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Chemical characterization of extracts from various parts of *Salvia hispanica* L. and their antibacterial activity

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Plants have been an important source of phytomedicines for thousands of years. *Salvia hispanica* L. distributed in Central and Southern Mexico and Guatemala has potential medicinal properties. The current research focused on the chemical characterization of the plant extracts from leaf, seed, and flower parts using gas chromatography-mass spectrometry (GC MS) analysis and evaluating its antibacterial effects. Results of antibacterial tests proved that the crude extracts could be potentially utilized in controlling some bacterial strains. The highest zone of inhibition (14.6 mm) was observed against the bacterium *Pseudomonas fluorescens* at 50 µg/100 µL ethyl acetate flower extract. GC-MS analysis confirmed the occurrence of 49 and 34 different compounds in the ethyl acetate flower and leaf extract respectively. Many of them are used in industry for various applications like flavour and fragrance agent, antioxidant, anti-inflammatory, antimicrobial, antitumor etc. These plant extracts which proved to be potentially effective can be used as natural alternative preventives to control food poisoning diseases and preserve foodstuff avoiding health hazards of chemical antimicrobial agent applications.

Keywords: Antibacterial activity, GC-MS, *Salvia hispanica* L.

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Introduction

Traditional medicine is the fusion of therapeutic knowledge of generations of routine systems of medicine. A natural drug has only those traditional medicines that primarily use medicinal plant preparations for therapy[1-2]. During the last century, a decline in the use of natural products was witnessed because of the application of synthetic drugs and it was assumed that these drugs would perhaps entirely substitute the use of traditional plant-based medicines. However, in the current scenario, the world is witnessing an era of herbal renaissance and the use of herbal drugs has risen significantly. This is due to the toxic effects of synthetic drugs on human beings and there is a realization that plant-derived medicines are safe with no side effects. Due to the growing interest in medicinal plants by researchers, many indigenous botanical resources have been screened for their pharmacologically important compounds[3].

The occurrence of multiple drug resistance has tremendously increased which has lead to the development of new synthetic antimicrobial drugs and the quest for new antimicrobial agents from different sources[4-5]. Current progress to invent new drugs from plant sources has resulted in the discovery of active compounds that are effective in treatment against resistant bacteria and viruses, cancer, and immunosuppressor disorders[6-7]. Secondary metabolites contain bioactive compounds which exhibit antimicrobial potency, and screening of these new bioactive compounds with pharmaceutical potential can lead to new medicinal drugs for treatment against different diseases.

Salvia hispanica L. popularly known as 'Chia' belongs to the family Lamiaceae. It is a native of Central and Southern Mexico and Guatemala and spans several countries of South America. It has been reported in *Codex Mendoza* that chia was cultivated by Aztecs during pre-Columbian times[8-9]. Chia oil contains the highest known content of α-linolenic acid up to 68%[10-11] compared with 57% in Flax seeds which makes it an excellent source of omega-3 fatty acid[12]. It also contains a major amount of primary and synergistic antioxidants, such as caffeic acid, myricetin, quercetin, chlorogenic acid, and kaempferol[13-14]. It is a rich source of other natural

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antioxidants *viz*. carotenoids, phytosterols, tocopherols which could be utilized as nutritional supplements[15]. The antioxidant potential of chia seeds is considerably more than vitamin C (ascorbic acid), vitamin E (α-tocopherol), ferulic acid, other flavonoid substances[16-17]. Recently, it has been discovered that chia seed contains high values of phytosterols[18] an important nutrient that helps to prevent cardiovascular diseases[19] and possess anticancer[20], antioxidants[21], anti diabetic[22], bactericidal, and antifungal properties[23-24].

Thus, in view of the high economic value of Chia as an industrial crop and functional food and owing to its pharmaceutical properties, the present research aimed to evaluate the antibacterial activity of the plant extracts against some bacterial strains and characterize chemically the crude extract of seed, flower, and leaf of *S. hispanica* L.

Materials and Methods

Collection and authentication of plant material

Seeds of *S. hispanica* L. were authenticated by the Canadian organic production system and USDA organic system (April, 2015) and a voucher specimen was deposited (RUBL211668) in the Herbarium, Department of Botany, University of Rajasthan. Initially, seeds were grown in pots. Later on, leaves, flowers, and seeds were used as experimental material. Mature, fresh, healthy and disease-free plant materials were properly washed in tap water, shade dried and then homogenized into a fine powder and stored in airtight bottles for further experimental purpose.

Extraction method

Flowers, leaves, and seeds powder of *S. hispanica* L. were subjected to hot soxhlet extraction method[25].

Solvent extraction using soxhlet apparatus (Hot extraction)

The air-dried and ground plant material (flower, leaves, and seeds) was extracted with organic solvents; ethyl acetate and methanol using Soxhlet apparatus for 1 h in each case. 20 g of powdered plant material was put in Soxhlet. Then, 200 mL of solvent was added to the flask and then extracted at 60 ^{o}C for 1 h. The solvent was evaporated using a rotator evaporator. Dried crude extract residues were resuspended in mother solvent and DMSO (500 mg/mL) and stored in a refrigerator at 4 ^{o}C for further use.

Bacterial strains

The test microorganisms were collected from the culture collection of the Institute of Microbial Technology (IMTECH), Chandigarh.The MTCC strains were *Bacillus coagulans* (MTCC 2449), *Bacillus megaterium* (MTCC 2412), *Escherichia coli* (MTCC 1696), and *Pseudomonas fluorescens* (MTCC 709).

Maintenance of bacterial strains

The microbial cultures were maintained by periodic sub culturing in nutrient agar slants[26] incubated at 37 ^{o}C for 24 h and stored at 4 ^{o}C.

Bacterial inoculum

Bacterial cultures were routinely maintained on nutrient agar slants at 4 ºC. For the experimental purpose, bacterial inoculums were prepared by taking a loopful of isolated colonies and inoculating into 4 mL of peptone water followed by incubating at 37 °C for 4 h. Peptone water was prepared by dissolving 10 g of peptic digest of animal tissue with 5 g of Sodium chloride and final volume was adjusted to 1000 mL of distilled water at pH 7.2±0.2. The actively growing bacterial cultures were then adjusted with this peptone water till turbidity visually comparable to that of 0.5 McFarland standard[27] was obtained.

***In vitro* antibacterial assay**

The plant extracts prepared from the discontinuous extraction method were used for antibacterial screening. It was performed by the Disc diffusion method[28]. Sterile liquid Mueller Hinton Agar media (pH 7.4±2) was poured into a sterile Petri dish and after media solidification, the inoculum of different bacterial strains was swabbed with a sterile needle under aseptic conditions. Each disc was impregnated with 100 µL of crude plant extract. A disc with mother solvent was used as control. Discs were put on the surface of the medium. The plates were incubated at 37 ^{o}C for 24 h to obtain the zone of inhibition. Experiments were repeated thrice and the average zone of inhibition was determined. Mother solvent was taken as negative control and Cefixime (5 µg/disc) procured from Hi-Media, Mumbai, was used as positive control.

