PHYTOCHEMICAL SCREENING, ANTIMICROBIAL POTENTIAL AND GC-MS ANALYSIS OF CHLOROFORM LEAF EXTRACT OF *HYPTIS SUAVEOLENS* (L.) POIT.

ABSTRACT

Bioactive compounds possessing therapeutic value are used for long time by vast group of researchers to treat various diseases for the goodness of the mankind. Most of the commercially available bioactive compounds are derived from plants because of their affirmative property on human health with minimum side effects. *Hyptis suaveolens* is one of the most important traditional and therapeutic plant belongs to Lamiaceae family. The Biochemical test of the leaf extract of *Hyptis suaveolens* using different solvent shows the presence of significant compounds. Thereby, the Antimicrobial properties of leaf extract of *H. suaveolens* carried out against six different bacterial pathogens and three fungi pathogens. Most potential activity was observed in the crude extract of Hexane, Ethyl acetate and Chloroform against E.*coli, Enterococcus faecalis* and *Streptococcus pyrogenes* respectively when compared to *Klebshilla pneumonia.* Among the other crude extract, the chloroform crude extract resulted with more number of molecules in the phytochemical analysis and also it exhibits superior antibacterial activity. Hence, the Gas Chromatography-Mass Spectrometry (GC-MS) analysis of chloroform extract was achieved and 17 different bioactive compounds were discovered. Neophytadiene, (S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol, 2,3-Diazabicyclo[2.2.1]hept-2-ene, 7,7-dime and Stigmasterol are the significant molecules present in the chloroform leaf extract it has the highest value for the mankind.

Keywords: GC-MS, Antimicrobial property, phytochemical analysis, Medicinal Plant and *Hyptis suaveolens*.

1. INTRODUCTION

Medicinal plants and aromatic plants are well-known, most important and unavoidable life form in human life since, they are used for food and the treatment of various illnesses. The plant products are used as therapeutic and pharmacological replacements are inexpensive and widely accepted in our culture. Plants based compounds are the fundamental and potential source of new antibiotic drugs with safe and no side effects [1]. Ayurvedic, Unani and Sidha are the ancient system of medicine recommends the plants or their extracts having the potential of controlling the different type of diseases. *Hyptis suaveolens* (L. poit) is native of tropics of America commonly called American Mint and considered as a weed worldwide [2]. India preserves 20% plant species are having medicinal value and used for medicinal purposes [3, 4]. *Hyptis suaveolens* is remarkably used in Maharashtra to cure different parasitical cutaneous diseases, sudorific in catarrhal condition, uterus infection, stomach ache, headache and snuff to stop bleeding of the nose. According to WHO (World Health Organization), 80% of the population of Asian and African countries are presently practices to use herbal medicines for their primary health care. The secondary metabolites from *H. suaveolens* contain plenty of structural arrangements with economic and medicinal properties [5]. *H. suaveolens* comprises different composition of volatile compounds and essential oil based on their geographic origin and its growth phase. Essential oil isolated from the aerial parts of *H. suaveolens* has antibacterial, antifungal and wound healing properties [6]. *H. suaveolens* is being used by various tribal communities in Maharashtra, Marathwada region to control and inhibit diseases. They have been regarded generally as man's first knownmedicine, are extensively used for bactericidal, fungicidal, anti-parasitic and cosmetic applications, in the pharmaceutical, cosmetic, agricultural industries [7] stimulant carminative, antiseptic, sudorific and galactagogue [8,9]. The essential oil derived from the leaves holds the significant antimicrobial and antifungal properties. The root extract of *H.suaveolens* has anti-retroviral compound which is called as urosolic acid, it is a triterpenoid hence may target retroviral integrates and proteases blocking the replication of retroviruses such as HIV. Most of these bioactive compounds derived from of *H. suaveolens* used for therapeutic purposes and also it is being used as a precursors of useful drugs. The essential oil of *H. suaveolens* has sabinene, limonene, bicyclogermacrene, phellandrene and 1,8-cineole Isolated dehydroabietinol. The oldest and most wide spread diseases known to man is the kidney stone formation, this plants shows powerful effect to treat urinary tract infection, diuretic and kidney

disorders [10,11].

