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BIOMEDICAL POTENTIAL OF CYMBOPOGON FLEXUOSUS(LEMON GRASS)LEAVES

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Abstract

Central India is one of the largest biodiversity regions in Asia, characterized by a great range of medicinal and aromatic plants, whose therapeutic use reflect a longstanding heritage across several cultures. The genus Cymbopogon possesses significant medicinal characteristics and is recognized for its remarkable potential, including its use in cancer treatment in certain regions, thereby offering an alternative to allopathic therapies with natural remedies. This study aims to evaluate the antibacterial efficacy of the chemical and identify its specific constituents. The antimicrobial profile of hydro-alcoholic, aqueous, methanol, ethanol, hexane, chloroform, and petroleum ether extracts of Cymbopogon flexuosus demonstrated that the methanol and ethanol extracts exhibited significant antibacterial efficacy against pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa, Brevibacillus brevis, Enterococcus faecalis, Vibrio cholerae, Aspergillus niger, Candida albicans, Schizosaccharomyces sp., and Saccharomyces cerevisiae. The petroleum ether extract of Cymbopogon flexuosus demonstrated minimal efficacy against the test pathogens. FTIR and HPLC analyses have shown Quercetin as the predominant bioactive metabolite.

Keywords: Antimicrobials, HPLC Analysis,Cymbopogon, Phytochemical, TLC Profile

Introduction

Recently, contemporary science and culture have acknowledged the therapeutic properties of indigenous herbs as an alternative to allopathic synthetic medications. Currently, the emergence of resistance in microbial populations to standard therapeutic agents poses a significant worry in the healthcare sector (Alviano and Alviano, 2009). Plants are a rich source of bioactive compounds, specifically secondary metabolites such as alkaloids, flavonoids, phenols, polyphenols, saponins, steroids, and tannins, which may serve as effective antimicrobial agents against pathogenic bacterial strains due to their biological efficacy. These naturally produced bioactive compounds have significantly contributed to the pharmacological sector in the development of various healthcare goods, such as antibacterial, anti-tumor, and anti-hepatotoxic medications. Gortzi et al. (2007). A variety of herbs, shrubs, trees, and climbers have been investigated for bioactive compounds for diverse medical purposes in the healthcare industry. (Ezzatadeh et al., 2012). Medicinal plants are regarded for their potential use in preventing and treating certain microbial diseases(Ncube et al, 2012). The active compounds derived from

medicinal plants using organic solvents are more substantial (Mitra and Sur, 1997). The interaction among active secondary metabolites may have elicited the therapeutic effects of medicinal plant extracts (Jaiswal and Jain, 2017). Biological mechanisms produce deleterious free radicals and superoxide as by-products, which may lead to mutations and related diseases (Adiguzelet al., 2009). Secondary metabolites, specifically phenolic acids and flavonoids, possess antioxidant properties that may neutralize free radical activity, hence alleviating adverse effects on human health (Choi et al., 2019).

Conventional medicinal herbs are acknowledged as sources of several bioactive chemicals. The demonstrated therapeutic efficacies have permitted pharmaceutical companies to utilize crude extracts in medication production (Meskin et al., 2002). Contemporary pharmaceutical companies are increasingly depending on research involving medicinal plants to uncover possible bioactive therapeutic agents and to synthesize medications derived from their chemical structures (Unnikrishnan 2010). In comparison to the vast potential of Earth's flora, just a minor portion has been investigated thus far (Chidozie et al., 2014). Therefore, more systematic initiatives are necessary based on bioassay evaluations of natural compounds derived from medicinal flora.

Cymbopogonflexuosus(Syn. CymbopogoncitratusfamilyPoaceae)(Chweya and Mnzava,1997)is an orphan leafy, herbaceous plant also known as citronella grass or lemongrass in English and Nimbughaasor Cochin ghaasin Hindi. Plants belonging to this genus are typically C_4 plants having widespread populations in tropical areas of the world(Van Den Bergh, 2014). In several communities of India and especially Chhattisgarh the plant is cultivated in home gardens. The leaves and young shoots are often consumed as pot herbs adding in soups and tea. Sometimes, the leaves are blanched and dried for preservation also (Flyman and Afolayan, 2006).

