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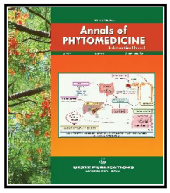

Original Article : Open Access

GC-MS analysis of bioactive compounds, standardization, and assessment of antimicrobial efficacy of himalayan *Juniperus communis* L. stems

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Abstract

Juniperus communis L. (Family: Cupressaceae) is traditionally practiced in treating abdominal disorders, skin affections, arthritis, respiratory affections, and as diuretics. Detailed anatomical characteristic features of *J. communis* stems have been worked up along with physicochemical variables such as extractive value, ash value, moisture content, fluorescence analysis, and TLC fingerprinting by standard procedures. Preliminary phytochemical analysis indicated the presence of terpenoids, flavonoids, tannins, and alkaloids. The air-dried stems were powdered, extracted with ethanol using a Soxhlet extractor, and analysed by GC-MS. The GC-MS analysis identified 24 different bioactive compounds in methanolic extract. The peak area percentage of major bioactive compounds were 54.46% (Lup-20(29)-en-3β-ol, acetate), 6.67% (Lupeol), and 2.57% (24-Norursa-3,12-diene), respectively. The agar well diffusion method for the determination of antibacterial activity was used. According to the antimicrobial test results, aqueous extract exhibited more inhibitory action against the *E. coli* strain than ethanolic extract and the results were compared with standard ciprofloxacin.

1. Introduction

Gas chromatography-mass spectrometry (GC-MS), plays an important role in qualitative and quantitative analysis of phytoconstituents in plant extracts. It is essential for detailed and accurate chemical analysis of plant-based medications. Volatile components are identified and evaluated by their mass spectra. These include terpenoids, alkaloids, flavonoids, *etc*.

These bioactive components play a significant role as antimicrobials due to their ability to inhibit the growth of or kill microorganisms. *E. coli* is a leading cause of urinary tract infections, which can lead to symptoms like frequent urination, pain during urination, and lower abdominal pain. Other infections may be gastrointestinal and respiratory infections (Ralte *et al*., 2022; Zhou *et al*., 2023).

The essential oil present in the *J. communis* has medicinal value. This pale-yellow oil has a distinctive aroma produced by terminal twigs, berries, and needles-shaped leaves and contains cadinene, d-pinene, and camphene. Ascorbic acid, resin, and esters are abundant in juniper needles (Bais *et al*., 2014; Orav *et al*., 2010). The stem is bitter and reported as a purgative, styptic, diuretic, emmenagogue, and aphrodisiac. The tonic enriches the blood and is useful in stomatitis, bronchitis, piles, labor pain and is also used in liver complaints (Khare, 2007; Alam and Sharma, 2022). Different ayurvedic formulations containing *Juniper* berries, such as Hingvadi churna, Hinguvachadi churna, Narayana churna, Chavikasavam, Nityanand ras, Erand paka, Kumaryasava, Pradarantak lauha, Saptavinshati guggulu, Trayodasanga guggulu, are available in the market for abdominal disorders, urinary tract infection, menstrual disorder, skin diseases, heart ailments.

The work aims to carry out studies on stems of the valuable *J. communis* based on histological parameters, physicochemical, phytochemical, fluorescence analysis, and chromatographic analysis by using TLC, GC-MS, and antimicrobial activity against *E. coli*.

2. Materials and Methods

2.1 Chemicals

All the chemicals purchased from Thermo Fisher Scientific India Pvt. Limited, Rankem laboratory reagent, and Finar Chemicals, India were of analytical grade.

2.2 Plant material

Fresh stems of *J. communis* were collected from the campus of Kumaun University, Bhimtal (Uttarakhand) in November and authenticated from the National Botanical Research Institute (NBRI) Lucknow (U.P.). The voucher specimen provided by CSIR-NBRI herbarium (LWG) with Accession No. 10211 has been deposited for future reference. For later usage, the stems were dried and kept in an airtight container.

2.3 Organoleptic parameters and macroscopic studies

The color, odor, and taste of stem powder were evaluated for organoleptic parameters. The size, shape, and texture were determined for macroscopic studies.

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2.4 Microscopic studies

Microscopic studies on the stem part (powder and transverse section) were carried out using the Labomed Vision 2000 (LED) Research microscope with a 45 X objective lens and an eyepiece at 10 X. The dried stems were softened by boiling water with them. Free-hand thin transverse sections were observed on slides using cover slip after cutting by the razor blade and mounted in glycerine-aqueous solution (50% v/v) (Rao *et al*., 2015). Lignified tissues were identified by staining with phloroglucinol and hydrochloric acid (Foster, 1949). Powdered *J. communis* (# 60) was also stained and mounted in glycerine.

2.4.1 Scanning electron microscopy (SEM)

Scanning electron microscopy is an effective technique for obtaining high-magnification images. It operates at a high vacuum. A high-energy electron beam is generated by a source (a field emission gun) which is accelerated through a high voltage (*e.g*., 20 kV). The samples were put inside the SEM chamber (Duarte and Empinotti, 2012). The focussed beam of electrons impinged on the stem sample and scanned the surface. Electrons were bounced back from the specimen and collected by a suitably positioned detector. ZEISS EVO 18 Research (Germany), an analytical microscope was used for the microscopical analysis.

