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Preliminary phytochemical investigation, Antimicrobial activity and GC-MS analysis of leaf extract of *Capparis zeylanica* Linn

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Abstract

The traditional medicinal plant *Capparis zeylanica* Linn., belonging to the family Capparidaceae, common name is indian caper. The present study deals with the preliminary phytochemical screening, determination of antimicrobial activity (aqueous, acetone, ethyl acetate and methanol extract) and GC-MS analysis (methanol extract) of the leaf of *Capparis zeylanica*. The extracts were tested against 5 pathogenic bacteria and 5 fungal organisms were tested by agar well diffusion method at various concentrations (25–100 µl). The results revealed the presence of alkaloids, Anthraquinones, flavonoids, phenol, Phlobatannins, protein, saponins, steroids, tannins and terpenoids in the extracts. The leaf extracts were found to be a rich source of phytochemicals. All the extracts exhibited dose dependent antimicrobial activity, however, highest antibacterial activity was observed against *Staphylococcus aureus* with inhibition zone was 24.66 ± 0.28 at 100µl, similarly highest antifungal activity was recorded against *Aspergillus niger* with the zone of inhibition of 14.50 ± 0.50 at 100µl. Both the results were recorded in methanol extract of leaf provided to be more powerful antibacterial sensitivity when compared with other extracts. As per the best result of preliminary phytochemical analysis, the methanol extract was chosen for identification of major phyto-constituents by GC-MS analysis. Nineteen compounds were detected. These results indicate the methanol extract of leaf possess potent antioxidant, anti-inflammatory, anticancer, antitumour, cancer preventive, antibacterial effects. Therefore, Methanol extract of *Cappariszeylanica* proves as a potential source of bioactive compounds of pharmacological importance.

Keywords: Bioactive compound, Antimicrobial activity, GC-MS, Caryophyllene

1. Introduction

Human beings have been contingent on natural products as a resource of drugs for thousands of years. Plant-based drugs have shaped the basis of traditional medicine systems that have been used as traditional medicines for their primary health care [1]. According to the prevailing reports, it is estimated that about 70–80% of world population, particularly in developing countries, depend on herbal medicine prevent and cure diseases. In addition, it has been reported that about 25% of the synthesized drugs are being derived from medicinal plants [2-3]. Medicinal plants have been used for centuries as remedies for human disease as they contain components of therapeutic value. There are plentiful plant natural products which have antifungal, antibacterial, antiprotozoal activity that could be used either systematically or locally [4]. Conventionally used medicinal plant produce a variety of compound of known therapeutic properties possessed by these medicinal plants are antimicrobial. These scarcity of infective disease in plants is in itself a suggestion of the successful defence mechanism developed by them [5]. Today in this modern world, even though synthetic drugs are readily available and highly effective in curing various diseases, there are people who still favour using traditional folk medicines because of their less harmful effects. There is a wide diversity of phytochemicals, especially secondary metabolites, found in plants and studies have exposed that these compounds have anticancer, antibacterial, anti-inflammatory, analgesic antitumor, antiviral and many other activities to a greater or lesser extent [6-7]. Recently, several studies have reported that ethnomedicinal plants are having diverse pharmacological and biological properties [8]. In the traditional ayurvedic system of medicine, this shrub is used as “Rasayana drug”. Leaves are used as counter-irritant, febrifuge and as cataplasm to treat swellings and piles in North India, most of the parts like root, bark, fruit, leaves and seeds are used for the treatment of various ailment categories [9]. Plant extracts and their components have been known to exhibit biological activities, especially antimicrobial [10], antifungal [11] and antibacterial [12] *Capparis zeylanica* Linn., belonging to the family Capparidaceae

