Available online on www.ijcpr.com
International Journal of Current Pharmaceutical Review and Research; 6(3); 153-163

ISSN: 0976 822X

Research Article

Screening of Chemical Composition and Antimicrobial Activity of Essential Oil Isolated From Flower Heads of *Sphaeranthus Indicus* Linn Against MRSA, ESBL, AMP_C, and Carbapenamase Producing Bacteria

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Available Online: 1st May, 2015

ABSTRACT
The aim of present study is to isolate and identify essential oils by hydro distillation method from flower head of *Sphaeranthus indicus* Linn and evaluates their antimicrobial activity against pathogenic bacteria through disc diffusion method. The essential oil was obtained from by hydro distillation method using Clevenger type apparatus. Essential oils Isolate were identified by GC–MS and determine their *in vitro* antimicrobial activity against pathogenic bacteria through agar plate method. Twenty three bioactive ingredients with different percentage were identified based on GC retention time. The predominant 5 bioactive ingredients with high percentage in essential oil were identified as 10-ep-γ eudesmol, 2,5-dimethoxy-p-cymene,Longifoene,Selin 11-en-4α-ol, 2 pentadecanone, 6,10,14,tri methyl. Essential oils isolated by hydro distillation were used for the study of antimicrobial activity against pathogenic resistance (gram positive and gram negative). Almost all bacterial strains give activity against the employed essential oils at different concentrations. Therefore, the obtained results show that essential oils could be needed further extensive biological study and their mechanism of action.

Keywords: Hydro distillation, flower head, *Sphaeranthus indicus* Linn, Antimicrobial activity, GC–MS

INTRODUCTION

Essential oils have been traditionally used for treatment of infections and diseases all over the world for centuries .[1] Today the use of essential oils is a growing market and there are a considerable range of applications. The oils are used, for example, in the food and beverages industry and as fragrances in perfumes and cosmetics, but the oils also cover a broad spectrum of biological activity which has lead to an increased interest among researchers. In recent years there has been extensive research to explore and determine the antimicrobial activity of essential oils. All oils tested to date have displayed some antimicrobial activity and some have been shown to be more effective than others. In the last decade there has also been an increased interest in essential oils and their antimicrobial activity due to the spread of antibiotic resistance. Since the discovery of penicillin by Alexander Fleming in 1929 many new classes of antibiotics have become available for treatment of bacterial infections, but due to excessive and often unnecessary use of antibiotics in humans and animals, bacterial resistance has now been reported against every currently available antibiotic.[2] Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE) and resistant strains of *Pseudomonas* are examples of multiresistant bacteria that are becoming an alarming problem within the healthcare system. MRSA is probably the most common antibiotic resistant bacterium found in hospitals throughout the world and it naturally colonises skin and infects wounds. Today the prevalence of MRSA is between 25-50 percent in parts of the world, including the USA, Australia, South America and central parts of Europe. Even in Scandinavian countries, where MRSA rates have been low, the frequency is beginning to rise.[3] VRE has also spread throughout the world since it was first discovered and isolated in the late 80's and can now be found in every continent. *Enterococci* can cause bacteremia, wound infection and urinary tract infection, but serious infections of VRE usually only occurs in patients with significantly compromised host defences .[4] *Candida* and *Pseudomonas* are other opportunistic pathogens that usually only lead to serious infections in immunocompromised individuals. Therapies for *Candida* have been difficult because of the limited number of antifungal agents, and for *Pseudomonas* even drug-susceptible strains have considerable defences against antibiotics.[5, 6] *Sphaeranthus indicus* Linn is a common annual spreading herb found in rice field throughout India, Srilanka, Ausralia and Africa. Essential oil [7], obtained by steam distillation of the whole herb contains ocimen, α- terpine, a- citral, geranion, a-ionone, β-ionone, d- cadinene, p- methoxy cinnamaldehyde [8], and an alkaloid spearanthine [9]. The alcoholic extract of

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Table 1: Strain Of Gram Positive Bacteria

S.no	Strain of gram positive bacteria	Specimen
1	*Staphylococcus aureus*	pus
2	*Staphylococcus aureus* , *Staphylococcus aureus* (MRSA)	ET (Endotrachial)
3	*Staphylococcus aureus*	blood
4	*Enterococcus faecalis*	urine

Table 2: Strain of gram negative bacteria

S.no	Strain of gram negative bacteria	Specimen
1	*Klebsiella pneumonia* , *Klebsiella pneumonia (AmpC), Escherichia coli , E scherichia coli(Carbapenamase), Pseudomonas aeruginosa, proteus mirabilis , Candida krusei*	ET (Endotrachial)
2	*Acinetobactor spp. Pseudomonas aeruginosa , Klebsiella pneumonia , Klebsiella pneumonia (Carbapenamase)*	pus
3	*Candida albicans , Escherichia coli*	urine
4	*Pseudomonas aeruginosa (Carbapenamase) , Klebsiella pneumonia(ESBL) ,*	TT (Trachiotomy)
5	*Escherichia coli(AmpC)*	blood
6	*Escherichia coli(ESBL)*	stool

Table 3: Strain of fungi

S.no	Strain of fungi	Specimen
1	*Candida krusei*	ET (Endotrachial)
2	*Candida albicans*	urine

powdered caputula contains stigmasterol, β- sitosterol, hentriacontane, sesque- terpinelactone[10], sphaeranthanolide [11], flavone and isoflavone glycoside. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world.[12-15] Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine.[16] Considering the aforesaid, one of the traditionally used medicinal plants belonging was screened for their antimicrobial properties,*Sphaeranthus indicus* which belongs to *Asteraceae* family. It is used in homeopathic medicine for the treatment of insomnia, epilepsy, tetanus, muscle spasms and leaves presented anxiolytic activity[17,18]. *Sphaerathus indicus* Linn is an annual spreading herb used to treat hemicraniasis [19],