2.5 Physicochemical evaluation

2.5.1 Moisture content

The loss on drying method was used for the determination of moisture content and calculated on a dry weight basis. The experiment was done in triplicate. The procedure was followed given in the Indian Pharmacopoeia (1996).

2.5.2 Extractive/ash values

Following the procedures described in the Indian Pharmacopoeia (1996), the extractive values of the dried, powdered plant stems that were both alcohol- and water-soluble, total ash, acid-insoluble ash, and water-soluble ash were calculated. A muffle furnace (Narang Scientific Works, New Delhi) was used to prepare the ash (Hussain *et al*., 2015).

2.5.3 Qualitative phytochemical screening

Usually, secondary metabolites present in crude drugs are responsible for pharmacological effects. Powdered crude drug extracts were prepared in ethanol and water by soaking in respective solvents for seven days with occasional stirring. After filtration, extracts were concentrated and dried at 40-50°C in an oven. The presence of phytoconstituents was screened by performing different qualitative chemical tests for flavonoids, phytosterols/terpenoids, polyphenols, alkaloids, carbohydrates, tannins, and proteins in both extracts (Harborne, 1984; Khandelwal, 2002).

2.5.4 Fluorescence analysis

Small amounts of stem powder were treated with various types of reagents for fluorescence analysis and identified under visible, short, and long UV wavelengths *via* changes in color under ultraviolet light (Roshchina *et al*., 2017; Rahman and Hussain, 2017).

2.6 TLC fingerprint profiles

Dried powdered stems of *J.* communis (2 g) were macerated for 3 days in ethanol. The extract was recovered by Digital Rotary Evaporator, (Gentek India Pvt. LTD.). Thin-layer chromatography was carried out on a TLC plate pre-coated with silica gel 60-F_{254} aluminium sheets (Merck KGaA, 64271 Darmstadt, Germany) with 0.2 mm of layer thickness. Using a capillary tube, band-shaped sample spots were placed 1.5 cm above from the bottom. Before inserting the plate into the chromatographic tank, which was immediately covered, the sample that had been spotted on the plate was allowed to dry. The plate was taken out, marked, and dried when the solvent reached the top of it. The bands were counted under visible, UV light at 365 nm and 254 nm wavelengths (Ahamed *et al*., 2017).

2.7 Gas chromatography-mass spectroscopic (GC-MS) analysis

2.7.1 Extraction of plant material

The 100 g of crushed dried stem was packed in the soxhlet apparatus using thimble with 350 ml of ethanol at a temperature of 50-60°C. Continuous extraction was done till a clear solvent was attained. Afterward, the solvent was evaporated to get the dried extract (Ukwubile *et al*., 2019). The color and yield of the extract were reddish brown and 7.31 g, respectively.

2.7.2 Instrument and running conditions

For the identification of compounds through GC-MS analysis, the ethanolic extract of the *Juniper* stem was carried out by utilizing a GC-MS-QP 2010 Ultra instrument (Shimadzuβ). The ethanol was used to dissolve the sample and injected into a 30 m x 0.25 mm x 0.25 m SH-I-5Sil MS capillary column with a splitless injection technique. The GC-MS used for the analysis was run under the following conditions: Oven temperature was raised from 45°C to 140°C at a rate of 5°C every minute until reaching 280°C and remaining there isothermally for 10 min. The sample injection volume was 2 µl, and the carrier gas utilised was helium at a 1 ml/min flow rate. At 70 eV energy of electron impact, sample components were ionized from 9.10 min to 52.0 min. NIST Mass spectral library (2020) was then used for fragmented compounds to compare the structures with those of the NIST database. These compounds were then characterized based on the peak area (%) and retention time (Jubie and Dhanabal, 2012; Sharma *et al*., 2021).

2.8 Antibacterial activity in extracts of *J. communis* stem

2.8.1 Bacterial strains

J. communis stem extracts (JME and AJC) were tested for their antibacterial efficacy against the Gram-negative bacteria *Escherichia coli* (ATCC25923) that cause urinary tract infections. The bacterial strains were obtained from the Biosciences Department of Integral University Lucknow, India.

2.8.2 Agar well diffusion technique

The antimicrobial activity of two samples was evaluated against the *E. coli* by the method of Well Diffusion method. 100 µl of the culture broth was added to the pre-casted EMB agar (HIMEDIA) by using a sterile metal spreader. After ten minutes of spreading, using a sterile well borer the wells were punched into the plates. The samples were

filled in the wells and then allowed to diffuse into the surrounding media. After sealing with parafilm the plates were kept for incubation at 37ºC for up to 24 h. The plates had 5 wells, the first well was for positive control that was having ciprofloxacin (standard) of 50 ig, the second well was loaded with distilled water (DW) as a negative control, the third well was loaded with 40 µg concentration of the sample, the fourth was loaded with 60 µg concentration of the sample and fifth was loaded with 80 µg concentration of the sample. On completion of the incubation period, a clear inhibition zone was observed and the diameter (in mm) in plates around the well was measured (Mahire and Patel, 2020; Imran *et al*., 2017).

2.9 Statistical analysis

The average zone of inhibition excluding well on each treatment triplicate plate was recorded. The mean ± standard deviation for each measurement was provided.

3. Results

3.1 Organoleptic evaluation of stem

The sensory-based organoleptic characteristics of the stems showed a brownish color due to the presence of bark, a characteristic taste with a strong, pleasant, aromatic, and long-lasting odor.

