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Estimation of Phytoconstituents of *Picrorhiza kurroa* Rhizome by Phytochemical Screening, FTIR, and GC-MS and Antimicrobial Analysis

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ABSTRACT:
Bioactive compounds and other plant constituents have been shown to vary across cultivation regions, species, environmental conditions, and extraction methods, among other factors. *Picrorhiza kurroa* is a wonderful medicinal plant, a member of the Plantaginaceae family, indigenous to the upper Himalayan region of India. The present research aimed to screen for phytochemicals and characterize the ethanolic rhizome extracts of *P. kurroa* collected from Uttarakhand, India. Rhizome extract obtained using the Soxhlet apparatus with ethanol, then preconcentrated in a vacuum rotary evaporator. The extracts were characterized using a Fourier transform infrared spectrometer (FTIR) and Gas chromatography-mass spectrometry (GC-MS) to identify key functional groups and phytoconstituents. FTIR spectra showed the signature absorption bands of functional groups, including alkyl halides, vinyl groups, primary amines, phenols, alcohols, and nitro compounds. GC-MS analysis identified 47 phytoconstituents in the extracts. The extract exhibited significant antibacterial and antifungal activities. The findings suggest that *P. kurroa* will facilitate the development of herbal medicines, potentially leading to the discovery of new therapeutic agents.

Keywords: *Picrorhiza kurroa*, Kutki, Phytochemical Compounds, FTIR, GC-MS, Cis-Vaccenic Acid

INTRODUCTION

Fig 1. *Picrorhiza kurroa*

Nature always stands as a golden mark exemplifying the outstanding phenomenon of symbiosis, the plant being man's only chemist for ages (Kokate et al., 2008). Medicinal Plants have been utilized on Earth for aeons as a reliable source for treating various diseases in humans and animals (Varshney et al., 2022). The wide range of phytoconstituents present in plants significantly contributes to the discovery and development of novel drugs (Rani & Kapoor, 2019 & Singh et al., 2015).

Picrorhiza kurroa Royle ex Benth (Family: Plantaginaceae) is a well-known medicinal herb (Fig.1), popularly known as "Kutki" or "Kurro" and 'Indian gentian' (Soni and Grover, 2019). Picrorhiza is a perennial herb well distributed in the upper Himalayas and also in China. It is found naturally in Kashmir, Himachal Pradesh, Punjab and Sikkim. These can grow well at higher altitudes in temperate or tropical climates (Kokate et al., 2008).

A total 22 iridoid glycosides present in the genus Picrorhiza, *P. kurroa* posseses 7 distinct iridoid glycosides namely kutkin, kutkoside, picroside V, pikuroside, mussae- nosidic acid, bartsioside and boschnaloside (Kumar et al., 2017). *Picrorhiza kurroa* has an pharmacological action as an anti-arthritic (Kumar et al., 2015), anti-asthmatic (Sehgal et al., 2013), anti-carcinogenic (Soni and Grover, 2019 & Rathee et al., 2013), anti-diabetic (Husain et al., 2014), anti-fibrotic (Raut et al., 2023), anti-inflammatory (Kumar et al., 2016),

anti-leishmanial (Guilherne et al., 2021) anti-microbial (Rathee et al., 2016 & Thapa et al., 2022), anti-oxidant (Navya et al., 2018 & Rajkumar et al., 2011), collagen synthesis- promoting and collagenase inhibitory (Morikawa et al., 2020), hepatoprotective (Sakamoto et al., 2023), immuno-modulatory (Kumar et al., 2016), etc. Therefore, it was essential to screen the ethanolic extract of *P. kurroa* rhizome for the detection of phytochemicals, to identify and characterize phytoconstituents in its crude extracts for chemical profiling using gas chromatography-mass spectrometry (GC-MS) analytic technique and FTIR, to evaluate its antimicrobial activity (in vitro) for the production of modern drugs from phytoconstituents. GC-MS plays an essential role in the phytochemical analysis and chemotaxonomic studies of medicinal plants containing biologically active components (Olivia et al., 2021)

MATERIALS AND METHODS

PLANT MATERIAL COLLECTION AND AUTHENTICATION

The plants of *Picrorhiza kurroa* were procured from the High Altitude Plant Physiology Centre, HNB Garhwal University, Srinagar, Uttarakhand, and authenticated by Dr. K.C. Bhatt, Principal Scientist, Taxonomic Division, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic and Resources (NBPGR), PUSA Campus, New Delhi, India, with the reference number AC-332/2025.

PREPARATION OF SAMPLE

The collected plant material root and rhizome, was manually rinsed with tap water followed by distilled water to eliminate the surface dust particles and other contaminants. It was then dried in the shade at room temperature for 4 weeks. The shade-dried rhizome was pulverized into a coarse powder using an electric grinder.

Fig 2. Different steps followed for sample preparation

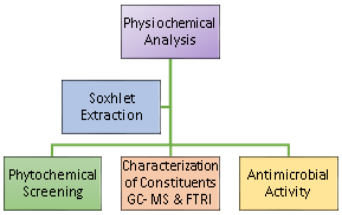


Fig 3. Outline of work

PHYSIOCHEMICAL EVALUATION

PHYSICOCHEMICAL ANALYSIS

The physicochemical parameters of ash analysis, acid-insoluble ash, and extractive values were determined as per standard Ayurvedic pharmacopoeial procedures (Government of India, n.d.).

