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Extraction and Characterization of Bioactive Compounds from Plant, *Michelia champaca* (L) in Polar Solvents: GC- MS and Antibacterial properties

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ABSTRACT

For thousands of years, medicinal plants were used to cure many common infectious diseases and health issues. An extremely important medicinal plant called *Michelia champaca* L.(Magnoliaceae) has long been used to treat various ailments, including inflammatory ones. The current study was designated to explore the antibacterial properties of the alcoholic and methanolic extract of *Magnolia champaca* leaves and its different fractions. Antibacterial activity was evaluated against a panel of gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*, *Staphylococcus saprophyticus*) and gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Acinetobacter baumannii*, and *Salmonella paratyphi*) bacterial strains using the disc diffusion method. According to the results, methanolic extract showed slightly higher antibacterial activity than acetone extract. The highest zone of inhibition in the methanolic extract was *Staphylococcus aureus* followed by *Staphylococcus saprophyticus* and *Proteus mirabilis*. The acetone extract shows significant efficiency in gram-positive strains such as *Staphylococcus* spp. The FTIR spectroscopic studies revealed the presence of various functional compounds such as alkanes, carboxylic acid, aromatic compounds, esters, amines, nitro, alkyl halide, and phenols. Acetone and methanol extracts showed the presence of halo compounds, anhydride, isothiocyanate, alcohol, and phenol. GCMS analysis of both extracts shares five biocomponents: Alpha copaene, beta-elemene, beta-caryophyllene, and phytol showed antibacterial activity. This investigation showed that *M. champaca* leaf extracts had prospective therapeutic properties in terms of their antibacterial properties.

Key words: *Magnolia champaca, Antibacterial activity, FTIR, GC-MS analysis*

Introduction

Since ancient times, people have employed plants to treat widespread infectious ailments. The long-term ineffectiveness of synthetic drugs, like that seen with microbial resistance, is now a serious threat to treating microbiological infections. Researchers are looking for new sources of antibiotics with a broad spectrum of activity against Gram-negative and Gram-positive bacterial strains and with the fewest possible side effects (Masoumian and Zandi, 2017; Saleem *et al*., 2010). Most plant genera provide a regenerative source of antibacterial, antifungal, and antiparasitic compounds. Plants are distinguished by various secondary metabolite compounds produced in all morphological parts and have multiple

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survival-related roles. Plants are the only sources of extraction of some compounds since many of the secondary metabolites cannot be synthesized chemically, which may be crucial for biological activity (Kpoviessi *et al*., 2014). The therapeutic benefit of plants is due to their presence of bioactive substances. These compounds fall into one of the major groups of compounds like phenols, quinones, flavonoids, tannins, terpenoids, alkaloids, and other mixtures (Bassey *et al*., 2016). Each functional class of phytochemicals comprises a variety of compounds, sometimes with multiple activities and different potencies (Agunu *et al.,* 2011). Many current pharmaceuticals, cosmetics, and food industries are built on an awareness of the medicinal plant's properties.

Michelia champaca L., commonly known as 'champakam' is a sizable evergreen tree belonging to the family Magnoliaceae. It naturally grows in the Eastern Sub Himalayan tract and belongs to a genus with at least 12 members in India and Myanmar (Burma). It is 18 to 21 m long straight-bole and a closely tapered crown of ascending branches with leaves that are generally lanceolate, occasionally oval, delicately acuminate, and shining above, glabrescent underneath. Flowering is during summer, and fruiting is in winter (Troup, 1921; Bor, 1953; Negi and Gupta, 1987). It is well-known for various ailments, including fever, colic, leprosy, postpartum care, eye diseases, and many more. It has several pharmacological properties, including antipyretic, anti-inflammatory, insecticidal, antibacterial, antioxidant, insecticidal, anti-uretic, carminative, antidiabetic, and antiulcer (Nayak *et al*., 2004). The present study focuses on the efficacy of the extraction process to determine various compounds and evaluate the antimicrobial activity of *M. champaca* leaf extracts against a panel of nine bacterial strains.

Antimicrobial-active plants have attracted interest as a source of potential novel medications; therefore, there is a massive need for study in this area to discover newer bioactive chemicals with antibacterial action. The unrivalled abundance of the chemical diversity of natural products, whether pure or standardized extracts, provides limitless prospects for finding novel drugs (Cos *et al*., 2006). Identifying these key natural bioactive components would greatly aid in designing and formulating naturally derived medications (Davison and Brimble, 2019). However, there is still a considerable research study gap, and many prospective herbs have not yet been thoroughly investigated.

