR (400 MHz, $CDCl_3$) 4.68 (m, 1H), 4.58 (m, 1H), 3.78 (dd, J =10.2Hz, 1H), 3.31 (dd, J =10.8 Hz, 1H), 3.18 (dd, J =10.8 and 5.1 Hz, 1H), 2.38 (m, 1H), 1.68 (s,3H).

^{13}C-NMR (100 MHz, $CDCl_3$)- 150.6 (C), 109.7 (CH2), 78.9 (CH), 60.5 (CH 2), 55.2(CH), 50.3(CH), 48.6 (CH), 47.7 (CH), 47.7 (C), 42.6 (C), 40.8 (C), 39.8 (CH 2), 38.7 (C),38.6(CH 2), 37.2 (CH), 37.0 (C), 34.1 (CH2), 29.6 (CH2), 27.8 (CH3), 27.2 (CH2), 26.9 (CH2), 25.0(CH2), 20.7 (CH2), 18.9 (CH3), 18.1 (CH2), 15.9 (CH3), 15.8 (CH3), 15.2 (CH3), 14.6 (CH3).

HR-ESIMS m/z 443.35237 corresponding to molecular formula as $C_{30}H_{50}O_2$

The IR spectra displayed major absorption bands at 3433, 3073, 2942, 1641, 1485, 1466, 1453, 1389, 1347, 1285, 1190, 1131, 1061 cm^{-1}.

From the data the compound was assigned as triterpene compound betulin (Elvira, 2009; Himanshu Joshi, 2013).

3.4.2 Characterization of compound 2 from methanol extract fraction F17- 23

^{1}HNMR ($CDCl_3$, 300 MHz) 1.02 (1H,m), 1.57 (1H,m), 3.24 (1H,dd,j= 10.5Hz, H-3), 0.88 (1H,m), 1.58 (1H,m), 1.39 (1H,m), 1.53 (1H,m), 1.36 (1H,m), 1.71 (1H,m), 1.96 (1H,m), 5.28 (1H,s), 1.72 (1H,m), 2.19 (1H,m), 2.12 (1H,m), 1.96 (1H,m), 2.83 (1H,dd, j=13.8, 4.5Hz), 1.83 (1H,m), 1.32 (1H,m), 1.46 (1H,m), 1.23 (1H,m), 1.82 (1H,m), 2.04 (1H,m), 1.00 (3H,s), 0.79 (3H,s), 0.93 (3H,s), 0.77 (3H,s), 1.15 (3H,s), 1.15 (3H,s), 0.92 (3H,s) and 0.95 (3H,s).

The ^{13}CNMR (75 MHz, $CDCl_3$) 15.3 (C-25), 15.4 (C-24), 17.1 (C-26), 18.3 (C-6), 22.9 (C-11), 23.6 (C-30), 23.4 (C-16), 25.9 (C- 27), 27.2 (C-2), 28.1 (C-23), 28.4 (C-15), 30.7 (C-20), 32.4 (C-22), 32.6 (C-29), 33.1 (C-7), 33.8 (C-21), 37.1 (C-10), 38.4 (C-1), 38.8 (C-4), 39.3 (C-8), 41.0 (C18), 41.6 (C-14), 45.9 (C-19), 46.7 (C-17), 47.6 (C-9), 55.2 (C-5), 79.6 (C-3), 122.6 (C-12), 143.6 (C-13), 182.9 (C-28).

The IR spectra showed major absorption bands at 3423, 2922, 2851, 1691, 1463, 1383 and 1182 cm-1.

ESIMS spectrum *m/z* 456 $[M+]^+$ and its molecular formula was found to be $C_{30}H_{48}O$.

From the data the compound was assigned to be a pentacyclic triterpenoid which was oleanolic acid (Qinhua Chen *et al.,* 2011; Castellano *et al.,* 2022).

3.4.3 Characterization of compound 3 from methanol extract fraction F33- 39

^{1}H-NMR (400 MHz, pyridine-d5): γ 4.97 (1H, br s, Hβ-29), 4.79 (1H, br s, Hα-29), 3.56 (1H, m, H-19), 3.48 (1H, t, J = 8.2 Hz, H-3), 2.76 (1H, t, J = 9.3 Hz, H-13), 2.65 (1H, d, J = 12.6 Hz, H-16), 2.26 (1H, br s, H-21), 1.81 (3H, s, Me-30), 1.25, 1.09, 1.08, 1.03, 0.84 (Me- 23, Me-27, Me-26, Me-24, Me-25).