C. flexuosusleaves has been traditionally used in Indian medical practices like Ayurveda for several years. The leaves are still used as disinfectants, for eye wash and earache. The whole plant has been reported to have remarkable antimicrobial, antihelminthic and antifungal properties(Sridhar et al. 2014). Recent studies have also demonstrated antiproliferative, antineoplastic and anticancer activities of C. flexuosuson various cell lines(Rajendran et al.,2014, Pettit et al, 2005, Bala et al 2010).

Keeping the above facts in mind, the present study was designed and proceeded accordingly.

Materials and Methods

Sample collection and Processing

Cymbopogon flexuosus specimens were gathered from Raipur district, with final identification conducted by Dr. P.K. Joshi, Principal Scientist at the Centre of Excellence on MAPs and NTFP, IGKV, Raipur. The leaves of the gathered plant material were subsequently processed, specifically oven-dried and pulverized into powder. The extracts were produced using the cold percolation method. The specified fraction of ground powder was immersed in (50% w/v) hydro-alcohol, aqueous methanol, ethanol, hexane, chloroform, and petroleum ether for 72 hours. The

produced combinations were agitated with a sterilized glass rod at 24-hour intervals. The extracts were filtered using Whatman filter paper No. 1 (Dulger and Gonuz, 2004). The filtrates were subsequently concentrated in a water bath.

Antimicrobial Profile

The antimicrobial profile of each extract was assessed against a range of Gram-positive and negative pathogenic bacteria utilizing the Kirby-Bauer method as outlined by NCCLS standards(NCCLS, 2002), along with fungi through the Poisoned food technique (Pundir et al, 2010). The test pathogens were sourced from the Microbial Type Culture Collection at IMTECH in Chandigarh, India. The following bacterial strains were acquired: Alcaligens faecalis (MTCC 9780), Brevibacterium brevis (MTCC 3136), Bacillus cereus (MTCC- 1272), Enterococcus faecalis (MTCC- 2729), Staphylococcus aureus (MTCC-3160), Escherichia coli (MTCC- 3221), Klebsiella pneumoniae (MTCC- 9544), Pseudomonas aeruginosa (MTCC- 3163), Salmonella typhi (MTCC- 733), and Vibrio cholerae (MTCC-3904). The fungal strains acquired include Aspergillus niger (MTCC-478), Candida albicans (MTCC-183), Saccharomyces cerevisiae (MTCC-170), and Schizosaccharomyces japonicus (MTCC-3061). The bacterial strains were cultivated on Soyabean Casein Agar at a temperature of 37 degrees Celsius. The fungal strains were cultivated on Sabouraud's Dextrose Agar at 25°C. The inoculums of each pathogenic bacterium were meticulously spread over the surface of the Muller-Hinton agar in an even manner. Each disc, measuring 6.0 mm in diameter, was infused with various plant extracts at specified concentrations and subsequently dried in an oven at 400°C. The impregnated discs were aseptically placed onto the surface of the Muller-Hinton agar. The incubation conditions for the bacterial plates were set at 35±20C, while the yeast plates were maintained at 28±20C. The plates underwent examination at intervals of 24 to 48 hours. The clear zones were measured in millimeters using a ruler (Mathur et al., 2011). Both the positive and negative controls were implemented. The positive control employed consisted of Azithromycin (1µg/ml) for the bacterial plates and Fluconazole (1 µg/ml) for the fungal plates. The experiments were conducted in triplicate.

Phytochemical Profile

Major phytochemical viz., alkaloids, flavonoids, glycosides, reducing sugars,saponin, steroids and tannins were examined(Kamal, 2014).