Table 4: Physical parameter of volatile oil of flowers of *S. indicus*

S.No	Characteristics	SIEO
1	Appearance	Dark Pale yellow
2	Odour	Characteristic, pleasant
3	Yield %	0.2
4	Solubility	Soluble in alcohol and oils, insoluble in water

jaundice, diabetes, hernia, haemorrhoids, helmenthiasis, skin diseases, nervine tonic etc. The bark ground and mixed with whey, is said to be useful in treating piles. Leaf juice is boiled with milk and sugar-candy and prescribed for cough. An aqueous extract of the whole plant was slightly toxic to American cockroaches [20]. *Sphaeranthus indicus* was found to possess powerful medicinal properties to cure skin infections, diseases of the liver, jaundice, bronchitis. In view of the medicinal importance of *Sphaeranthus indicus* in the indigenous system, it was decided to work on the phytochemistry and antimicrobial investigations on *Sphaeranthus indicus* Linn. The aim of this study was to evaluate the antimicrobial activity of plant volatile oils and to determine how the inhibition was effected by different exposure times to the essential oil vapours. Essential oils were test,*stayphylococcus aureus* and *Candida albicans*. tested against five different microorganisms: MRSA, and *Pseudomonas aeruginosa .kleseilla pneumonie E.coli beta lactamase producing and non beta lactamase* producing bacteria

MATERIALS AND METHOD

A- Selection and authentication of plant material

Whole plants of *Sphaeranthus indicus* Linn. was authenticated by Dr. H. B Singh, Raw Materials Herbarium and Museum (RHMD) of NISCAIR, New Delhi. (Ref. letter No NISCAIR/RHMD/Consult/-2009-10/1249/53)

B-Collection and processing of plant material

Sphaeranthus indicus collected from local area of Hoshangabad Bhopal M. P. The collected plant materials were naturally dried under shade and subjected to size reduction using hand grinder. The powder so obtained was passed through sieve and then used for evaluation.

C-Isolation of volatile oil from flowers of S. indicus

The volatile oil of flower heads of *S. indicus* was obtained by hydro distillation for 3 hr in a Clevenger type apparatus and obtained oil was collected in vial and dried over anhydrous Na_2SO_4, filtered by cotton and the oil stored at 4 -8^{0}C until analysis and was marked as SIEO (*S. indicus* essential oil)

D- Identification and quantification of compounds present in volatile oil by GC-MS Chromatography

Instrumentation – GC-MS analysis was carried out on Perkin Elmer autosystem XL with turbo mass, instrument employing the following conditions:

- Column PE-5 MS, column length 30 m.
- Carrier gas – Helium was used as carrier gas.
- Injection volume - 0.5 µl (split ratio – 1:60)
- Injector temperature – 250 ^{0}C
- Ion-source temperature – 220 ^{0}C

Table 5: Area percent report chromatogram of volatile oil of flowers of *Sphaeranthus indicus* Linn.

No.	RT	Area	Height	BL	Conc.	Area/ Conc.	m/z	Area%
1	7.319	9,499.9	50,559	Bb	0.00	0.00	TIC	0.19
2	8.932	29,501.0	215,830	Bb	0.00	0.00	TIC	0.58
3	9.885	11,257.0	89,838	Bb	0.00	0.00	TIC	0.22
4	10.307	15,136.4	123,302	MM	0.00	0.00	TIC	0.30
5	10.747	19,831.2	159,541	MM	0.00	0.00	TIC	0.39
6	11.150	8,422.1	58,332	Bb	0.00	0.00	TIC	0.17
7	11.554	40,450.9	278,436	Db	0.00	0.00	TIC	0.80
8	11.957	9,190.5	53,193	Bb	0.00	0.00	TIC	0.18
9	12.215	18,913.8	150,939	Bb	0.00	0.00	TIC	0.37
10	12.507	0.5	0	MM	0.00	0.00	TIC	0.00
11	13.185	32,341.1	256,930	MM	0.00	0.00	TIC	0.64
12	13.699	320,518.3	2,367,683	Db	0.00	0.00	TIC	6.30
13	14.029	1,813,131.3	11,460,249	Bb	0.00	0.00	TIC	35.67
14	15.624	135.6	-4	MM	0.00	0.00	TIC	0.00
15	15.862	100,889.3	798,604	MM	0.00	0.00	TIC	1.98
16	16.247	1,644,037.0	7,874,653	Bb	0.00	0.00	TIC	32.34
17	16.926	354,097.2	1,991,670	Db	0.00	0.00	TIC	5.00
18	17.109	118,808.9	1,000,868	Bb	0.00	0.00	TIC	2.34
19	17.256	120,507.2	985,460	Bb	0.00	0.00	TIC	2.37
20	17.751	25,149.2	202,850	MM	0.00	0.00	TIC	0.49
21	18.997	277,718.5	2,146,547	MM	0.00	0.00	TIC	5.46
22	19.639	104,546.1	700,312	Bb	0.00	0.00	TIC	2.06
23	20.226	109,644.8	494,612	MM	0.00	0.00	TIC	2.16

- Oven temperature – The oven temperature was programmed from 70 ^{0}C (isothermal for 5 min), with an increase of 10 ^{0}C/min. up to 290 ^{0}C
- Scan range – 30 amu – 350 amu
- Total GC running time – 30 min

E- Collection of clinical strain by different specimen of patient (Gram positive, Gram negative bacteria and fungi)

The clinical isolates (bacteria and fungi strain was collected during march-may month