^{13}C-NMR (100 MHz, pyridine-d5): ä C: 39.8 (C-1), 28.9(C2), 78.3 (C-3), 39.8 (C-4), 56.1 (C-5), 19.7 (C-6), 35.1 (C-7), 41.3(C-8), 51.2 (C-9), 37.7 (C10), 21.4 (C-11), 26.3 (C-12), 39.5 (C-13), 43.1 (C-14), 31.4 (C-15), 33.1 (C-16), 56.9 (C-17), 48.0 (C-18), 50.0 (C-19), 151.6 (C-20), 30.5 (C-21), 38.8 (C-22), 28.5 (C-23), 19.7 (C-24), 19.6 (C-25), 16.6 (C-26), 15.1 (C-27), 179.1 (C-28), 110.2 (C-29), 21.4 (C-30).

HR-ESIMS m/z 457.35237 corresponding to molecular formula $C_{30}H_{48}O_3$

From the data the compound was assigned to be a pentacyclic triterpenoid which was betulinic acid (Chiedozie *et al.,* 2020; Prabhjit Kaur *et al.,* 2022)

3.4.4 Characterization of compound 4 from methanol extract fraction F40-48

^{1}H NMR (CDCl3, 300 MHz) demonstrated shifts at ä ppm 6.97 (1H, d, J=1.8Hz), 6.73 (1H, d, J = 8.1Hz), 6.85 (1H, dd, J=1.8 & 8.1Hz), 6.12 (1H, d, J=15.8Hz), 7.48 (1H, J=15.8Hz).

^{13}CNMR (CDCl3, 75MHz) demonstrated signals at ä 128.0 (C-1), 115.2 (C-2), 145.9 (C-3), 146.5 (C-4), 117.2 (C-5), 123.2 (C-6), 144.9 (C-7), 116.5 (C-7),116.5 (C-8), 171.5 (C-9).

ESI Mass spectrum at m/z 179 [M-H] corresponding to molecular formula as $C_9H_8O_4$

The IR spectra displayed major absorption bands at 3407, 3229, 2923, 1645 and 1449 cm^{-1}.

Based on NMR, Mass, IR spectral data, the structure of compound 1 was assigned as phenolic compound caffeic acid. It was confirmed from the literature (Yun Wei, 2010; Jiaqi Yuan, 2021).

3.4.5 Characterization of compound 5 from methanol extract fraction F61-68

^{1}HNMR (CD_3OD, 300 MHz) γ 7.1(S, 2H).

^{13} CNMR (CD_3OD,75MHz) at γ 170.4(S), 146.3(S), 139.5(S), 121.9(S), 110.3(S).

HR-ESIMS m/z 170.0 with the formula $C_6H_2(OH)_3CO_2H$

From the data it was easily identified that it would be a is a trihydroxybenzoic acid which was a phenolic acid known as gallic acid (Patrick Iwuanyanwu *et al.,* 2014; Felipe *et al.*, 2016).

3.4.6 Characterization of compound 6 from methanol extract fraction F69- 76

^{1}H NMR (CD_3OD, 300MHz, 7.85 (2H, d, J = 8.9 MHz, H-2/H-6) 6.60 (1H, s, H-3), 6.93 (2H, d, J = 8.9 MHz, H-3/H-5).6.25 (1H, d, J = 2.2 MHz, H-6), 6.46 (1H, d, J = 2.2 MHz, H-8),)

^{13}C NMR (CD_3OD, 75 MHz) 182.2 (C-4),164.2 (C-2), 164.(C-7) 162.4(C-4), 161.1 (C-5), 157.9 (C-9), 128.3 (C-2/C-6) ,122.3 (C-1), 115.9(C-3/C-5), 104.3 (C-10), 103.1 (C-3), 98.8 (C-6), 93.8(C-8).

HR-ESIMS m/z 270.2 corresponding to the molecular formula $C_{15}H_{10}O_5$

From the data the compound was assigned to be a flavone apigenin (Kumar 2018; Ana Cruz *et al.,* 2022).