Since *H. suaveolens* has many therapeutic value, it creates an interest to analyse the chemical composition, Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed to conform the presences of significant chemo-types present in the plant extract.

2. MATERIALS AND METHODS

2.1. Plant Collection and Identification

The fresh leaves of *Hyptis suaveolens* was collected from Anaikuttam village, located at Sivakasi Taluk of Virudhunagar district, Tamil Nadu, India. The plant was identified and authenticated by Dr.A.Sarvalingam M.Sc., M.Phil., Ph.D, Assistant professor, Department of Botany, NGM College, Pollachi, Tamilnadu, India. The shade dried leaf powder (10 mgs) of *Hyptis suaveolens* was packed well in Soxhlet apparatus and the serial extraction was carried out for three hours at 55-85^0 C using different solvents and the crude extract was stored under 4^0 C for further analysis.

2.2. Biochemical test for Leaves Extract

Phytochemical examinations were carried out using eight solvent extracts (Hexane, Petroleum Ether, Ethyl acetate, Chloroform, Acetone, Ethanol, Methanol and Aqueous extracts) as per the standard method to analyze the presence of pharmacologically active constituents such as Tannins, Phlobatannins, Saponins, Coumarins, Flavonoids, Terpenoids, Cardiac glycoside, Glycosides, Phenolic groups, Amino acids, Essential oil, Aromatic acid and Gum & Mucilage, Alkaloids, Steroids, Reducing sugars, Phlobatannins, Anthraquinones, Quinones, Xanthoproteins, Carbohydrates, Anthocyanins, Leucoanthocyanins and Emodins [12].

2.3. Antibacterial Activity

Antibacterial activity was performed by well diffusion method using Nutrient Agar medium [13]. The medium was prepared, sterilized and poured in the petriplate under sterile condition. After solidification of the medium, Bacterial pathogens was spread on the medium using sterile cotton swap (Spread plate method). Wells (6mm) were made on the medium to load the plant extract and the plates were incubated at 37^O C. The pH of the medium was maintained with the pH 7 ± 0.2. Different concentrations of plant extract were tested against different Bacterial pathogens for its anti-bacterial activity [13].

2.4. GC-MS Analysis

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of chloroform leaf extract of *H. suaveolens* was performed (Shimadzu GCMS-QP2010). The oven temperature was programmed from 100 ºC to 320º C at100 ºC/min and hold for 10 min using Helium carrier gas at flow rate of 1.0 mL/min. The injector temperature was 250 ºC, injection size 1 µL neat, with split ratio 1:10. The interface and MS ion source were maintained at 320º C and 200º C respectively and the mass spectra were taken at 70eV with a mass scan range of 40-700 amu (atomic mass unit). Data handling was done using GC-MS solution software [14].

3. RESULTS

3.1. Biochemical test for Leaves Extract

The phytochemicals analysis of the eight solvent extracts such as Hexane, Petroleum Ether, Ethyl acetate, Chloroform, Acetone, Ethanol, Methanol and Aqueous extracts were tested and reported with the presence of 23 phytochemicals and the Hexane leaves extract alone positively resulted with 12 phytochemicals (Table 1). Alkaloids, Steroids, Reducing sugars, Phlobatannins, Anthraquinones, Quinones, Xanthoproteins, Carbohydrates, Anthocyanins, Leucoanthocyanins and Emodins were not identified in the hexane extract. Whereas, the Petroleum ether extract shows 13 phytochemicals such as Steroids, Tannins, Phlobatannins, Saponins, Coumarins, Flavonoids, Terpenoids, Cardiac glycosides, Glycoside, Phenolic group, Essential oil, Aromatic acids and Gum & Mucilage. The remaining 10 phytochemicals were absent such as Alkaloids, Reducing sugars, Anthraquinons, Quinones, Amino acids, Xanthoproteins, Carbohydrates, Anthocyanins, Leucoanthocyanins, Emodins. The Ethyl acetate leaves extract of *Hyptis suaveolens* contains only 11 phytochemicals such as Alkaloids, Tannins, Phlobatannins, Saponins, Flavonoids, Cardiac glycosides, Glycosides, Phenolic group, Essential oil, Aromatic acids and Gum & Mucilage. The remaining 12 phytochemicals were absent such as Steroids, Reducing sugar, Coumarins, Terpenoids, Anthraquinons, Quinones, Amino acid, Xanthoproteins, Carbohydrates ,Anthocyanins ,Leucoanthocyanins and Emodins (Table 1).