3.2 Macroscopic characters of the stem

According to morphological observations, *J. communis* is an evergreen shrub that grows up to 8-9 meters with multiple stems and extensive branching. The green-colored needle-like leaves have been present in whorls of three on twigs about 0.4 to 2.2 cm in size. The stems were 6-35 cm long, 2-3 mm wide; cylindrical, rough surface with thin dark brown bark woody texture and yellow color inner side. The stems were found very hard to break and fibrous. Figure 1 (a-c) shows the shrub, green-colored needle-shaped small leaves, and its stems.

Figure 1: (a) *J. communis* plant, (b) stems with needle-shaped leaves, (c) its stems.

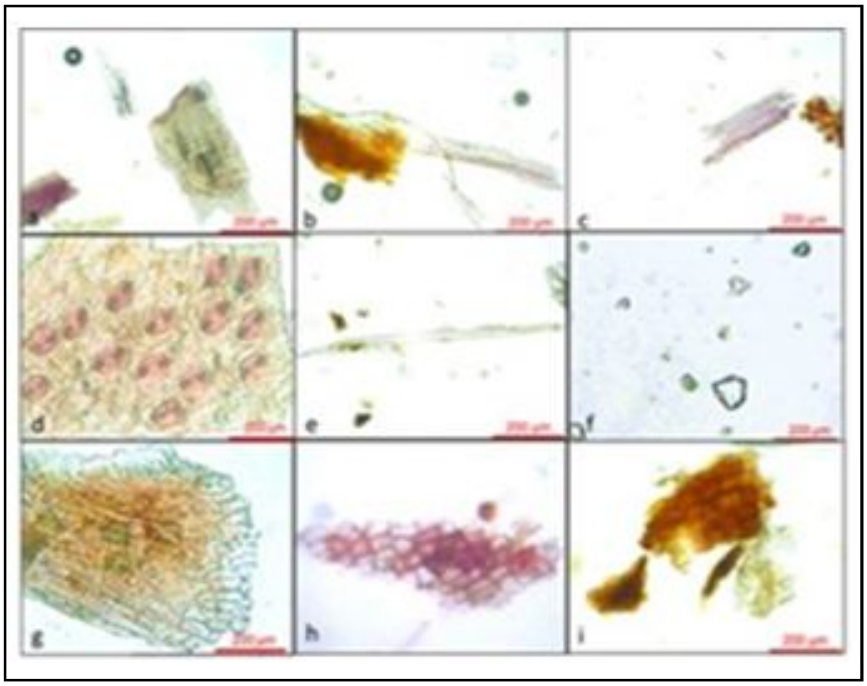

Figure 2: *J. communis* stem powder characteristics (a) ray parenchyma with pits and transverse wall, (b) xylem tracheids, (c) uniseriate rays, (d) stomata, (e) phloem fibre, (f) calcium oxalate crystals, (g) parenchyma cells, (h) cork cells, (i) fragment of cork cambium.

3.3 Microscopic characters of the stem

3.3.1 Powder characteristics

The color of the stem powder was found light yellowish brown with a specific aromatic odor. It was characterized by the presence of ray parenchyma with pits and transverse wall in Figure 2 (a), xylem tracheids in Figure 2 (b), uniseriate rays in Figure 2 (c), stomata in fragments of epidermal cells in Figure 2 (d), phloem fibres in Figure 2 (e), microscopic calcium oxalate crystals in Figure 2 (f), group of parenchymatous cells in Figure 2 (g), cork cells with polygonal in shape Figure 2 (h) and cells of cork cambium Figure 2 (i) (Bar = 200 µm).

3.3.2 Transverse section (T.S.) of stem

The microscopical observations play an important role in authenticating herbal crude drugs (Pawaskar and Sasangan, 2018). The observations were made on a stem cross-section for the anatomical characteristics of the *Juniper* stem. Transverse sections of *J. communis* stems are shown in Figure (3-5). The parts of the cross-section of the stem showing unstained and stained lignified xylem tracheids in Figure 3 (a, b), the triangular pith of heterocellular parenchyma cells in Figure 3 (c), an abundance of phloem fibres in the form of bands in Figure 3 (d), resin duct in Figure 3 (e), and cortex with cork cell in Figure 3 (f). Conifers have secondary growth, and annual growth rings distinguish the earlywood from the latewood transition. The transverse section consists of outer cork cells followed by cork cambium and cortex in Figure 4 (a), medullary rays along with narrow, thick-walled lignified flattened xylem tracheids in Figure 4 (b, c) which were radially arranged. Xylem vessels were absent. The form of the ray parenchyma was found to be one cell in width. The secondary phloem was characterized by parenchyma cells and phloem fibres. The complete transverse section of the stem showed a periderm which is composed of (1) phellem, (2) phellogen or cork cambium, and (3) phelloderm (secondary cortex of parenchyma-like cells). The layer of rectangular cells is arranged tangentially in phellogen. The cortical parenchyma was found with the organization of continuous cells with resin channels in the phloem. Phloem consists of sieve tubes and phloem parenchyma. Distinct annual ring boundaries showing tracheids radially arranged with medullary rays of parenchyma cells. Pith was well defined as parenchyma cells as shown in Figure 5.