Alkaloids- Warmed in advance Five milliliters of 1% hydrochloric acid was combined with 0.5 grams of the plant extract and subsequently filtered. 1.0 ml of the filtrate was combined with Dragendroff's reagent. The emergence of turbidity or precipitation was noted in relation to the presence of alkaloids.

Flavonoids- A mixture of methanol and 0.2 g of the plant extract was subjected to heating. Upon the introduction of magnesium metal and a small amount of concentrated hydrochloric acid, a red/orange coloration was noted. In the preparation of glycosides, 2.0 ml of glacial acetic acid was combined with a few drops of 1% $FeCl_3$ and 0.5 g of plant extract. Upon the addition of concentrated H_2SO_4, a brown ring was observed at the interphase.

Reducing Sugars-Fehling's solutions I and II (1 ml) were combined with 2.0 ml of the plant extract and placed in a boiling water bath for five minutes. The observation of a brick red precipitate was noted for the confirmation of reducing sugars.

Saponin-The distilled water was combined with 0.5 g of the plant extract and subjected to vigorous shaking. The formation of froth upon heating validated the presence of saponin.

Steroids- The Salkowaski method was employed. A mixture of chloroform (3.0 ml) and 0.5 g of plant extract was prepared and subsequently filtered. Concentrated H_2SO_4 was introduced to the filtrate. The emergence of a reddish-brown colored ring was noted in relation to the presence of steroids.

Tannins- A mixture was prepared by combining 10 ml of boiling distilled water with 0.5 g of the plant extract, followed by filtration. A small volume of $FeCl_3$ (6.0%) was introduced. The emergence of a dark green hue validated the presence of tannins.

Separation and purification of bioactive compound from plant extract

Thin Layer Chromatography

The Thin Layer Chromatography analysis was conducted on a potent plant extract exhibiting maximum antimicrobial activity, utilizing a silica gel bed on a glass plate to detect steroids, alkaloids, flavonoids, and polyphenol groups (Sani et al, 2018). The samples were applied to one end of the glass plate. The solvent system employed consisted of n-Hexane and Ethyl acetate in a 7:3 ratio. To detect alkaloids, flavonoids, and polyphenols, Dragendorff reagent, Sitrobaric reagent, $FeCl_3$, and UV light were utilized as spraying reagents.

Identification and structure elucidation of purified compounds

Identification of purified compounds was done using the combination high performance liquid chromatography (HPLC) and Fourier transform infrared (FTIR) spectrometry.

High Performance Liquid Chromatography Analysis

The HPLC analysis of the plant extract was conducted using the Shimadzu LC-2010 HPLC system, sourced from NCS Green Earth Pvt. Ltd., located in Nagpur, Maharashtra, India. The HPLC equipped with a Shimadzu LC 2010 UV-VIS detector A C-18 column block (heating-type Shim-pack VP-ODS, with an interior diameter of 4.6 mm and a length of 150 mm), featuring a particle size of 5.0 µm, was utilized. The mobile phase was composed of 50% acetonitrile and 50% phosphate buffer, with a flow rate of 3.0 ml/min at a temperature of 25°C. The volume of the sample was 40 µl. The wavelength corresponding to maximum absorbance was determined through UV absorption spectra of the purified plant extract utilizing a UV-VIS Spectrophotometer.

FTIR Analysis

The FTIR analysis of the plant extract was conducted by NCS Group, located in Nagpur, Maharashtra, India, using equipment from Perkin Co., Germany.

The FTIR spectrum was acquired over a range of 4000-400 cm-1 utilizing the KBr pellet technique.
The outcome derived from FTIR spectrometry of the purified compound (isolated from the plant extract) was compared with values found in the literature.

Statistical Analysis

All the experiments were done in triplicates and standard error mean was calculated. The tabulation of data and statistical analysis was done by MS-Excel 2010.