SOXHLET EXTRACTION

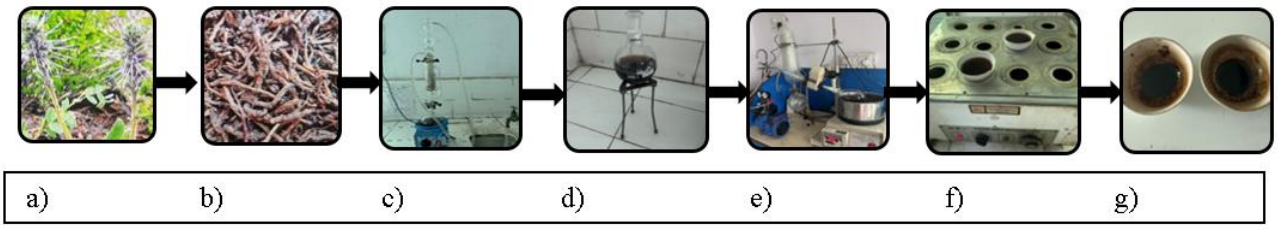


Fig. 4. a), b), c), d), e), f) & g) showing the full extraction procedure

35 g of coarsly powdered *Picrorhiza kurroa* was loaded into a thimble in the Soxhlet apparatus, then the ratio of plant material to solvent was 1:10 (w/w). 350ml of 95% (v/v) ethanol was used. Extractions were conducted at 60 °C for 12 hours, in a triplate (Bontzolis et al., 2024). The extract was evaporated and concentrated by using a rotatory evaporator (SCI 100-Pro; SCILOGEX, USA) at 40°C and transferred into a weighed porcelain dish. Afterward, the extract evaporated to dryness on a copper water bath, dried at 50°C, to yield a viscous brown residue (6.8 gm). The extract was stored at 4°C till further analysis (Mustafa et al., 2021).

PHYTOCHEMICAL ANALYSIS

The extract was screened for the presence of alkaloids, glycosides, phytosterols, saponins, tannins, and others were carried out by using a standard procedure (Trease and Evans, 2002 & Soni and Sosa, 2013).

FTIR ANALYSIS

Fourier Transform Infrared (Shimadzu- IR Spirit X) in the range 350 cm^{-1} to 4700 cm^{-1} was used for this

analysis. The instrument was calibrated. About 1mg dried ethanolic extract was placed in the sample holder and mounted on the FTIR machine, ready for analysis. The FTIR spectrum for transmittance against wavelength was taken, and an interpretation was performed to identify various pronounced peaks of interest (Patel and Kanaki, 2021).

GC-MS ANALYSIS

GC-MS analysis of ethanolic extract was performed at the University Science Instrumentation Centre, AIRF, Jawaharlal Nehru University, Delhi, India. The analysis was carried out on a Shimadzu GC-MS-QP2010 Plus System equipped with an auto injector (AOC- 20s), and a mass selective detector with an ion source (220 °C) and interface (280 °C). Sepration was achieved using an Rtx-5 Ms capillary column (Restek Company, Bellefonte, USA) with dimensions of 30m x 0.25mm x 0.25 μm. The mass range was set to 40-600 m/z with a threshold of 1000ev. The injector was operated in split injection mode at 260 °C. The oven temperature was programmed to start at 70 °C (2min), followed by a ramp rate of 10 °C/min to 300 °C. Helium (>99.99%) was used as the carrier gas at a linear velocity of 40.3 cm/s. The total flow rate was set to 16.3mL/min, with a column flow rate of 1.21mL/min, (Das et al., 2014)

Identification of the components

Components in the rhizome ethanolic extract were identified based on retention time (RT) by Gas Chromatography. Mass spectral fragmentation was interpreted by comparing the obtained spectra to the NIST 1.1 LIB and Wiley8. LIB databases.

ANTIMICROBIAL ASSAY

The following bacterial strains were used in the antimicrobial tests. Gram positive bacteria was *Staphylococcus aureus*, Gram negative bacteria was *Escherichia coli*. Fungus *Aspergillus niger* was used. In vitro antibacterial activity was determined by using Nutrient agar, McConkey's agar, Vogel Johnson's agar and Sabouraud's dextrose agar. Each medium was autoclaved at 121°C, 15 psi for 15 min before inoculation. The bacteria used in the tests were obtained from 24 h cultures, whereas *A. niger* inocula were prepared by suspending colonies from 48 and 72 h cultures, respectively and suspended in sterile saline solution to obtain concentrations.