The Chloroform leaves extract of *Hyptis suaveolens* significantly shows the presence of contains 14 different phytochemicals such as Steroids, Reducing sugar, Tannins, Phlobatannins, Saponins, Coumarins, Flavonoids, Terpenoids, Glycoside, Anthraquinons, Phenolic group,

Essential oil, Aromatic acid and Gum & Mucilage. Whereas the Alkaloids, Cardiac glycosides, Quinones, Amino acids Xanthoproteins, Carbohydrates, Anthocyanins, Leucoanthocyanins and Emodins were not identified in the biochemical tests performed (Table 1). Comparatively, Acetone extract contains eight Phytochemicals and Ethanolic and Methanolic extract shows the presence of seven and eleven Phytochemicals respectively.

Water is the universal polar solvent therefore water also used for the extraction of phytochemicals. The aqueous extract of *Hyptis suaveolens* contains 12 different phytochemicals such as Tannins, Phlobatannins, Saponins, Coumarins, Flavonoids, Terpenoids, Cardiac glycosides, Glycosides, Phenolic group, Quinones, Aromatic acid and Carbohydrates and other phytochemicals were not identified (Table 1).

Table 1. Qualitative analysis of phytochemicals constituents of chloroform leaves extract of *Hyptis suaveolens*

S.NO	TEST NAME	HE	PE	EA	CM	AE	EL	ML	AS
1.	Alkaloids	-	-	+	-	-	-	-	-
2.	Steroids	-	+	-	+	-	-	-	-
3.	Reducing sugars	-	-	-	+	-	-	-	-
4.	Tannins	+	+	+	+	+	+	+	+
5.	Phlobatannins	-	+	+	+	+	+	+	+
6.	Saponins	+	+	+	+	+	+	+	+
7.	Coumarins	+	+	-	+	-	-	+	+
8.	Flavonoids	+	+	+	+	-	-	+	+
9.	Terpenoids	+	+	-	+	+	-	-	+
10.	Cardiac glycosides	+	+	+	-	+	+	+	+
11.	Glycosides	+	+	+	+	+	+	+	+
12.	Anthraquinones	-	-	-	+	-	-	-	-
13.	Phenolic groups	+	+	+	+	-	-	+	+
14.	Quinones	-	-	-	-	-	-	-	+
15.	Amino acids	+	-	-	-	-	-	-	-

16.	Essential oils	+	+	+	+	-	-	-	-
17.	Aromatic acids	+	+	+	+	+	+	+	+
18.	Xanthoproteins	-	-	-	-	-	-	-	-
19.	Carbohydrates	-	-	-	-	-	-	+	+
20.	Anthocyanins	-	-	-	-	-	-	-	-
21.	Leucoanthocyanins	-	-	-	-	-	-	-	-
22.	Emodins	-	-	-	-	-	-	-	-
23.	Gum and Mucilage	+	+	+	+	+	+	+	-
Total No. of Phytochemicals		**12**	**13**	**11**	**14**	**08**	**07**	**11**	**12**

(+) Indicates present; (-) Indicates Absent

[HE – Hexane, PE – Petroleum Ether, EA- Ethyl Acetate, CM – Chloroform, AE – Acetone, EL – Ethanol, ML – Methanol, AS – Aqueous].