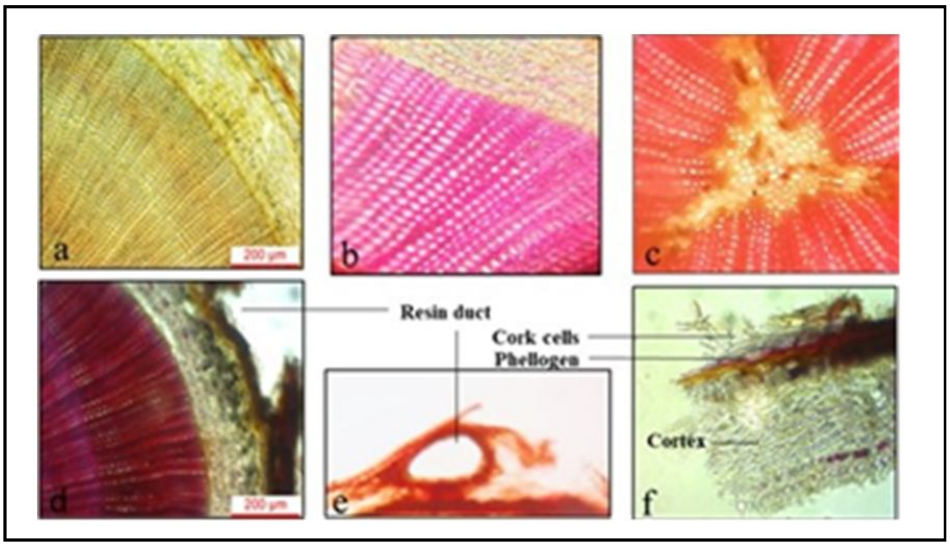

Figure 3: ***J. Communis*** **stem showing transverse section (T.S.) (a and b) without staining and with staining, (c and d) central pith and secondary growth of stem cortex with cork cells and resin duct, (e and f) showing resin duct and cortex (100 X).**

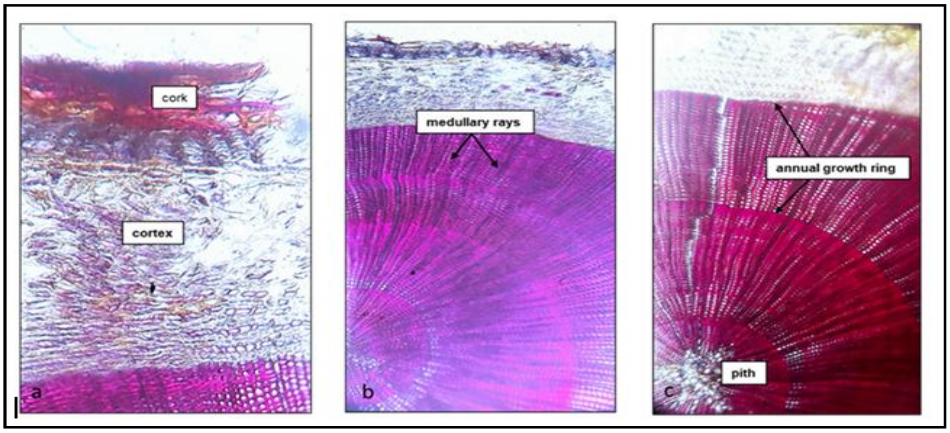

Figure 4: **T.S. of stem shows (a) cork cells with portion of cortex, phloem, and xylem tracheids, (b) medullary rays, (c) pith and secondary xylem growth (100 X).**

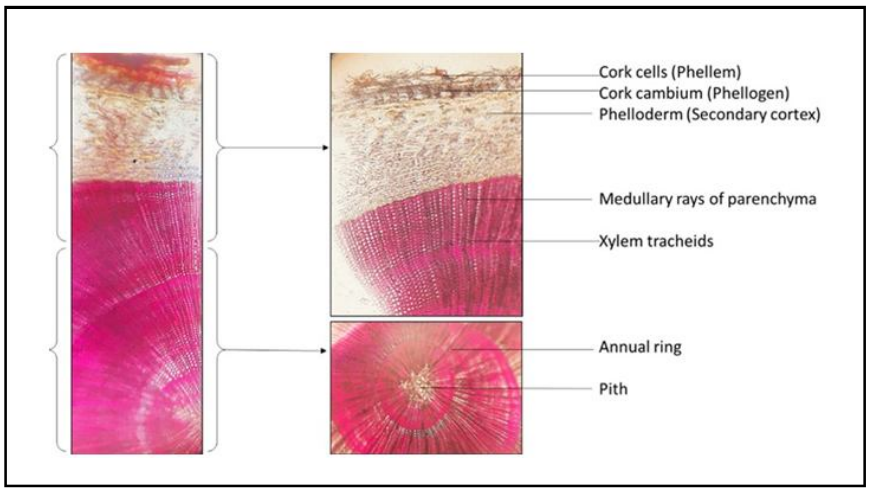

Figure 5: T. S. of *J. communis* stem (100 X).