F-Culture of bacterial strain obtained by different patient specimen on to the different agar media

The specimen sample of patients was taken with the help of sterile wire loop and was streak on to the different type of agar medium according to the sample require and then plate was incubated at 37^0 C for 24 hr in incubator

G-Confirmatory test for cultured micro organism strains By visualization

Cultured microorganism strain was confirmed on the basis of color of colonies developed on to the different medium of agar plate

Test For Gram Positive Bacteria

Catalase and Coagulase test

This test was used for the confirmation of *Staphylococcus aureus*

Catalase test

Slide catalyze test results. Hydrogen peroxide was added directly to the culture on a microscope slide. A positive reaction produced by *Staphylococcus aureus* is indicated by bubbling

Coagulase test

The Coagulase Test is used to differentiate *Staphylococcus aureus* from other species of this genus. To perform the Slide Coagulase test, place a drop of coagulase reagent human plasma onto a clean microscope slide, and then add several colonies of the unknown Staphylococcus. Mix well. If fine Grains of sand or small clumps were seen, the coagulase test is positive. If the mixture remains smooth (milky looking), the coagulase test is negative. The Slide Coagulase Test detects bound coagulase only. Bound coagulase is an enzyme in the cell wall of *Staphylococcus aureus.*

MRSA (Methicillin resistant Staphylococcus aureus detection test

a strain of *Staphylococcus aureus* that is resistant to antibiotics. Specifically, Beta-lactamase Antibiotics, which includes penicillin's and its derivatives. For the detection firstly prepared a plate culture for the organism to be tested and emulsify a colony from the plate by using a sterile loop in the sterile saline Solution. Mix thoroughly until the making sure that no solid material from the colonies is visible. Repeat procedure until the turbidity of the saline solution matches with the McFarland turbidity standard and then dip the swab into the broth culture of the organism. The swab streak on Mueller-Hinton agar plate in one direction, then streaking at right angles to the first streaking, and finally streaking diagonally. End by using the swab to streak the outside diameter of the agar and Plates was dry for about 5 minutes. After that oxacillin disc was placed on to the agar media surface, Incubate for 18-24 hr at 37°C

Test For Gram Negative Bacteria

Oxidase Disc Test

This test was used for the confirmation of *Pseudomonas aeruginosa.* The oxidase test is based on the bacterial production of an oxidase enzyme. Collect the 1-2 colonies

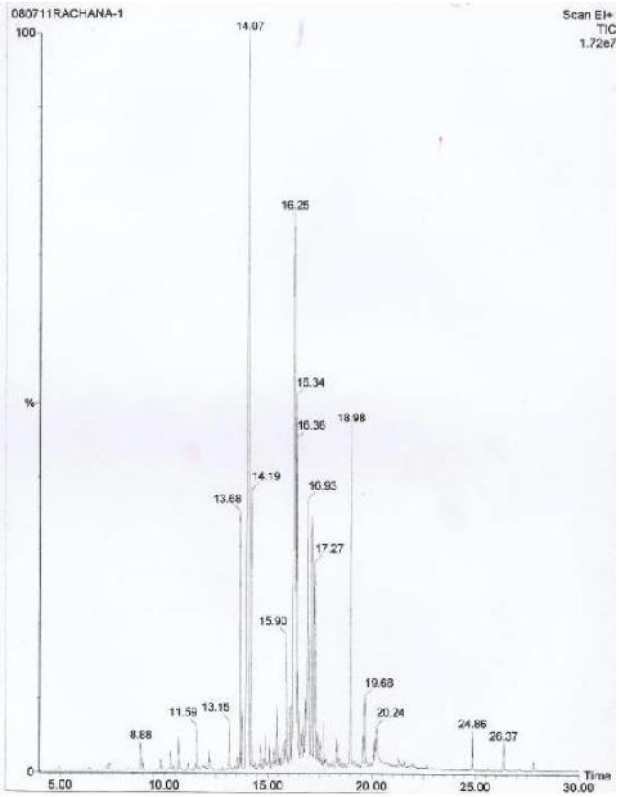

Figure 1: Chromatogram of volatile oil of flowers of *Sphaeranthus indicus* Linn.

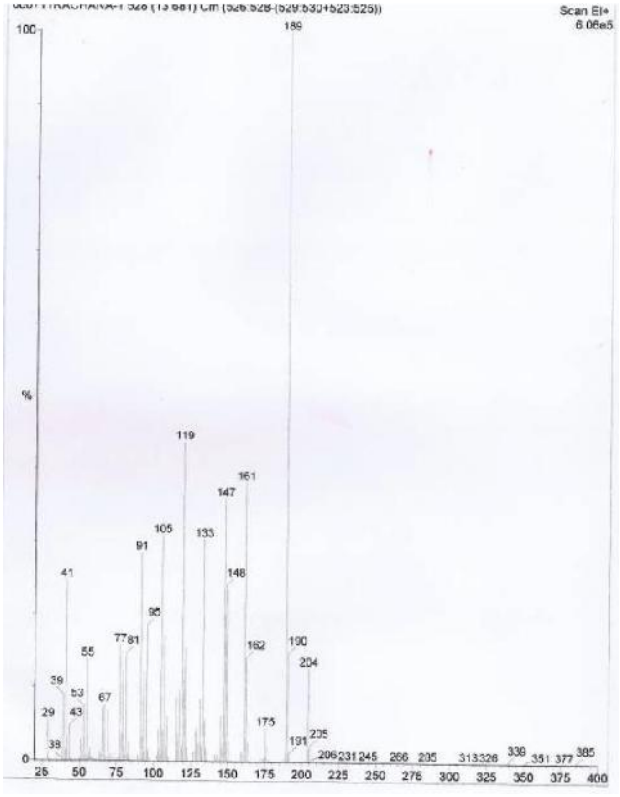

Figure 2: Mass spectra of peak no.12 (RT-13.699) of chromatogram of volatile oil of flowers of *Sphaeranthus indicus* Linn.