Antimicrobial activity of the ethanolic extract was determined using the agar well diffusion method. About 15ml of sterilized selective agar-based medium was added aseptically to sterile plates to prepare a basal layer. The plates were incubated at 37°C + 0.5 °C for 24 hrs. The basal layer was seeded the next day with 7ml of sterilized selective agar-based medium containing 1ml of suspension of standard inoculums. The plates were allowed to set. Each petridish was divided into f sectors, and in each sector a bore of 6mm diameter was made using a sterilized borer in the solidified medium. Using sterilized dropping pipettes, each bore in a different sector was carefully loaded with 75μl of test compound and allowed to diffuse at room temperature for 2 h. The plates were then incubated at 37°C for 24 h for bacteria and 72 h for *Aspergillus niger*. The ethanolic extract was 2-fold serially diluted in dimethyl sulphoxide (DMSO) to obtain concentrations from 5 mg – 20 mg/ 75 μl. Wells with equal volumes of DMSO were used as negative controls (Tabassam et al., 2021).

RESULT & DISCUSSION

PHYSICOCHEMICAL EVALUATION

The result of the physicochemical evaluation of *Picrorhiza kurroa* rhizome was summarized in Table 1 and Fig. 5. The physicochemical evaluation was performed, and the observed values were compared with Ayurvedic Pharmacopoeia of India (API) standard values, which include all parameters within the specified limits.

Table 1. Physicochemical Evaluation of *Picrorhiza kurroa* rhizome

S/N	Physiochemical Parameters	Observed Values (%)	Standard Values (%)
1.	Total Ash	4.3%	Not more than 7 %
2.	Acid Insoluble Ash	0.6%	Not more than 1 %
3.	Alcohol Soluble Extractive	18%	Not less than 10 %
4.	Water Soluble Extractive	32%	Not less than 20 %

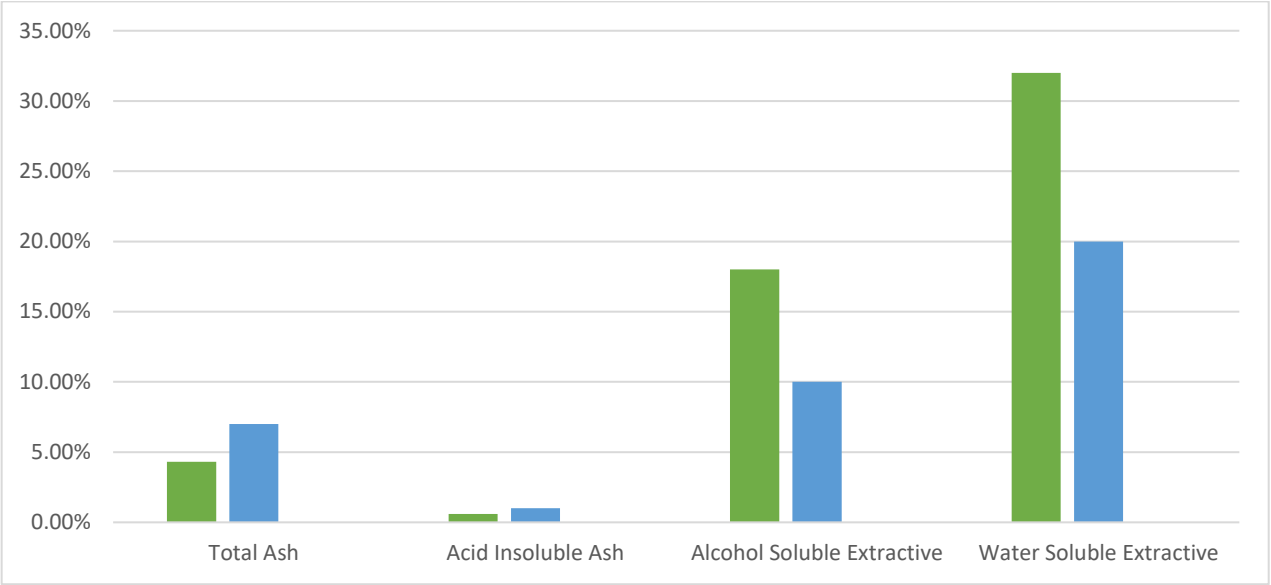


Fig.5. Graph showing comparison of observed values and standard values of *Picrorhiza kurroa* rhizome

PHYTOCHEMICAL ANALYSIS

Ethanolic extracts of *P. kurroa* (**Table 2**) had shown the presence of various phytochemical compounds, including alkaloids, glycosides, flavonoids, tannins and phenols, carbohydrates, phenolic compounds, saponins, terpenoids, proteins, oil and fats. Phytochemicals such as fixed oils was not detected in *P. kurroa* extract. These results confirmed to those of Rathee et al. 2016 and Sharma et al. 2018 who have reported similar phytochemical constituents from *P. kurroa*.

