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COMBATING MULTI-DRUG RESISTANCE IN *E. COLI* AND *S. AUREUS* WITH METHANOLIC FLOWER EXTRACTS OF *SPILANTHES OLERACAE* AND ESTIMATING ITS PHYTOCHEMICAL CONSTITUTES

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ABSTRACT

Background and Purpose: At present, combating multidrug resistance in bacteria remains a major challenge globally. There is an urgent need to replace currently available antibiotics. However, plant based drugs have been proved to be an effective alternative for the treatment of infectious diseases caused by bacteria. In the present study, we have evaluated the antibacterial activity of methanolic extract of flower of *Spilanthes oleraceae* by using agar well diffusion and serial micro dilution method against drug resistant strains of *Staphylococcus aureus and Escherichia coli*. ***Methods***: The antioxidant activity of the crude extract was determined by using the DPPH method. Further, phytochemical investigation was done by GC-MS analysis. ***Results*:** Remarkable inhibition of the bacterial growth was observed against the tested organisms and minimum inhibitory concentration (MIC) of crude extract of *S. oleraceae* flower was found to be 0.75 and 0.25 mg/ml against *S. aureus and E. coli, respectively*. The antioxidant results indicated that crude methanolic extract

exhibited DPPH radical scavenging activity comparable to ascorbic acid (IC_{50} of ascorbic acid = 0.25 mg/ml; IC_{50} of crude extract = 0.27 mg/ml). Moreover, GC-MS results showed that the major groups present in the extract are aromatic (50%) and aliphatic compounds (30%). The presence of wide range of phytochemicals in the methanolic extract of *S. oleraceae*, might be considered responsible for its antibacterial and antioxidant potential. ***Conclusions*:** Thus, crude extract of this plant can be used to discover bioactive natural compounds that may serve as leads in the development of novel agents of biomedical importance especially against multidrug resistant *E.coli* and *S. aureus*.

KEYWORDS: Multi drug resistance, *Spilanthes oleraceae*, Antibacterial, Antioxidants, Bioactive compounds, GC-MS.

INTRODUCTION

The large number of synthetic drugs produced from pharmaceutical industries from time to time has led to the development of multi drug resistance microorganisms that become major global issue in the treatment of infectious diseases.[1] At present, there is an urgent need to discover new therapeutic compounds with novel mechanisms of action and diverse chemical structures which could work effectively against new and re-emerging infectious diseases.[2] Therefore, scientists all over the world are looking for new compounds from various biological sources like fungi, algae, and higher plants to develop better drugs.[3] Among them, higher plants play an important role by producing large number of secondary metabolites which can act as chemotherapeutic, bactericidal, and bacteriostatic agents.[4] Plant based drugs have been proved to be effective in the treatment of infectious diseases simultaneously with lesser side effects, which are often associated with synthetic drugs.[5]

Medicines derived from plants have been utilized for different purposes, particularly in medical care such as anti-asthmatic, anti-rheumatic, anti-cariogenic, etc. [6, 7] Moreover, medicinal plants bearing bioactive compounds are of due scientific interest which can be employed for therapeutic and health care purposes. Interestingly, different medicinal plants have been extensively studied for their antibacterial, anticancer and antioxidant potential [8, 9] but only few studies has been carried out on wild plants. However, due to lack of scientific proof of effectiveness of wild plants, the validity of these remedies remains questionable and their use have been locally restricted.

Spilanthes species commonly known as "akarkara" or "toothache plant" is a traditional medicinal herb of Asteraceae family, occurring in the tropics and sub-tropics such as, Africa, America, Borneo, India, Sri Lanka.[10] It can be found in damp pastures, at swamp margins, on rocks near the sea and as a weed of roadsides and has been well documented for its anti-inflammatory, antibacterial, antimicrobial and antifungal properties.[10-12] Flowers and leaves of the plant have a pungent taste and have been used as a spice for appetizers and as folk medicine for stammering, toothache, stomatitis, throat complaints and fever.[13] Furthermore, analgesic, antifungal, antihelminthic, antibacterial, immunomodulatory, adaptogenic, lithotriptic, antiscorbitic and digestive properties of this plant have also been reported.[14]

The aim of this study was to determine the antibacterial potential of methanolic extract of flower of *Spilanthes oleraceae* against multidrug resistant *Escherichia coli* and *Staphylococcus aureus*. It may provide a possible alternative for the treatment of bacterial infections by chemically synthetic drugs to which these infectious microorganisms have become resistant. In the recent years the interest for the characterization of the organic compounds from plants has been developed. GC–MS is an ideal technique for qualitative and quantitative analysis of volatile and semi volatile compounds. Therefore, in the present study phytochemical constituents in the methanolic crude extract of flower of *Spilanthes oleraceae* were also characterized by GC-MS analysis.