GC-MS analysis

GC/MS is a hyphenated technique, which combines the separating power of Gas Chromatography (GC), with the detection power of mass spectrometry. The Gas Chromatography/Mass Spectrometry (GC/MS) instrument separates chemical mixtures (GC component) and identifies the components at a molecular level (MS component). For identification of active compounds showing

antibacterial potentials, the samples were subjected to GC-MS analysis. GC-MS analysis was carried out at Advanced Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi, India. Gas-Chromatography Mass Spectrometry was performed using Shimadzu GCMS-QP-2010 plus system. The column used for experiment purpose was RTx-5 MS column- (30 m X 0.25 mm id X 0.25 film thickness). The instrument was set to an initial temperature of 80 ºC and maintained at this temperature for 3 minutes. Next, the temperature was maintained at 250 ºC at the rate of an increase of 10 ºC/min. The temperature of the oven was elevated to 280 ºC at the rate of an increase of 15 ºC/min and maintained for 23 minutes. Injection port temperature was maintained as 250 ºC and column flow rate as 1.21 mL/min. The samples (2 µL) were injected in split mode at 10:0.

Identification of components

Identification as well as the proportionate percentage of each component was done by comparing its average peak area to the total area. The identification of compounds was performed by comparing their mass spectra with data from NIST08 (National Institute of Standards and Technology, US and WILEY 8) libraries. The spectrum of unknown compound was compared with the spectrum of known compounds stored in data libraries and their molecular formula, molecular weight and the number of hits was used to identify the name of components from data library[29].

Statistical analysis

Each sample was evaluated individually in triplicates (n=3) and the results are expressed as mean value±standard deviation of the mean. Two way ANNOVA was applied to test the difference in means of different plant parts (flowers and leaves) and antibiotic (cefixime) (SPSS statistics version 26 software).

Results

Different parts of *S. hispanica* L. flower, leaf and seeds (Fig. 1) were chosen for the extraction purpose which was carried out by using Soxhlet extraction unit in two solvents viz. methanol as polar and ethyl acetate as non-polar to screen the antimicrobial activity. These extracts were tested against two Gram-positive and two Gram-negative bacteria.

% Yield of *S. hispanica* L. plant extracts by Soxhlet extraction method

Methanolic and ethyl acetate crude extract obtained was evaporated to get dry yield. The highest percentage yield was observed in methanolic leaf extract, i.e., 11.25% and the lowest in ethyl acetate flower extract, i.e., 3.18%. The final results in the form of percentage of all plant extracts on the basis of their dry weights are given in Table 1.

Antibacterial assay

Solvent extracts prepared by Hot continuous extraction method (Soxhlet extraction method) were used for the biological assay. In the present study, a total of 6 plant extracts were evaluated. All these plant extracts were analyzed against 4 bacterial strains which included MTCC bacterial strains. Mother

Table 1 — Percent yield of methanolic and ethyl acetate plant extracts of *S. hispanica* L.

S. No.	Solvents	Dry weight of the extracts (g)		% Yield
		Plant part	Dry weight	% Yield = Dry weight of the plant extract/ mother solvent (dissolved in 1: 10 ratio) x 100
1	Methanol	Flower	0.470	4.70
		Leaf	1.125	11.25
		Seed	0.980	9.80
2	Ethyl acetate	Flower	0.318	3.18
		Leaf	0.700	7.00
		Seed	0.653	6.53

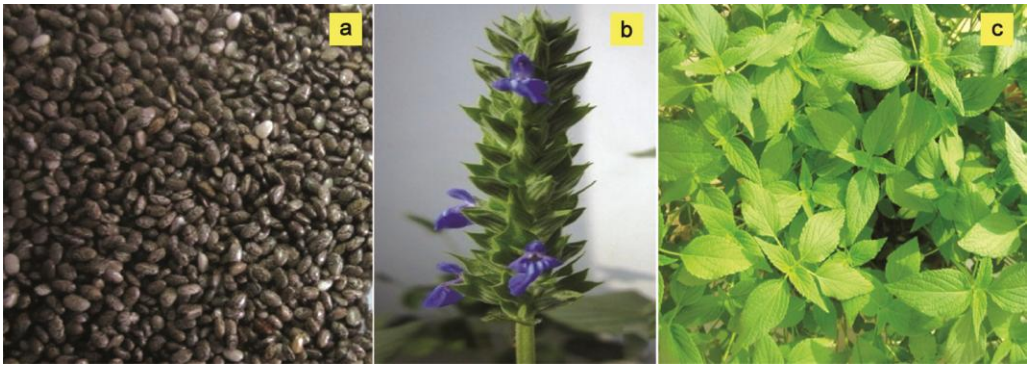

Fig. 1 — Morphological features of *S. hispanica* L. (a, b, and c depicting the seed, flower, and leaves respectively).

solvents in which respective samples were prepared used as negative control and the antibiotic (Cefixime) was used as positive control against all selected bacterial strains. Various levels of dilution of plant extracts were taken i.e., 25 and 50 µg/100 µL in which the dilution at 25 µg/100 µL did not display any antibacterial activity.

In general, the results revealed that some plant extracts showed significant antibacterial activity in terms of zone of inhibition and some were found to be ineffective against the same bacterial strains. The results of the antimicrobial screening are shown in Table 2. Antimicrobial activity of plant extracts was also compared with the positive control i.e., antibiotic (Cefixime). Ethyl acetate flower extract (Fig. 2) of *S. hispanica* L. showed maximum activity against *E. coil* with 11.20 mm value as compared to its leaf extract while control, cefixime antibiotic displayed a value of 15.06 mm. Maximum activity of flower extract was obtained against *P. fluorescens* (14.60 mm) which was nearly comparable to positive control (17.30 mm) (Table 3) and the lowest mean value against this bacteria was displayed by leaf extract (7.20 mm). The highest mean value against *B. coagulans* was found in the flower extract with a 9.36 mm value and the lowest activity was exhibited by leaf extract (Fig. 2) with a mean value of 7.06 mm against cefixime (15.16 mm). Flower extract also

Table 2 — Antibacterial activity in ethyl acetate flower, leaf, and seed extracts of *S. hispanica* L. by Disc diffusion method

Samples	Sample concentration (µg/100 µL)	Bacterial strains			
		Escherichia coli	*Pseudomonas fluorescens*	*Bacillus coagulans*	*Bacillus megaterium*
Cefixime (+C)	25	7.2 ± 0.13^{a}	9.76 ± 0.7^{b}	6.81 ± 0.14^{a}	10.02 ± 0.3^{bc}
	50	15.0 ± 0.05^{cb}	17.3 ± 0.23^{d}	15.1 ± 0.5^{c}	19.3 ± 0.25^{ed}
Ethyl acetate	25	-	-	-	-
(-C)	50	-	-	-	-
Flower	25	-	-	-	-
	50	11.2 ± 0.26^{bd}	14.6 ± 0.36^{ca}	9.3 ± 0.32^{bc}	8.3 ± 0.26^{b}
Leaf	25	-	-	-	-
	50	7.3 ± 0.26^{ab}	7.2 ± 0.26^{ab}	7.0 ± 0.11^{ac}	6.8 ± 0.32^{a}
Seed	25	-	-	-	-
	50	-	-	-	-

+C=Positive control
-C= Negative control
- = No zone of inhibition
The value represents mean±SD of ZOI, significant at P =0.05 level (Two way ANOVA); Value followed by the same letter in a column are not significantly different; The experiment was performed in triplicates.