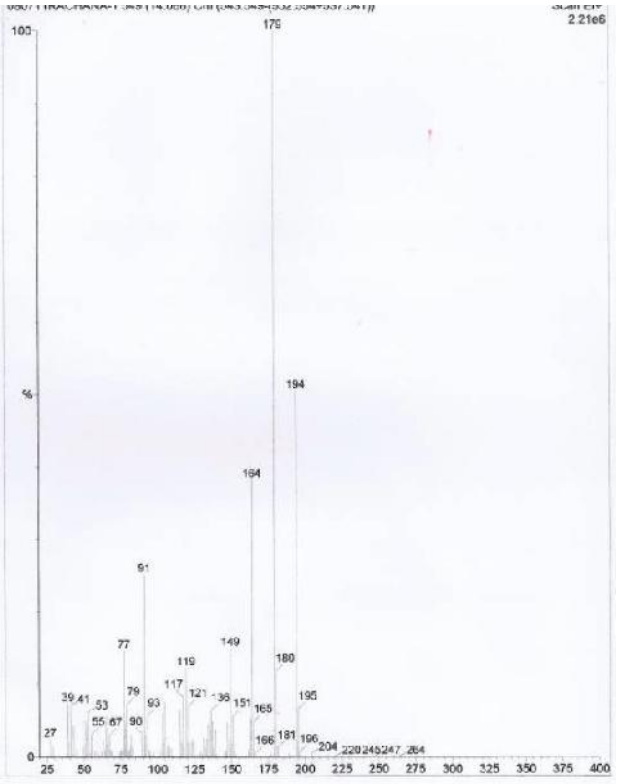

Figure 3: Mass spectra of peak no.13 (RT-14.029) of chromatogram of volatile oil of flowers of *Sphaeranthus indicus* Linn.

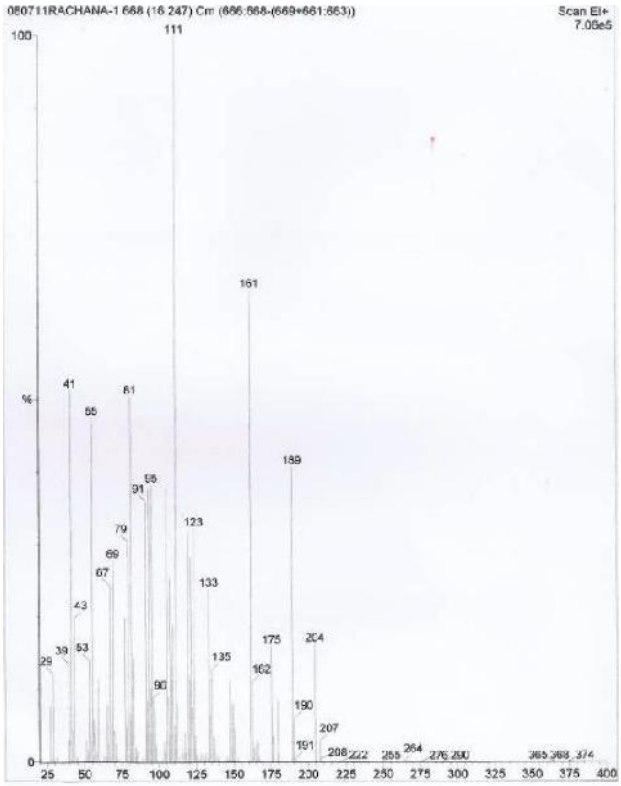

Figure 4: Mass spectra of peak no.16 (RT-16.247) of chromatogram of Volatile oil of flowers of *Sphaeranthus indicus* Linn.

on agar plate with the help of toothpick and touch the oxidase disc then color was observed

ESBL (Extended Spectrum Beta Lactamase) confirmation test for Klebsiella pneumonia and Escherichia coli

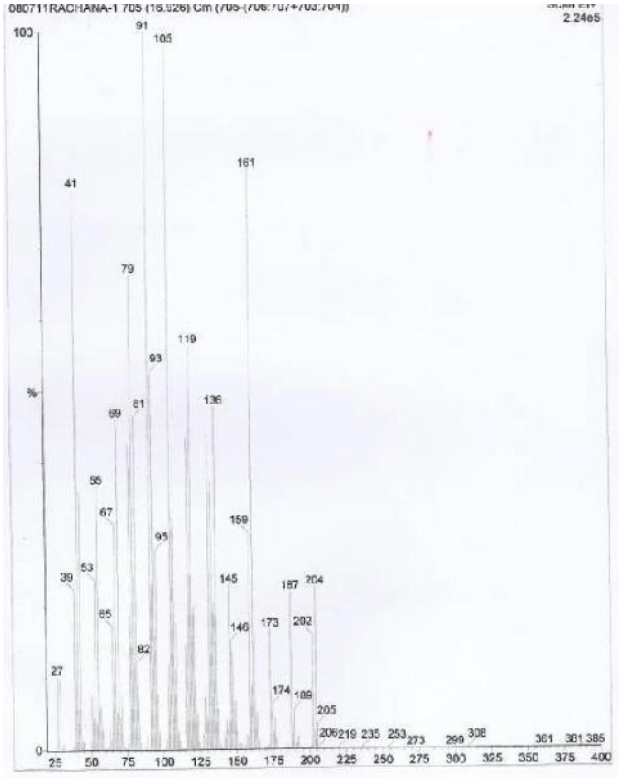

Figure 5: Mass spectra of peak no.17 (RT-16.926) of chromatogram of volatile oil of flowers of *Sphaeranthus indicus* Linn.