Table 2 : Preliminary phytochemical screening of Ethanolic extracts of *Picrorhiza kurroa* Pk E

S/N	Compounds	Tests	Inference	Pk E
01	Alkaloids	Dragendorff's test	Orange red precipitation	+
		Mayer's test	White precipitation	+
		Wagner's test	Reddish brown precipitation	+
		Hager's test	Prominent yellow precipitation	+
02	Carbohydrates	Molish's test	Violet ring	+
		Fehling's test	Red precipitation	+
		Benedict's test	Characteristic coloured precipitation	+
03	Glycosides	Borntrager's test	Pink colour	+
		Legal's test	Pink colour	+
04	Saponin	Froth test	2cm layer of foam	+
		Foam test	Foam persists for ten minutes	+
05	Proteins and Amino acids	Biuret test	Pink colour	+
		Xanthoproteic test	Yellow colour	+
06	Phytosterols	Libermann – Burchard's test	Colour changes	+
07	Fixed oils	Spot test	Oil strain on the papers	-
08	Phenolic compounds	Ferric chloride test	Dark green colour	+
		Lead acetate test	Bulky white precipiteation	+
		Alkaline reagent test	Yellow fluorescence	+

FT-IR

The spectrum of the ethanolic extract of *Picrorhiza kurroa* revealed multiple absorption bands, indicating the presence of diverse functional groups. The spectrum peaks at 3627.89 cm-1 (free O-H stretching) and 3307.99 (N-H stretching), indicative of hydroxyl group and aliphatic primary amines. The FTIR spectrum included absorption bands at 1692.73 cm-1, attributed to the presence of imines or oximes, and the peak at 1632.48cm-1 (N-H bending) (Firdous et al., 2016) reported a similar peak, earlier. the stretching vibrations of carbon-carbon bonds in conjugated alkenes (1600.92 cm-1), and the stretching vibrations of nitrogen-oxygen bonds in nitro compounds (1514.85 cm-1). Finally, the absorption band corresponding to the bending vibrations of carbon-hydrogen bonds in alkanes, 1364.23 cm-1, which has been reported in cis-

Vaccenic acid, (Anandhan et al., 2019). The spectrum also exhibited an absorption band at 1282.46 cm-1 was indicative of the presence of vinyl ethers. Additionally, the absorption bands at 1225.08 cm-1 and 1159.09 cm-1 correspond to the stretching vibrations of alkyl/aryl ethers and carbon-oxygen bonds in tertiary alcohols, respectively. The absorption band at 923.83 cm-1 was attributed to the presence of a vinyl group. Conversely, the spectral data also revealed the presence of aromatic compounds, as evidenced by the absorption band at 873.62cm-1, characteristic of 1,3-disubstituted benzene. The absorption band at 764.60 cm-1 was indicative of a carbon-chlorine bond, typically found in alkyl halides. Furthermore, the presence of a metal-oxygen stretching vibration at 437.53 cm-1 suggested the presence of an inorganic compound. This data is comparable with the spectral data for β-sitosterol reported by (Ibrahim et al., 2020).

Table 3. FTIR peak height obtained from the ethanolic extract spectrum of *Picrorhiza kurroa* rhizome

S/N	Peak Value	Functional Group	Class
1.	437.53	M-O stretching	Metal-oxygen (inorganic)
2.	764.60	C-Cl streaching	Alkyl halide
3.	873.62	C-H bending	1,3- disubstituted benzene
4.	923.83	=C-H out of plane bending	Vinyl group
5.	1159.09	C-O stretching	Tertiary alcohol
6.	1225.08	C-O stretching	Alkyl, aryl ether
7.	1282.46	C-O stretching	Vinyl ether
8.	1364.23	C-H bending	alkanes
9.	1514.85	N-O stretching	Nitro compounds
10.	1600.92	C=C stretching	Conjugated alkene
11.	1632.48	N-H bending	primary amines
12.	1692.73	C=N stretching	Imine/oxime
13.	3307.99	N-H stretching	Aliphatic primary amine
14.	3627.89	O-H stretching (free)	Phenols, alcohols