Results and Discussion

The present investigation was performed to assess the antimicrobial efficacy of secondary metabolites of Cymbopogonflexuosus. The isolation and identification of potential purified bioactive compound from plant extract was done using FTIR and HPLC.

Antimicrobial Profile

The antimicrobial profile of hydro-alcohol, aqueous methanol and ethanol, hexane, chloroform, and petroleum ether extract of Cymbopogon flexuosus was assessed (Table I). Observations revealed that the methanol extract demonstrated notable antibacterial effectiveness against pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, and Enterococcus faecalis. The extracts based on non-polar solvents, specifically hexane and chloroform, demonstrated effectiveness against Escherichia coli and Enterococcus faecalis, respectively, while the antimicrobial efficacy against Brevibacillus brevis and Vibrio cholerae was somewhat diminished. The petroleum ether extract of Cymbopogon flexuosus demonstrated minimal activity against the test pathogens. Nearly all extracts of Cymbopogon flexuosus demonstrated significant activity against Aspergillus niger, Candida albicans, Schizosaccharomyces japonicus, and S. cerevisiae, with the exception of the petroleum ether-based extract. The methanolic extract demonstrated the highest anti-fungal and yeast activity against Schizosaccharomyces, Aspergillus niger, and Candida albicans, while showing the least activity against S. cerevisiae.

Phytochemical Profile

The analysis of Cymbopogon flexuosus extracts revealed the presence of notable phytochemicals, including alkaloids, flavonoids, steroids, saponins, and reducing sugars (Table II).

Table 1: Antimicrobial activity of Cymbopogon flexuosusextract against human pathogenic bacteria and fungi/yeast through Disk Diffusion Method.

Test Pathogen Bacterial	Hydro-alcoholic	Methanol extract	Ethanol extract	Hexane extract	Chloroform extract	Petroleum ether extract	Ref. (Azithromycin)

	extract						
A. faecalis	10.50±0.08	13.83±0.00	ND	ND	9.00±0.28	ND	35±0.06
B. cereus	11.16±0.02	10.12±0.02	10.0±0.14	15.5± 0.99	8.00±0.01	6.63±0.05	32.1±0.09
B. brevis	18.50±0.02	17.16±0.15	21.00±0.10	7.63±0.11	6.63±0.05	NA	33.0±0.21
E. faecalis	19.16±0.05	15.00±0.00	24.80±0.09	16.2±0.08	9.50±0.07	NA	29.2±0.18
S. aureus	21.83±0.11	21.00±0.10	25.83±0.00	11.00±0.04	8.80±0.07	NA	26.25±0.02
E. coli	19.00±0.00	NA	18.00±0.14	19.0±0.12	16.16±0.01	NA	36.25±0.02
K. pneumoniae	ND	12.50±0.04	10.67±0.20	NA	NA	NA	30.01±0.09
P. aeruginosa	22.83±0.10	23.00±0.07	20.83±0.84	15.83±0.20	11.50±0.30	ND	34.8±0.07
S. typhi	10.0±0.04	11.83±0.00	NA	ND	ND	ND	28.15±0.15
Vibrio cholerae	ND	12.50±0.10	ND	8.50±0.04	ND	ND	26.18±0.20
Test Pathogen Fungal/Yeast	**Hydro-alcoholic extract**	**Methanol extract**	**Ethanol extract**	**Hexane extract**	**Chloroform extract**	**Petroleum ether extract**	**Ref. (Flucanazol)**
A.niger	13.00±0.80	17.00±0.019	18.20±0.02	12.16±0.27	NA	NA	16.18±0.00
C. albicans	16.00±0.05	12.00±0.33	10.00±0.09	13.0±6.01	ND	ND	15.00±0.01
S. cerevisiae	NA	11.83±0.15	NA	ND	ND	ND	14.58±0.33
S. japonicus	11.50±0.15	17.67±0.15	17.0±0.36	8.00±0.11	14.0±0.01	ND	16.00±0.20