Table 3 — Comparative antibacterial study between plant extracts, antibiotic and bacterial strains

Bacteria		N	Mean	Std. deviation	Std. Error	95% Confidence interval for Mean		Minimum	Maximum
						Lower bound	Upper bound		
E. Coli	Leaf	3	7.300	.264	.152	6.642	7.957	7.00	7.50
	Flower	3	11.200	.264	.152	10.542	11.857	11.00	11.50
	Cefixime	6	15.066	.051	.021	15.012	15.120	15.00	15.10
	Total	12	12.158	3.365	.971	10.019	14.296	7.00	15.10
Pseudomonas fluorescens	Leaf	3	7.200	.264	.152	6.542	7.857	7.00	7.50
	Flower	3	14.600	.360	.208	13.704	15.495	14.30	15.00
	Cefixime	6	17.300	.236	.096	17.051	17.548	17.00	17.50
	Total	12	14.100	4.324	1.248	11.352	16.847	7.00	17.50
Bacillus coagulans	Leaf	3	7.066	.115	.066	6.779	7.353	7.00	7.20
	Flower	3	9.366	.321	.185	8.568	10.165	9.00	9.60
	Cefixime	6	15.166	.258	.105	14.895	15.437	15.00	15.50
	Total	12	11.691	3.734	1.078	9.318	14.064	7.00	15.50
Bacillus megaterium	Leaf	3	6.866	.321	.185	6.068	7.665	6.50	7.10
	Flower	3	8.300	.264	.152	7.642	8.957	8.00	8.50
	Cefixime	6	19.333	.258	.105	19.062	19.604	19.00	19.50
	Total	12	13.458	6.164	1.779	9.541	17.374	6.50	19.50

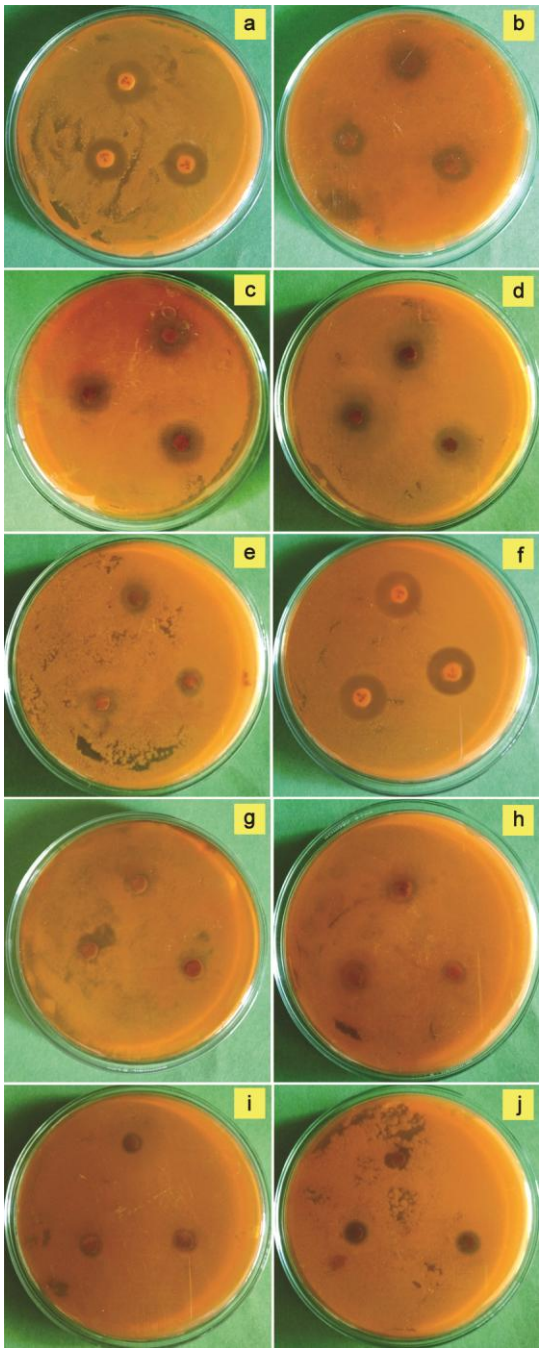

Fig. 2 — Antibacterial activity of ethyl acetate flower and leaf extract against various MTCC bacterial strains (a, f) Cefixime (Positive control), (b, g) *E. coli*, (c, h) *P. fluorescens*, (d, i) *B. coagulans*, (e, j) *B. megaterium*.

showed the highest antibacterial activity against *B. megaterium* bacteria with a mean value of 8.30 mm and the lowest found in leaf extract with the mean value of 6.86 mm (Fig. 3). Among all the tested samples, flower extract showed the highest activity against all bacterial strains but specifically against

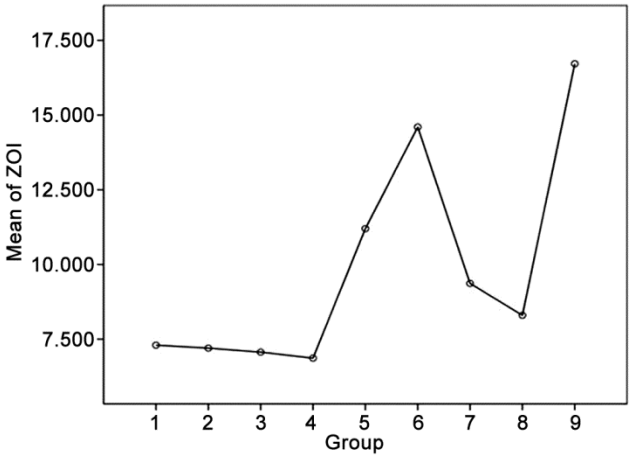

Fig. 3 — Comparative mean of ZOI of various tested samples. 1) Leaf + *E.coli* 2) Leaf + *P. fluorescens* 3) Leaf + *B. coagulans* 4) Leaf + *B. megaterium* 5) Flower + *E.coli* 6) Flower + *P. fluorescens* 7) Flower + *B. coagulans* 8) Flower + *B. megaterium* 9) Cefixime (antibiotic).

gram-negative bacteria and it was found to be equally active in positive control. In both parts, the flower was exhibiting more activity than the leaves. The ethyl acetate seed extract and all methanolic extracts were rejected for further study because they did not exhibit any zone of inhibition.

GC-MS

In the present study, GC-MS analysis revealed the presence of various phytochemical constituents in two plant samples which accounts for the pharmacological activity of the plant. The identification of the phytoconstituents was confirmed based on peak, peak area in percentage, retention time, and molecular formula.