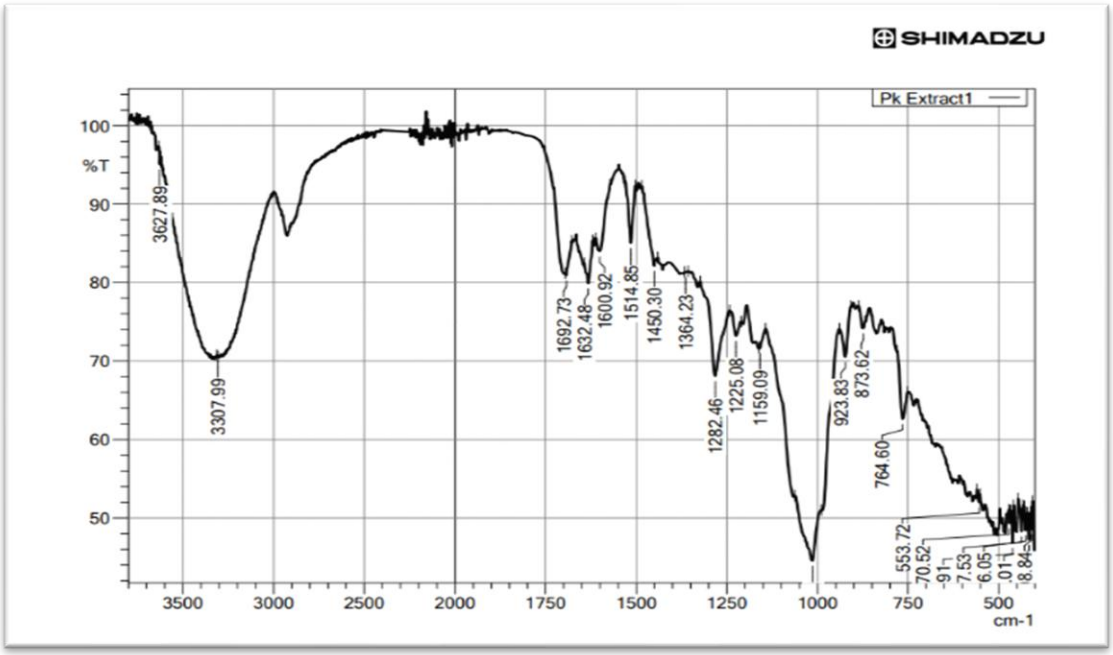


Fig.6. FTIR of ethanolic extract of *Picrorhiza kurroa* rhizome

GC-MS

The GC-MS analysis yielded results comprising the active principles, including retention time, molecular formula, molecular weight, peak area percentage and composition of bioactive components in P. kurroa, as summarized in Table 4. The Corresponding GC-MS Chromatogram is depicted in Fig. 7. A compound, namely β-sitosterol, is studied as a tumor inhibitor anticancer agent in vivo (Abu-Lafi et al., 2019). Cis-vaccenic acid has been linked to numerous health benefits, particularly concerning cardiovascular risk factors and cholesterol regulation. Additionally, research suggests that cis-vaccenic acid can help reduce triglyceride levels and potentially

minimize the risk of atherosclerosis, partly due to its influence on hepatic lipid metabolism and fatty acid oxidation processes (Raina et al., 2025). 2-Methoxy-4-vinylphenol (2M4VP) is a natural anti-inflammatory compound (Asami et al., 2023)

Table. 4 Showing GC-MS results of *P.kurroa*

Peak	Name	R.Time	Molecular Formula	M. W.	Area%
1	Cyclopropane, Nonyl-	8.067	$C_{12}H_{24}$	168	0.24
2	Dodecane	8.194	$C_{12}H_{26}$	170	0.34
3	2,3-Dihydro-Benzofuran	8.690	C_8H_8O	120	1.27
4	2-Methoxy-4-Vinylphenol	9.939	$C_9H_{10}O_2$	150	5.84
5	1-Pentadecene	10.931	$C_{15}H_{30}$	210	0.63
6	Pentadecane	11.036	$C_{15}H_{32}$	212	0.60
7	2-Propenoic Acid, 3-Phenyl-	11.775	$C_9H_8O_2$	148	9.62
8	Guanosine	12.117	$C_{10}H_{13}N_5O_5$	283	6.32
9	Phenol,2,4-Bis(1,1-Dimethylethyl)-	12.517	$C_{14}H_{22}O$	206	1.23
10	2h-Benzocyclohepten-2-One, 3,4,4a,5,6,7,8,9-Oc	13.071	$C_{11}H_{16}O$	164	0.22
11	N-Propyl Cinnamate	14.019	$C_{12}H_{14}O_2$	190	4.00
12	Methyl(3-Oxo-2-Pentylcyclopentyl)Acetat	14.185	$C_{13}H_{22}O_3$	226	1.18
13	1-Heneicosanol	15.748	$C_{21}H_{44}O$	312	0.37
14	Isopropyl Myristate	16.057	$C_{17}H_{34}O_2$	270	0.41
15	Neophytadiene	16.209	$C_{20}H_{38}$	278	0.48
16	(2-Isopropenyl-5-Methylcyclopentyl)Meth	16.331	$C_{15}H_{26}O_2$	238	1.03