METHODS

Plant material

Spilanthes oleracea plants were obtained from the fields of Pharmacy Herbal Garden, Integral University, Lucknow. Plant was authenticated by Dr. Tariq Husain, Senior Scientist, National Botanical Research Institute (NBRI), Lucknow, India and voucher specimens are maintained at the NBRI herbarium.

Preparation of Plant Extract

The flowers of *Spilanthes oleracea* were washed thoroughly under running tap water and dried under shade. They were then finely ground to a powder in an electric blender. Dried flower powder (1 gm) was homogenized in 10 ml methanol (HPLC grade, Merck, India) and was extracted on a rotator shaker in an Erlenmeyer flask at 40 rpm overnight.[15] The crude extract was then filtered through Whatman No. 1 filter paper and concentrated in vacuum at 40 °C using a rotary evaporator. The concentrated extract was then dried aseptically with the help of drier.

Antibacterial Assay

Agar well diffusion method

Multidrug resistant *Staphylococcus aureus* (NCIM 2079) and *Escherichia coli* (NCIM 2571) were used as test bacteria in the present study. For antibacterial testing fresh inoculum was prepared for each bacterium and incubated at 37 ± 2 °C for 24 h. The inoculum was adjusted with nutrient broth to obtain turbidity comparable to that of MacFarland 0.5 standard (10^8 CFU/ml) according to NCCLS (NCCLS, 1997). The cell suspension in each case was swabbed onto MH agar with the help of a sterile cotton swab and incubated at 37 ± 2 °C for 20 minutes. Fifty microlitre (50 µl) of different concentration of extract were prepared from 100 mg extract/ml of DMSO stock and poured into the well on agar plate (DMSO without extract was used as a control). Further, each plate was incubated at 37 ± 2 °C for 18 h. All of the experiments were performed in triplicate and the results (millimeters of the inhibition zone) were expressed as mean values.

Micro dilution method

Minimum inhibitory concentration (MIC) was determined by using micro broth-dilution bioassay of only selected extracts having potential antibacterial activity.[17] Crude plant extract was dissolved in DMSO to make stock solution of 100 mg/ml. 100 µl of extract stock solution was twofold serially diluted with sterile nutrient broth in a 96-wells of microtitre plates for each bacteria. Thereafter, 100 µl inoculum (1.5×10^8 CFU / ml bacterial suspension) was added to each well. The microtitre plates were incubated at 37±2°C for 24 h. Fifty microlitre (50 µl) of 2 mg/ml p-iodo-nitrotetrazoleum chloride (INT) to each well was added and incubated at 37 °C for 30 minutes. The reddish-pink colour indicates growth of bacteria in the microtitre plate and clear wells indicates the inhibition by extract. The MIC values were taken as the lowest concentration of extract in the well that showed no color. The minimum bactericidal concentration (MBC) was determined by sub culturing 50 µl from each well showing no apparent colour and the least concentration of extract showing no visible growth on agar plate was taken as MBC.

Determination of free radical scavenging activity

The free radical scavenging activity of methanol crude extract was measured by using DPPH (2, 2- diphenyl-1-picrylhydrazyl) radical.[18] DPPH radicals have strong absorption maximum at 515 nm which decreases due to the reduction by antioxidants. The DPPH solution in methanol (6×10^{-5} M) was prepared freshly and 3 mL of this solution was mixed with 100 µl

of various concentrations (0-0.5 mg/ml) of crude extract. The samples were incubated for 20 min at 37 °C in a water bath, and then the decrease in absorbance at 515 nm was measured (Ae). A blank sample containing 100 µl of methanol in the DPPH solution was prepared and its absorbance was measured (Ab). All tests and analyses were run in triplicates and the results obtained were averaged.

Radical scavenging activity was calculated using the following formula:

% inhibition = [(Ab-Ae)/Ab] × 100

Where Ab = absorbance of the blank sample, and Ae = absorbance of the methanol extract.

GC-MS analysis

For the identification of metabolites showing antibacterial activity, the samples were subjected to GC-MS analysis. The crude extract (1 µl) was injected into a RTX-5 column (60 m X 0.25 mm i.d., film thickness 0.25 µm) of GC-MS (model GC-MS-QP-2010 plus, Shimadzu Make). Helium was used as carrier gas at a constant column flow 1.2 ml/min at 173 kpa inlet pressure. Temperature programming was maintained from 10 0°C to 200 °C with constant rise of 5 °C/min and then held isothermal at 200 °C for 6 min; further the temperature was increased by 10 °C/min up to 290 °C and again held isothermal at 290 °C for 10 min. The injector and ion source temperatures were 270 °C and 250 °C, respectively. The sample was injected with a split ratio of 1:10. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 950 Dalton. The final confirmation of constituents was made by computer matching of the mass spectra of peaks with the Wiley and National Institute Standard and Technology (NIST) libraries mass spectral database.