(Syn: *C. horrida* Linn.) is a rigid, wiry and many branched climbing shrub grow in a moist habitat and is commonly disseminated throughout the major parts of India, China, Nepal, Bangladesh, Sri Lanka, Malaysia and some parts of Pakistan [13]. In India, the plant is widely distributed in Mumbai, Delhi, Dehradun, South of Himalayas and Andaman [14]. The plants are 2-3 metres in height and armed with 3-6 mm long recurved thorns. *C. zeylanica* is a scandent shrub with young parts clothed with reddish-brown tomentum and hairy, which are soon falling off. Stem is woody, rough, young parts green, rusty tomentose with pungent smell. Leaves are ellipticoblong, 3-10 cm long on 10-15 mm long petiole, rounded base and apex mucronate. Flowers are supra axillary, usually 2, sometimes 1 or 3, 2-4 cm across on up to 4 cm long pedicel. Sepals are subequal and tomentose. Petals are up to 18 mm long, white or slightly yellowish and hairy outside. Stamens are many, twice as long as petals and purple tinged in upper part. Fruits are globose, 3-4 cm in diameter and embedded in white pulp. *Capparis zeylanica* has been stated to possess anthelmintic, antimicrobial [15], antidiabetic activity [16], antioxidant activity [17], anticovulsant activity [18], Analgesic [19], antipyretic activity and immunostimulant activity [20]. Modern phytochemical screening of the plant has shown the presence of fatty acids [21], flavonoids [22] and it contains alkaloids, phytosterols, mucilaginous substances and water-soluble acid [23]. The objective of this study was to evaluate the preliminary phytochemistry constituents, antimicrobial activity of various extract and GC-MS analysis of leaf of *Capparis zeylanica*.

2. Materials and methods

2.1. Plant material

The collected plant identified as *Capparis zeylanica*. L botanically identified and authenticated with the help of floras, such as Flora of Presidency of Bombay [24], Flora of British India, Flora of presidency of Madras [25] and Flora of Karnataka [26] by Dr. S. SOOSAIRAJ, St. Joesph's College, Thiruchirappalli (Accession No: SJCOT 2561). The herbarium specimen was prepared and deposited at Department of Botany and Microbiology, A.V.V.M Sri Pushpam College, Poondi, Thanjavur, Tamilnadu, India.

2.2. Preparation of extracts

The leaf samples were washed sensibly with water to remove dust and foreign materials. Then the washed leaves samples (200gm) were dried under shade at temperature (25 C) for 7 days. After drying the leaf samples were ground into a powder form using a grinder for 30 s. The powdered material was subjected to successive Soxhlet extraction by various solvents namely Aqueous, Acetone, Ethyl acetate and Methanol were used. After that, extract was concentrated and stored at 4 °C until further use in the equipment [27].

2.3. Preliminary phytochemicals screening

Phytochemical screening was carried out on the plant extracts using different solvents to identify the major natural chemical groups [28-30] such as alkaloids, Anthraquinones, flavonoids, phenol, Phlobatannins, protein, saponins, steroids, tannins and terpenoids.

2.4. Antimicrobial study

2.4.1. Microorganism

The antimicrobial properties of *Capparis zeylanica*. L was investigated against Three-gram negative bacterial strains; *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas*

aeruginosa, Two-gram positive bacterial strains; *Bacillus cereus* and *Staphylococcus aureus*, five fungal; *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Fusarium* sp and *Penicillium* sp. A total of ten pathogenic microorganisms were obtained from the culture collection of Microbiology Laboratory, Thanjavure government medical college, Thanjavure, Tamilnadu.

2.4.2 Inoculation of microorganism

The pure cultures of microorganisms were streaked onto nutrient agar plate (Merck) and potato dextrose plate and incubated at 37°C for 24-28hrs and 28°C for 48-72 hours for bacteria and fungi separately. The well-isolated colonies were aseptically transferred to the nutrient broth (NB) and potato dextrose broth (PDB) and again incubated at appropriate temperature. The optical densities of incubated bacteria and fungus were measured using a UV spectrophotometer (Shimadzu, Japan) at 600 nm (OD₆₀₀) and 530 nm (OD₅₃₀) wavelength respectively. The desired optical density (OD) were obtained as 0.45–0.55, represented 0.5 McFarland standards (10⁸ CFU/mL) and 1.0 – 5.0 represented 5.0 McFarland standards (10⁶ CFU/mL). The turbidity of each pathogen was used to the desired range by dilution if OD values were higher than the standards or then incubated again if OD values were less than the standards.