- Data are multiple of three observations
- Values ± standard error
- Ref: Reference antibiotic (Azithromycin and Flucanazol)
- ND: Not detectable, NA-No activity

Table 2: Phytochemical screening of leaves extracts of Cymbopogonflexuosus

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Solvent extracts	Phytochemical constituents						
	Alkaloids	Flavanoids	Glycosides	Reducing sugars	Saponin	Steroids	Tannins
Hydro-alcoholic extract	+	+	-	+	+	-	+
Methanol extract	+	+	-	+	+	-	-
Ethanol extract	+	+	-	+	+	-	+
Hexane extract	-	+	-	+	+	+	+
Chloroform extract	-	-	-	+	+	+	+
Petroleum ether extract	-	-	-	+	+	-	+

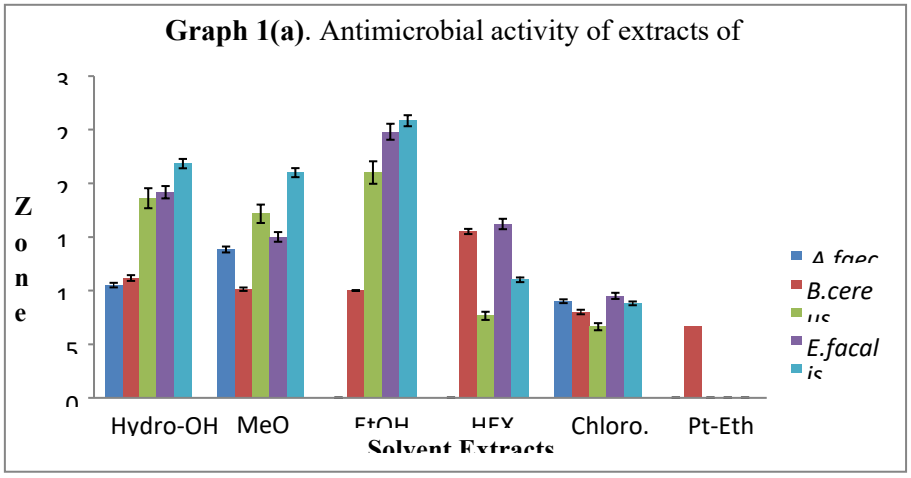
+: present; -: absent.

TLC Analysis

The analysis through Thin Layer Chromatography has yielded valuable insights for detecting various phytochemicals in potent plant extracts. Flavonoid emerged as the predominant phytochemical in nearly all extracts, with the exception of petroleum ether. The comprehensive examination is outlined in Table III.

Table III: Thin Layer Chromatography Analysis

Solvent System	Spray Reagent	R_f Value	Metabolites
nHexane: Ethyl acetate	UV Light	0.37, 0.60, 0.74	Steroids
	UV light	0.32,0.56, 0.92	Terpenoids
	Dragendorff Reagent	0.53,0.74	Alkaloids
	Sitroboric Acid	0.20, 0.86, 0.90	Flavonoids
	$FeCl_3$	0.34, 0.63	Polyphenols