GC-MS analysis of ethyl acetate extract of *S. hispanica* L. flower exhibited the 49 peaks in which some components were repeatedly isolated (Fig. 4). There were 44 components identified individually and many of the components showed antibacterial, antifungal, antioxidant, anti-inflammatory, vasodilator, anti-tumor, antidiarrheal, antidepressant, antiseptic, anti-allergen, and used as flavoring and fragrance agents. Based on their maximum percentage of peak area and retention time the name of few important components are described. At 44.703 retention time and 25.16% of peak area, the component was identified as Pentatriacontane at 42.322 (14.25%) as Dotriacontane, at 39.968 (7.67%) as 3,8-Dimethylundecane, at 47.820 (4.62%) as 2-methyloctacosane and 35.958 (4.32%) as 3,3-Dimethylheptane. The detail of components with their molecular weight, molecular formula, the peak area of

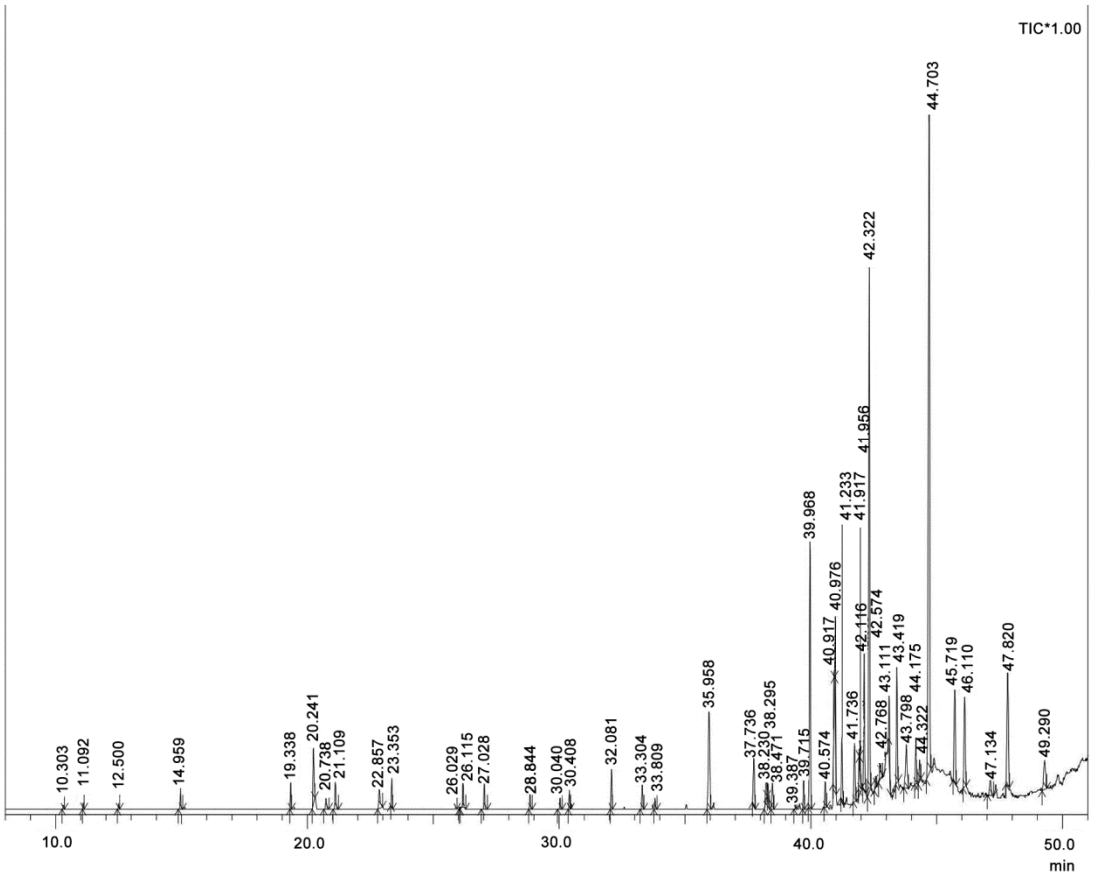

Fig. 4 — GC-MS chromatogram of ethyl acetate flower extract of *S. hispanica* L.

molecular weight, molecular formula, the peak area of percentage, retention time, and structure are given in Table 4.

GC-MS analysis of ethyl acetate extract of *S. hispanica* L. leaves revealed 34 peaks and showed the presence of 27 components with various biological activities (Fig. 5). The most prevailing compounds based on retention time and maximum percentage of peak area were Octacosane at 44.721 (28.33%) , as Pentatriacontane at 42.340 (14.28%), as Alpha-Tocopherol (Vitamin E) at 42.604 (7.39%), as Citronellyl acetate at 20.247 (7.18%), as Heptadecane at 39.966 (4.75) and as 2,6,11-Tri methyldecane at 43.425 (4.49%). The detail of components with their molecular weight, molecular formula, peak area in percentage, retention time (RT), and structure are given in Table 5.

Discussion

With increasing public health awareness all over the world, demand for functional food with a plethora of nutritional benefits has also increased. The use of plant-based medicines to prevent diseases has also gained momentum. Herbal medicines serve as the representatives of a traditional medicinal system that has emerged as an important field in modern-day pharmaceutical industries. A large number of antibacterial components have already been reported and isolated from plants[30], there is a need to evaluate more and more plants to promote their use as herbal medicines and discover phytoconstituents present in them for developing a novel drug moiety[31]. Extraction of various plant parts viz. leaves, flowers, and seeds of *S. hispanica* L. was done by the Soxhlet apparatus. Two solvents, methanol and ethyl acetate were used for extraction purposes. The maximum amount of dry yield (11.25%) was obtained in methanolic leaf extract which showed the presence of those phytochemicals which extract out in polar solvents and a minimum 3.18% dry yield was obtained in flower ethyl acetate extract, which clearly shows the affinity of phytoconstituents in the plant samples for non-polar solvents. However, maximum dry yield (33.55%) was obtained from n-hexane seed extract of *S. hispanica* L. while the dry yield of isopropanol seed extract was 23.98%[32]. The presence of