Methodology

Collection and extraction of plant material

M. champaca leaves were collected in and around the Calicut University campus (Fig. 1), and the plant was taxonomically identified by Dr. A. K. Pradeep, Department of Botany, University of Calicut.

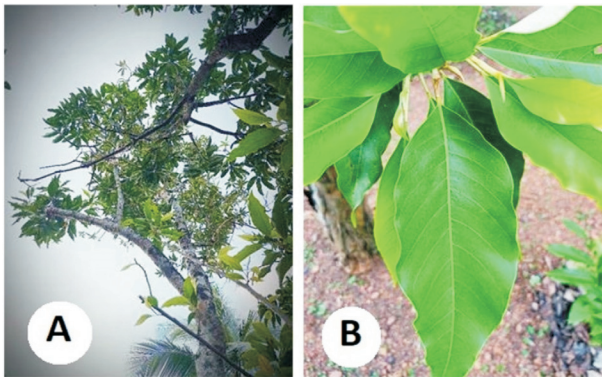

Fig. 1. A: *Michelia champaca* tree, B: Leaf

The *M. champaca* leaves were collected and washed thoroughly in tap water, followed by distilled water. The leaves were shade-dried at room temperature for 2-3 weeks. The dried plant materials were powdered using a grinder. A known quantity (30g) of powdered plant material was taken in a thimble and incorporated into the main chamber of the Soxhlet apparatus using different organic solvents such as methanol and acetone. The process continued for 50 cycles, and the procedure for acetone (boiling point 56 ^{o}C) was repeated. After the extraction, the extracts were kept in reagent bottles under refrigeration. The thimble with residue after extraction was kept in the oven overnight for drying. Then, the thimble was weighed and subtracted from the initial weight to calculate the amount of extractable solvent.

Phytochemical screening

The estimated extract volume was transferred into a beaker and concentrated using an evaporator (60 ^{0}C) to get an equal concentration of each extract. One ml of the corresponding solvent was added to the dried plant extract and centrifuged at 5000 rpm for about 20 minutes. The supernatant is subjected to further analysis. The preliminary qualitative phytochemical analysis was carried out using standard protocol

(Shaikh and Patil, 2020).

Media and microorganisms used

The pure bacterial cultures were maintained in Nutrient agar (Mueller Hinton Agar). A single colony was picked and inoculated from pure cultures in peptone water. The bacterial strains were incubated for 24 hours at 37 °C in nutrient agar plates. The pure bacterial cultures were inoculated in 5 ml nutrient broth (peptone water) and incubated for 20 minutes at a temperature of 37 °C until light to moderate turbidity developed. The bacterial strains were collected from the neighboring clinic (Table 1) for the study. The selected bacterial strains of food poisoning, urinary tract, and respiratory tract infections, are given below. After the experiments, all the strains and glassware were sterilized as per standard protocol.

Table 1. Bacterial strains used for the study

Gram-positive bacteria	Gram-negative bacteria
Staphylococcus aureus	*Escherichia coli*
Enterococcus faecalis	*Pseudomonas aeruginosa*
Staphylococcus saprophyticus	*Klebsiella pneumoniae*
	Proteus mirabilis
	Acinetobacter baumannii
	Salmonella paratyphi

Antimicrobial assay

The antimicrobial activity of the extracts of *Michelia champaca* was determined by Kirby Baur's disc diffusion method. Both gram-positive and gram-negative bacterial strains were used for the test. Discs impregnated with extracts were placed on the plates along with the antibiotic controls (Amikacin, Cotrimoxazole, Vancomycin, and Ciprofloxacin). The plates were kept in the incubator for 24 hours. Then the diameter of the zone of inhibition was measured.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Fourier Transform Infrared Spectrophotometer (FTIR) is the most effective tool for determining compounds' chemical bonds (functional groups). The wavelength of light absorbed is characteristic of the chemical bond, as observed in the annotated spectrum. The chemical bonds of a molecule can be identified by reading the infrared absorption spectra. The powdered samples were blended with KBr using mortar and pestle and formed pellets using a pelletizer. The pelletized sample of each plant specimen was loaded in an FTIR spectroscope (Agilent Cary 600 series), with a Scan range from 400 to 4000 cm^{-1} with a resolution of cm^{-1}.