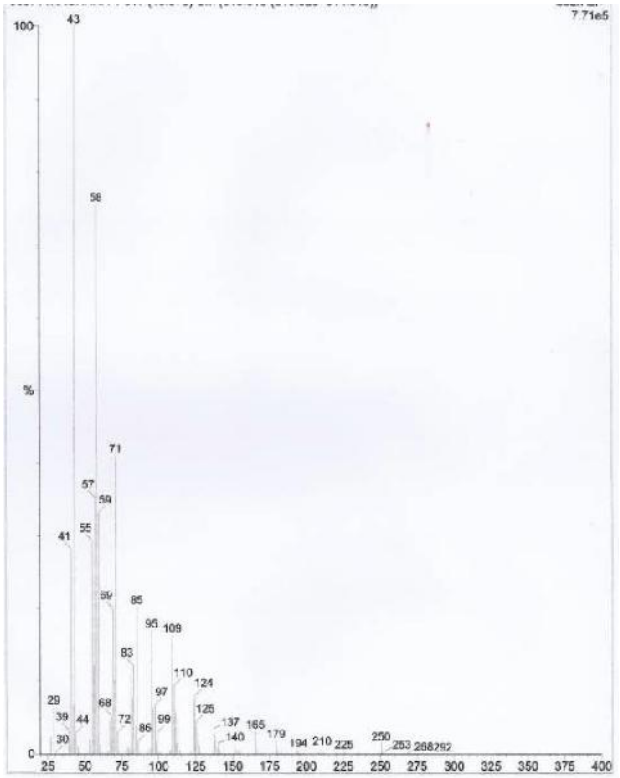

Figure :6 Mass spectra of peak no.21 (RT-18.997) of chromatogram of volatile oil of flowers of *Sphaeranthus indicus* Linn.

Bacteria were suspended equivalent to McFarland 0.5 turbidity standard in nutritional broth with peptone water as test medium. After inoculating a Mueller-Hinton Agar plate, disks containing ceftazidime (CAZ, 30 µg) and ceftazidime + clavulanic acid were placed 25 mm (centre to centre) respectively, incubated at 37°C over night. If there is synergy (combination of paper near the edge of the side appears to expand or enhance), determine the strains producing ESBL.

Amp C confirmation test for Klebsiella pneumonia and Escherichia coli

Three dimensional extract test

Inoculate a heavy inoculum of bacteria (10-15mg weight) in 2 ml of sterile pepton water add cox (cloxacillin) and incubate at 37^0C for 24 hours. Freeze and thaw the cell pellet for 5 time.then prepared inoculum of *Escherichia coli* (ATCC 25922) and compare with *0.5* mcfarland turbidity standard. Swab it on MHA plate.Centrally placed cefoxitin (30µg/disc) .Get a slit beginning 5mm from edge of disc in out word radial direction. Dispense 25-30 microlitre of the extract in the slit .Incubate for 18-24 hr at 37°C.The plate is than observed for a zone of blunting.

Carbapenamase confirmation test for Klebsiella pneumonia, Escherichia coli and Pseudomonas aeruginosa

HODGE Test

Firstly prepared inoculum of *Escherichia coli* (ATCC 25922) and compare with *0.5* McFarland turbidity standards. Swab it on MHA plate and place meropenem disc in the cetre of the plate. Then collect the 2-3 colonies with the help of wireloop, mark a straight line peripheral to redial on media surface. Plate incubates overnight at 37^0C for 24 hours The plate is than observed for a zone of blunting.

H- Preparation of inoculums

Using a sterile inoculating loop, touch four or five isolated colonies of the organism to be tested.

1. Suspend the organism in 2 ml of sterile peptone water
2. Adjusted the turbidity of this suspension to a 0.5 McFarland standard by adding more organisms.
3. Use this suspension within 15 minutes of preparation.

Inoculation on MHA plate

1. MH agar plate (one for each organism to be tested) allowed to come to room temperature
2. If the surface of the agar has visible liquid present, set the plate inverted, ajar on its lid to allow the excess liquid to drain from the agar surface and evaporated. Plate placed in a 37°C incubator or in a laminar flow hood at room temperature until dry (usually 10 to 30 minutes).
3. Label each MH agar plate for each organism to be tested.
4. Dip a sterile swab into the inoculum tube.
5. Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid.
6. The dried surface of a MH agar plate was inoculated by streaking the swab
7. Rim the plate with the swab to pick up any excess liquid.
8. The swab was discarded into an appropriate container.
9. The plate was allowed to stand at room temperature at least 3 to 5 minutes.

Figure 7: White colonies appeared

Figure 8: A positive reaction produced by *Staphylococcus aureus* shown as bubbling

Figure 9: A positive reaction produced by *Staphylococcus aureus* was indentified by fine grains of sand or small clumps on the slide

Figure 10: No zone of inhibition was found on oxacillin disc against MRSA .