2.4.3 Antimicrobial screening tests

The antimicrobial activities of the crude extracts were determined by agar well diffusion method. Immediately after autoclaving, the media was allowed to cool at 45 to 50 °C. The freshly prepared and cooled media was poured into flat-bottomed Petri dishes (90 mm in diameter) and placed on a level and horizontal surface to give a uniform depth of almost 4 mm. The agar media was allowed to cool and solidify at room temperature and the plates were incubated at 35 °C for 18–20 h before they were used to confirm sterility. Then 0.1 mL of the tested inoculum was evenly spread on the surface of the solidified agar by using sterile spreader. Four intermediate wells of 3 mm in depth and 8 mm in diameter were made on the agar plate. About 25 µL -100 µL of the plant extracts were filled into the wells depend upon the concentration chosen. Amoxicillin was used as a positive bacterial control and fluconazole used for fungal positive control, whereas DMSO was used as a negative control. The incubation of bacterial agar plates aerobically at 37 °C for 24 h. The fungal plates were incubated for 48 h at 30 °C. The antimicrobial activities were determined by measuring the diameters of inhibition zones (mm). The test was performed in triplicates with controls.

2.5 Identification of phyto components by GC-MS analysis

The plant extract was filtered by using what mann No.1 filter paper with sodium sulphate to remove the sediments and traces of water in the filter paper. Before filtering, the filter paper along with sodium sulphate was moisturized with absolute alcohol. 2 µl leaf extract of the sample of the solutions was employed in Gas chromatogram and mass spectroscopy analysis to identify different compounds [31]. Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were determined. The spectrum of the unknown component was

compared with the spectrum of the component stored in the NIST library version (2005), software, Turbomas 5.2.

Table 1: Qualitative phytochemical analysis of *Capparis zeylanica* L.

Phytochemical compounds	Aqueous	Acetone	Ethyl acetate	Methanol
Alkaloids	+	-	+	+
Antro quinines	-	+	-	-
Flavanoids	+	-	-	+
Phenol	-	-	+	+
Phlobatannins	-	-	-	-
Protein	-	-	-	-
Saponnins	+	-	-	-
Steroids	-	+	+	-
Tannins	-	-	+	+
Terpenoids	-	-	-	+

Table 2: Antibacterial activity of *Capparis zeylanica* L.

Extracts	Extract Conc.	Test organisms				
		<i>Bacillus cereus</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
Aqueous	25µl	14.16±0.28	5.00±0.0	5.66±0.28	12.33±0.28	7.66 ± 0.28
	50µl	14.30±0.0	6.33±0.28	5.66±0.28	13.66±0.28	11.33 ± 0.50
	75µl	15.00±0.50	8.50±0.50	7.00±0.50	14.00±0.0	12.66 ± 0.28
	100µl	21.5±0.50	9.33±0.28	8.33±0.28	14.00±0.50	13.33 ± 0.50
Acetone	25µl	11.76±0.25	12.00±0.0	12.83±0.28	9.00±0.0	11.00 ± 0.0
	50µl	13.00±0.0	13.00±0.50	13.50±0.50	10.33±0.28	14.00 ± 0.50
	75µl	13.33±0.28	14.00±0.0	14.00±0.0	14.66±0.28	14.66 ± 0.28
	100µl	13.50 ±0.0	15.00±0.5	19.33±0.28	15.00±0.0	22.00 ± 0.50
Ethyl acetate	25µl	6.43±0.40	7.33±0.28	12.00±0.0	14.66±0.28	15.00 ± 0.50
	50µl	18.00±0.0	12.33±0.28	17.33±0.28	15.00±0.0	16.33 ± 0.28
	75µl	21.43±0.40	13.33±0.28	21.50±0.50	16.00±0.86	16.66 ± 0.28
	100µl	22.00±0.0	19.00±0.0	23.00±0.50	20.00±0.50	19.66 ± 0.28
Methanol	25µl	7.33±0.28	12.33±0.28	5.33±0.28	8.33±0.28	16.33 ± 0.28
	50µl	10.00±0.50	13.63±0.32	6.33±0.28	11.00±0.0	17.00 ± 0.50
	75µl	10.67±0.17	14.33±0.28	6.66±0.28	13.00±0.50	17.66 ± 0.28
	100µl	11.67±0.57	16.00±0.0	7.50±0.50	13.16±0.28	24.66 ± 0.28

Table 3: Antifungal activity of *Capparis zeylanica* L.