3.4.7 Characterization of compound 7 from methanol extract fraction F77-84

^{1}H NMR ($CDCl_3$+CD_3OD, 500 MHz) 7.57 (1H, dd, *J* = 9.918 Hz, 15.569 and 25.787Hz), 7.06 (H, t, *J* = 2.316 Hz and 4.425 Hz), 6.94 (H, td, *J* = 1.831 Hz and 3.662 Hz), 6.81 (H, dd, *J* = 1.608 Hz and 8.087 Hz), 6.26 (H, t, *J* = 16.479 Hz and 32.501 Hz), 5.37 (H, m) 4.20 (H, m), 3.73 (H, m), 2.32 (H, m), 2.19 (H, s), 2.15 (H, t, *J* = 3.357 Hz and 5.798 Hz), 2.05 (H, m).

^{13}C NMR ($CDCl_3$+CD_3OD, 100 MHz) 175.260, 167.294, 147.492, 145.497, 144.721,125.941, 121.377, 114.768, 113.507, 113.507, 74.698, 72.563, 70.135, 69.975, 37.836, 36.459.

ESI Mass spectrum at *m/z* 354[M-H], it corresponds to the molecular formula $C_{16}H_{18}O_9$.

From the data the compound was assigned to be a polyphenol ester that may confirmed to be chlorogenic acid Rakesh Jaiswal *et al.*, 2010; Ivana Tomac *et al*., 2017).