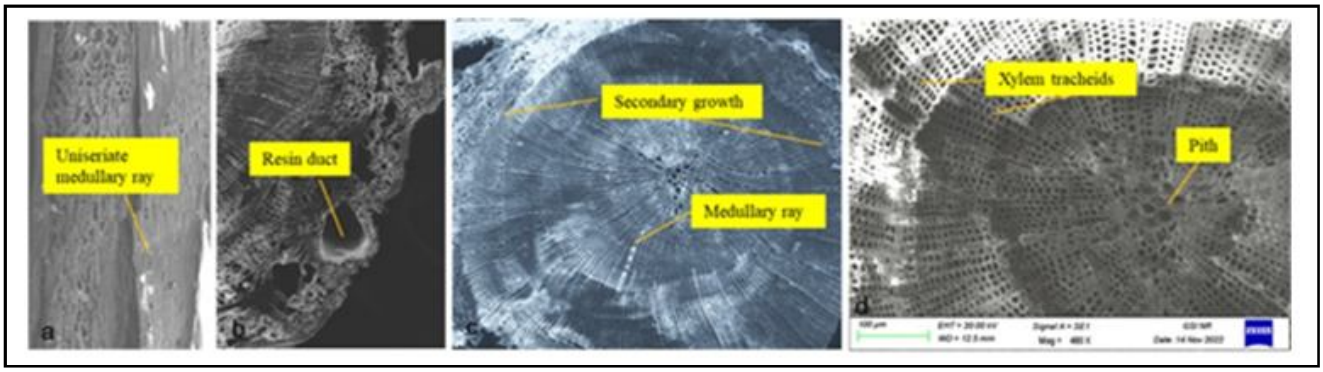

Figure 6: SEM micrographs of *J. communis* stem (a) longitudinal section through xylem and phloem showing tangential pits, (b-d) transverse section showing resin duct, secondary growth, xylem, tracheids, medullary ray, and pith at magnification 260 X, 377 X, and 622 X.

3.3.3 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) is used to authenticate the species by examining the structures at high resolution (Joshi, 2008). SEM imaging shows the high magnification images of the uniseriate medullary rays in the longitudinal sections of the stem samples in Figure 6 (a). The transverse showed resin duct in Figure 6 (b), secondary growth rings, uniseriate medullary ray in Figure 6 (c), xylem tracheids, and pith in the center in Figure 6 (d) of the sample. The images were taken at different magnification ranges from 260 X to 622 X.

3.4 Physicochemical evaluation

For the physicochemical evaluation, the different variables like moisture content (Loss on drying), extractive values (ethanol and water-soluble), and ash values (total, acid insoluble, and water-soluble) were assessed to determine the purity of the *J. communis* stems. The presence of high moisture content signifies the poor efficacy and quality of the herbal crude drug as moisture may affect the bioactive components by hydrolysing them. Quantitative ash value determination is also important in standardization as it indicates any possible adulteration, inorganic salts, sand, *etc*. It was calculated in terms of total ash by igniting a measured quantity of crude drug completely till constant weight and residue left after boiling with acid and after treatment with water (Kunle, 2012). All these quantitative measurements are given in Table 1.

Table 1: Physicochemical parameters

Parameters	Results
Moisture content*	9.20% w/w
Ethanol soluble extractive value*	11.6% w/w
Water soluble extractive value*	3.92% w/w
Total ash*	9.65% w/w
Acid insoluble ash value*	4.92% w/w
Water soluble ash value*	8.78% w/w

n=3: *dry weight basis

3.5 Qualitative phytochemical screening

Phytochemical screening provides the presence of a confirmed class of compounds like alkaloids, flavonoids, tannins, and terpenoids/ steroids by performing specific tests for them (Dubale *et al*., 2023) Initial phytochemical screening of ethanolic and aqueous extracts revealed the presence of triterpenoids, fatty acids, phytosterols, carbohydrates, tannins, alkaloids, flavonoids, and tannins. Table 2 displays the outcomes.

Table 2: Phytochemical screening of both ethanolic and aqueous extracts of *J. communis* stem

Class of phytoconstituents	Ethanolic extract	Aqueous extract
Terpenoids	+	+
Phytosterols	+	-
Proteins	-	-
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Tannins	+	+

(+) Present, (-) Absent

3.6 Fluorescence analysis

Fluorescence analysis is the assessment of crude drug powder qualitatively for pharmacognostic study because in UV light or during daylight some compounds after treatment with reagents can show fluorescence (Majid *et al*., 2021). The change in color of the powdered crude drug after treatment with various reagents such as acids, picric acid, iodine, *etc*., was recorded in an ultraviolet chamber under visible, UV light (254 nm and 365 nm) as shown in Table 3. In this study, picric acid showed the green fluorescence.

Table 3: Fluorescence analysis of *J. communis* stem powder

Treatment	Visible light	Under UV light	
		Short wavelength(254 nm)	Long wavelength (366 nm)
Powder as such	Light brown	Light brown	Light brown
Powder + Water	Light brown	Dull cream	Dark brown
Powder + HCl	Light brown	Dark brown	Black
Powder + HNO_3	Pinkish brown	Yellowish brown	Dark brown
Powder + H_2SO_4	Pinkish brown	Yellowish brown	Black
Powder + 1N Me NaOH	Dark brown	Black	Dark brown
Powder + Picric acid	Yellowish brown	Fluorescent green	Brownish Black
Powder + Iodine	Dull brown	Dull brown	Dark brown
Powder + $FeCl_3$	Yellowish brown	Brownish cream	Dark brown
Powder + Lead acetate	Light brown	Light brown	Dark brown