Extracts	Extract Conc.	Fungal Test organisms				
		<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Aspergillus terreus</i>	<i>Fusarium</i> sp.	<i>Penicillium</i> sp.
Aqueous	25µl	-	6.33± 0.28	-	-	5.33 ± 0.28
	50µl	-	9.00 ± 0.0	-	-	-
	75µl	-	9.33± 0.28	-	-	6.33 ± 0.50
	100µl	-	10.33 ± 0.28	-	-	8.66 ± 0.28
Acetone	25µl	-	-	-	-	5.00 ± 0.0
	50µl	-	5.00 ± 0.50	-	6.50 ± 0.50	5.66 ± 0.28
	75µl	-	5.33± 0.28	-	-	6.00 ± 0.50
	100µl	12.66 ± 0.28	9.0 ± 0.50	6.00 ± 0.0	6.66 ± 0.28	7.66 ± 0.50
Ethyl acetate	25µl	6.33± 0.28	7.33± 0.28	-	-	-
	50µl	6.66 ± 0.28	7.66 ± 0.28	6.33± 0.28	-	-
	75µl	7.00 ± 0.50	7.66 ± 0.28	7.00 ± 0.50	-	-
	100µl	7.66 ± 0.28	8.00 ± 0.50	11.16 ± 0.28	-	13.66 ± 0.50
Methanol	25µl	6.33± 0.28	9.00 ± 0.50	6.33± 0.28	-	-
	50µl	6.33± 0.28	12.33± 0.28	6.33± 0.28	6.33± 0.28	-
	75µl	6.50 ± 0.50	13.33± 0.28	6.50 ± 0.50	9.16 ± 0.28	6.00 ± 0.50
	100µl	7.63± 0.28	14.50 ± 0.50	8.00 ± 0.50	14.00 ± 0.50	7.33 ± 0.50

Table 4: GC-MS Analysis of *Capparis zeylanica* L.

S. No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	4.989	Silacyclopentane,	C ₄ H ₁₀ Si	86	0.191
2	15.244	Caryophyllene	C ₁₅ H ₂₄	204	0.155
3	17.861	Pseudosolasodine diacetate	C ₃₁ H ₄₉ NO ₄	499	0.450
4	19.461	Phenol, 2,4-bis(1,1-dimethylethyl)	C ₁₄ H ₂₂ O	206	2.206
5	21.766	Diethyl Phthalate	C ₁₂ H ₁₄ O ₄	222	2.582
6	25.116	Hexadecanoic acid	C ₁₈ H ₃₆ O ₂	284	0.146
7	26.602	Hexadecanoic acid, 15-methyl-, methyl ester	C ₁₈ H ₃₆ O ₂	284	0.709
8	26.804	1-Iodo-2-methylundecane	C ₁₂ H ₂₅ I	296	0.851
9	27.597	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	1.063

10	29.634	Pentadecanoic acid, 14-methyl-, methyl ester	C ₁₇ H ₃₄ O ₂	270	1.063
11	31.184	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	1.108
12	32.769	9,12-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294	0.615
13	32.947	7-Hexadecenoic acid, methyl ester, (Z)	C ₁₇ H ₃₂ O ₂	268	7.081
14	33.158	Phytol	C ₂₀ H ₄₀ O	296	12.777
15	33.441	Hexadecanoic acid, 15-methyl-, methyl ester	C ₁₈ H ₃₆ O ₂	284	16.106
16	34.734	Cyclopentane undecanoic acid	C ₁₆ H ₃₀ O ₂	254	11.294
17	40.100	Bis(tridecyl) phthalate	C ₃₄ H ₅₈ O ₄	530	2.662
18	41.915	Sulfurous acid, butyl heptadecyl ester	C ₂₁ H ₄₄ O ₃ S	376	1.106
19	44.600	2-Piperidinone, N-[4-bromo-n-butyl]	C ₉ H ₁₆ BrNO	233	0.270