3.2 Antimicrobial Activity

The Hexane leaves extract *of Hyptis suaveolens* shows the zone of inhibition against E.*coli* (21 mm), *Enterococcus faecalis* (8 mm) and *Klebshilla pneumonia* (6 mm). Followed by the Ethyl acetate the zone of inhibition against *Enterococcus faecalis* (11 mm), *Shigella flexneri* (11 mm), *Klebsnilla pneumonia* (6 mm). The Chloroform leaf extract has inhibition zone against *Streptococcus pyogenes* (21 mm), *Klebsnilla pneumoniae* (16 mm). The Acetone shows bacterial inhibition zone shows in *E.coli* (16 mm) (Fig. 1). The Petroleum ether, Ethanol, Methanol and Aqueous samples leaf extract of *Hyptis suaveolens* has no inhibition activity against human bacterial pathogens and fungal pathogens (Table 2).

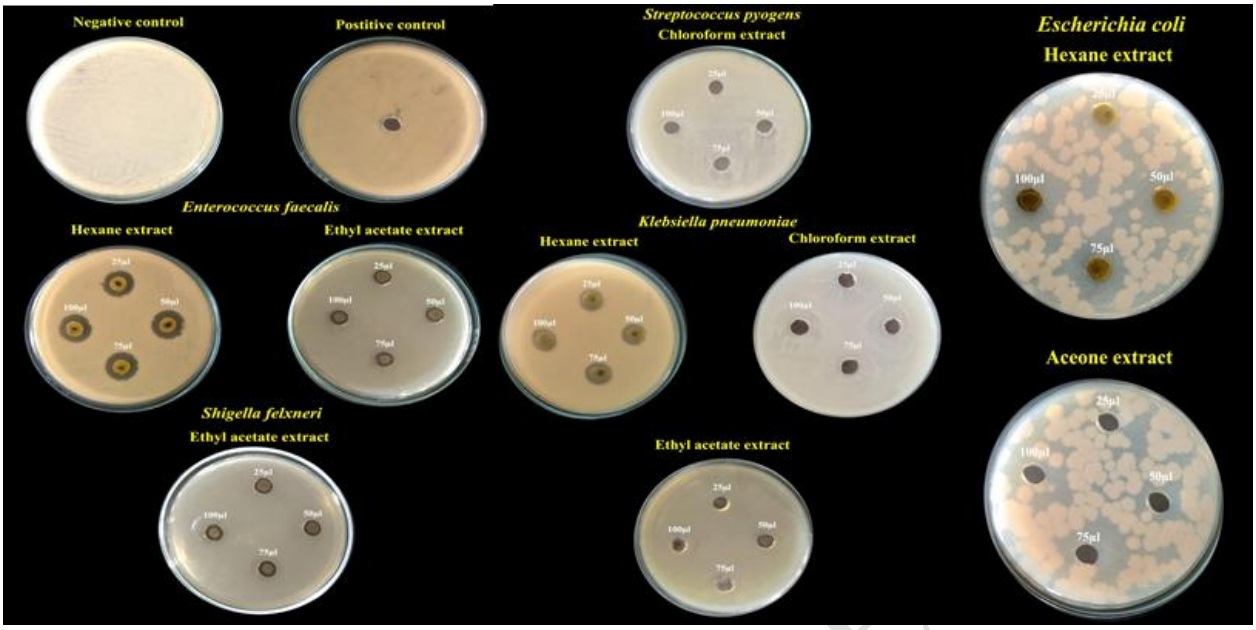

Figure 1. Antibacterial activities of leaf extract of *Hyptis suaveolens* against different pathogens.

Table 2. Antimicrobial Activity of Leaves extracts of *Hyptis suaveolens* (L.) Poit.

S. No.	Name of Pathogens	Name of the Solvents							
		HE	PE	EA	CM	AE	EL	ML	AS
1.	*Enterococcus faecalis*	+	--	++	--	--	--	--	--
2.	*Shigella flxneri*	--	--	++	--	--	--	--	--
3.	*Streptococcus pyogens*	--	--	--	+++	--	--	--	--
4.	*Klebshilla pneumonia*	+	--	++	+++	--	--	--	--
5.	*Pseudomonas* sp.	--	--	--	--	--	--	--	--
6.	*E.coli*	+++	--	--	--	+++	--	--	--
7.	*Rhizoctonia solani*	--	--	--	--	--	--	--	--
8.	*Sclerotium rolfsii*	--	--	--	--	--	--	--	--
9.	*Macrophomina*	--	--	--	--	--	--	--	--

(+) Indicates below 9 mm, (++) Indicates 12 – 15 mm and (+++) Indicates 16 mm & above
[HE – Hexane, PE – Petroleum Ether, EA- Ethyl Acetate, CM – Chloroform, AE – Acetone, EL – Ethanol, ML – Methanol, AS – Aqueous].