phytochemicals in various plant extracts of

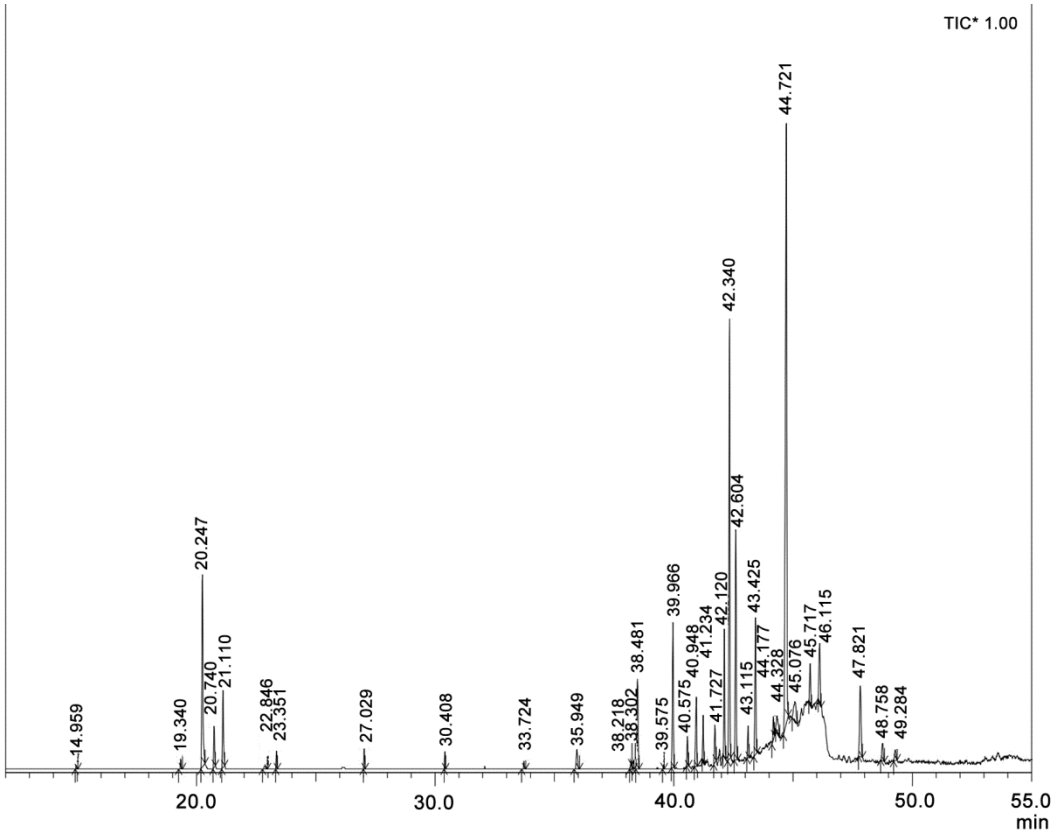

Fig. 5 — GC-MS chromatogram of ethyl acetate leaf extract of *S. hispanica* L.

Table 4 — The GC-MS profile of compounds identified in Ethyl acetate flower extract of *S. hispanica* L.

Peak No.	R time	Area	Area %	Name	Molecular formula	Molecular weight
1	10.303	5083	0.09	2-Nitropropane	$C_3H_7NO_2$	89
2	11.092	6200	0.12	Butanenitrile	C_4H_7N	69
3	12.500	5375	0.10	Acetonitrile	C_2H_3N	41
4	14.959	21094	0.39	1-Decanol	$C_{10}H_{22}O$	158
5	19.338	28796	0.53	1-Dodecanol	$C_{12}H_{26}O$	186
6	20.241	94682	1.76	Octadecanal	$C_{18}H_{36}O$	268
7	20.738	16208	0.30	3-Methylbutanenitrile	C_5H_9N	83
8	21.109	41163	0.76	2-Undecen-1-ol	$C_{11}H_{22}O$	170
9	22.857	42554	0.79	Tridecanoic acid	$C_{13}H_{26}O_2$	214
10	23.353	33975	0.63	1-Tetradecanol	$C_{14}H_{30}O$	214
11	26.029	1008	0.02	Dihydrocitronellol	$C_{10}H_{22}O$	158
12	26.115	5713	0.11	Pyrrole	C_4H_5N	67
13	27.028	32453	0.60	Propionic acid, 3,3'-thiodi-, didodecyl ester	$C_{30}H_{58}O_4S$	514
14	28.844	19075	0.35	2-Methylbutyl nitrite	$C_5H_{11}NO_2$	117
15	30.040	12802	0.24	1-Bromo-2-Methylbutane	$C_5H_{11}Br$	150
16	30.408	21039	0.39	Undecyl ester of Dodecanoic acid	$C_{23}H_{46}O_2$	354
17	32.081	57684	1.07	Pentadecane	$C_{15}H_{32}$	212
18	33.304	33264	0.62	Pentadecane	$C_{15}H_{32}$	212
19	33.809	14239	0.26	2,6-Dimethylheptan-4-one	$C_9H_{18}O$	142
20	35.958	232453	4.32	3,3-Dimethyhleptane	C_9H_{20}	128
21	37.736	91519	1.70	2-Propyldecan-1-ol	$C_{13}H_{28}O$	200

Table 4 — The GC-MS profile of compounds identified in Ethyl acetate flower extract of *S. hispanica* L. (*Contd*.)

Peak No.	R time	Area	Area %	Name	Molecular formula	Molecular weight
22	38.230	44620	0.83	1,5-Dimethyltricyclo [3.3.0.0(2,6)]octane	$C_{10}H_{16}$	136
23	38.295	24252	0.45	Docosane	$C_{22}H_{46}$	310
24	38.471	50759	0.94	Bromocyclopentane	C_5H_9Br	148
25	39.387	4046	0.08	2,3-Pentanedione	$C_5H_8O_2$	100
26	39.715	40933	0.76	Propanoic Acid, 3,3'-Thiobis-, Didodecyl Ester	$C_{30}H_{58}O_4S$	514
27	39.968	413259	7.67	3,8-Dimethylundecane	$C_{13}H_{28}$	184
28	40.574	36678	0.68	Octadecane	$C_{18}H_{38}$	254
29	40.917	64621	1.20	3-Methyldecane	$C_{11}H_{24}$	156
30	40.976	127125	2.36	Patchoulane	$C_{15}H_{26}$	206
31	41.233	85111	1.58	Octadecane	$C_{18}H_{38}$	254
32	41.736	109076	2.03	2,2-Dimethyl-3-(3-methyl-1-Pentynyl)Cyclopropyl Isopropyl Ether	$C_{14}H_{24}O$	208
33	41.917	17337	0.32	2-Propyldecan-1-ol	$C_{13}H_{28}O$	200
34	41.956	23709	0.44	4, 4-Dimethyltetra cyclo[5.2.1.0 2,6 .0 3,5]decane	$C_{12}H_{18}$	162
35	42.116	194123	3.60	Cholesterol methyl ether	$C_{28}H_{48}O$	400
36	42.322	767678	14.25	Pentatriacontane	$C_{35}H_{72}$	492
37	42.574	14495	0.27	2,2,5,7-Tetramethyl-4,6-Octadiene-3-one	$C_{12}H_{20}O$	180
38	42.768	31051	0.58	α-4-Dimethyl-3 cyclo hexene-1-acetaldehyde	$C_{10}H_{16}O$	152
39	43.111	69619	1.29	2,6,10,15-Tetramethyl heptadecane	$C_{21}H_{44}$	296
40	43.419	165284	3.07	Tetracosane	$C_{24}H_{50}$	338
41	43.798	107368	1.99	Artemiseole	$C_{10}H_{16}O$	152
42	44.175	70496	1.31	2, 4, 4- tri methyl hexane	C_9H_{20}	128
43	44.322	34063	0.63	Docosane	$C_{22}H_{46}$	310
44	44.703	1355432	25.16	Pentatriacontane	$C_{35}H_{72}$	492
45	45.719	184007	3.42	Spiro[Cyclopentane-1,2'(1'H)-Quinoxaline], 3'-(4-Morpholinyl)-6',8'-Dinitro-	$C_{16}H_{19}N_5O_5$	361
46	46.110	173036	3.21	Sulfurous acid, 2-ethylhexyl isohexyl ester	$C_{14}H_{30}O_3S$	278
47	47.134	31173	0.58	Docosane	$C_{22}H_{46}$	310
48	47.820	248626	4.62	2-methyloctacosane	$C_{29}H_{60}$	408
49	49.290	81930	1.52	Dihexylsulfide	$C_{12}H_{26}S$	202
		5386286	100			

Table 5 — The GC-MS profile of compounds identified in Ethyl acetate leaf extract of *S. hispanica* L.