RESULTS

Antibacterial Activity of Extract

Table 1 shows the dose dependent effect of crude extract of *S. oleracea* against *E. coli* and *S. aureus*, whereas, Table 2 shows Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of this crude extract against *E. coli* and *S. aureus*. It is interesting to notice that *S. oleracea* flower extract was more effective against *E. coli* than *S. aureus*. At 600 µg/well concentration, methanolic extract of flower of *S. oleracea* showed 14 mm inhibition zone against *E. coli*. In contrast, at the same concentration of extract, inhibition zone was found to be 13 mm against *S. aureus*. MIC and MBC of *S. oleracea* flower extract against *E. coli* were found to be 0.37 and 1.25 mg/ml, respectively. While, same for *S. aureus* were determined to be 0.75 and 1.25 mg/ml, respectively.

DPPH free radical scavenging activity

For evaluation of antioxidant properties of crude extract of *Spilanthes oleracea* DPPH radical scavenging assay was used and the results are shown in Fig 1. DPPH was reduced with the addition of methanolic extracts of flower of *S. oleracea* in a concentration-dependent manner (Fig1). Crude extract was effective radical scavenger with the inhibition of 70.13% at 0.5 mg/ml concentration while scavenging activity of control ascorbic acid was found to be 95.62 % inhibition at 0.5 mg/ml concentration. However, IC_{50} of ascorbic acid and crude extract were found to be 0.25 mg/ml and 0.27 mg/ml, respectively.

GC-MS analysis

In the present investigation a total of 80 volatile compounds have been identified from the methanolic crude extract of flower of *S. olearace* by GC-MS analysis (Table 3; Fig 2). The components listed in Table 3 can be divided into five main groups. The first group (I) consists of aromatic compounds, comprising of phenols, aromatic carboxylic acids and esters. While, second group (II) comprises of aliphatic compounds, represented by acidic, alcoholic, amide and aldehyde compounds of aliphatic series, which included the saturated and unsaturated mono, dicarboxylic and hydrocarboxylic acids, alcohols, amides and aldehydes. In contrast, the third group (III) contains aliphatic hydrocarbons, which had n-alkanes and alkenes, while, the fourth group (IV) includes terpenoids based compounds. Lastly, fifth group (V) consists of phytosterols.

The group composition of GC-MS analysis components has been shown in Table 4. Aromatic compounds (I) were the major group (50%) present in the methanolic extract of flower of *S. oleracae*. In contrast, the total percentage of aliphatic compounds (II) was 30 in the crude extract. The percentage of aliphatic hydrocarbons (III), terpenoids (IV) based compounds and phytosterols (V) were observed to be 2.5, 7.5 and 10, respectively, in the crude extract.

The major components present in the methanolic crude extract of flower of *S. olearace* were 1,2,3-Propanetriol (RT: 10.086), Isopentyl alcoho (RT: 15.473), 1-Dodecanol (RT: 20.208), 2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl (RT: 24.604), 8,8,9-Trimethyl-deca-3,5-diene-2,7-dione (RT: 26.840), Globulol (RT: 27.580), 2,6,10-Trimethyl,14-ethylene-14-pentadecne (RT: 28.201), 2-Methyl-2-(3-Methyl-2-oxobutyl) cyclohexanone (RT: 28.829), 4H-1,3,2-Dioxaborin, 2-ethyl-4-methyl-4,6-dipropyl (RT: 29.146), N-Isobutyl-2(E),6(Z),8(E)-decatrienamide (RT: 29.610), N-Methyl-2-furohydroxamic acid (RT: 30.335), n-Hexadecanoic acid (RT: 31.013), [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl

ester (RT: 32.773), 9,12-Octadecadienoic acid (RT: 34.240), 4-(7-Methoxy-3,3,7-trimethyl-oxepan-2-ylidene)-butan-2-one (RT: 35.973), Cyclohexanol, 2-methyl-3-(1-methylethenyl)(RT: 36.873), Hex-5-enamide, N-(2-phenylethyl) (RT: 38.484) and Lariciresinol (RT: 50.564) (Table 3).

Table1. Antibacterial activity of methanolic extract of *Spilanthes oleracae* against *Staphylococcus aureus* and *Escherichia coli*

Concentration (ug/well)	Zone of inhibition in mm*	
	Staphylococcus aureus	*Escherichia coli*
600	13	14
400	12	12
200	11	11

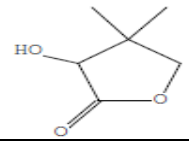
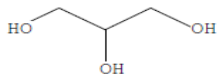
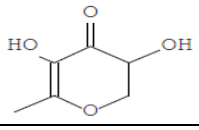
*The data refer to mean value of three replicates