17	Octadecanoic Acid, Methyl Ester	17.121	$C_{19}H_{38}O_2$	298	0.50
18	Hexadecanoic Acid, Ethyl Ester	17.795	$C_{18}H_{36}O_2$	284	0.92
19	1-Octadecanol	18.707	$C_{18}H_{38}O$	270	1.18
20	2-Methyltetracosane	18.826	$C_{25}H_{52}$	352	0.96
21	Cis-Vaccenic Acid	19.315	$C_{18}H_{34}O_2$	282	15.76
22	9,12-Octadecadienoic Acid (Z,Z)-	19.380	$C_{18}H_{32}O_2$	280	0.35
23	Ethyl Oleate	19.439	$C_{20}H_{38}O_2$	310	2.81
24	Oleyl Alcohol, Trifluoroacetate	20.186	$C_{20}H_{35}F_3O_2$	364	1.12
25	13-Octadecenal, (Z)-	20.893	$C_{18}H_{34}O$	266	0.61
26	Oleoyl Chloride	21.686	$C_{18}H_{33}ClO$	300	1.31
27	9,12-Octadecadienoic Acid (Z,Z)-	22.043	$C_{18}H_{32}O_2$	280	0.48
28	9-Octadecenoic Acid (Z)-, Oxiranylmethyl Ester	22.084	$C_{21}H_{38}O_3$	338	3.81
29	Glycidyl (Z)-9-Nonadecenoate	22.217	$C_{22}H_{40}O_3$	352	2.32
30	Hexadecanoic Acid, 2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester	22.416	$C_{19}H_{38}O_4$	330	1.18
31	9-Octadecen-1-Ol, (E)-	22.667	$C_{18}H_{36}O$	268	1.26
32	Oleoyl Chloride	23.611	$C_{18}H_{33}ClO$	300	0.13
33	9-Octadecenoic Acid (Z)-, 2,3-Dihydroxypropyl Ester	23.813	$C_{21}H_{40}O_4$	356	9.53
34	Octadecanoic Acid, 2,3-Dihydroxypropyl Ester	23.986	$C_{21}H_{42}O_4$	358	0.44
35	9-Eicosyne	24.490	$C_{20}H_{38}$	278	0.54
36	Squalene	24.640	$C_{30}H_{50}$	410	0.49
37	Hexadecane, 2,6,10,14-Tetramethyl-	25.223	$C_{20}H_{42}$	282	0.97
38	Oleic Acid, 3-(Octadecyloxy)Propyl Ester	25.361	$C_{39}H_{76}O_3$	592	2.06
39	Cholest-3,5-Diene	26.243	$C_{27}H_{44}$	368	2.46
40	(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5-Ethyl-6-Meth	26.679	$C_{30}H_{52}O$	428	0.90
41	Triacontane, 1-Iodo-	26.829	$C_{30}H_{61}I$	548	1.04
42	.Beta.-Sitosterol Acetate	27.014	$C_{31}H_{52}O_2$	456	4.55
43	Ergost-5-En-3-Ol, (3.Beta.,24r)-	28.428	$C_{28}H_{48}O$	400	0.50
44	Stigmast-5-En-3-Ol, (3.Beta.)-	29.514	$C_{29}H_{50}O$	414	3.46
45	Stigmasta-3,5-Dien-7-One	30.842	$C_{29}H_{46}O$	410	0.67
46	.Gamma.-Sitostenone	31.437	$C_{29}H_{48}O$	412	1.83
47	Phytyl Linoleate	35.299	$C_{38}H_{70}O_2$	558	2.07