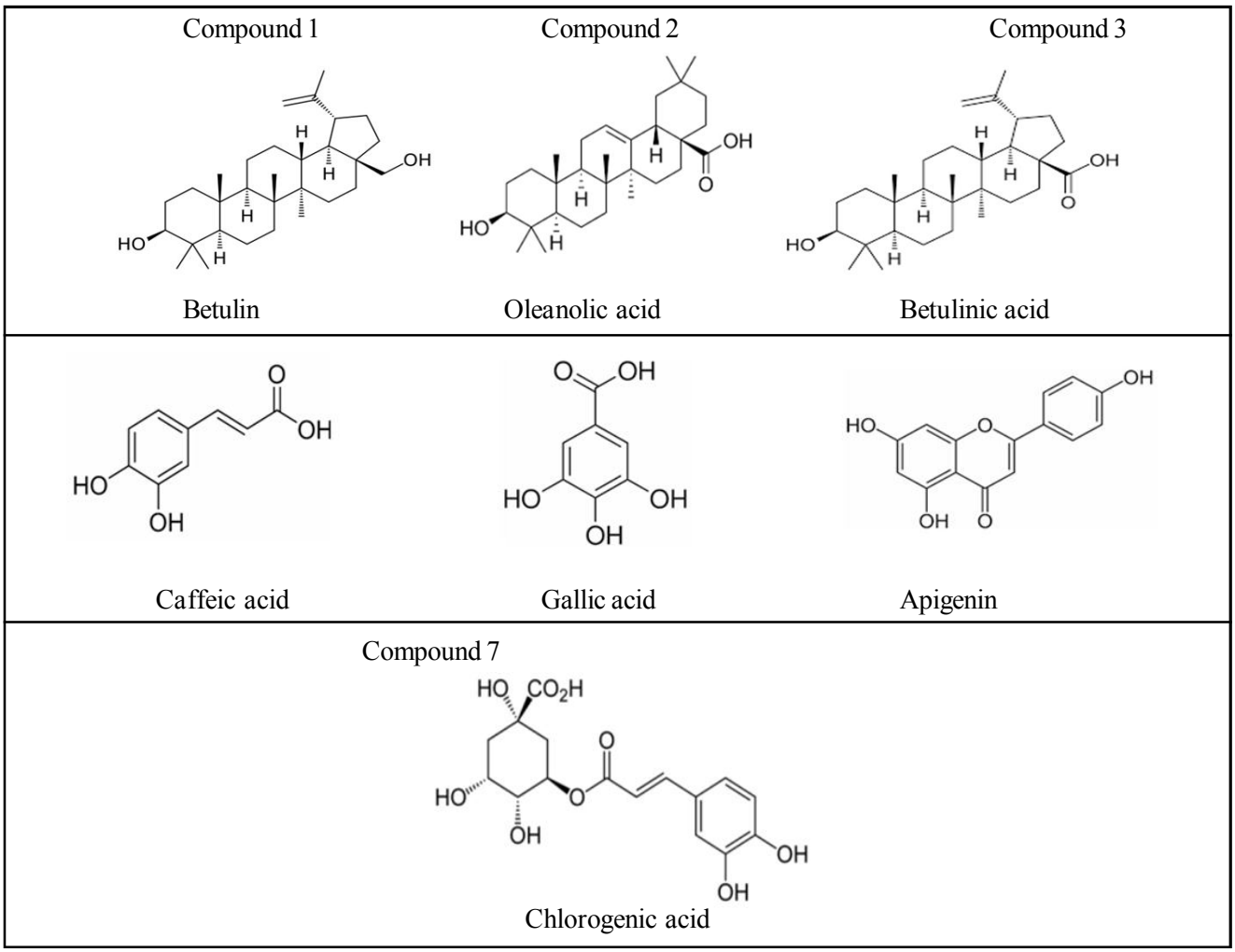


Figure 7: Structures of isolated compounds.

4. Discussion

Herbal medicine is becoming the choice because it normally has no adverse side effects, leaves a long lasting curative impact on human health and is often cost effective, although the duration of treatment may be a little longer (Bushra Parveen *et al.,* 2020). Individuals use a variety of herbal remedies, and various indigenous medications are regularly introduced into modern therapeutics (Sanjeev Singh and Divya Singh, 2021). Apart from the various biological activities like antioxidant activity, hepatoprotective activity, antiinflammatory, analgesic and antipyretics of *I. parviflora* (Sunitha *et al.,* 2015), it has recently studied for its ability to act as anticancer agent in the previous article (Srivani and Krishna Mohan, 2022). Hence, isolation of phytoconstituents from this plant and evaluating for its major pharmacological properties plays a key role in upcoming drug designing process. The plant possesses very crucial components in it which are now mainly used in cancer therapy. GC-MS analysis of the extracts revealed the presence of some active constituents in the plant. Using column chromatography fractionation of the methanol extract was done which lead to elution of many fractions which were developed for TLC for detection of compounds in the form of spots. Similar looking TLC fraction were pooled and rechromatographated. Continuous running of column resulted in isolation of seven compounds; namely, betulin (compound 1) oleanolic acid (compound 2), betulinic acid (compound 3), caffeic acid (compound 4), gallic acid (compound 5), apigenin (compound 6) and chlorogenic acid (compound 7). Gallic acid is one of the most abundant naturally derived drugs used to treat several diseases such as diabetes, cancer, inflammation, *etc* (Parvesh Kumar Dhama *et al.,* 2022). The compounds betulin (Sylwia Katarzyna *et al,.* 2015), oleanolic acid (Zhong *et al.,* 2022), betulinic acid (Simone Fulda, 2008; Wenkai Jiang *et al.,* 2021) and chlorogenic acid (Ashutosh Gupta et *al.,* 2022) are well known potent anticancer agents. Research is still on process about these compounds to find the potency of the compounds in various types of cancer.

5. Conclusion

From the above obtained data and information it was identified that *I. parviflora* has potent components. The compounds oleanolic acid, betulinic acid, caffeic acid, gallic acid were newly identified in this plant for the first time. Whereas apigenin and chlorogenic acid were newly identified in the leaf of the plant which were earlier found in flowers of *I. parviflora*. Apart from these major active compounds there are other components which are high in nutrition were also

present in the plant that were characterized by GC-MS. Compounds like tocopherol and its derivaties like vitamin E acetate were also found which are known potent antioxidants. Lupeol is well known for its antiinflammatory and antimicrobial properties. The different parts of this plant were extensively used traditionally but the diverse pharmacological activities have not been fully tested. Even though there are some articles describing the phytochemical and pharmacological properties of *Ixora* further investigation needs to be carried out in order to make use of these phytoactive components in formulations for their clinical applications, which can be used for the welfare of the mankind. Identifying such plants containing important anticancer agents makes the scientists easier for drug discovery and development process.

Conflict of interest

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

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