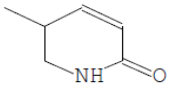
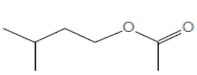
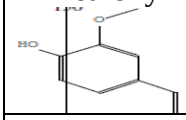
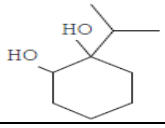
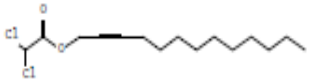
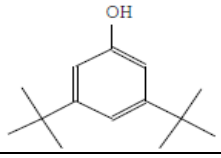
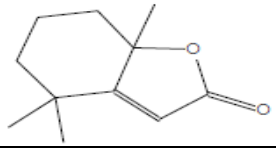
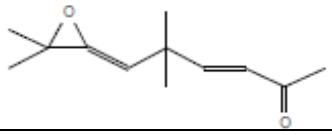
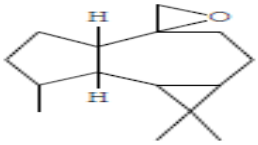
Table2. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of methanolic extract of *Spilanthes oleracae* against *Staphylococcus aureus* and *Escherichia coli.*

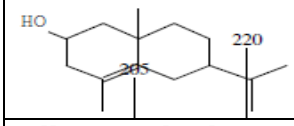
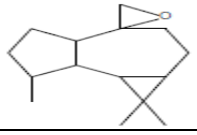
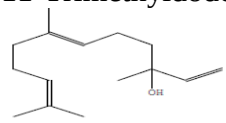
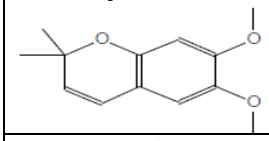
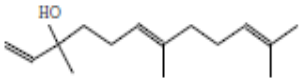
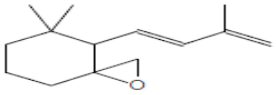
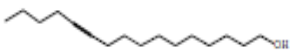
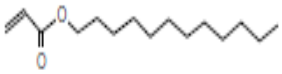
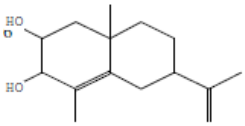
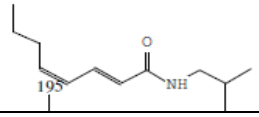
Bacteria	Concentration in (mg/ml)*	
	MIC	*MBC*
Staphylococcus aureus	0.75	1.25
Escherichia coli	0.37	1.25

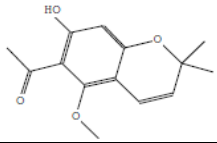
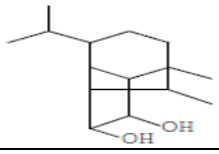
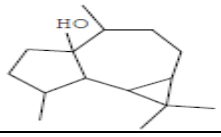
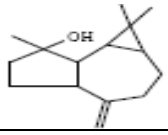
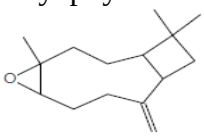
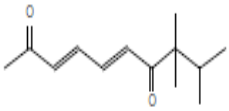
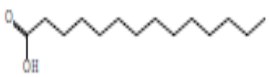
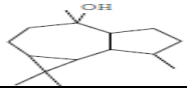
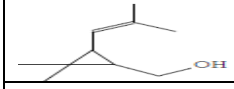
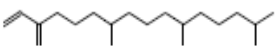
*The data refer to mean value of three replicates

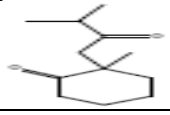
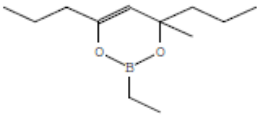
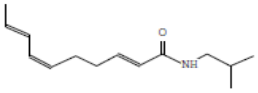
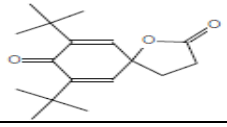
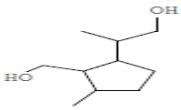
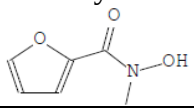
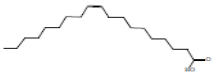
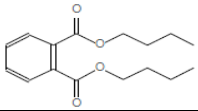
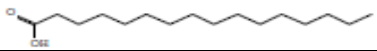
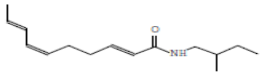
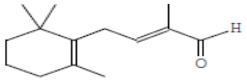
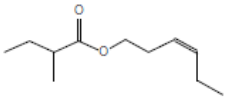
Table3. Bioactive compounds identified in the methanolic crude extract of the flower of *Spilanthes oleracea by* GC-MS.