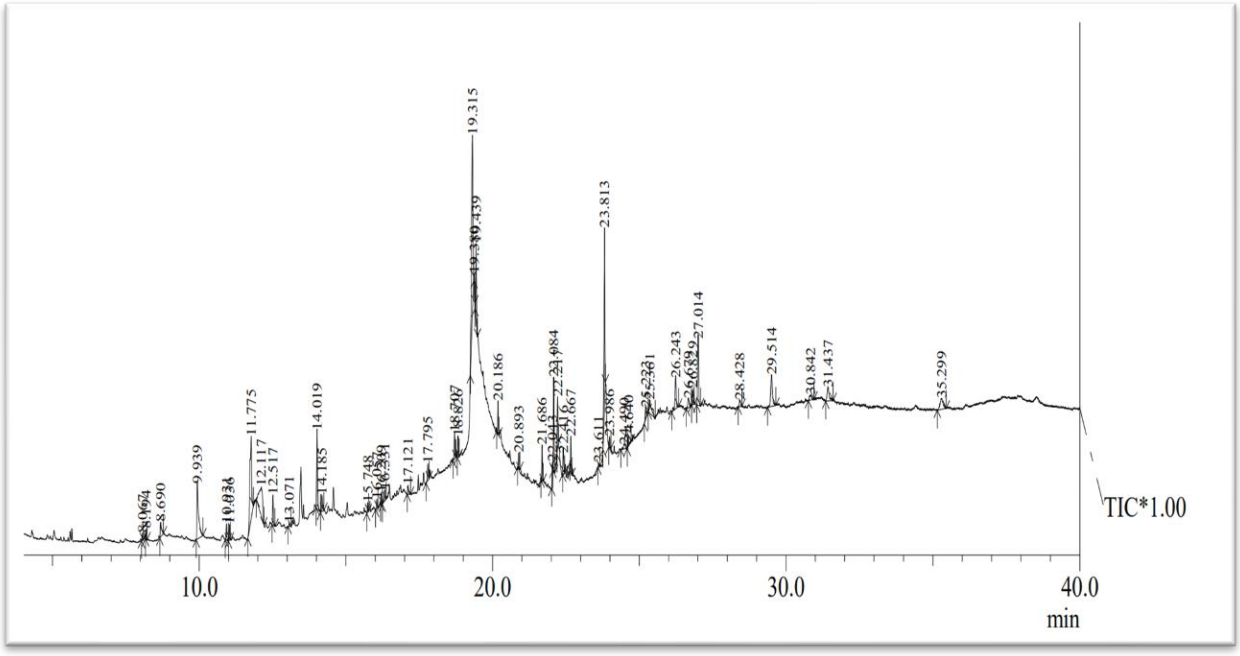
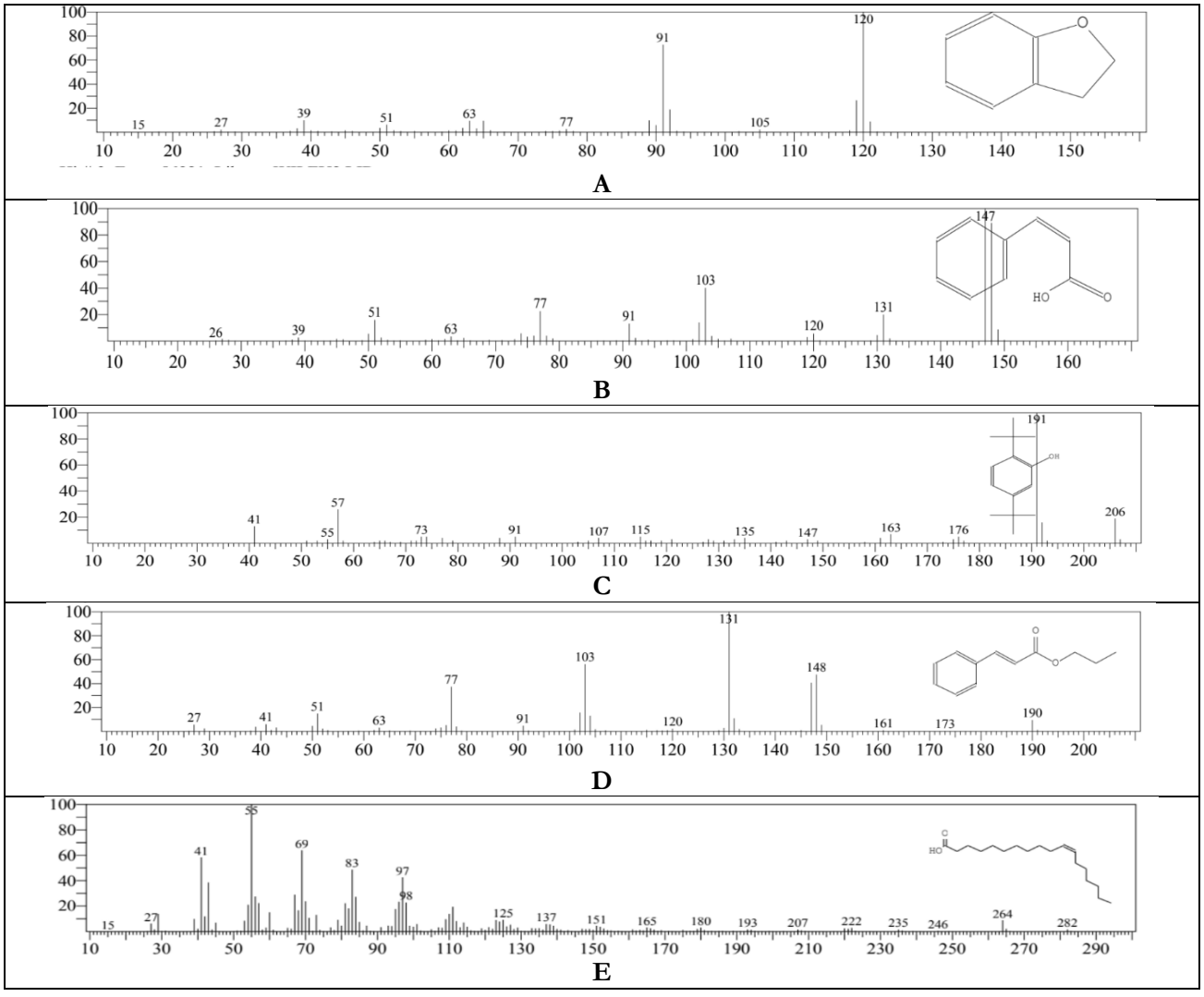