GC-MS analysis

The phytochemical constituents of acetone and methanol extracts were analyzed using GC-MS (Shimadzu type GCMS-QP2010S). The Phytocompounds present in the extract and the data obtained evaluated the nature of the compound. The injected volume was 1 µl per sample. The operating conditions were as follows: injector temperature, 260 °C; column flow rate,1.00 ml/min; splitting ratio, 20:0; Column Oven temperature 60 °C.

Statistical analysis

Each experiment was run in triplicate. The zones of inhibition for each test were recorded and tabulated as mean ± standard error of the mean (SEM). IBM SPSS was used to statistically analyze the results using a one-way analysis of variance (ANOVA). Results with $P < 0.05$ were considered to be statistically significant.

Results

The present study explored the efficacy of the medicinal plant *M. champaca L* against gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*, *Staphylococcus saprophyticus)* and gram-negative (*Escheria coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Acinetobacter baumannii, and Salmonella paratyphi*) bacterial strains using the disc diffusion method to evaluate its antibacterial potential.

Extraction efficiency of *M. champaca*

The Extraction efficiency of *M. champaca* L. leaf extracts was determined, and their extractive values are depicted in Table 2. Methanol as the extractive solvent produced a higher value than acetone. The high extractive value may be due to the polarity of methanol and the high solubility of plant secondary metabolites in methanol.

Phytochemical analysis

The results of the preliminary phytochemical analysis of the methanolic and acetonic extracts of *Michelia champaca* leaves are presented in Table 3.

Table 2. Extraction efficiency of methanol and acetone leaf extracts

Solvents	Loss of weight (g)	Total volume gained(ml)
Acetone	6.9225	110
Methanol	7.2916	130

Table 3. Qualitative phytochemical screening of *M. champaca*

Phytochemicals	Methanol extract	Acetone extract
Phenol	+	+
Tannins	+	-
Flavonoids	+	-
Saponins	-	-
Alkaloids	+	+

Positive results were obtained for both extracts.

Antibacterial activity

The antibacterial screening of acetone and methanol extract of *Michelia champaca* against the tested bacteria revealed that significant activity was observed at 40µl for both samples and control. According to the results of the present study, methanolic extract shows slightly higher antibacterial activity than acetone extract. The highest zone of inhibition in the methanolic extract was *Staphylococcus aureus,* followed by *Staphylococcus saprophyticus* and *Proteus mirabilis*. The extract showed significant to moderate antimicrobial activity towards tested strains except for *Enterococcus faecalis*. The acetone extract shows significant efficiency in gram-positive strains such as *Staphylococcus* spp. The maximum inhibition was shown against *Staphylococcus saprophyticus* by the acetone extract, with 28.67mm, which is higher than that of antibiotic control (vancomycin 24.67 mm). There is no zone of inhibition in *P. aeruginosa*, *E. coli*, and *P. mirabilis*. However, neither extract was effective against *Enterococcus faecalis*. There was no/little zone of inhibition in the case of acetone and methanol, which is used as a negative control. The zone of inhibition is higher in antibiotics used as the positive control. The highest zone was shown by Cotrimoxazole in *Staphylococcus aureus* (33.33) (Fig. 2, 3). (Table 4). Methanol and acetone leaf extracts of *M. champaca* against nine bacterial strains are represented in (Fig. 4).

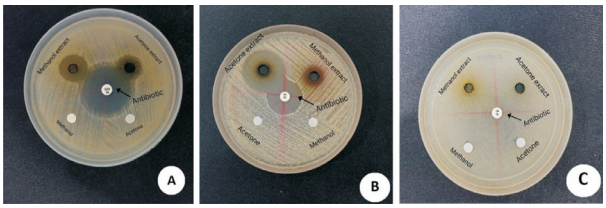

Fig. 2. Zone of inhibition of Acetone and Methanol leaf extracts of *M. champaca* for Gram-positive bacterial strains. A – *Staphylococcus aureus, B - Staphylococcus saprophyticus, C - Enterococcus faecalis*

FTIR

Fourier-transform infrared spectroscopy analysis is used to identify the functional groups present in the leaf extract. The FTIR spectroscopic studies revealed the presence of different characteristic peak values with various functional compounds such as alkanes, carboxylic acid, aromatic compounds, esters, amines, nitro, alkyl halide, and phenols.