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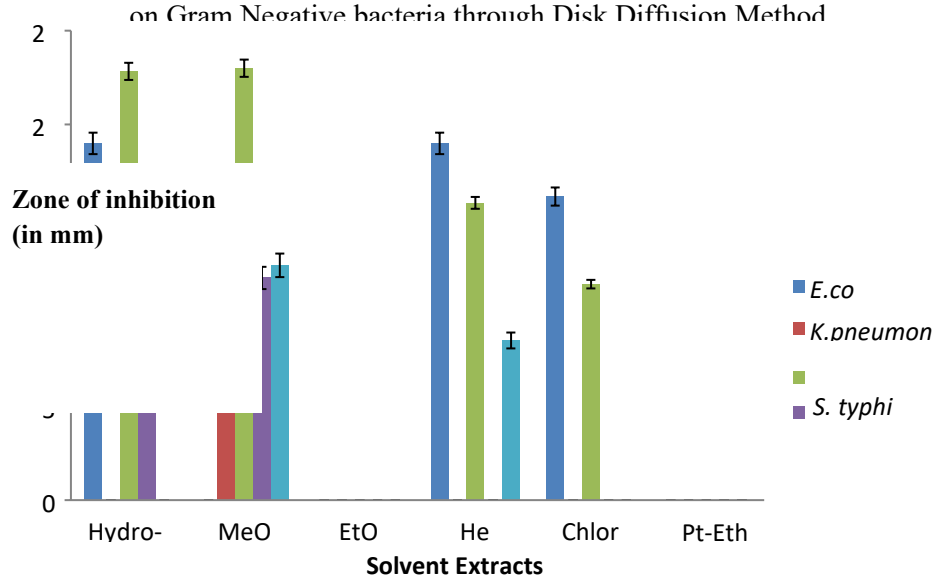
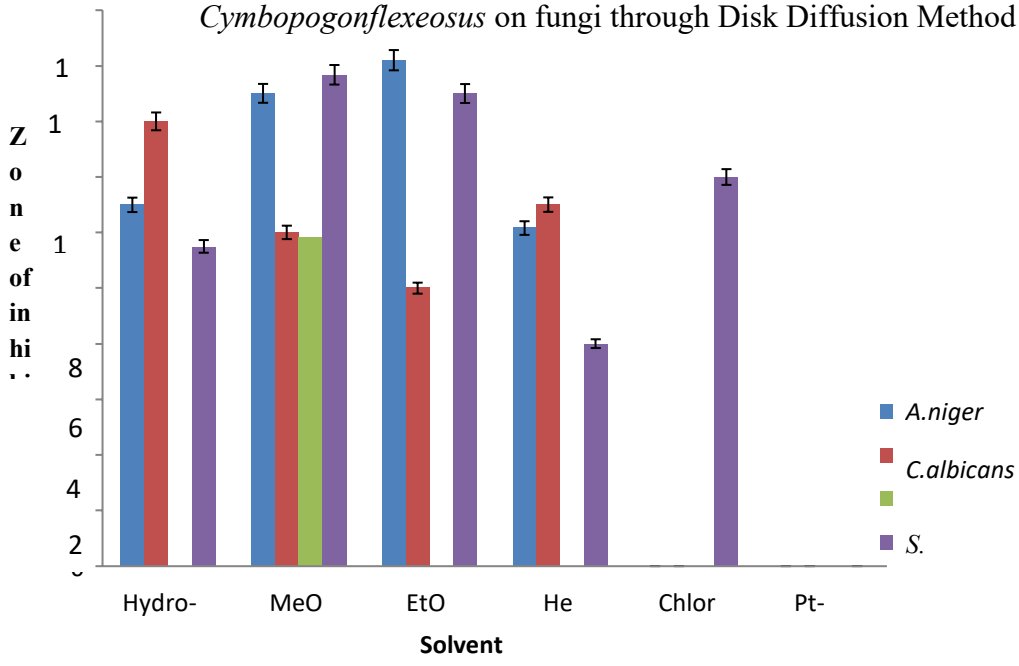
Graph1(b). Antimicrobial activity of extracts of *Cymbopogonflexeosus* leaves on Gram Negative bacteria through Disk Diffusion Method



Graph 1 (c) Antimicrobial activity of extracts of leaves of *Cymbopogonflexeosus* on fungi through Disk Diffusion Method



