Original Research Article

Isolation of Antibacterial compound from *Ocimum sanctum* (Tulsi) against enteropathogenic bacteria

ABSTRACT:

Hexane, petroleum ether, chloroform, ethyl acetate, acetone and methanol extracts of Tulsi (*Ocimum sanctum*) were analyzed for determination of antibacterial activity against enteric-pathogenic bacteria *Escherichia coli*, *Shizella dysenteriae*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Vibrio cholerae* using agar well diffusion method. Only acetone extract of all of them exhibited an inhibiting effect on the development of the tested strains. By using the disc diffusion method, the chosen pathogenic bacteria were examined for their pattern of antibiotic susceptibility. ~~Among all, only acetone extract had the inhibitory effect on the growth of tested strains. The selected pathogenic bacteria were tested for antibiotic susceptibility pattern by disc diffusion method.~~ A comparison of antibiotics and acetone extract was performed. It was discovered that ~~A comparative study was done between the antibiotics and acetone extract. It was observed that~~ acetone extract found to be remarkable sensitivity against the test pathogens, total 37 fractions were obtained from the fractionization of acetone extract through column chromatography and found 4 major and 13minor compounds were obtained through Thin Layer Chromatography method. Antibacterial activity of different fractions was determined by spot assay technique. The fractions 1, 7, 13, and 21 demonstrated the greatest antibacterial activity when tested against the test pathogens out of all the fractions collected. Octadecanoic acid and methyl ester were found in the fraction number 13 according to the results of the Gas Chromatography Mass Specrometry. ~~From all the fractions obtained fraction number 1, 7, 13, 21 showed maximum antibacterial activities against the test pathogens. The Gas Chromatography Mass Specrometry of the fraction number 13 exposed that it contained Octadecanoic acid, methyl ester.~~ Stearic acid methyl ester obtained from the acetone extract of *Ocimum sanctum* has been recommended for human trials against various bacterial infections. ~~It is suggested that Stearic acid methyl ester isolated from the acetone extract of *Ocimum sanctum* can be recommended for human trials against different bacterial pathogens.~~

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Key Words: Compound isolation, *Ocimum sanctum, E. coli, S. dysenteriae S. typhi, P. aeruginosa and V. cholera.*

Introduction

"Tulsi is the medicinal plant and three types of Tulsi are found in India; the green leafed (Sri or Rama Tulsi) is the most common; the second type (Krishna Tulsi) bears dark to purple leaves and the third type is a forest variety (Vana Tulsi) that often grows in wild. India is the richest source of medicinal plants, which constitutes an effective source of both traditional and modern medicine. Our rural population has great faith in herbal medicine; about 80% of the population depends on it as their primary health care" (Maimes, 2004). (Bradu,2000) found that "among the enormous number of medicinal plants, members of Genus *Ocimum sanctum* belonging to the

Family Lamiaceae is a group of aromatic shrub distributed mainly in tropical and warm temperate regions of the world". "Among the 176 species of *Ocimum* identified, only six species have been reported from India, *O. gratissimum* L., *O. basilicum* L., *O. canum* L. and *O. adscendens* L. (Only four out of six have been listed). Of these only *O. gratissimum* L., *O. basilicum* L. and *O. sanctum* have been reported to have medicinal properties" (Nadkarni, 1954).

Tulsi (Holy basil or sweet basil) in Sanskrit means "one that is incomparable" one that does not tolerate or permit similarity. The extract of tulsi is commonly known for the treatment of cold, headache, stomach disorders etc (Kumar V *et al.*, 2018). "Tulsi has been recognized for thousands of years to be one of India's greatest healing herbs that nourishes a person's growth to a perfect health and nurtur for long-life. The goal of Ayurveda is to develop good health and protect the health of the healthy and alleviate disorders in the diseased person. Herbal products are produced to maintain good health and to treat disease" (Mukherjee PK *et al.*, 2017). "Tulsi has a long history of medicinal use; it is mentioned in the oldest ancient Ayurvedic Charak Samhita and also mentioned in Puranas and Rig Veda. For its religious sanctity, tulsi has been grown in Indian sub-continent" (A. Choudhary et. al., 2020). The diverse medicinal properties of tulsi such as hypoglycemic, anti-hyperlipedimic, hypotensive, anti-inflammatory and antimicrobial are mentioned in an Indian traditional medicine scripture "Charak Samhita". "The various compounds like beta-caryophyllene, derivatives of eugenol, rosmarinic acid, vanillin, gallic acid, ursolic acid and vanillic acid are present in tulsi leaves" (A. Choudhary *et. al.*, 2020). "The Tulsi has ties with the Hindu God Vishnu and his worship. Tulsi is grown in every house of Hindus in India and the leaves of Tulsi are used in the routine worship. Similarly, the rosary beads made from the stem of Tulsi plant is used for prayers and meditation" (Maimes, 2004).

"About 85% population of the whole world partially or wholly is dependent on herbal medicines for the treatment of primary health related issues. According to traditional medical system, herbal medicines are the major remedies" (Raghav PK & Saini M 2018). "*Ocimum* is used in medicine to treat different diseases of respiratory tract infection, diarrhea, headache, ophthalmic, skin diseases, and pneumonia, also as a treatment for cough, fever and conjunctivitis. Methanol extract an aqueous suspension of *O. sanctum* leaves have been shown to exhibit anti-inflammatory activity against carrageenan induced paw edema in rats and analgesic activity. Antihistaminic, antispasmodic and resistant stress induced cholesterol lowering activities of *Ocimum sanctum* are available" (Singh, 1996). "In addition to therapeutic significance of *O. sanctum* reports on their antimicrobial and antibacterial varieties of *Ocimum* species against different pathogenic bacteria i.e. *E. coli*, *Shigella* sp. and *Salmonella typhi* etc. were reported by" (Elujoba, 2000). Tulsi contains vitamins A, C and also four marker compounds, *viz.*, eugenol, luteolin, ursolic acid and oleanolic acid in the leaf of green and black varieties of *O. sanctum.* One important component of Tulsi, eugenol was shown to lower blood levels of stress hormone in rats.