3.7 Thin layer chromatography (TLC)

Thin-layer chromatography (TLC) is a simple, useful, and easy analysis for identifying various phytoconstituents present in a mixture by separating them using a mobile phase and developing a fingerprinting profile (Puri, 2019). On a precoated silica gel TLC plate, a standard solution of ethanolic extract was made and quantitatively loaded. The plate was exposed to 0.5% anisaldehyde-H_2SO_4 for development before being examined in the chamber under UV light at 365 nm and 254 nm, respectively, in Figure 7 (a-c). On spraying with 0.5% anisaldehyde- sulphuric acid and heating the plate for ten minutes at 110°C seven spots were detected at R_f 0.10, 0.18, 0.29, 0.45, 0.55, 0.78, and 0.82 using the mobile phase toluene: ethyl Acetate (9:1). Under UV (254 nm) one fluorescent zone at R_f 0.55 (light blue) was observed among 5 spots.

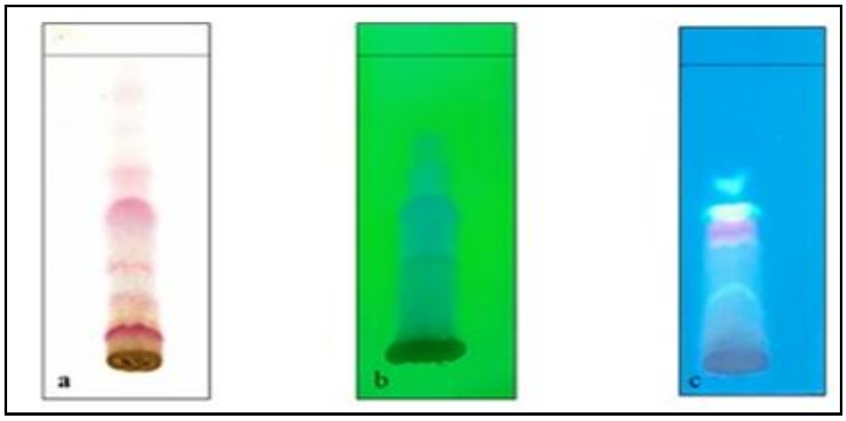

Figure 7: (a) Thin-layer chromatography of ethanolic extract of the stem in visible light after spraying with anisaldehyde-sulphuric acid reagent, (b) TLC of ethanolic extract in UV-long wavelength, (c) TLC of ethanolic extract in UV-short wavelength.

3.8 GC-MS analysis of an ethanolic extract of *J. communis* stem

Gas chromatography is an important analytical technique to identify volatile chemical compounds (Shulammithi, 2016). GC-MS chromatogram of the ethanolic extract of *J. communis* stem showed 25 compounds (Table 4 and Figure 8). Confirmation of their presence was based on retention time (RT), peak area (%), and molecular formula. It was found that main constituents lup-20(29)-en-3β-ol, acetate (54.46%), lupeol, 24-norursa-3,12-diene (6.67%), podocarpa-6,8,11,13-tetraen-12-ol,13-isopropyl (2.50%), 9Z,12Z-octadecadienoic acid (1.03%) present in high quantities. In previous studies, lup-20(29)-en-3β-ol acetate and lupeol displayed antimicrobial, anti-inflammatory, antitumor, and diuretic activities (Gallo and Sarachine, 2009). Lupeol acts as an antioxidant, lowers calcium-oxalate, and cadmium levels in the kidney, and is considered nephroprotective (Sharma *et al*., 2020). Lupeol reestablished the level of renal enzymes and increased urinary excretion (Malini *et al*., 1995).

Table 4: Major and minor compounds identified by GC-MS in the ethanolic extract of *J. communis*