3.3. Gas Chromatography and Mass Spectroscopy Analysis

The GC-MS analysis of Chloroform leaves extract of *Hyptis suaveolens* reported with seventeen bioactive compounds with their Retention time, Molecular weight, Molecular formula and Molecular structure for alfa.-Copaene, Neoalloocimene, (1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-m, (1R,2R,4S,6S,7S,8S)-8-Isopropyl-1-methyl-, 2H-Cycloprop[c]indene-2,3(3ah)-dione, hexa, 2H-Cycloprop[c]indene-2,3(3ah)-dione, hexa, Neophytadiene, 2-Pentadecanon, 6,10,14-Trimethy, 14-.BETA.-H-Pregna Hexadecanoic Acid, Methyl Este (S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol, 2,3-Diazabicyclo[2.2.1]hept-2-ene, 7,7-dime, gamma.-Dodecalactone (33.138 RT), 2-Hexadecen-1-OL, 3,7,11,15-Tetram, Palmitaldehyde, Diallylacetal, 17-Octadecene-9,11-Diynoic Acid, 8 Hexadecanoic acid, 2-hydroxy-1-(hydroxym (40.628 RT) and Stigmasterol (Table 3, Figure 2).

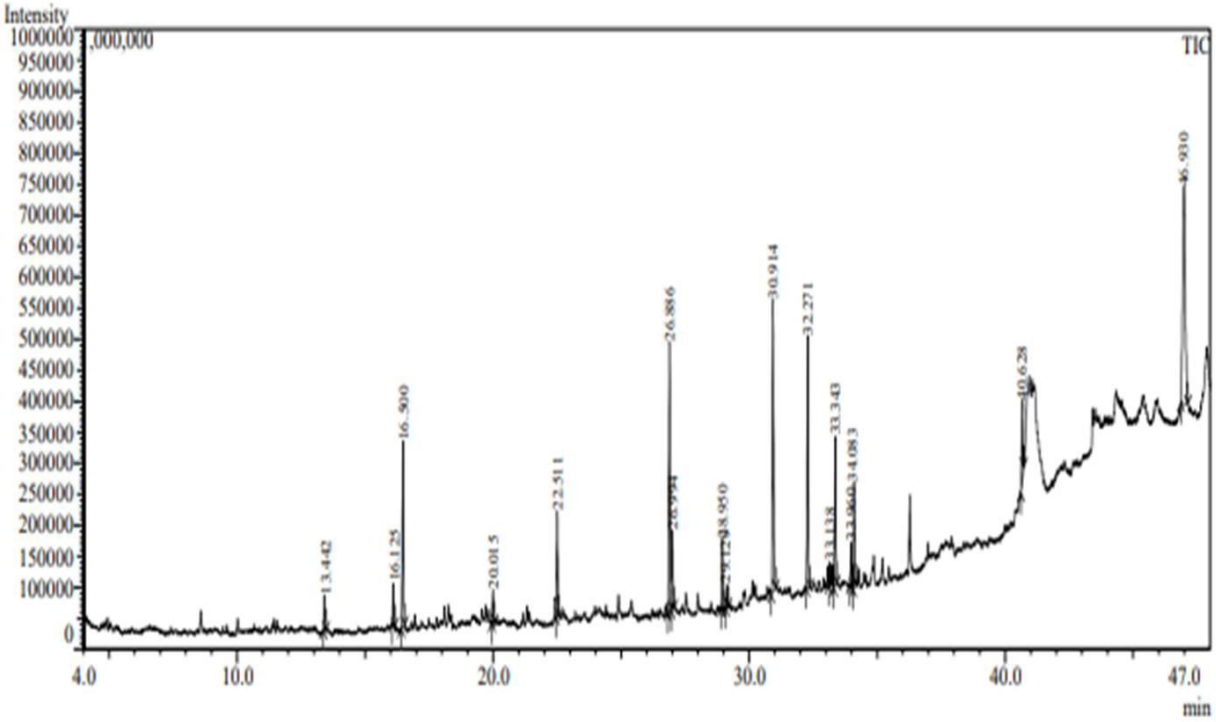

Figure 2. GC-MS analysis of chloroform leaves extract of *Hyptis suaveolens*

Table No 3. GC-MS analysis of Chloroform leaves extract of *Hyptis suaveolens* (L.) Poit.

Peak	R. Time	Area %	Name of the Compound	Molecular Formula	Molecular Weight (g/mol)	Isomeric / Canonical SMILES
1.	13.440	1.44	(-)-alpha-Copaene	$C_{15}H_{24}$	204.35	CC1=CC[C@H]2[C@H]3[C@@H]1[C@@]2(CC[C@H]3C(C)C)C
2.	16.125	1.72	Neoalloocimene	$C_{10}H_{16}$	136.23	C/C=C(\C)/C=C/C=C(C)C
3.	16.500	7.85	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decane-rel	$C_{15}H_{24}$	204.35	CC(C)[C@@H]1CC[C@]2([C@H]3C1C2CCC3=C)C
4.	20.015	1.39	(1R,2R,4S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decan-4-ol	$C_{15}H_{24}O$	220.35	CC(C)C1CCC2(C3C1C2C(=C)C(C3)O)C