Figure 11: Light pink mucoid colonies was appeared on MacCONKEY AGAR plate

Figure 12: Pink colonies was appeared on MacCONKEY AGAR plate

Figure 13: Color less colonies was appeared on MacCONKEY AGAR plate

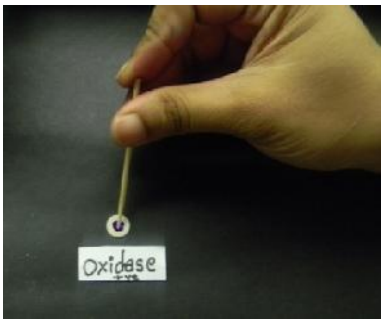

Figure 14: Purple color was appeared on oxidase disc,show confirmation of *Pseudomonas aeruginosa*

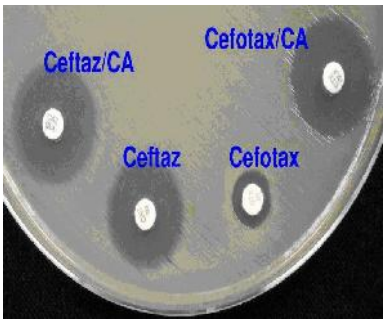

Figure 15: The results was shown differences between CAZ and CAZ+CA zone

I-Antimicrobial screening

Disc diffusion method was employed for the determination of antimicrobial activities of the essential oils. The MICs of the essential oils against the test microorganisms were determined by disc diffusion method.

Disc diffusion method:[21, 22]

Essential oils were diluted in dimithylsulphoxide(DMSO) to the test concentration (1, 0.500, 0.250, 0.125, 0.0625 µl/100µl). Antimicrobial tests were carried out by the disc-diffusion method using 1000 µl of suspension containing 2.0 x 10^5 CFU/ml of bacteria and 2.0 x 10^4 CFU/ml of fungal spores spread on Mueller- Hinton agar (MHA) in sterilized Petri dishes . The discs (10 mm in diameter, HiMedia Laboratories Pvt. Limited) were impregnated with 100 µl of the oil dilution (1, 0.500, 0.250, 0.125, and 0.0625) and placed on the inoculated agar. Negative control was prepared using the same solvent (DMSO). For use dissolve the essential oil .Streptomycin (30 µg), Vancomycin for Gram positive bacteria and colistin, polymyxin –B, and ciprofloxacin for Gram negative bacteria Nystatin (30 µg) for fungi were used as positive reference standards to determine the sensitivity of a strain of each tested microbial species. The inoculated plates were kept at 4^0C for 2 h and incubated at 37^0C for 24 h for bacterial strains, and at 28^0C for 48 h for fungi strains. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms.

Determination of minimum inhibitory concentration (MIC) [23, 24, 25]

The minimal inhibitory concentration (MIC) is the lowest concentration of volatile oil that will inhibit the visible growth of a micro-organism after overnight incubation. For the determination of MIC, which represents the concentration that completely inhibits the growth of microorganism; a disc diffusion method was used. The

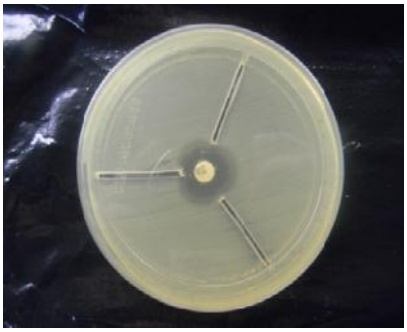

Figure 16: AmpC producing bacteria *(Klebsiella pneumonia* and *Escherichia coli)* was shown the blunting , give positive test

Figure 17: Carbapenamase producing bacteria *(Klebsiella pneumonia , Escherichia coli and Pseudomonas aeruginosa)* was shown the blunting , give positive test.

Figure 18: *Staphylococcus aureus-3 and 4*

Figure 20: *Klebsiella pneumonia (ESBL) & Klebsiella neumonia(AmpC)*

Figure 21: *Klebsiella pneumonia (Carbapenamase)*

Figure 22: *Escherichia coli3(ESBL) & Escherichia coli4(AmpC)*

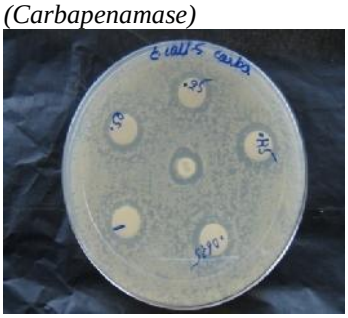

Figure 23: *Escherichia coli5 (Carbapenamase)*

Figure 24: *Pseudomonas aeruginosa(Carbapenamase) 1 & 2*

dilution was carried out with DMSO 10 µl , 5 µl , 2.5 µl , 1.25 µl , 0.625 µl of Essential oil were add to 990 µl, 995 µl, 997.5 µl, 998.75, 9 µl 99.375 µl of DMSO. The inoculum containing 10^5 CFU/ml bacteria was swabed on to the MH Agar plate.5 blank disc was placed on the inoculated MH agar plate and then each disc loaded with the prepare different concentration of Essential oil and in the center of the plate the positive control disc was placed. The result was observed and zone of inhibition calculated after 24 hr at 37^0Cof incubation.

Experimental design and statical analysis

Table 6*:* Antimicrobial activity of Essential oil of Sphaeranthus indicus flower heads by disc diffusion method

Tested organisms (Clinical strain)	Zone of inhibition in diameter (mm)					
	Vancomycin	Flower head volatile oil t for Sphaeranthus indicus µl/disc				
	10 µg/disc	1	0.50	0.25	0.125	0.0625
Gram +ve Bacteria Staphylococcus aureus (AMRSA producing)	17	20	19	19	18	13

Table 7: Antimicrobial activity of Essential oil of *Sphaeranthus indicus* flower heads by disc diffusion method

Tested organisms (clinical strain)	Zone of inhibition in diameter (mm)					
	colistin					
	10 µg/disc	1	0.50	0.25	0.125	0.0625
Gram -ve Bacteria						
Klebseilla pneumonia 3 (ESBL Producing)	15	15	16	16	16	16
Klebseilla pneumonia 4 (AmpC Producing)	15	14	14	16	16	15
Klebseilla pneumonia 5 (Carbapenamase Producing)	14	15	17	16	17	17