The results of the present study showed some variations in the peak values of plant powder and solvents. The obtained peaks are almost similar in the case of plant powder and solvent extracts. Both

Table 4. Zone of inhibition of Acetone and Methanol extracts of *M. champaca* against nine bacterial strains

Tested bacterial strains	Zone of inhibition (average ± SE)					
	Extracts		Control			
	Acetone	Methanol	Antibiotic		Acetone	Methanol
E. coli	0	10±0.577*	AK	23.67±1.202	0	0
S. aureus	15.67±0.333	17.67±0.677	Cot	33.33±1.202	0	0
P. aeruginosa	0	11.33±0.677*	AK	26±0.577	0	0
E. faecalis	0	0	VA	17±1	0	0
K. pneumonia	11±0.577	13±0.577	AK	22.67±1.202	0	0
P. mirabilis	0	13.67±0.333*	AK	23.33±0.667	0	0
A. baumannii	11.33±0.882	12.67±0.333	AK	23±1.155	0	0
S. paratyphi B	12.33±0.882	13±0.557	Cip	21.67±0.882	0	0
S. saprophyticus	28.67±0.882*	15±0.557*	VA	24.67±0.882	0	0

Values are triplicate and represented as mean ± SE. Significance is represented as (*P< 0.05).

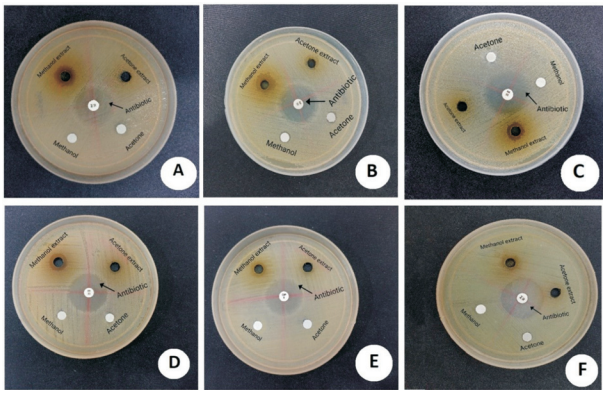

Fig. 3. Zone of inhibition of Acetone and Methanol leaf extracts of *M.champaca* for Gram-negative bacterial strains. A- *Proteus mirabilis*, B- *Klebsiella pneumonia*, C - *Escherichia coli*, D - *Acinetobacter baumannii*, E- *Salmonella paratyphi*, F-*Pseudomonas aeruginosa*

showed the presence of halo compounds, anhydride, isothiocyanate, and alcohol; acetone and methanol solvents also contained phenol.

Acetone solvent extract revealed the presence of isothiocyanate (N=C=S), representing amino acid groups. The band at 3402cm^{-1} represents O-H stretching, suggesting that it may be alcoholic or phenols. The peaks in methanolic solvent extract contain alkyl, amino, andcarbonyl peaks and aromatic heterocyclic compounds (Fig. 5).

GC-MS analysis

GC-MS analysis was conducted with methanolic and acetonic leaf extracts of *Michelia champaca*. Methanol extract showed more antimicrobial activity compared to acetone in a broad spectrum. Thirteen phytocompounds were recorded in methanol extract (Table 5), and their % of peak height varies.

The highest % of peak height was shown by 5-Oxatricyclo [8.2.0.0(4,6)] dodecane,12-trimethyl-9-methylene (44.07). The lowest % of peak height is shown by Dodecamethylcyclohexasiloxane (0.93). The highest retention time is taken by 3,7,11,15-

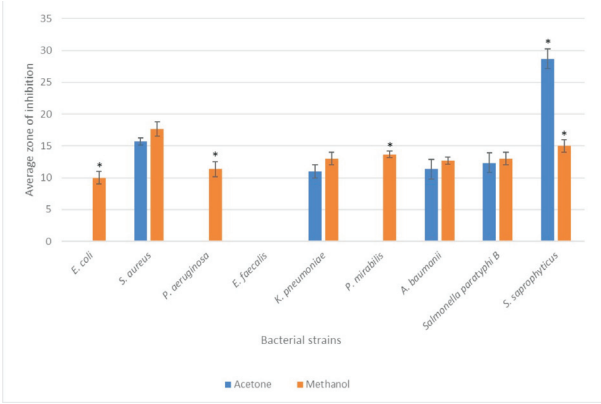

Fig. 4. Methanol and acetone leaf extracts of *M. champaca* against nine bacterial strains (Mean ± SE) (*P<0.05).