5.	22.510	4.64	2H-Cycloprop[c]indene-2,3(3ah)-dione, hexahydro-3a,7,7-trimethyl	$C_{13}H_{18}O_2$	206.28	CC1(CCCC2(C13CC3C(=O)C2=O)C)C
6.	26.885	12.93	Neophytadiene	$C_{20}H_{38}$	278.5	CC(C)CCCC(C)CCCC(C)CCCC(=C)C=C
7.	26.995	3.55	"2-Pentadecanone, 6,10,14-trimethyl-"	$C_{18}H_{36}O$	268.5	CC(C)CCCC(C)CCCC(C)CCCC(=O)C
8.	28.950	2.93	14-.Beta.-H-Pregna	$C_{21}H_{36}$	288.5	CC[C@H]1CC[C@H]2[C@@]1(CC[C@H]3[C@H]2CCC4[C@@]3(CCCC4)C)C
9.	29.130	0.90	Hexadecanoic Acid, Methyl Este	$C_{35}H_{56}O_5$	556.8	CCCCCCCCCCCCCCCC(=O)OCC1=CC(=C(C(=C1C=O)O)C/C=C(\C)/CCC=C(C)C)OC
10.	30.915	13.62	(S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol	$C_{17}H_{24}O$	244.37	CCCCCCC/C=C\CC#CC#C[C@H](C=C)O
11.	32.270	10.01	2,3-Diazabicyclo[2.2.1]hept-2-ene, 7,7-dimethyl-5-phenyl-, (1.alpha.,4.alpha.,5.beta.)-	$C_{13}H_{16}N_2$	200.28	CC1(C2CC(C1N=N2)C3=CC=CC=C3)C
12.	33.140	0.90	gamma.-Dodecalactone	$C_{12}H_{22}O_2$	198.3	CCCCCCCCC1CCC(=O)O1

13.	33.345	6.22	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}O$	296.5	CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C
14.	33.960	1.69	Palmitaldehyde, Diallyl Acetal	$C_{22}H_{42}O_2$	338.6	CCCCCCCC/C=C\CCCCCCCCCCCC(=O)O
15.	34.085	4.19	17-Octadecene-9,11-Diynoic Acid, 8-Hydroxy-, Methyl Ester	$C_{19}H_{28}O_3$	304	C[C@]12CC[C@@H](CC1=CC[C@@H]3[C@@H]2CC[C@]4([C@H]3C[C@H](C4=O)O)C)O
16.	40.630	3.32	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.5	CCCCCCCCCCCCCCCC(=O)OC(CO)CO
17.	46.930	22.70	Stigmasterol	$C_{29}H_{48}O$	412.7	CC[C@H](/C=C/[C@@H](C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CC[C@@H](C4)O)C)C)C(C)C