S. No.	Compound name	Molecular formula	Molecular weight g/mol	R. time (RT)	Peak area%	Nature of compounds
1.	2,3-dihydro-3,5-dihydroxy-6-methyl -4H-pyran-4-one	$C_6H_8O_4$	144	14.564	0.17	Dihydropyranones
2.	Vanillin	$C_8H_8O_3$	152	21.612	0.10	Phenolic aldehyde
3.	1,4,7-Cycloundecatriene, 1,5,9,9-te- tramethyl-	$C_{15}H_{24}$	204.35	23.467	0.08	Sesquiterpenoid
4.	Humulenol-II	$C_{15}H_{24}O$	220	29.453	0.09	Sesquiterpene
5.	Benzenepropanol, 4-hydroxy-3-methoxy-	$C_{10}H_{14}O_3$	182	29.723	0.07	Phenylpropanoid
6.	Thunbergol	$C_{20}H_{34}O$	290	30.113	0.02	Monocyclic diterpene
7.	Nootkatone	$C_{15}H_{22}O$	218	32.512	0.03	Sesquiterpenoid
8.	Incensole oxide	$C_{20}H_{34}O_3$	322.5	32.749	0.21	Diterpenoid oxide
9.	Thujol	$C_{10}H_{18}O$	154	33.272	0.05	Monoterpene alcohol
10.	Cedran-diol	$C_{15}H_{26}O_2$	238	33.885	0.14	Sesquiterpene alcohol
11.	n-Hexadecanoic acid	$C_{16}H_{32}O$	238	34.623	0.75	Fatty acid
12.	D-Fructose, 3-O-methyl-	$C_7H_{14}O_6$	194	35.116	2.52	Sugar
13.	9Z,12Z-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	37.272	1.03	Fatty acid
14.	Oleic Acid	$C_{18}H_{34}O_2$	282	37.395	0.92	Fatty acid
15.	Phenanthrene,1,2,3,4,4a,10a-hexa hydro-7-methoxy-1,1,4a-trimethyl- 8(1-methylethyl)-	$C_{21}H_{30}O$	298.5	38.65	1.53	Tricyclic diterpenoid
16.	Podocarpa-6,8,11,13-tetraen-12-ol, 13-isopropyl	$C_{20}H_{28}O$	284	40.824	0.23	Tricyclic diterpene alcohol
17.	Totarol	$C_{20}H_{30}O$	286.5	40.996	2.50	Diterpenoid phenol
18.	Eicosanoic acid	$C_{20}H_{40}O$	312.5	41.322	0.33	Fatty acid
19.	1-Heptatriacotanol	$C_{37}H_{76}O$	537	41.407	0.75	Alcoholic compound
20.	γ-Sitosterol	$C_{29}H_{50}O$	414	42.417	0.59	Steroid
21.	Podocarpa-8,11,13-trien-3-one, -13-hydroxy-14-isopropyl-, acetate	$C_{22}H_{30}O_3$	342.48	42.777	0.21	Abietane diterpenoid
22.	13,27-Cyclours-11-en-3-ol, acetate	$C_{32}H_{50}O_2$	466.7	43.827	1.06	Triterpene
23.	24-Norursa-3,12-diene	$C_{29}H_{46}$	394.6	45.129	2.57	Nortriterpene
24.	Lupeol	$C_{30}H_{50}O$	426.7	45.362	6.67	Pentacyclic triterpenoids
25.	Lup-20(29)-en-3β-ol, acetate	$C_{32}H_{52}O_2$	468.75	50.224	54.4	Triterpenoid

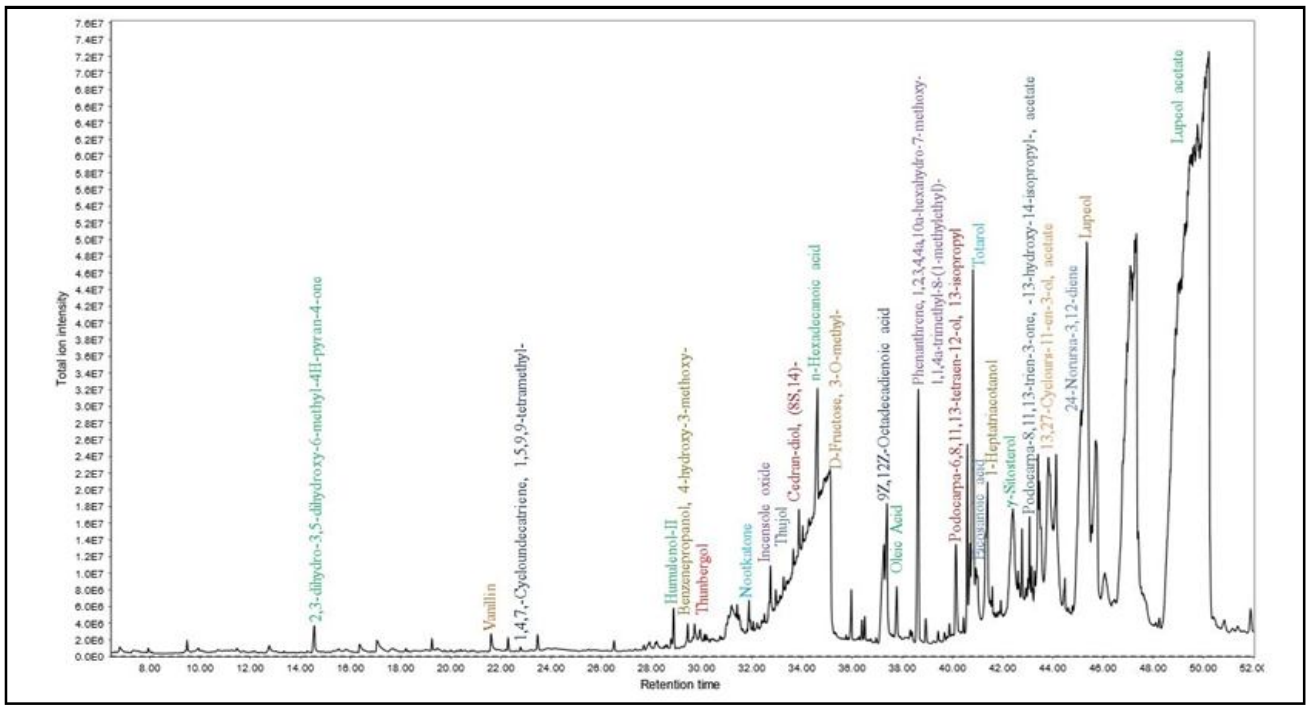

Figure 8: GC-MS chromatogram of the ethanolic extract of *J. communis* stem.