Peak No.	RT	Area	Area %	Name	Molecular formula	Molecular weight
1	14.959	6951	0.10	2-Nitropropane	$C_3H_7NO_2$	89
2	19.340	16426	0.23	2-Nonenal, 2-Pentyl-	$C_{14}H_{26}O$	210
3	20.247	510745	7.18	Citronellyl acetate	$C_{12}H_{22}O_2$	198
4	20.740	95354	1.34	Citronellyl acetate	$C_{12}H_{22}O_2$	198
5	21.110	184118	2.59	Citronellyl acetate	$C_{12}H_{22}O_2$	198
6	22.846	10674	0.15	2-Nitropropane	$C_3H_7NO_2$	89
7	23.351	32880	0.46	1-Tetradecanol	$C_{14}H_{30}O$	214
8	27.029	37422	0.53	Propanoic acid, 3,3'-Thiobis-, Didodecyl Ester	$C_{30}H_{58}O_4S$	514
9	30.408	28394	0.40	Propanoic acid, 3,3'-Thiobis-, Didodecyl Ester	$C_{30}H_{58}O_4S$	514
10	33.724	13570	0.19	2-Nonenal, 2-Pentyl-	$C_{14}H_{26}O$	210
11	35.949	63990	0.90	Pentadecane	$C_{15}H_{32}$	212
12	38.218	16344	0.23	1-(2-Methylenecyclohexyl)-1-phenylethanol	$C_{15}H_{20}O$	216
13	38.302	18750	0.26	*Dioctyl disulfide*	$C_{16}H_{34}S_2$	290
14	38.481	249094	3.50	2,6-Dimethyl-1,5-Heptadiene	C_9H_{16}	124
15	39.575	6543	0.09	Allyl 2-Methylacrylate	$C_7H_{10}O_2$	126
16	39.966	337791	4.75	Heptadecane	$C_{17}H_{36}$	240

(*Contd*.)

Table 5 — The GC-MS profile of compounds identified in Ethyl acetate leaf extract of *S. hispanica* L. (*Contd*.)

Peak No.	RT	Area	Area %	Name	Molecular formula	Molecular weight
17	40.575	57731	0.81	Caryophyllene Diepoxide	$C_{15}H_{24}O_2$	236
18	40.948	179438	2.52	1,1'-Bicyclopropyl, 2,2,2',2'-tetramethyl-	$C_{10}H_{18}$	138
19	41.234	87781	1.23	Octadecane	$C_{18}H_{38}$	254
20	41.727	88793	1.25	Caryophyllene Diepoxide	$C_{15}H_{24}O_2$	236
21	42.120	258889	3.64	Stigmastan-3,5-diene	$C_{29}H_{48}$	396
22	42.340	1015880	14.28	Pentatriacontane	$C_{35}H_{72}$	492
23	42.604	525631	7.39	Alpha-Tocopherol (Vitamin E)	$C_{29}H_{50}O_2$	430
24	43.115	66767	0.94	Dihexylsulfide	$C_{12}H_{26}S$	202
25	43.425	319305	4.49	2,6,11-Trimethyldodecane	$C_{15}H_{32}$	212
26	44.177	48719	0.68	2,3,4-Trimethyldecane	$C_{13}H_{28}$	184
27	44.328	74123	1.04	2-Methyl-1-octanol	$C_9H_{20}O$	144
28	44.721	2015193	28.33	Octacosane	$C_{28}H_{58}$	394
29	45.076	113359	1.59	Vinyl myristate	$C_{16}H_{30}O_2$	254
30	45.717	99683	1.40	3,8-Dimethylundecane	$C_{13}H_{28}$	184
31	46.115	177081	2.49	3,8-Dimethylundecane	$C_{13}H_{28}$	184
32	47.821	260110	3.66	Sulfurous acid, 2-ethylhexyl isohexyl ester	$C_{14}H_{30}O_3S$	278
33	48.758	57132	0.80	14-Heptadecenal	$C_{17}H_{32}O$	252
34	49.284	38299	0.54	Oxalic acid, dineopentyl ester	$C_{12}H_{22}O_4$	230
		7112960	100			

S. hispanica L. showed antibacterial activity against pathogenic microorganisms. Two dilutions of plant extracts were tested i.e., 25 and 50 µg/100µL but dilution 25 µg/100µL did not show any antibacterial activity. Therefore, 50 µg/100µL dilutions of plant extract were taken for the present study, and the positive control was taken to compare the antibacterial activity of various plant parts. In our experimental work, the methanolic extract of flower, seed, and leaves did not show any activity against selected bacterial strains and was not included in the analysis. Ethyl acetate flower extract exhibited the best activity against *P. fluorescens* (14.06 mm) as compared to the mean value of positive control (17.30 mm). The mean values against *E. coli*, *B. coagulans* and *B. megaterium* are 11.20, 9.36, and 8.30 mm, respectively. Ethyl acetate leaf extract exhibited activity against *E. coli*, *P. fluorescens*, *B. coagulans,* and *B. megaterium* with the mean values of 7.30, 7.20, 7.06, and 6.86 mm, respectively. Ethyl acetate extract of seeds did not show any activity against the selected bacterial strains. The activity of ethyl acetate extract is due to the presence of various secondary metabolites which include phenolic compounds, flavonoids, and tannins. In contrast, ethanolic and aqueous seed extracts of *S. hispanica* L. indicated antimicrobial activity against microbes causing periodontal diseases like *Porphyromonas gingivalis, Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans*. Aqueous extracts were found highly effective against *A. actinomycetamcmitans* with 18 mm zone of inhibition[33]. Minimum inhibitory concentration for aqueous extract of chia seeds against *A. actinomycetamcomitans* and *P. gingivalis* was established at 50% and for *F. nucleatum* at 12.5%. The minimum inhibitory concentration of the ethanolic extract for *A. actinomycetamcomitans*, *P. gingivali,* and *F. nucleatum* was 12.5, 6.25, and 50% respectively. Antibacterial activity present in methanolic extracts of *Salvadora persica* L. (leaves)[34], *Oxalis corniculata*, *Artemisia vulgaris*, *Cinnamomum tamala*, and *Ageratina adenophora*[35].

GC-MS analysis of ethyl acetate flower extract of *S. hispanica* L. revealed the presence of 44 compounds. The identified components possess many biological properties like antibacterial, antifungal, antioxidant, anti-inflammatory, vasodilator, anti-tumour, antidiarrheal, antidepressant, antiseptic, anti-allergen, and used as flavouring and fragrance agents. Pharmacologically active components were identified based on the percentage of peak area. The first component identified was Pentatriacontane, which is a linear hydrocarbon that has antimicrobial activity[36-37]. Pentadecane was another identified component that has a linear alkane hydrocarbon and possesses anti-inflammatory and antimicrobial activity[38]. 2,6,10,15-Tetramethyl heptadecane component has antibacterial, antiviral, antifungal properties[39] and also acts as a sex hormone in algae (*Kappaphycus alvarezii*, *Caulerpa lentillifera,* and *Sargassum Polycystem*)[40].

Major compounds 2-Undecen-1-ol, 1-Tetradecanol, Dihydrocitronellol, Propionic acid, 3,3'-thiodi-, didodecyl ester, Pentadecane, 2,3-Pentanedione, 3-Methyldecane, 2,2,5,7-Tetramethyl-4, 6-Octadiene-3-one and α-4-Dimethyl-3 cyclo hexene-1-acetaldehyde were used as a flavouring and fragrance agent in cosmetic and food industries[41-42].