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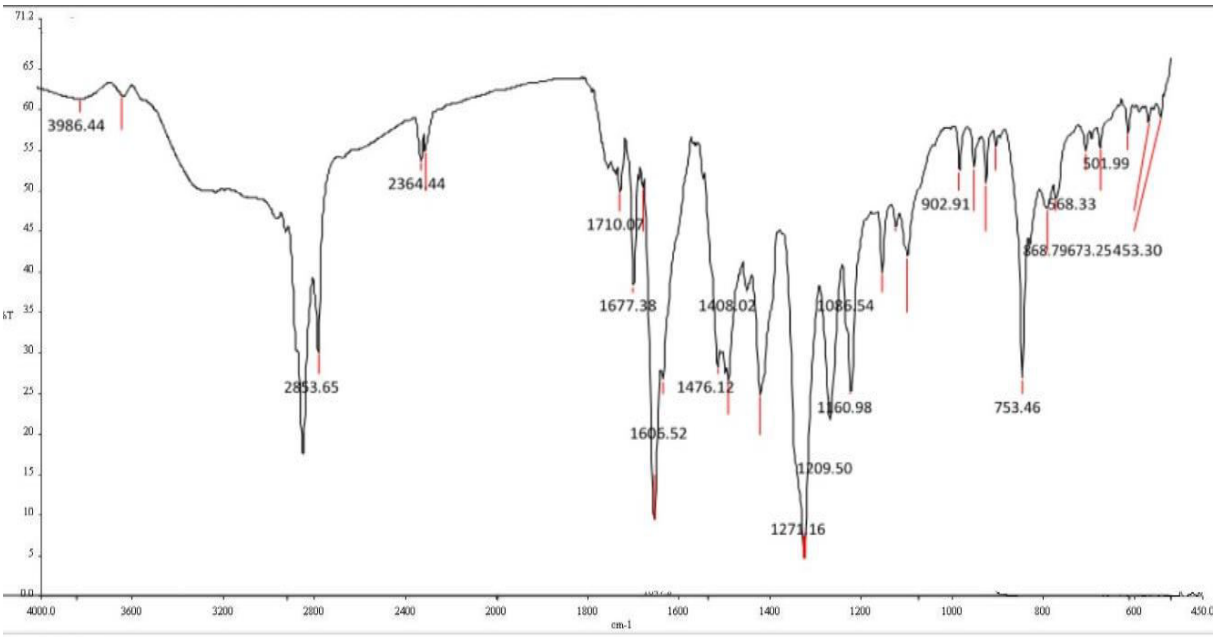
Identification and structure elucidation of purified compounds

The examination of potent plant extract using FT-IR spectroscopy revealed the presence of functional groups, including aromatic ring structures, keto groups, alkanes, aryl ethers, aromatic-OH, secondary alcohols, and benzene rings (Fig. 1). The comprehensive examination is illustrated in Table IV. The effective methanol and ethanol plant extracts underwent separation through column chromatography and were identified as Quercetin via HPLC analysis (Fig. 2).

Table 3: FTIR Analysis

Functional Group	**FTIR Bands(cm^{-1})**	**Assignment of Peaks**
Aromatic Ring Structure	2925,2853	=C-H Stretching
	2364,2345	Overtone of CH bending possible in side chain structure
	3784	O-H Stretching
Keto group	1710	C=O stretching
Alkane	1476,1452, 1376	C=C ring stretching
	1350-1376	Secondary-OH bending
Aryl Ether	1271	--C—O stretching
Aromatic-OH	1209	With-OH stretching
Secondary Alcohols	1160	With C-O stretching
Benzene Ring	680,694,810	Meta Disubstitutional benzene ring