4. DISCUSSIONS

For many decades, researchers were used plants and plant extract for many purposes like body pain, pathogens infections, malfunctions, inflammations and nutritional supplements [15, 16]. Phytochemical tests, Antimicrobial activity of the plant extract and GC-MS analysis are the source to discover the new novel drugs for different purposed with varied age groups [17]. *Hyptis suaveolens* is distributed all over the country and many workers were focused on its phytochemical analysis and its potential activity. Different kind work was carried out using this plant collected from Bhopal, Madhya Pradesh [18] and Mahodari Ghat Region, Nashik (Maharashtra) during the month of November to march based on the work need [19]. Leaf extract of *Hyptis suaveolens* was isolated by Soxhlet apparatus using different solvent systems, the plant was collected from the Anaikuttam village, located at Sivakasi Taluk of Virudhunagar district, Tamil Nadu, India where the plant mass is high in the wild area. Phytochemical investigation of Leaf extracts of *Hyptis suaveolens* illustrates the varied range of compounds, in the chloroform, Petroleum Ether and Hexane extract shows 14, 13 and 12 compounds respectively. The results show the same number molecules present in the hexane and water extract. Among the eight solvent system used for the extraction, the chloroform extract exhibits significant antimicrobial activity against *Streptococcus pyogens* and *Klebshilla pneumonia* when compared to the solvent extracts. Whereas, *E.coli* was highly inhibited in the hexane and acetone. *Streptococcus pyogens* and *Klebshilla pneumonia* are said to be the most toxic human pathogens, which were inhibited the chloroform Leaf extracts of *Hyptis suaveolens.* It shows the significance of the presence of the molecules present in the chloroform leaf extracts, thereby these pathogens were highly inhibited.

Phytochemical comparative analysis:

The essential oil from the leaf oil of *H. suaveolens* showed antimicrobial activity against *Enterococcus faecalis*, *Candida albicans* and *Bacillus cereus* [20,21]. The chloroform extract of our work not showed any significant results against fungal pathogens, hence it decided that, the extract is effective against only on this bacterial pathogens. Ethyl acetate leaf extract of *H. suaveolens* has inhibition against *Enterococcus faecalis*, *Streptococcus pyogens* and *Klebshilla pneumonia.* The overall results showed the *Klebshilla pneumonia* was inhibited by chloroform, ethyl acetate and hexane. Next to this *E.coli* was strongly inhibited by acetone and hexane. The antimicrobial properties of the oil sample contests positively with data of *H. suaveolens* essential oil sample [13, 14] and the inhibitory activity also recorded from the flower

oil of *H. suaveolens* [22]. Ethnolic extract of *H. suaveolens* leaves results showed Thirty compounds and 5,5-dimethylimidazolidin-2,4- diamine (20.35%) was the major compound followed by 1-hexadecene (8.43%) [23]. The GC-MS spectrum of the extract showed the presence of more long-chain hydrocarbons [24]. Various aliphatic acids, aromatic compounds and ketones were also identified. Stigmast -5-en-3-ol, oleate shows highest peak at retention time of 26.014 with 20.89% of area followed by gamma-sitosterol with peak retention time at 27.766 with 16.61% of area and butyl 11-eicosenoate with retention of 28.984 with 14.12 % of area [25, 26]. This study supports and recommends the plant *H. suaveolens* has got potential drug for antibacterial activity and purified compound with pharmacological properties could be used as an alternative to commonly used synthetic drug having various side effects.

5. CONCLUSION

Present study revealed that, the leaves extract *of Hyptis suaveolens* shows potential antibacterial activity and the phytochemicals were present in the extract chloroform were confirmed by the different biochemical tests. Based on the biochemical, antibacterial and GC-MS analysis, we concluded that, potential bioactive compounds were present in *H. suaveolens* act as a good medicinal plant for the preparation of different new novel drugs.

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