Table 8: Antimicrobial activity of Essential oil of *Sphaeranthus indicus* flower heads by disc diffusion method

Tested organisms clinical strain	Zone of inhibition in diameter (mm)					
	colistin					
	10 µg/disc	1	.50	.25	.125	.0625
Gram -ve Bacteria						
Escherichia coli 3 (ESBL Producing)	14	15	15	16	17	15
Escherichia coli 4(AmpC Producing)	14	14	14	14	15	16
Escherichia coli 5 (Carbapenamase Producing)	13	14	15	15	16	15

Table 9: Antimicrobial activity of Essential oil of *Sphaeranthus indicus* flower heads by disc diffusion method

Tested organisms clinical strain	Zone of inhibition in diameter (mm)					
	Polymyxin-B					
	10 µg/disc	1	.50	.25	.125	.0625
Gram -ve Bacteria						
Pseudomonas aeruginosa 2	12	16	15	17	15	17
Pseudomonas aeruginosa (Carbapenamase)	13	15	15	15	16	15

Table 10: Antimicrobial activity of the Essential oil of flower heads part of *Sphaeranthus indicus* by disc diffusion method (mean value of zone of inhibition)

Tested organisms clinical strain	Zone of inhibition in diameter (mm)					
	Positive control	Flower head volatile oil t for Sphaeranthus indicus µl/disc				
	10 µg/disc	1	0.50	0.25	0.125	0.0625
Staphylococcus aureus & MRSA	15	18	19	19	19	14
Klebsiella pneumonia & Klebsiella pneumonia(ESBL, AmpC, Carbapenamase)	14	15	16	16	17	16
Escherichia coli & Escherichia coli(ESBL, AmpC, Carbapenamase)	14	15	15	15	16	15
Pseudomonas aeruginosa & Pseudomonas aeruginosa (Carbapenamase)	13	15	15	16	16	16
Negative control	No inhibition was recorded					

Table 11: Minimum inhibitory concentration (MIC) of Essential oil of flower heads part of *Sphaeranthus indicus* by disc diffusion method

Tested organisms clinical strain	Minimum inhibitory concentration (MIC)
Staphylococcus aureus & MRSA	0.125
Klebsiella pneumonia & Klebsiella pneumonia(ESBL, AmpC, Carbapenamase)	0.0625
Escherichia coli & Escherichia coli(ESBL, AmpC, Carbapenamase)	0.125
Pseudomonas aeruginosa & Pseudomonas aeruginosa (Carbapenamase)	0.0625

This experiment was performed as a completely randomized block with three replicate (3). statical analysis was conducted using standard deviation

RESULTS AND DISCUSSION

C- Physical parameter of volatile oil of flowers of S. indicus

D- Identification and quantification of compounds present in volatile oil

In GC-MS chromatogram of volatile oil we got a total of 23 retention peaks (RT) of different compounds. This showed that a lot of compounds are present in the sample with different abundance, but for identification we preferred the 5 mass spectra of those compounds whose abundance appeared more then 90%. The selected mass spectral plots of the compounds were compared with the mass spectra of reference compounds through library search by prior spectral interpretation and previously reported literature review data.

F &G Culture of bacterial strain obtained by different patient specimen on to the different agar media and their confirmatory test

Gram Positive Bacteria

Growth of Staphylococcus aureus on blood agar

Coagulase test: MRSA (Methicillin resistant Staphylococcus aureus-

Gram Negative Bacteria

I. Growth of Klebsiella pneumonia on MacCONKEY AGAR

Growth of Escherichia coli on MacCONKEY AGAR

Growth of Pseudomonas aeruginosa on MacCONKEY AGAR

Oxidase Test

ESBL (Extended Spectrum Beta Lactamase) test

AmpC producing confirmation Three dimensional extract test

Carbapenamase producing confirmation *HODGE Test*

I- Antimicrobial screening.

Disc diffusion method

For Gram positive bacteria - *Staphylococcus aureus-*

The streptomycin positive control was not show any zone on MRSA culture plate and vancomycin positive control was show zone on MRSA culture plate because vancomycin inhibit the production of beta- lactamase

For Gram negative bacteria - *Klebsiella pneumonia*

For Gram negative bacteria - *Escherichia coli*

For Gram negative bacteria - *Pseudomonas aeruginosa*

DISCUSSION

The volatile oil of flowers of *Sphaeranthus indicus* was isolated by hydro distillation technique and physical parameter were evaluated shown in table no 4. The volatile components of oil were identified by GC/MS spectroscopy showed 23 components (Table No 5) and the major 5 compound were identify whose abundance appeared more than 90% and the MASS spectral plots of these compounds were compared with the spectra of the reference compounds through library search by prior spectral interpretation and on the basis of review of literature and were identified as 10-ep-γ eudesmol, 2,5-dimethoxy-p-cymene,Longifoene,Selin 11-en-4α-ol, 2 pentadecanone, 6,10,14,tri methyl (Figure No. 1 to 5). Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens. *In vitro* studies in this work showed that the essential oils inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of *Sphaeranthus indicus* essential oils has been previously reviewed and classified as strong, medium or weak. In our study, *Sphaeranthus indicus* essential oil exhibited strong activity against the selected bacterial strains. The anti-bacterial activity of *Sphaeranthus indicus* essential oil was carried out against 8 bacterial species and 2 fungi . The results revealed that the *Sphaeranthus indicus* essential oils showed antibacterial activity with varying magnitudes.The zone of inhibition above 7 mm in diameter was taken as positive result. Generally most of the tested organisms were sensitive to *Sphaeranthus indicus* essential oil. Essential oil showed antibacterial activity against one or more bacteria.*Sphaeranthus indicus* essential oil showed maximum activity against the *E.coli* bacterial species . Both gram-positive and gram-negative bacteria were sensitive to the *Sphaeranthus indicus* essential oils. In general *Sphaeranthus indicus* essential oil showed significant inhibitory effect against *P. aeruginosa* (16 mm), K. *pneumoniae* (17.5 mm) and *S. aureus* (19 mm), . *serratia marcescens(16mm), Escherichia coli(16mm) and highly effective on MRSA and candida spp.* No obvious difference in susceptibility was found between gram-negative and gram-positive bacteria. There was no inhibition of growth with the vehicle control (DMSO). Minimum inhibitory concentration (MIC) for *Sphaeranthus indicus* essential oil from 0.125 to 0.0625µl/ml. The results obtained in the present study indicate that the selected plant may possess high therapeutic value. They can be exploited for discovery or development of new therapeutic agents. We also conclude that these finding will contribute to the new source of economically important material with high pharmacological activity of phytoconstituents present and evaluated in these plants selected for present study.