Table 5. GC-MS analysis of Methanol leaf extract of *Michelia champaca*

Peak	# R. Time	Area	Area %	Height	Height %	Name	Base m/z
1	14.293	354333	0.58	150426	0.93	Dodecamethylcyclohexasiloxane	73.05
2	15.836	406730	0.67	154241	0.95	Alpha-Copaene	105.05
3	16.245	1248566	2.04	400545	2.47	Beta-Elemene	81.05
4	17.010	1111461	1.82	400958	2.47	Beta-Caryophyllene	93.05
5	17.891	487437	0.80	188854	1.16	1,4,8-Cycloundecatriene,2,6,6,9-tetramethyl-,(E,E,E)	93.05
6	18.737	470342	0.77	168368	1.04	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.05
7	21.111	26973210	44.12	7146379	44.07	5-Oxatricyclo [8.2.0.0(4,6)] dodecane, 12-trimethyl-9-methylene	79.05
8	21.731	4701442	7.69	1556342	9.60	12-Oxabicyclo [9.1.0] dodeca-3,7-diene, 1,5,5,8-tetramethyl	67.10
9	26.459	5553727	9.08	1668881	10.29	2,6,10-Trimethyl,14-Ethylene-14-Pentadecane	68.05
10	27.203	4176685	6.83	1074090	6.62	1,2-Benzenedicaboxylic acid, Bis (2-Methylprophy) ester	149.00
11	28.241	2930568	4.79	598665	3.69	Hexadecanoic acid, Methyl ester	74.05
12	31.802	9652192	15.79	2275015	14.03	Phytol	71.10
13	38.718	3074597	5.03	432288	2.67	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	68.05
		61141290	100.00	16215052	100.00		

Tetramethyl-2-hexadecen-1-ol (38.718), and the easily eluted compound is Dodecamethylcyclohexasiloxane (14.293). The maximum area in % is shown by 5-Oxatricyclo [8.2.0.0(4,6)] dodecane, 12-trimethyl-9-methylene, which is 44.12 and has an area of 26973210 cm^2. Dodecamethylcyclohexasiloxane shows the lowest % area. Based on GC-MS analysis, several bioactive molecules/drugs were identified from methanolic leaf extract. By analyzing the chemical component out of 13 identified compounds, four showed isomeric properties, i.e. Alpha-Copaene, Beta-Elemene, Beta-Caryophyllene, and1,4,8-Cycloundecatriene, 2,6,6,9-tetramethyl-,(E, E, E) and their molecular formula is the same ($C_{15}H_{24}$) (Fig. 6).

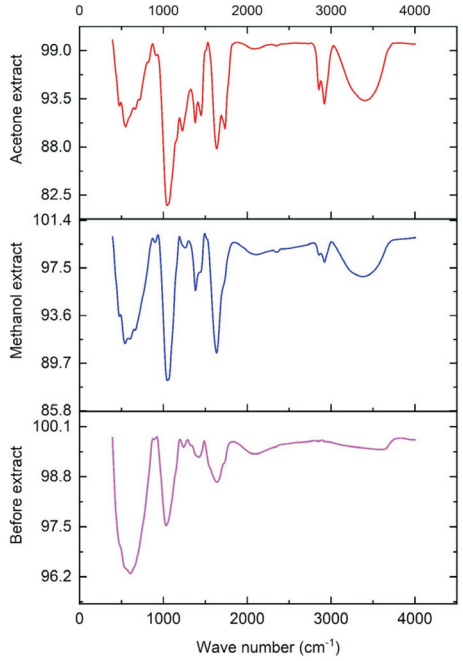

Fig. 5. FTIR spectra of plant powder, methanol and acetone extracts.

Similarly, fourteen phytocompounds were recorded in the Acetone extract (Table 6). The highest % peak height showed Beta-Caryophyllene epoxide (36.61 %). The lowest % of peak height is indicated by 3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane (1.20). The highest retention time is taken by Phytol (20.082), and the most easily eluted compound is Alpha-Copaene (12.073).

Based on structural information, PubChem provides the functional aspect and the previously submitted bioactivity. Few identified compounds were associated with antimicrobial activity and have been reported previously. Both extracts share five biocomponents: Alpha copaene, beta-elemene, beta-caryophyllene, 3butoxy,1,1,1, 7,7,7, hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane and phytol. Others exhibit antimicrobial activity except for 3 butoxy,1,1,1, 7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloane.