Fig. 1: FTIR spectra of purified active compound from Cymbopogonflexuosus



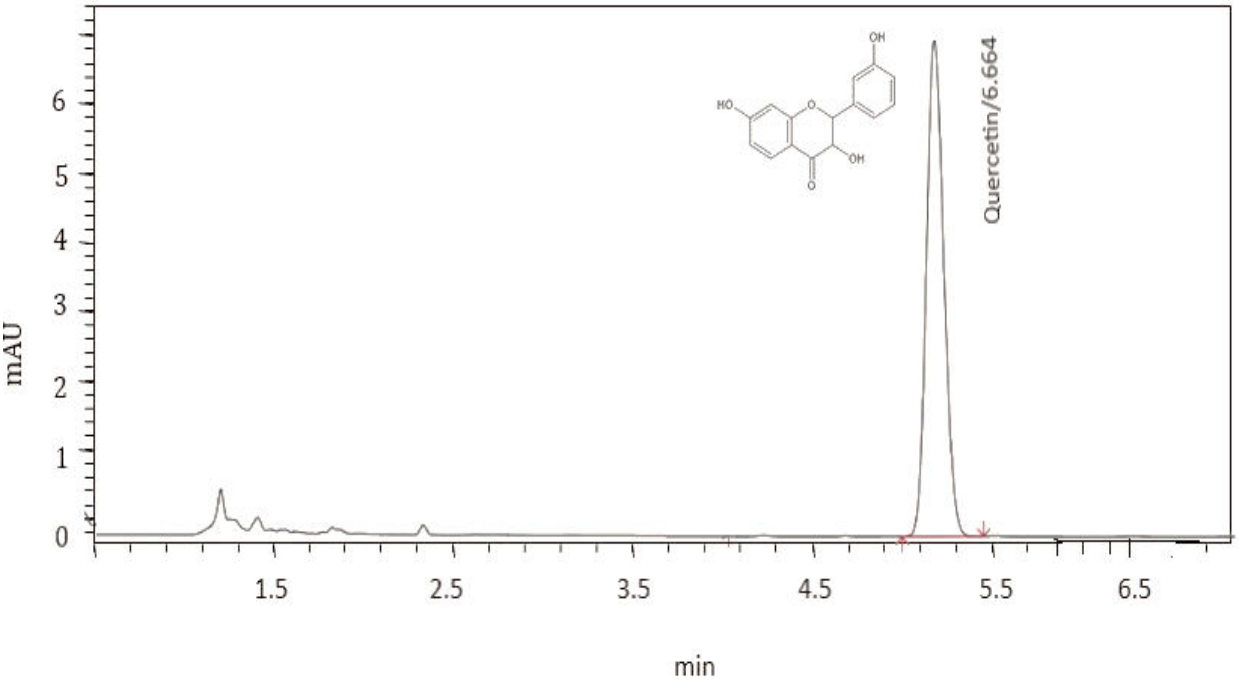


Fig. 2.HPLC chromatogram of purified active compound (Quercetin) of Cymbopogon flexuosus

Discussion

Species within the genus Cymbopogon have a rich history of application in traditional medicinal practices. This information has been utilized in the current study. Compounds such as alkaloids,

flavonoids, terpenoids, and saponins have been identified in the plant extracts. Comparable findings were also presented by Borgioet al, 2008, whose reports aligned with those of Ajaiyeobaet al, 2000.

Table 1 and 2 demonstrate that all the extracts displayed significant antimicrobial properties, with the exception of petroleum ether, across both bacterial and fungal strains. Comparable findings were reported by Thenmoziet al, 2017. The activities noted against B. cereus, B. subtilis, S. aureus, P. aeruginosa, and E. coli align with the findings presented by Kanimathi et al., 2019.The flavonoid Quercetin was identified as the purified bioactive compound. This fact is supported by the work done by Kujumgieve, 1999 and Erdman, 2007.

Conclusion

The Cymbopogon flexuosus plants were chosen for their extensive pharmacological applications and their prevalence in the Chhattisgarh region of central India. This study concentrated on evaluating the antimicrobial effectiveness of Cymbopogon flexuosus, followed by the purification and identification of its promising bioactive compound. Advanced analytical tools and equipment such as TLC, HPLC, and FTIR were employed to acquire a valid and authentic database for Cymbopogon flexuosus. Quercetin, a flavonoid, has been identified as a bioactive compound present in Cymbopogon flexuosus, functioning as both an antimicrobial agent and an antioxidant. The current findings may potentially enhance the selection of candidates for drug development applications, such as antibiotics and antioxidants, within the pharmaceutical sector.

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Conflict of interest

The authors declare none.

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