3.9 Antibacterial activity of *J. communis* extracts

The most common urinary tract infection is caused by *E. coli* bacteria, associated with several risk factors. Individuals show antibiotic resistance by using frequent antimicrobial medicines (Flores-Mireles, 2015; Bhavsar *et al*., 2022). There is a need for natural antimicrobials for fighting such type of infection. The antibacterial activity against *E. coli* of the ethanolic and aqueous extracts was tested using a microbiological assay. Both ethanol and aqueous extract of *J. communis* stems were observed to be moderately effective against *E. coli* (Table 5). The results were compared with the standard drug ciprofloxacin. The well-known antibiotic ciprofloxacin displayed a zone of inhibition at 20 ± 0.79 at the concentration of 50 µg, whereas the ethanolic extract displayed a zone of 12.1 ± 1.02 mm, 13 ± 0.73 mm, and 14.1 ± 0.35 mm at 40, 60 and 80 µg/ml, respectively. The aqueous extract displayed an inhibitory zone measuring 15 ± 0.2 mm, 15.2 ± 0.7 mm, and 15.4 ± 0.17 at 40, 60, and 80 µg/ml, respectively. This outcome shows that the effectiveness of aqueous extracts at all concentrations was moderately antimicrobial when compared to standard ciprofloxacin.

Table 5: Antibacterial activity of ethanol extract and aqueous extract against *E. coli* using well diffusion method

S. No.	Sample	Bacteria	Concentration µg/ml	Diameter of zone of inhibition (mm)
1.	Ethanol extract	*E. coli*	40	12.1 ± 1.02
			60	13.0 ± 0.73
			80	14.1 ± 0.35
2.	Aqueous extract		40	15.0 ± 0.2
			60	15.2 ± 0.7
			80	15.4 ± 0.17
3.	Positive control (Ciprofloxacin)		50	20.0 ± 0.79
4.	Negative control (Distt. water)		-	Nil

4. Discussion

In various diseases, herbal medicines are used as alternative and safe medicines because of the presence of bioactive components. The development and production of herbal medicines should be quality controlled. For manufacturing medicinal herbal products, every step has an important role from the correct identification of raw material to the last step of the manufacture by using quality control methods for standardization that assure the quality, purity, and efficacy (Balekundri and Mannur, 2020). For assessing their quality, the WHO guideline (WHO, 2011) described a series of tests for assessing the quality of medicinal plant materials. To establish the identity and purity of the crude drug, the first step is macroscopic and microscopic examination before carrying out any further tests (Ichim *et al*., 2020). This study explored detailed morphological and

anatomical descriptions. Epidermal cells of the stem are covered with a cuticle and the pith is surrounded by the xylem and phloem (Lakušiæ, and Lakušiæ, 2011). The present study also showed the cork cells, resin ducts, xylem tracheids, phloem fibres, and central pith in the transverse section of the stem.

According to the WHO, moisture content determination is important for quality control. In the presence of excess water, the crude drug can deteriorate due to the growth of mites, mold, and insects. The average moisture content was found 9.20% w/w in the stem powder. Other physicochemical parameters were also determined which can be used as a reference for quality assurance. The ethanolic and aqueous extracts showed positive results for the phytoconstituents given in Table 2. Several biological activities of *J. communis* are due to these phytochemicals (Živiæ *et al*., 2019).

As a qualitative method for authentication, powder fluorescence analysis is employed. The study reveals a certain color for a particular compound even a non-fluorescent compound with different reagents can convert to fluorescent compounds (Kamble and Gaikwad, 2019). Table 3 shows the characteristic fluorescent properties of *J. communis* stem powder. Thin-layer chromatography (TLC) is an invaluable technique used for the fingerprint profiling of phytoconstituents (Ahamed *et al*., 2017). TLC of the ethanolic extract showed 7 bands which denote the presence of 7 phytochemicals.

Gas chromatography, which is rapid and precise, is best for analysing and identifying volatile oils, and heat-sensitive components. It aids in the exact determination of the phytoconstituents and the profiling of complex structures (Qadir *et al*., 2022; Akbar, 2020). In this study, the ethanolic extract revealed mostly terpenoids, their alcohol, and fatty acids were found to be present.

E. coli is the causative pathogen for complicated and non-complicated urinary tract infections. In case of antibiotic resistance and fear of surgery, someone takes steps for alternative medicines. Natural substances exhibit positive effects due to their enormous potential (Zhou *et al*., 2023). Because of this, the antimicrobial assay was done against ciprofloxacin to determine the antibacterial activity of the ethanolic and aqueous extract against *E. coli*. This result concludes the moderate efficacy of the extracts. The efficacy may be due to the bioactive constituents present in the stems such as flavonoids, phenolics, and terpenoids, but further investigation is required for the specificity of the compounds.

5. Conclusion

The pharmacognostic analysis of the stem will provide some guidelines for accurate identification in further works. The GC-MS method was used to identify the phytoconstituents in the ethanol extract, in which 25 compounds were recognized with dominant constituents such as lupeol and its acetate. The qualitative analysis of the *J. communis* stem showed the presence of terpenoids, phytosterols, alkaloids, flavonoids, and tannins. According to the antimicrobial investigation against the strain of *E. coli* the aqueous extract showed better activity than the ethanolic extract of *J. communis* stem. Previous studies show that triterpenoids exhibit antibacterial activity. Juniper stems show the presence of triterpenoid compounds which may be responsible for the antimicrobial activity against *E. coli*, so further research is required to confirm specific responsible compounds in this plant part.

Conflict of interest

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

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