Phytol is widely distributed as a constituent of chlorophyll and exhibits high antibacterial properties. Phytol has antimicrobial activity against *Staphylococcus aureus*, which is present more in methanol (Area% is 15.79). Acetone also possesses antimicrobial activity due to the presence of humulene epoxide, spathulenol, neophytadiene, germacrene, and bicyclogermacrene, which are more antioxidant antipyretic than antibacterial. They are also anti-inflammatory. Germacrene D (1.40%), â-caryophyllene (12.06%), spathulenol (1.77%), and humulene epoxide (6.61%) were identified as the major compounds that showed antibacterial activity against the important human pathogenic gram-positive *Staphylococcus* spp. The bioactive components unique to methanol are 2,6,10-Trimethyl, 14-Ethylene-14-Pentadecane (9.08%), 1,2-benzedicarboxylic

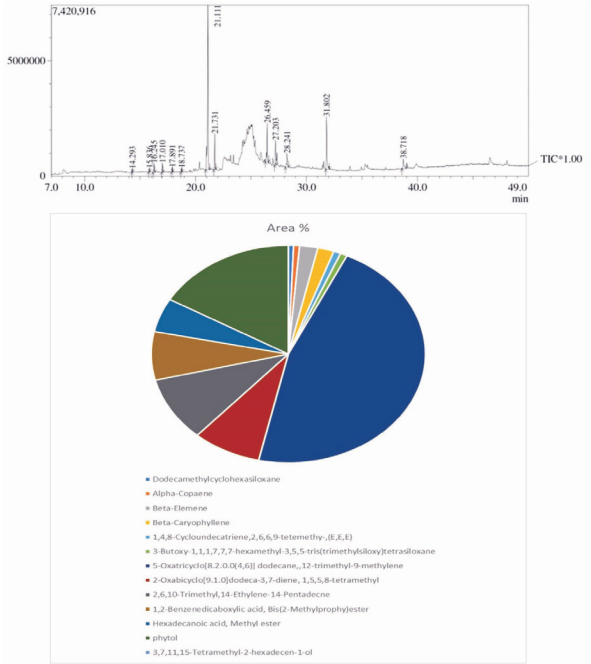

Fig. 6. GC-MS chromatogram and Area % of bioactive molecules in the Methanol leaf extract of *M. champaca*

acid (6.83%), and hexadecenoic acid methyl ester (4.9%). The current study also enfolds the identification of a wide range of bioactive compounds that may involve various other biological activities (Fig. 7).

Discussion

In this study, we explored the potentiality of the polar solvents (acetone and methanol) leaf extracts of *Michelia champaca* on the antimicrobial activity. Studies have shown that differences in the yields of different extracts can be linked to the polarities of distinct plant compounds, and the extraction yield is greatly influenced by the solvent utilized (Hayouni *et al*., 2007). A relatively broader spectrum activity of the methanol extract over the acetone extract is difficult to explain since all the extracts contained the same phytochemicals. The phytochemical analysis conducted on the plant extracts revealed the presence of constituents known to exhibit medicinal properties. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, glycosides, and alkaloids. Tannins, flavonoids, and saponins are absent in acetone extract. These findings are correlated with the chemical composition of leaf extracts of *M. champaca* (Ruwali *et al*., 2019). Phenolic compounds are one of the significant and widespread groups of plant metabolites (Singh *et al*., 2007). It provides biological properties such as anti-inflammation, anti-apoptosis, anti-aging, anti-carcinogen, anti-microbial, and inhibition of angiogenesis and cell proliferation (Han *et al.*, 2007). The methanolic extracts of leaves, seeds, and bark possess a broad spectrum of antibacterial activity and reported the antiulcerogenic activity of leaves and flowers of *M. champaca* (Kumar *et al*., 2011). It also possesses anti-inflammatory and diuretic activity. The high extractive value may be due to the polarity of methanol and the high solubility of plant secondary metabolites in methanol (Gami and Parabia, 2011).