Ethyl acetate leaf extract of *S. hispanica* L. showed the presence of 27 components with many biological activities including antimicrobial, anaesthetic, anticarcinogenic, antifeedant, antitumor, insecticide, anthelmintic, cytotoxic, and anti-ageing which may further be used in drug formulation. Octacosane (acyclic hydrocarbon) was identified as the major component that has efficacy as an antibacterial agent, antioxidant and cytotoxic activities[43]. The most important component of ethyl acetate leaf extract is α-Tocopherol (Vitamin E) having antioxidant[44], anti-ageing and anti-cancer activities and is also used to overcome vitamin E deficiency, improving metabolism, prevent cardiovascular disease, reduce and delay the growth of cataract[45]. Several compounds having antimicrobial activities were found in ethyl acetate leaf extract - Sulfurous acid, 2-ethylhexyl isohexyl ester, 14-Heptadecenal, 2,6,11-Trimethyldodecane, 1,1'-Bicyclopropyl, 2,2,2', 2'- tetramethyl-, Vinyl myristate and Octadecane[46-47]. Caryophyllene Diepoxide was also identified, which is a sesquiterpene hydrocarbon having anti-inflammatory[48], anti-microbial[49], central and peripheral analgesic[50]. Stigmastan-3, 5- diene is a sterol compound that was present in a high amount in the extract of leaves. It was used in the formation of vegetable oils from beta-sitosterol during the refining process and also has antioxidant and antimicrobial activities[51].

Conclusion

The worldwide increase in awareness about the negative impact created by the use of synthetic chemical treatments for microorganisms necessitates the need for further search for other natural alternatives such as plant extracts. The abundance of plant origin bioactive metabolites and their value in medicine is undisputed. This has generated tremendous interest and optimism among scientists and created unprecedented opportunities in the field of biotechnology and rapidly expanding natural product industries. One of the successful strategies for the investigation of medicinal agents from this plant includes pharmacological screening followed by a bioassay-guided fraction leading to the isolation of pure constituents. Thus, in the present investigation mainly the leaves and flowers of *Salvia hispanica* L. were found to be a potent source of antimicrobial agents. Based on our observations we assume that flowers had almost similar potency as that of antibiotics and thus can be replaced and used as a herbal drug to treat various disorders. There is still a lot of scope for further research, especially towards deciphering the mechanism of biological activity of phytochemicals from this plant.

Conflict of interest

There is no conflict of interest regarding the publication of this paper.

References

1 Samy R P and Gopalakrishnakone P, Current status of herbal and their future perspectives, *Nat Preced*, 2007, **10101**(1176.1), 1-13.

2 Rodrigues T, Reker D, Schneider P and Schneider G, Counting on natural products for drug design, *Nat Chem*, 2016, **8**, 531-541.

3 Sen S and Chakraborty R, Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future, *J Tradit Complement Med*, 2017, **7**, 234-244.

4 Gupta C, Garg A P, Uniyal R C and Kumari A, Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on some food-borne microbes, *Afr J Microbiol Res*, 2008, **2**(9), 247-251.

5 Boy H I A, Rutilla A J H, Santos K A, Ty A M T, Yu A I, *et al*., Recommended medicinal plants as source of natural products: A review, *Digit Chin Med*, 2018, **1**(2), 131-142.

6 Amghalia E, Nagi A A, Shamsudin M N, Radu S, Rosli R, *et al*., Multiplex PCR assays for the detection of clinically relevant antibiotic resistance genes in *Staphylococcus aureus* isolated from Malaysian hospitals, *Res J Biol Sci*, 2009, **4**(4), 444-448.

7 Mawalagedera S M, Symonds M R, Callahan D L, Gaskett A C and Rønsted N, Combining evolutionary inference and metabolomics to identify plants with medicinal potential, *Front Ecol Evol*, 2019, **7**, 267.

8 Elshafie H S, Aliberti L, Amato M, Feo V D and Camele I, Chemical composition and antimicrobial activity of chia (*Salvia hispanica* L.) essential oil, *Eur Food Res Technol*, **244**, 2018, 1675-1682.

9 Cahill J P, Ethnobotany of Chia, *Salvia hispanica* L. (Lamiaceae), *Econ Bot*, 2003, **57**, 604-618.

10 Ayerza R, Oil content and fatty acid composition of chia (*Salvia hispanica L*.) from five northwestern locations in Argentina, *J Am Oil Chem Soc*, 1995, **72**, 1079-1081.

11 Hrncic M K, Ivanoski M, Cor D and Knez K, Chia Seeds (*Salvia Hispanica* L.): An overview-phytochemical profile, isolation methods, and application, *Molecules*, 2020, **25**(1), 1-19.

12 Sultana C, Oleaginous flax, *Oils and fats manual*, edited by Karleskind A & Wolff J P, (Paris: Lavoisier), 1996, 157-160.

13 Rahman M J, de Camargo A C and Shahidi F, Phenolic and polyphenolic profiles of chia seeds and their *in vitro* biological activities, *J Funct Foods*, 2017, **35**, 622-634.

14 Alcantara M A, Polari I de L B, Meireles B R L de A, de Lima A E A, da Silva Junior J C, *et al*., Effect of the solvent composition on the profile of phenolic compounds extracted from chia seeds, *Food Chem*, 2019, **275**, 489-496.

15 Kulczynski B, Kobus-Cisowska J, Taczanowski M, Kmiecik D and Gramza-Michałowska A, The chemical composition and nutritional value of chia seeds-current state of knowledge, *Nutrients*, 2019, **11**(6), 1242.

16 Ayerza R and Coates W, Omega-3 enriched eggs: The influence of dietary alinolenic fatty acid source on egg production and composition, *Can J Anim Sci*, 2001, **81**, 355-362.

17 Grancieri M, Martino H S D and de Mejia G E, Chia Seed (*Salvia hispanica* L.) as a source of proteins and bioactive peptides with health benefits: A review, *Compr Rev Food Sci Food Saf*, 2019, **18**(2), 480-499.

18 Ciftci O N, Przybylski R and Rudzinska M, Lipid components of flax, perilla, and chia seeds, *Eur J Lipid Sci Technol*, 2012, **114**(7), 794-800.

19 Poudyal H, Panchal S K, Waanders J, Ward L and Brown L, Lipid redistribution by *α*-linolenic acid-rich chia seed inhibits stearoyl-CoA desaturase-1 and induces cardiac and hepatic protection in diet-induced obese rats, *J Nutr Biochem*, 2012, **23**(2), 153-162.

20 Kumar D G, Perumal P C, Kumar K, Muthusami S and Gopalakrishnan V K, Dietary evaluation, antioxidant and cytotoxic activity of crude extract from Chia seeds (*Salvia hispanica* L.) against human prostate cancer cell line (PC-3), *Int J Pharmacogn Phytochem Res*, 2016, **8**(8), 1358-1362.

21 Capitani M I, Spotorno V, Nolasco S M and Tomas M C, Physicochemical and functional characterization of byproducts from chia (*Salvia hispanica* L.) seeds of Argentina, *LWT-Food Sci Technol*, 2012, **45**(1), 94-102.