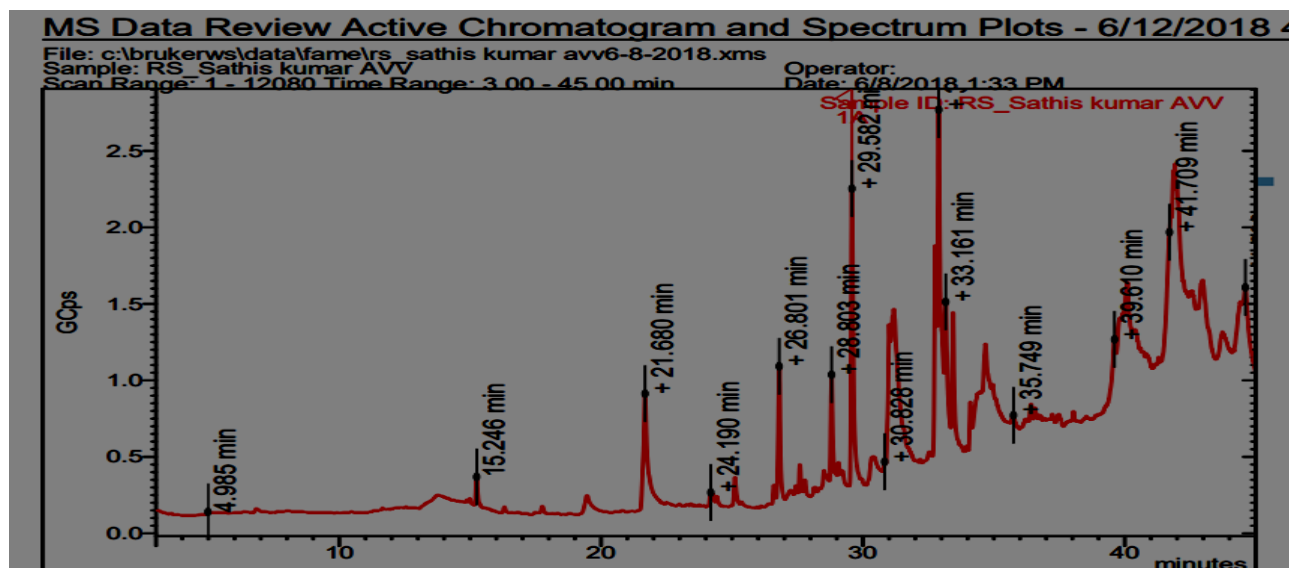
Fig 1: MS Chromatogram and spectrum plots of *C.zeylanica*

Table 5: Bioactive compound and their biological activity

S. No	RT	Name of the compound	Nature of the compound	Activity of the compound
1	4.989	Silacyclopentane,	Phenyl group	Antifungal and antagonist activity
2	15.244	Caryophyllene	Sesquiterpenes	Antimicrobial, anticarcinogenic, anti-inflammatory, antioxidant and anaesthetic activity
3	17.861	Pseudosolasodine diacetate	alkaloid compounds	Antioxidant, Antimicrobial
4	19.461	Phenol, 2,4-bis(1,1-dimethylethyl)	Polyphenol derivatives	Antioxidant, antibacterial, anti-inflammatory and anthelmintic.
5	21.766	Diethyl Phthalate	phthalic acid derivatives	Antimicrobial activity
6	25.116	Hexadecanoic acid	Saturated fatty acids	Antioxidant, hypocholesterolemic, nematicidal, pesticidal.
7	26.602	Hexadecanoic acid, 15-methyl-, methyl ester	Saturated fatty acids	Antibacterial and antifungal
8	26.804	1-Iodo-2-methylundecane	Iodo compound	Antimicrobial, Enhance reproductive activities
9	27.597	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Diterpene	Cancer-Preventive Antimicrobial anti-inflammatory anti-diuretic Antioxidant
10	29.634	Pentadecanoic acid, 14-methyl-, methyl ester	Palmitic acid methyl ester	Anti-tumor, analgesic
11	31.184	n-Hexadecanoic acid	saturated fatty acid	Anti-inflammatory, Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor, potent mosquito larvicide.
12	32.769	9,12-Octadecadienoic acid, methyl ester	Steroid	Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritis, anti-asthama, diuretic.
13	32.947	7-Hexadecenoic acid, methyl ester, (Z)	Palmitic acid ester	Antioxidant, Hemolytic, Hypocholesterolemic, Flavor, Nematicide, Anti-androgenic.
14	33.158	Phytol	Diterpene	Cancer-preventive
15	34.734	Cyclopentaneundecanoic acid	Fatty acid	Antimicrobial (28)
16	40.100	Bis(tridecyl) phthalate	Diester of phthalic acid	Not reported
17	41.915	Sulfurous acid, butyl heptadecyl ester	Ester compound	Antibacterial
18	44.600	2-Piperidinone, N-[4-bromo-n-butyl]	Alkaloid compound	Antimicrobial Antioxidant Antiinflammatory