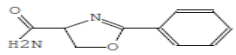
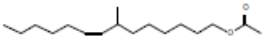
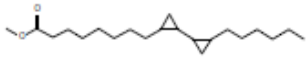
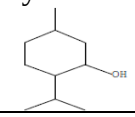
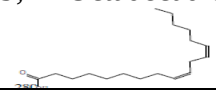
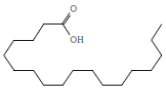
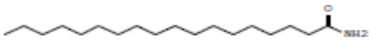
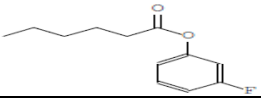
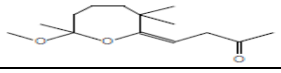
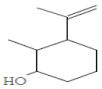
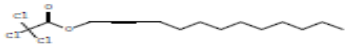
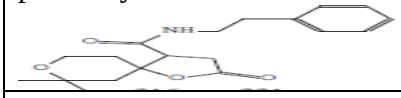
Identified Compounds*	Group**	RT (min)	Chromatogram % area
3-Hydroxy-4,4-Dimethyldihydro-2(3H)-Furanone 	I	8.525	0.07
1,2,3-Propanetriol 	II	10.086	4.89
Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one 	I	11.750	0.14
1,2,5,6-Tetrahydropyridin-2-one	I	11.899	0.08

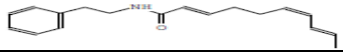
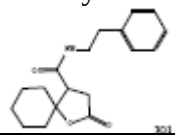
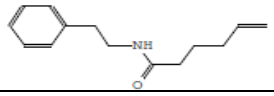
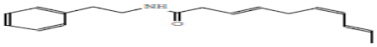
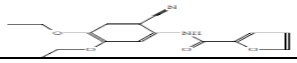
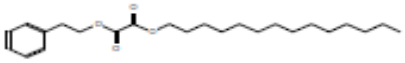
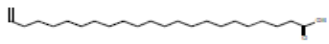
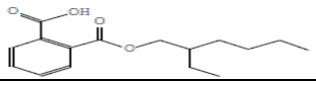
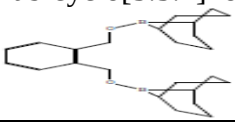
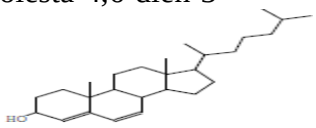
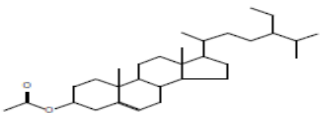
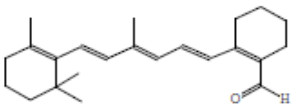
			
Isopentyl alcohol 	II	15.473	8.95
2-Methoxy-4-Vinylphenol 	I	16.244	1.95
1-Isopropyl-1,2-cyclohexanediol 	I	16.612	0.12
1-Dodecanol 	II	20.208	2.97
Dichloroacetic acid, tridec-2-ynyl ester 	II	20.626	0.30
Phenol, 3,5-bis(1,1-dimethylethyl) 	I	21.253	0.21
2(4H)-Benzofuranone, 5,6,7,7A-Tetrahydro-4,4,7A-Trimethyl 	I	21.723	0.17
Longifolenaldehyde 	I	22.253	0.71
6-(3,3-Dimethyl-2-Oxiranylidene)-5,5-Dimethyl-3-Hexen-2-One 	II	23.472	0.12
Aromadendrene oxide 	I	23.629	0.49

1-Naphthalenol, 1,2,4a,5,6,7,8,8a-Octahydro-3-Methyl-8-Methylene-5-(1-Methylethyl) 	I	23.781	0.11
Alloaromadendrenoxide 	I	23.923	0.46
3,7,11-Trimethyldodeca-1,6,10-Trien-3-ol 	II	24.063	0.25
2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl 	I	24.604	4.22
1,6,10-Dodecatrien-3-ol, 3,7,11-Trimethyl 	II	24.770	0.11
1-Oxaspiro[2.5]octane, 5,5-dimethyl-4-(3-methyl-1,3-butadienyl) 	I	24.881	0.67
11-Hexadecyn-1-ol 	II	25.101	0.26
2-Propenoic acid, Dodecyl ester 	II	25.200	0.10
6-Isopropenyl-4,8a-Dimethyl-1,2,3,5,6,7,8,8a-Octahydro-2,3-Naphthalenediol 	I	25.357	0.14
N-Isobutyl-(2E,4Z)-octadienamide 	II	25.452	0.28

Ethanone, 1-(7-Hydroxy-5-Methoxy-2,2-Dimethyl-2h-1-Benzopyran-6-yl) 	I	25.911	0.08
3-Isopropyl-6,7-dimethyltricyclo[4.4.0.0(2,8)]decane-9,10-diol 	I	25.983	0.08
Palustrol 	IV	26.292	0.67
Spathulenol 	IV	26.420	0.21
Caryophyllene oxide 	IV	26.582	0.70
8,8,9-Trimethyl-deca-3,5-diene-2,7-dione 	II	26.840	2.42
Tetradecanoic acid 	II	27.010	0.66
NONADECANE	III	27.294	0.25
Globulol 	IV	27.580	1.27
Cyclopropanemethanol, 2,2-dimethyl-3-(2-methyl-1-propenyl) 	II	28.072	0.13
2,6,10-Trimethyl,14-ethylene-14-pentadecne 	III	28.201	1.15