22 Vuksan V, Jenkins A L, Brissette C, Choleva L, Jovanovski E, *et al*., Salba-chia (*Salvia hispanica* L.) in the treatment of overweight and obese patients with type 2 diabetes: A double-blind randomized controlled trial, *Nutr Metab Cardiovasc Dis*, 2017, **27**(2), 138-146.

23 Alonso-Calderon A, Chavez-Bravo E, Antonio R, Montalvo-Paquini C, Arroyo-Tapia R, *et al*., Characterization of black chia seed (*Salvia hispanica L*) and oil and quantification of β sitosterol, *Int Res J Biol Sci*, 2013, **2**(1), 70-72.

24 Kobus-Cisowska J, Szymanowska D, Maciejewska P, Kmiecik D, Gramza-Michałowska A, *et al*., *In vitro* screening for acetylcholinesterase and butyrylcholinesterase inhibition and antimicrobial activity of chia seeds (*Salvia hispanica*), *Electron J Biotechnol*, 2019, **37**, 1-10.

25 Sukhdev S H, Suman P S K, Gennaro L and Dev D R, Extraction technologies for medicinal and aromatic plants, *Int Centre Sci High Technol*, 2008, **57**, 21-25.

26 Monica C, *Medical laboratory manual for tropical countries: Microbiology*, 1st edn, English Language Book Society, (London, Trop. Health Technol, Buttrworth), 1985.

27 McFarland J, Nephelometer: An instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines, *J Am Med Assoc*, 1907, **49**(14), 1176-1178.

28 Bauer A W, Kirby W M, Sherris J C and Turck M, Antibiotic susceptibility testing by a standardized single disk method, *Am J Clin Pathol*, 1966, **45**, 493-496.

29 Silverstein R M, Bassler G C and Morrill T C, *Spectrometric interpretation of organic compounds*, 4th edn, (Wiley: New York, NY), 1981.

30 Srinivasan D, Nathan S, Suresh T and Perumalsamy O, Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine, *J Ethnopharmacol*, 2001, **74**, 217-220.

31 Parekh J and Chanda S, Antibacterial and phytochemical studies on twelve species of Indian medicinal plants, *Afr J Biomed Res*, 2007, **10**, 175-181.

32 Silva C, Garcia V A S and Zanette C M, Chia (*Salvia hispanica* L.) oil extraction using different organic solvents: Oil yield, fatty acids profile and technological analysis of defatted meal, *Int Food Res J*, 2016, **23**(3), 998-1004.

33 Divyapriya G K, Veeresh D J and Yavagal P C, Evaluation of antibacterial efficacy of chia (*Salvia hispanica*) seeds extract against *Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans*-an *in-vitro* study, *Int J Ayu Pharm Res*, 2016, **4**(4), 22-26.

34 Mudzengia C P, Murwira A, Tivapasi M, Murungweni C, Burumu J V, *et al.*, Antibacterial activity of aqueous and methanol extracts of selected species used in livestock health management, *Pharm biol*, 2017, **55**(1), 1054-1060.

35 Manandhar S, Luitel S and Dahal RK, *In Vitro* Antimicrobial activity of some medicinal plants against human pathogenic bacteria, *J Trop Med*, 2019, **2019**, 1-5.

36 Tiwari P, Mishra B N and Sangwan S N, Phytochemical and pharmacological properties of *Gymnema sylvestre*: An important medicinal plant, *BioMed Res Int*, 2014, **21**, 1-18.

37 Paritala V, Chiruvella K K, Thammineni C, Ghanta R G and Mohammed A, Phytochemicals and antimicrobial potentials of mahogany family, *Rev Bras Farmacogn*, 2015, **25**, 61-83.

38 Martins C M, Nascimento E A, Morais S A L, Oliveira A, Chang R, *et al.*, Chemical constituents and evaluation of antimicrobial and cytotoxic activities of *Kielmeyera coriacea* Mart & Zucc essential oils, *Evid-Based Complement Altern Med*, 2015, **2015**, 1-9.

39 Manikandan G, Vimana R A, Divya C and Ramasubbu R, GC-MS analysis of phytochemical constituents in the petroleum ether leaf extracts of *Millettia peguensis*, *Int Res J Pharm*, 2017, **8**, 144-150.

40 Izzreen N Q M and Ratnam V R, Volatile compound extraction using solid phase micro extraction coupled with gas chromatography mass spectrometry (SPME-GCMS) in local seaweeds of *Kappaphycus alvarezii*, *Caulerpa lentillifera* and *Sargassum polycystem*, *Int Food Res J*, 2011, **18**(4), 1449-1456.

41 Morgan D L, Jokinen M P, Price H C, Gwinn W M, Palmer S M, *et al.*, Bronchial and bronchiolar fibrosis in rats exposed to 2,3- pentanedione vapors: Implications for Bronchiolitis obliterans in humans, *Toxicol Pathol*, 2012, **40**(3), 448-465.

42 Sasikumar K and Ghosh A R, Isolation of myristyl alcohol from *Hybanthus enneaspermus*, *Bangladesh J Pharmacol*, 2017, **12**(2), 113-114.

43 Luo H, Cai Y, Peng Z, Liu T and Yang S, Chemical composition and *in vitro* evaluation of the cytotoxic and

antioxidant activities of supercritical carbon dioxide extracts of pitaya (dragon fruit) peel, *Chem Cent J*, 2014, **8**(1), 1-7.

44 Roberts L J, Oates J A, Linton M F, Fazio S, Meador B P, *et al.*, The relationship between dose of vitamin E and suppression of oxidative stress in humans, *Free Radic Biol Med*, 2007, **43**(10), 1388-1393.

45 Mathew M C, Ervin A M, Tao J and Davis R M, Antioxidant vitamin supplementation for preventing and slowing the progression of age-related cataract, *Cochrane Database Syst Rev*, 2015, **6**, 1-62.

46 Rasekhi F, Tajick M A, Rahimian H and Sharifimehr S, Some of phytotoxic and antimicrobial compounds extracted from culture filtrates of *Fusarium proliferatum* FP85, *J Biodivers Environ Sci*, 2014, **4**(5), 245-251.

47 Li J, Xie S, Ahmed S, Wang F, Gu Y, *et al.*, Antimicrobial activity and resistance: Influencing factors, *Front Pharmacol*, 2017, **8**, 364-376.

48 Tung Y T, Chua M T, Wang S Y and Chang S T, Anti-inflammation activities of essential oil and its constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) twigs, *Bioresour Technol*, 2008, **99**(9), 3908-3913.

49 Yang D, Michel L, Chaumont J P and Clerc J M, Use of caryophyllene oxide as an antifungal agent in an *in vitro* experimental model of onychomycosis, *Mycopathologia*, 2000, **148**(2), 79-82.

50 Fidyt K, Fiedorowicz A, Strzadala L and Szumny A, β-caryophyllene and β- caryophyllene oxide-natural compounds of anticancer and analgesic properties, *Cancer Med*, 2016, **5**(10), 3007-3017.

51 Khan K, Firdous S, Ahmad A, Fayyaz N, Nadir M, *et al.*, GC-MS profile of antimicrobial and antioxidant fractions from *Cordia rothii* roots, *Pharm Biol*, 2016, **54**(11), 2597-2605.