Fourier-transform infrared spectroscopy analysis is used to identify the functional groups present in the leaf extract. The FTIR spectroscopic studies revealed the presence of different characteristic peak values with various functional compounds such as alkanes, carboxylic acid, aromatic compounds, esters, amines, nitro, alkyl halide, and phenols. The FTIR analysis was performed on solvent extract and residues to determine the efficiency of solvents on a preliminary basis. Extensive studies on FTIR spectral screening are still unclear on the comparative evaluation of methanolic and acetonic leaf extracts. The plant powder revealed the presence of isothiocyanate (N=C=S), representing amino acid groups and halo compounds possessing antimicrobial properties (Romeo *et al*., 2018; Bowman and Stretton, 1972). Both acetone and methanol solvent extracts exhibit the presence of phenol with high antioxidant and cell lysis properties (Sroka *et al.*, 2005). The two solvent extracts retained the charac-

Table 6. GC-MS analysis of Acetone leaf extract of *Michelia champaca*

Peak	#R.Time	Area	Area %	Height	Height %	Name	Base m/z
1	12.073	1363366	0.99	783938	1.26	Alpha-Copaene	105.10
2	12.261	8045901	5.83	4091307	6.60	Beta-Elemene	93.10
3	12.700	16653378	12.06	10219325	16.48	Beta-Caryophyllene	93.10
4	13.147	5500644	3.99	3018635	4.87	Alpha-Caryophyllene	93.10
5	13.359	1162079	0.84	745778	1.20	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73.05
6	13.488	1932547	1.40	1020393	1.65	Germacrene D	161.10
7	13.686	1969718	1.43	1187273	1.91	Bicyclogermacrene	121.10
8	14.803	58638062	42.48	22701906	36.61	Beta-Caryophyllene epoxide	79.10
9	15.111	9120370	6.61	4691348	7.57	Humulene epoxide II	67.10
10	15.364	1445526	1.05	796014	1.28	Beta-Resorcylic acid (TMS)	73.10
11	16.380	2448645	1.77	1100471	1.77	Spathulenol	93.05
12	17.350	11337010	8.21	5613426	9.05	Neophytadiene	68.05
13	17.794	6406946	4.64	2396437	3.86	Phthalic acid, isobutyl trans-dec-3-enyl ester	149.00
14	20.082	12008154	8.70	3642965	5.87	Phytol	71.10
		138032346	100.00	62009216	100.00		

teristics of functional groups in the plant powder.

The screening of the antibacterial activity on acetone and methanol extracts of *Michelia champaca* against some selected bacterial strains showed that the methanolic extracts have more antibacterial activity in a broad spectrum. The yield of a plant extract is essential in calculating the total activity of biocomponents (Eloff, 2000). The highest zone of inhibition in the methanolic extract was *Staphylococcus aureus* followed by *Staphylococcus saprophyticus* and *Proteus mirabilis*. The extract showed significant to moderate antimicrobial activity towards tested strains (Manhas and Dahiya, 2017) except for *Enterococcus faecalis*. The acetone extract shows significant efficiency in gram-positive strains such as *Staphylococcus* spp. There is no zone of inhibition in *P. aeruginosa, E. coli, and P. mirabilis*. The highest zone of inhibition was seen in *Staphylococcus saprophyticus* (28.67) than in the antibiotic control (Vancomycin-24.67). The antimicrobial activity was associated with the acetone fraction and was specific towards gram-positive bacteria (Lall and Meyer, 1999; Teles *et al*.,2019) except for *Enterococcus faecalis*, a Gram-positive bacterium. None of the plant extractions was as effective as standard antibiotics against the bacteria used in the study except in the case of *S. saprophyticus*. Many authors have reported that the antibacterial activity may be due to phytochemicals such as alkaloids and phenols (Amber *et al*., 2018). The FTIR results of our study support the above findings.

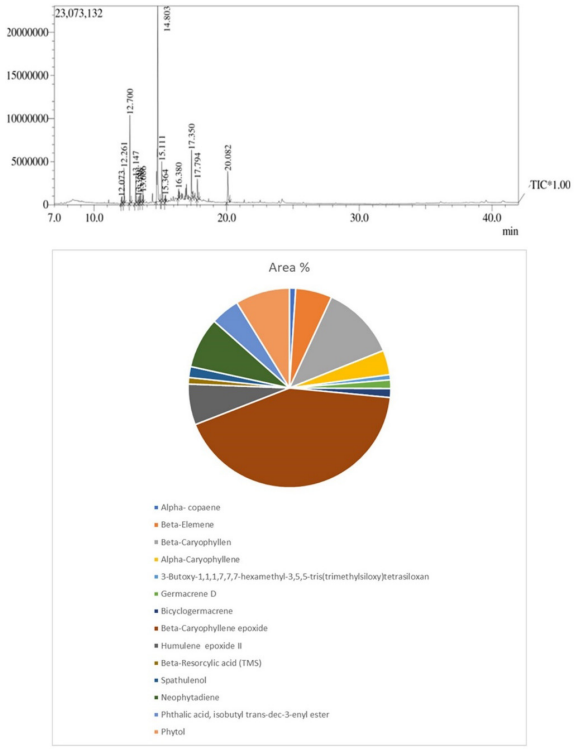

Fig. 7. GC-MS analysis and Area % of bioactive molecules in the Acetone leaf extract of *M. champaca.*