2-Methyl-2-(3-Methyl-2-oxobutyl) cyclohexanone 	I	28.829	1.13
4H-1,3,2-Dioxaborin, 2-ethyl-4-methyl-4,6-dipropyl- 	I	29.146	1.97
N-Isobutyl-2(E),6(Z),8(E)-decatrienamide 	IV	29.610	3.44
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione 	I	29.982	0.10
Iridodiol 	I	30.065	0.20
N-Methyl-2-furohydroxamic acid 	I	30.335	5.51
cis-10-Nonadecenoic acid 	II	30.575	0.29
Dibutyl phthalate 	I	30.797	0.50
n-Hexadecanoic acid 	II	31.013	4.65
N-(2-Methylbutyl)-(2E,6Z,8E)-decatrienamide 	II	31.673	0.25
2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl) 	I	31.910	0.17
Butanoic acid, 2-methyl-, 3-hexenyl ester 	II	32.125	0.77

4-Carbamoyl-2-phenyl-2-oxazoline 	I	32.354	0.95
7-Methyl-Z-tetradecen-1-ol acetate 	II	32.526	0.75
[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester 	II	32.773	1.84
Cyclohexanol, 5-methyl-2-(1-methylethyl) 	I	33.531	0.15
9,12-Octadecadienoic acid 	II	34.240	17.68
Octadecanoic acid 	II	34.624	0.39
Octadecanamide 	II	34.960	0.18
Hexanoic acid, 3-fluorophenyl ester 	I	35.208	0.72
4-(7-Methoxy-3,3,7-trimethyl-oxepan-2-ylidene)-butan-2-one 	I	35.973	10.42
Propanenitrile, 2-(2-trifluoromethylphenylhydrazono)-3-imino-3-(1-piperidinyl) 	I	36.477	0.31
Cyclohexanol, 2-methyl-3-(1-methylethenyl) 	I	36.873	4.63
Trichloroacetic acid, tridec-2-ynyl ester 	II	37.342	0.15
Spiro[4.5]decane-4-carboxylic acid, 7,7-dimethyl-2-oxo-1,8-dioxa-, phenethylamide 	I	37.618	0.47
N-(2-Phenylethyl)(2E,6Z,8E)-decatrienamide	I	38.063	0.15

			
2-Oxo-1-oxa-spiro[4.5]decane-4-carboxylic acid phenethyl-amide 	I	38.303	0.20
Hex-5-enamide, N-(2-phenylethyl)- 	I	38.484	1.12
N-(2-Phenylethyl)[3(E),6(Z),8(E)]-decatrienamide 	I	38.812	0.20
Furan-2-carboxylic acid (2-cyano-4,5-diethoxyphenyl)amide 	I	39.681	0.16
Oxalic acid, 2-phenylethyl tetradecyl ester 	I	39.827	0.19
22-Tricosenoic acid 	II	40.531	0.10
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester 	I	40.719	0.70
9-({2-[(9-Borabicyclo[3.3.1]non-9-Yloxy)methyl]Benzyl}oxy)-9-Borabicyclo[3.3.1]nonane 	I	43.033	0.31
Cholesta-4,6-dien-3-ol 	V	49.352	0.38
Stigmast-5-en-3-ol 	V	49.540	0.18
2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde 	I	50.313	0.13

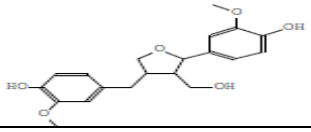
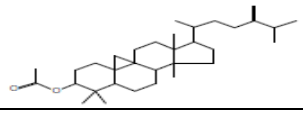
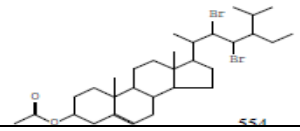
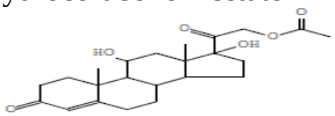
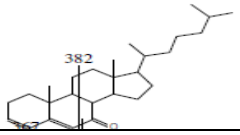
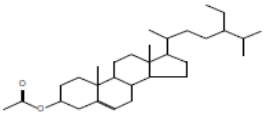
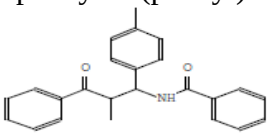
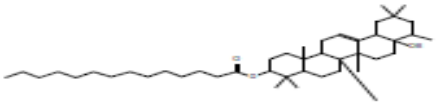
Lariciresinol 	IV	50.564	1.04
9,19-Cyclolanostan-3-ol, 24-methylene-, acetate 	V	51.056	0.13
22,23-Dibromostigmasterol acetate 	V	51.231	0.69
Hydrocortisone Acetate 	V	51.503	0.63
Cholesta-3,5-dien-7-one 	V	52.098	0.10
□-Sitosterol acetate 	V	52.341	0.10
2-Azapentane-1,5-dione, 4-methyl-1,5-diphenyl-3-(p-tolyl) 	I	52.683	0.29
3.beta.-Myristoylolean-12-en-16.beta.-ol 	V	53.867	0.19

Table 4. Group composition of GC-MS analysis components.