3. Results

3.1 Preliminary phytochemical analysis:

Qualitative phytochemical analysis of selected plant extracts was performed to detect the presence of phyto-constituents namely alkaloids, Anthroquinones, flavonoids, phenol, Phlobatannins, protein, saponins, steroids, tannins and terpenoids. Alkaloids found in methanol, ethyl acetate and aqueous extract, similarly flavonoids were present in aqueous and methanol extracts. Phenol found in ethyl acetate and methanol extract. Saponins was present only in aqueous extract and tannins were found in ethyl acetate and methanol extracts. The qualitative phytochemical analyses indicated the presence of above (Table.1) phyto-constituents. Out of 4 solvents, methanol extract shows well positivity of phytochemical other than 3 solvents extracts.

3.2 Antimicrobial activity

The antimicrobial activity of *C. zeylanica*. L leaf extract were investigated by using agar well diffusion method against 5 pathogenic bacteria namely *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Five fungal organisms were tested, such as *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Fusarium* sp and *Penicillium* sp. The result indicated that present investigated plant has positive antimicrobial activity. (Table.2 &3)

3.3 Antibacterial activity

The antibacterial activity of aqueous, acetone, ethyl acetate and methanol extracts against five pathogenic bacteria showed the variation of zone inhibition. The standard Amoxillin was used for positive control and DMSO was used as negative control. In aqueous extract the highest activity was recorded against *Bacillus cereus* with the zone of inhibition being 21.5 ± 0.5 at $100 \mu\text{l}$, while the lowest activity was noticed against *Klebsiella pneumoniae* with the clear zone of inhibition of 8.33 ± 0.28 at a dose of $100 \mu\text{l}$. The maximum activity showed in acetone extract with the zone inhibition was 22.00 ± 0.50 at $100 \mu\text{l}$ for *Staphylococcus aureus*, whereas minimum zone inhibition was recorded at $100 \mu\text{l}$ with inhibition zone was 13.50 ± 0.0 against *Bacillus cereus*. Ethyl acetate extract showed highest activity against *Klebsiella pneumoniae* with the zone of inhibition being 23.00 ± 0.5 at $100 \mu\text{l}$, whereas lowest activity on *E. coli* with inhibition zone of 19.00 ± 0.0 at $100 \mu\text{l}$. The leaf extract of methanol was maximum inhibition zone recorded against *Staphylococcus aureus* with inhibition zone was 24.66 ± 0.28 at $100 \mu\text{l}$ and minimum inhibition zone was noticed against *Klebsiella pneumoniae* with the inhibition zone being 7.50 ± 0.50 at $100 \mu\text{l}$. According to the above data obtained the leaf extract showed different activity level against pathogens. The present study indicated that methanol extract of leaf provided to be more powerful antibacterial sensitivity when compared with other extracts.

3.4 Antifungal activity

Antifungal activity of aqueous, acetone, ethyl acetate and methanol extracts of *C. zeylanica* leaf were determined against five pathogenic fungi in terms of zone of inhibition (mm). The common antifungal standard fluconazole was used as positive control whereas DMSO used as negative control. *Aspergillus niger* was medium susceptible in aqueous extract with the zone of inhibition of 10.33 ± 0.28 at $100 \mu\text{l}$ and least activity found against *Penicillium* sp with the zone of inhibition being 8.66 ± 0.28 at $100 \mu\text{l}$. In acetone extract the