Fig.7. GCMS chromatogram of ethanolic extract of *Picrorhiza kurroa* rhizome



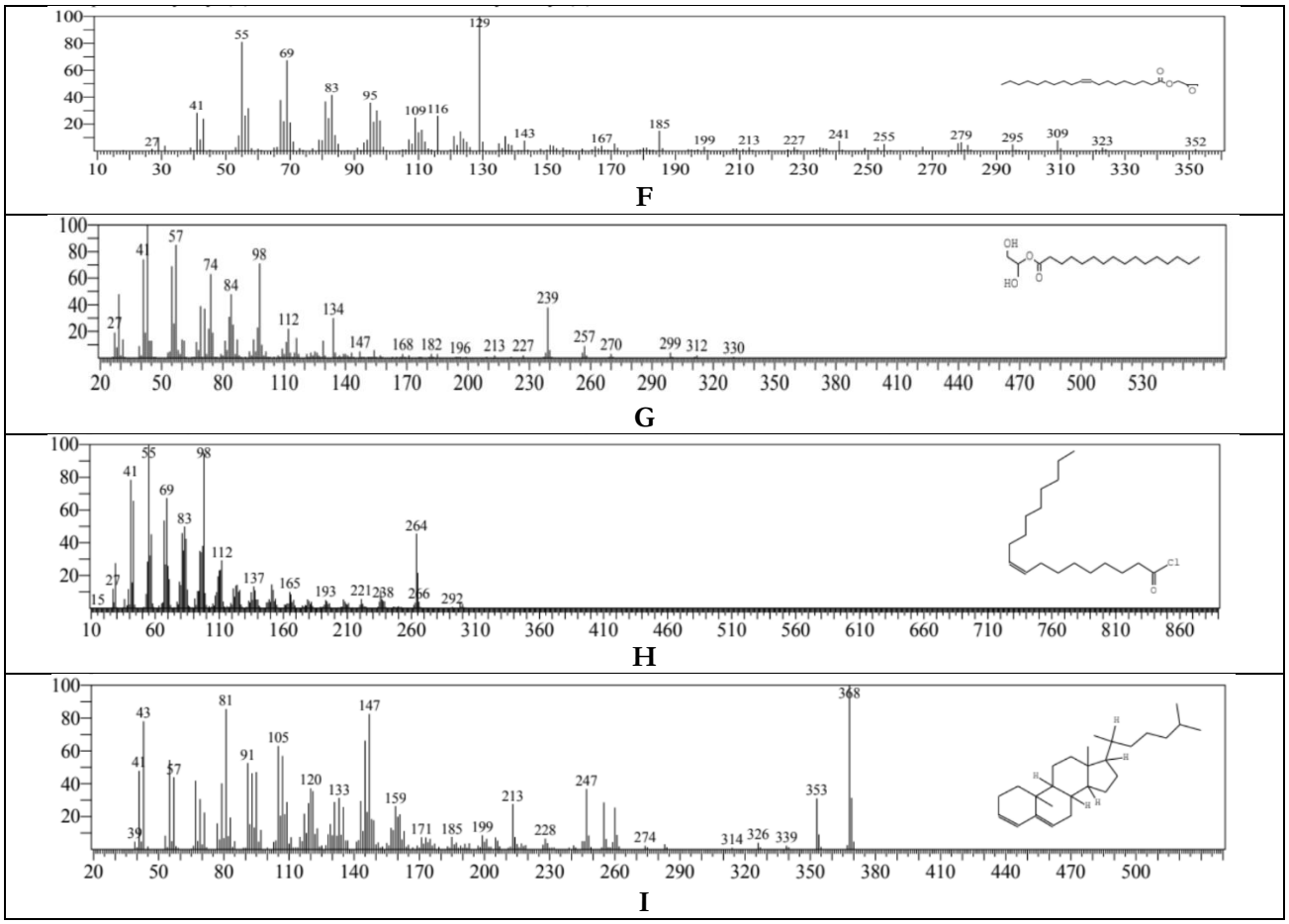


Fig. 8: GC-MS Mass Spectra of few compounds detected from Ethanolic Extract of P. kurroa rhizome. (A) Mass spectra of 2,3-Dihydro-Benzofuran (C_8H_8O) , (B) Mass spectra of 2-Propenoic Acid, 3-Phenyl ($C_9H_8O_2$), (C) Mass spectra of Phenol,2,4-Bis(1,1-Dimethylethyl) ($C_{14}H_{22}O$), (D) Mass spectra of N-Propyl Cinnamate ($C_{12}H_{14}O_2$), (E) Mass spectra of Cis-Vaccenic Acid ($C_{18}H_{34}O_2$), (F) Mass spectra of 9-Octadecenoic Acid (Z)-, Oxiranylmethyl Ester ($C_{21}H_{38}O_3$) , (G) Mass spectra of Hexadecanoic Acid, 2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester ($C_{19}H_{38}O_4$), (H) Mass spectra of Oleoyl Chloride ($C_{18}H_{33}ClO$), (I) Mass spectra of ERGOST-5-EN-3-OL, (3.BETA.,24R) ($C_{28}H_{48}O$)

ANTIMICROBIAL ACTIVITY

The antimicrobial properties of ethanolic extracts from *Picrorhiza kurroa* at different concentrations (100 mg/ml, 200 mg/ml, and 400 mg/ml) were evaluated using the well-diffusion method against three microorganisms: *Staphylococcus aureus*, *Escherichia coli,* and *Aspergillus niger*. The result of the antimicrobial study at a concentration of 400 mg/ml, PkE demonstrates higher inhibition zone towards *S. aureus* followed by *A. niger* and *E.coli* (Table 5). These findings are consistent with previous studies, which have reported the antimicrobial study of *Picrorhiza kurroa* ethanolic extract.

Table 5. Antibacterial activity of ethanolic extracts from *Picrorhiza kurroa* at different concentrations (100mg/ml, 200mg/ml, and 400mg/ml)

Name of the Organism	Doses (mg/ml)	*P. kurroa*
E. coli	**100**	3.7±0.14
	200	8.6±0.26
	400	16.7±0.36
S. aureus	**100**	2.4±0.31
	200	7.1±0.54
	400	18.5±0.63
A. niger	**100**	5.0±0.19
	200	12.2±0.11
	400	15.0±0.18
Ciprofloxacin	**100**	20.8±0.12
Fluconazole	**100**	22.3±0.11
DMSO	**100**	NIL

±: Standard deviation

CONCLUSION

The current study revealed that *P. kurroa* rhizome contain diverse secondary metabolites with potential pharmacological properties, notably antioxidant activity. GC-MS analysis identified 47 phytochemical constituents, which may contribute to antimicrobial, antioxidant, antidiabetic, anticancer, hypercholesterolemic, anti-inflammatory, and other activities. The presence of these phytochemicals likely underlies the therapeutic effects of *P. kurroa*. Further investigation is required to explore the development of novel drugs utilizing the bioactive compounds found in this plant.

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