Group No.	Group	Group % in crude extract of *S. oleracea*
I	Aromatic compounds	50
II	Aliphatic compounds	30
III	Aliphatic hydrocarbons	2.5
IV	Terpenoid compounds	7.5
V	Phytosterols	10

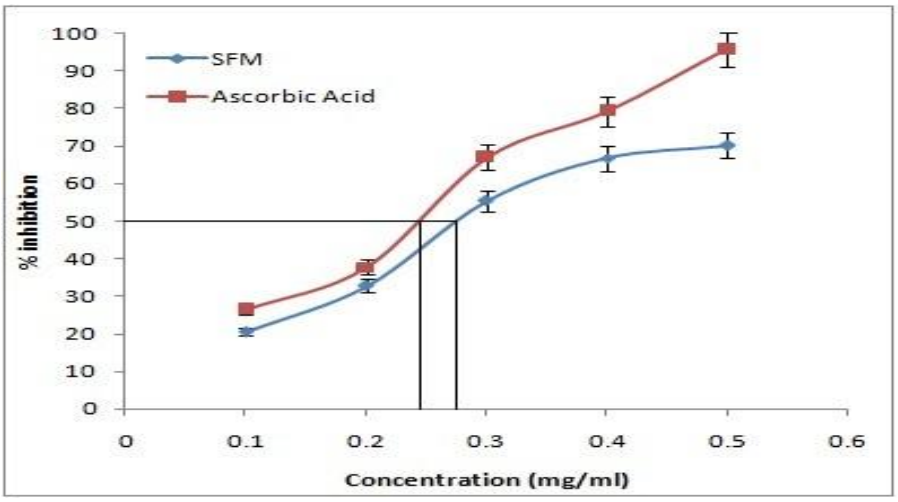


Figure1. Effects of crude extract of flower of *Spilanthes oleracea* on the scavenging of DPPH. Values are mean ± SE of three replicates. (SFM: *Spilanthes oleracea* Flower Methanolic extract).

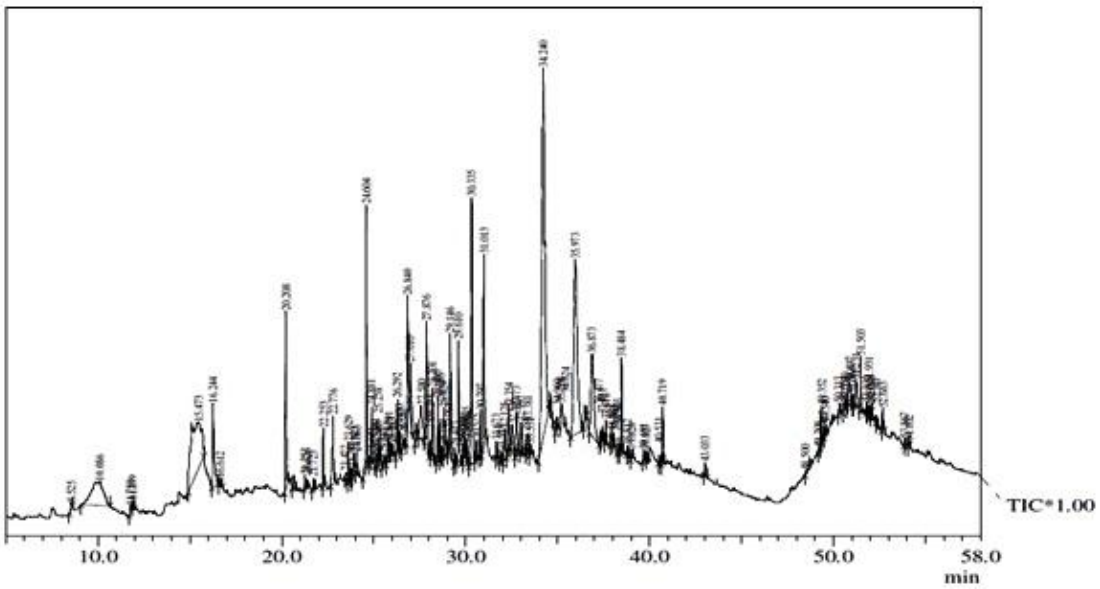


Figure2. Chromatogram of methanolic crude extract of flowers of *Spilanthes oleracea* by GC-MS.

DISCUSSION

Increasing bacterial resistance is prompting resurgence in research of the antimicrobial role of herbs against resistant strains.[19] Plants are known to produce secondary metabolites which are naturally toxic to bacteria, and a large body of literature has validated the antimicrobial activity of plant extracts, showing great potential especially against multidrug resistant bacteria.[20]

In view of the above, the present study was conducted to evaluate the antibacterial potential of methanol extracts of flower of *Spilanthes oleracea* against two drug resistant bacteria; *Staphylococcus aureus* and *Escherichia coli*.