Gas chromatography-mass (GCMS) analysis examined the next level of confirmation of plant extracts' antibacterial activity. It has been proven to be a valuable method for identifying various bioactive constituents, including non-polar compounds, alcohols, acids, esters, alkaloids, aromatic compounds, steroids, amino compounds, nitro compounds, and long-chain hydrocarbons (Iordache *et al.*,2009). Thirteen phytocompounds were recorded in the methanol extract, and compounds exhibited isomeric properties. Similarly, fourteen phytocompounds were recorded in the acetone extract, in which three compounds showed similar properties. Few identified compounds were associated with antimicrobial activity and have been reported previously. Both extracts share five biocomponents: Alpha copaene, beta elements, beta-caryophyllene, 3-butoxy,1,1,1, 7,7,7, hexamethyl, and phytol. Except for 3- butoxy,1,1,1, 7,7, hexamethyl, others exhibited antimicrobial activity. Phytol is widely distributed as a constituent of chlorophyll and exhibits high antibacterial properties (Lee *et al*., 2016), which is present more in methanol (15.49%). Acetone also possesses antimicrobial activity due to the presence of Humulene epoxide, Spathulenol, Neophytadiene Germacrene, and Bicyclogermacrene, which are more antioxidant, antipyretic, and anti-inflammatory than antibacterial. Unique bioactive molecules present in methanol are 2,6,10-Trimethyl,14-Ethylene-14-Pentadecne (9.08%) have high antioxidant and antibacterial activity (Naik *et al.*, 2020), and 1,2-benzedicarboxylic acid (6.83%), possess antibacterial activity (Garba *et al.*, 2016) and hexadecenoic acid methyl ester (4.9%) has antibacterial activity against multidrug-resistant bacteria (Shaaban Mohamed *et al*., 2021). From the above study, it is evident that the phytochemicals present in *Michelia champaca* indicate their potential as a source for the production of novel medicines; furthermore, isolation and characterization of bioactive components present in the *M. champaca* provide a newer avenue for health care. As drug resistance to currently available treatments worsens and spreads, there is an urgent need to find new antimicrobial agents for therapeutic uses in humans

and animals (Rios and Recio, 2005; Pauli, 2005).

The results of this work provide future insights associated with antibiotics that may be a more powerful tool to control microbes. This enables the synergistic action of plant extracts and antibiotics against bacterial infections when they are no longer effective and reduces their side effects during treatment. Active plant extracts may be used as organic in-feed supplements to replace antibiotic growth promoters, an economically significant application. Plants hold a unique position as a potential source of novel anti-infectives in treating and preventing human and veterinary diseases.

Conclusion

The study showed increased antibacterial activity in methanolic leaf extracts of *Michelia champaca*. FTIR results revealed the presence of several compounds, and the methanol extract displayed several functional groups compared to acetone, which must be further identified. The presence of bioactive molecules is confirmed by GCMS analysis. The biomolecules in *Michelia champaca* indicate the potential source for producing novel phytomedicines. Moreover, the isolation and characterization of the biocomponents present in *M. champaca* would create newer avenues in health care. This study may offer a new foundation for utilizing plants. However, as plant extracts are a rich source of antimicrobial agents, more research should be focused on developing the active components of these plant extracts for their better economic and therapeutic application. For the future discovery of low-cost medicines, extensive research still needs to be conducted on the phytochemicals of this plant. Although many synthetic pharmaceuticals now have undesirable side effects, developing new drugs utilizing plant-based chemicals might help address the demand for medicines with fewer adverse effects. Knowing the chemical components of plants helps comprehend their pharmacological and therapeutic properties. Future studies are essential for isolating and identifying the chemical elements in developing novel antimicrobial medications. To recover as many active chemicals as feasible, using different extraction methods with varying concentrations or extracting solvents may also be viable.

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Competing Interests

The authors have declared that no competing interest exists.

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