ACKNOWLEDGEMENTS

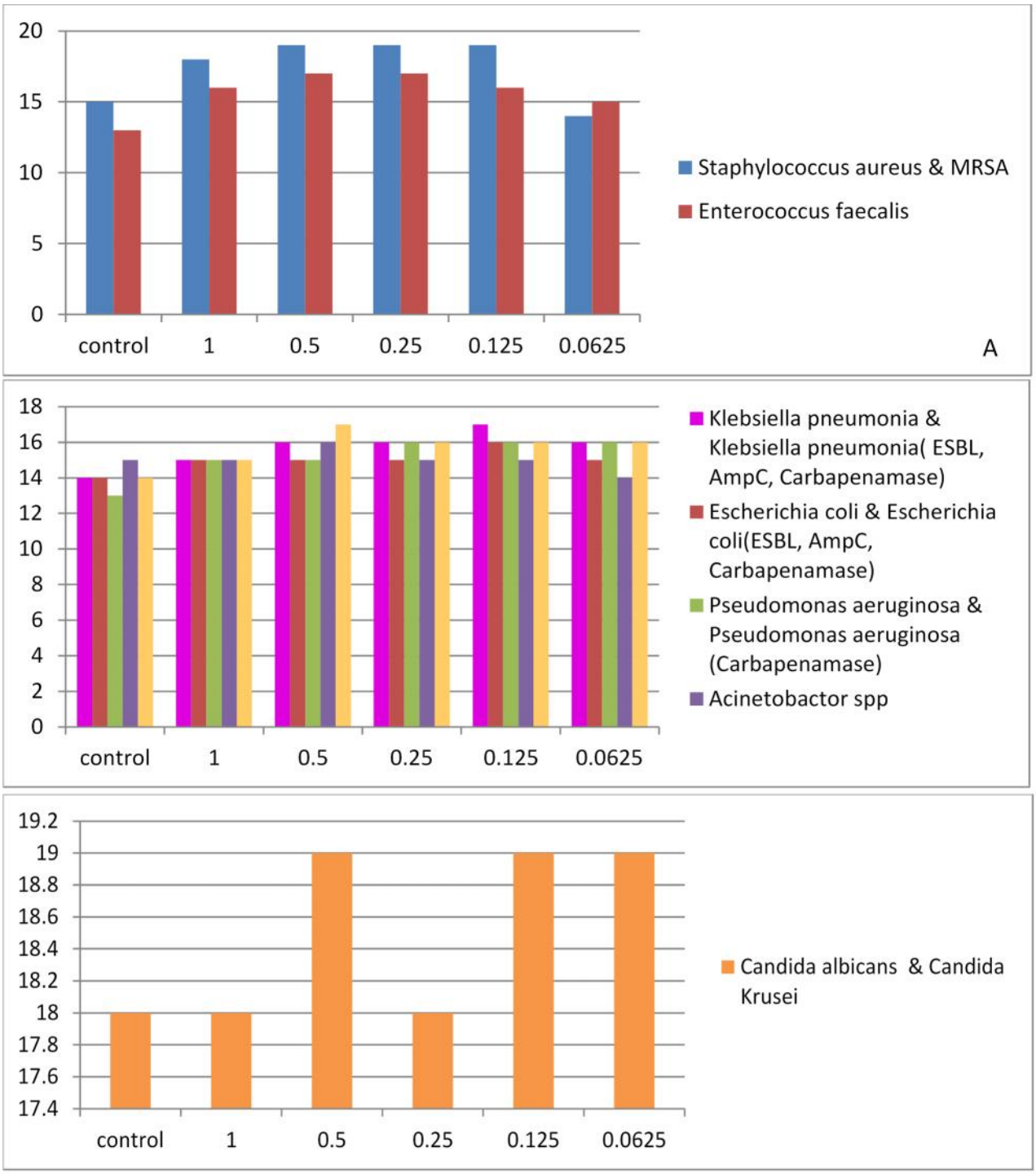

Figure 25: Antimicrobial activity evaluation of the essential oil *Spherenthus indicus* using agar disc diffusion method. Dilutions (1, 0.50,0.25,0.125,0.0625); (a) *S. aureus*, and *Enterococcus faecalis* (b) *Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, Acinetobactor spp and serratia marcescens*(c) *Candida albicans & Candida Krusei*

The author are thankful to the Department of Pharmacy Barkatullah University Bhopal ,School of studies in pharmaceutical sciences jiwaji university Gwalior. and dept. of microbiology, Bombay Hospital, Indore. Bombay hospital Indore.we are also thankful to SICART Anand Gujrat.

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