It is interesting to notice that *S. oleracea* flower extract was more effective against *E. coli* than *S. aureus*. MIC and MBC of *S. oleracea* flower extract against *E. coli* were found to be 0.37 and 1.25 mg/ml, respectively. While, same for *S. aureus* were determined to be 0.75 and 1.25 mg/ml, respectively. Concurrent to these results, a 2013 study also showed that the methanolic extract of flower head of *S. oleracea* expressed significant antibacterial activity against *Pseudomonas aeruginosa, Streptococcus salivarious, Streptococcus viridians* and *Streptococcus mutans*.[21]

There are many synthetic drugs which protect against oxidative damage but unfortunately they have adverse side effects on the body. Alternative solution to this problem is to consume natural antioxidants from traditional medicines.[22] However, many natural antioxidants have already been isolated from different plant materials.[23] In the present study, we have evaluated the free radical scavenging capacities of the methanolic extracts of flower of *S. oleracea* using DPPH (2, 2- diphenyl-1-picrylhydrazyl) assay. Relative stable DPPH radical has been widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and thus to evaluate the antioxidant activity.[24, 25]

The result of DPPH scavenging activity assay in this study indicates that flower of this plant was potently active and suggests that this plant extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. Bibhabasu *et al.*, (2008) reported the significant antioxidant and free radical scavenging activity of methanol extract of *Spondias pinnata* stem bark and further found that this activity might be due the presence of significant amounts of flavonoids and phenolic compounds in the extract.[26] It has also reported that the bark extract of *Ficus racemosa* have higher DPPH radical scavenging activity and this radical scavenging ability of extracts could be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating system.[27]

These investigations further supported the view that plants are promising sources of natural antioxidants and antibacterial. We have thus identified some promising antioxidant and antibacterial from methanolic flower extract of *S. oleracea* (family *Asteraceae*). Although,

additional study were required to characterize and identify the active component responsible for these biological activities of this plant extract. Consequently, we characterize the phytochemical components of the crude methanolic extract of flower of *S. oleracea* by GC-MS analysis. Exploring plant biochemical diversity by GC-MS [28, 29] has proved to be a fast and reliable approach, allowing identification of a large number of compounds. In some cases, the GC-MS screening of plant samples has revealed compounds with unknown MS spectra, which has resulted in the isolation and spectroscopic identification of new natural bioactive molecules.[30]

Aromatic, aliphatic, aliphatic hydrocarbons, phytosterols and terpenoids based compounds were present in the methanolic extract of flower of *S. oleracae*. All these classes of compounds have been reported as potent antibacterial and antioxidants.[31-36] Concomitant to our results, Naga *et al*., [37] also reported the presence of aliphatic compounds, such as hexadecanoic acid ethyl ester, 9,12,15- Octadecatrienoic acid and 9,12-Octadecanoic acid in the ethanolic extract of *Phragmytes vallatoria* leaf by GCMS analysis. On the other hand, aliphatic compounds- 10-Octadecenoic acid, 9, 12-Octadecenoic acid and 9, 12, 15-Octadecatrienoic acid has been reported for anti-bacterial, anti-inflammatory and antiarthritic property.[38-40] Moreover, several authors have shown that natural aromatic compounds possess important biological activities, such as antitumor, antihepatotoxic, antioxidant, anti-inflammatory, estrogenic and antibacterial activities.[41, 42] Further, terpenoids have also been reported to have anti-inflammatory, antioxidant and neuroprotective activities.[32]

All these reports suggested that the compounds present in the higher proportion may be responsible for the higher share of the antibacterial and antioxidant activity of methanolic crude extract of *S. olearace* flower while the involvement of the less abundant constituents should also be considered. In fact, the synergistic effects of the diversity of major and minor constituents present in the crude extract should be taken into consideration to account for their biological activity.[43]

CONCLUSIONS

In the present study, crude methanol extract of flower of *S. olearace*, can be seen as a potential useful bioactive compound against drug resistant *S. aureus* and *E.coli* as it is evident from the antibacterial assay. In addition, the crude extract also showed promising antioxidant potential. Moreover, the methanolic extract evaluated in this work has a great variety of phytochemicals as identified by GC-MS analysis, which could be considered responsible for

a larger or smaller part of the antibacterial and antioxidant activity. However, the presence of wide range of phytochemical constituents in the crude extract indicates that further studies are required to pinpoint the role of various minor and major compounds showing the antibacterial and antioxidant activities. Nevertheless, this study reflects a hope for the development of novel agents of biomedical importance from flowers of *S. oleraceae* especially against multidrug resistant *E.coli* and *S. aureus*.

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