maximum activity was recorded at $100 \mu\text{l}$ with the zone of inhibition of 12.66 ± 0.28 against *Aspergillus flavus*, whereas minimum activity was found against *Aspergillus terreus*. The zone of inhibition was 6.00 ± 0.0 at $100 \mu\text{l}$. Ethyl acetate extract showed medium susceptible against *Penicillium* sp with the zone of inhibition of 13.66 ± 0.50 at $100 \mu\text{l}$ and least susceptible against *Aspergillus flavus* and the inhibition zone was 7.66 ± 0.28 at a dose of $100 \mu\text{l}$. The maximum activity was recorded in methanol extract against *Aspergillus niger* with the zone of inhibition of 14.50 ± 0.50 at $100 \mu\text{l}$, whereas minimum activity was found against *Aspergillus flavus*, the zone of inhibition was 7.63 ± 0.28 at $100 \mu\text{l}$. no activity was recorded against *Aspergillus flavus*, *Aspergillus terreus*, *Fusarium* sp in aqueous extract, similarly *Fusarium* sp in ethyl acetate extract. According to the data mentioned above, the leaf extract showed different antifungal activity against pathogens. The present study indicated that methanol extract provided highest activity compared with other extracts.

3.5 GC-MS Analysis

As per the best result of preliminary phytochemical analysis, the methanol extract was chosen for identification of major phyto-constituents by GC-MS analysis. (Fig.1). Nineteen compounds were detected from the leaf extract based on their active principle with their retention time (RT), molecular weight (MW), molecular formula (MF), concentration of % and mass spectrum (MS) in methanol extract of leaf were given in the table (Table.4). The following phyto compound were found in methanol extract. Silacyclopentane, Caryophyllene, Pseudosolasodine diacetate, Phenol, 2,4-bis(1,1-dimethylethyl), Diethyl Phthalate, Hexadecanoic acid, 15-methyl-methyl ester, 1-Iodo-2-methylundecane, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Pentadecanoic acid, 14-methyl-, methyl ester, n-Hexadecanoic acid, 9,12-Octadecadienoic acid, methyl ester, 7-Hexadecenoic acid, methyl ester, (Z)Phytol, Cyclopentaneundecanoic acid, Bis(tridecyl) phthalate, Sulfurous acid, butyl heptadecyl ester, 2-Piperidinone, N-[4-bromo-n-butyl].

4. Discussion

Medicinal plants have long history of use and their uses are wide spread in both developed and developing countries. The potential of higher plants as source for new drugs is still largely unexplored. Medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines.

Numerous plants used in traditional medicine are effective in treating various ailments caused by bacterial and viral infections. Research has shown that medicinal plants exhibit antimicrobial activity [32]. In the present study an attempt has been made to screening of different solvent extracts for their antimicrobial activity against several pathogenic bacteria and fungi like *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Five fungal organisms, such as *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Fusarium* sp and *Penicillium* sp. By using agar well diffusion method. The Results showed that methanol extract has antimicrobial activity higher than aqueous, acetone and ethyl acetate extracts of leaf. These results are in accordance with that of [33]. Gas chromatography–Mass spectroscopy (GC-MS) is a potential tool for identification of phyto-compound. In the present studies, eighteen compounds were identified from the methano leaf extract. Silacyclopentane showed significant

broad-spectrum fungicidal activity^[34]. Caryophyllene present in a large number of plants worldwide. It possesses significant anticancer activities, affecting growth and proliferation of numerous cancer cells. The presence of Pseudosolasodine diacetate proved high antioxidant activity^[35]. Cancer-Preventive, Antimicrobial, anti-inflammatory, anti-diuretic and Antioxidant compound 3,7,11,15-Tetramethyl-2-hexadecen-1-ol reported^[36]. Pentadecanoic acid, 14-methyl-, methyl ester is a anti-tumor and analgesic phytochemical^[32]. Anticancer, hepatoprotective and anti-arthritis related compound 9,12-Octadecadienoic acid, methyl ester was identified^[37]. Phytol is a therapeutic activity have been reported in previous studies, inhibition of inflammatory response by reducing cytokine production and oxidative stress^[38], activity against mycobacteria^[39] and anti-convulsant^[40] anti-spasmodic^[41] and anti-cancer activities^[42].

5. Conclusion

The results of preliminary phytochemical screening suggest that the leaf extracts of *C.zeylanica* is a good source of beneficial phytochemicals. The antibacterial and antifungal activities of the prepared extracts indicated that leaf extracts have significant potent antimicrobial agent. Among the various prepared extracts of leaf, methanol extracts were most effective against tested bacterial and fungal species. According to the obtained data through GC-MS, there were eighteen phytochemicals identified. These compounds have significance of biological activity.

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