The aim of the present study was to compare the activity of extract obtained from fresh and dried leaves of *Ocimum sanctum* against clinical isolates of enteric bacteria, identify the antibacterial compound and compare the sensitivity of enteric pathogenic bacteria against antibiotics and extract.

Materials and Methods

Test organism: The test organisms in the study were obtained from the culture collection bank of the Department of Microbiology and Microbial Technology, SHUATS, Prayagraj, India. The enteric pathogens are given below (Table-1).

Table-1: Microbial culture from culture collection bank

S.No.	Test Organism	Culture Number
1	*Escherichia coli*	MCCB0017
2	*Salmonella typhi*	MCCB0038
3	*Shigella dysenteriae*	MCCB0042
4	*Pseudomonas aeruginosa*	MCCB0034
5	*Vibrio cholerae*	MCCB0049

Preparation of extract: Fresh leaves of *Ocimum sanctum* were collected and wash with distilled water and air dried. Twenty gram of powder was transferred in to the clean conical flask, 100 ml hexane added to it, left for 24 h and the extract was filtered, collected and dried at room temperature. The residue of the *Ocimum sanctum* powder dried and dispended in the petroleum ether. The extraction process was followed with petroleum ether, chloroform, ethyl acetate, acetone and methanol in a similar manner.

Antibacterial activity of *Ocimum sanctum* Extract: Antibacterial activity of Ocimum sanctum extracts was tested using Agar well diffusion technique as described by Agarry *et al*. (2005). For this melted and cooled sterile Nutrient Agar media was poured into sterilized Petri dishes and was allowed to solidify. Using sterilized cotton swabs, overnight broth culture of the test organisms was swabbed on the Nutrient Agar plates uniformly. Wells (5mm) were cut on the inoculated Nutrient Agar plates with the aid of a sterile stainless steel cork borer. About 0.1 ml of the *Ocimum sanctum* extracts were filled into each of the wells. These plates were then incubated at 37^{O}C. The zone of inhibition was recorded as the presence of antibacterial action. Each extract of *Ocimum sanctum* was tested against each organism in triplicates.

Isolation of antibacterial agents: The screening of major and minor compounds present in the extract was done by Thin Layer Chromatography (TLC) by using Silica gel G. Further the selection of solvent combination for mobile phase required to isolate the antibacterial agent through Column Chromatography by TLC.

Thin Layer Chromatography: TLC plates were prepared by pouring the slurry of silica gel G

prepared with distilled water on the sterilized glass plates and then drying at 60-80^{O}C. The plates have prepared to spot the sample with capillary. These plates were then kept in different concentrations of different solvent combinations (on the basis of polarity) in a vertical position to run the compound, the plates were dried and sprayed with H_2SO_4/ Methanol Solution (1:9v/v) and kept in oven at 60-80^{O}C for 5min to develop the TLC plates. The separated compounds on the TLC plates detected as clear spots (chromatogram). The distance of these compounds from the origin and distance of solvent front were calculated as retention factor (R_f) value (Wilson and Walker, 1997).

R_f value = Distance travelled by the solute (cm) / Distance traveled by the solvent (cm)

Thus, the total number of compounds in the particular extract as well as the extract solvent combination to isolate each compound was identified.

Column Chromatography: Column was packed with silica gel (60-120 mesh) slurry prepared in higher polar solvent and on the column the extract was loaded along with the slurry. Different concentrations of the solvent combinations were run through the column one after another till entire fraction of compound of particular mobile phase is accomplished. A different fraction obtained from column chromatography was used for analysis of their antibacterial properties (Wilson and Walker, 1997).

Evaluation of antibacterial activity of different compounds: The different fractions obtained from the column chromatography were allowed to stand at room temperature until complete evaporation of solvent and then the dried fraction was dissolved in di-methyl sulfoxide (DMSO) that were used for screening antibacterial activity against the selected pathogenic bacteria by using spot assay technique as described by Jack (1995)

Spot Assay Technique: The sterilized Nutrient Agar media was poured into the sterilized Petri dishes and was allowed to solidify and then the overnight broth culture of the selected pathogenic bacteria was swabbed on the surface of the media uniformly, with the sterile cotton swabs. The isolated dried fractions dissolved in DMSO and then spotted with the capillary. These plates were incubated at 37$\pm$1 ^{O}C for 24h. The presence of zones of inhibition was noted after 24h of incubation.

Identification of antibacterial compound: The maximum antibacterial activity against the test organisms was further identified with the help of Gas Chromatography Mass Spectroscopy (GCMS) and Proton Nuclear Magnetic Resonance (^{1}HNMR) for which the sample was sent to Central Drug Research Institute (CDRI), Lucknow. Identification of respective compound was done with the help of peak values and mass spectrum data generated during analysis (Kalsi 2006a, b).

Antibiotic susceptibility test: For conducting the Antibiotics susceptibility test the following antibiotic disc were used (Table-2)

Table-2: List of antibiotics with concentration

S.No.	Antibiotics	Concentrations

1	Ciprofloxacin	5 µg
2	Chloramphenicol	30µg
3	Gentamycin	10µg
4	Kanamycin	30µg
5	Norfloxacin	10µg
6	Oflaxacin	5 µg
7	Piperacillin	10µg
8	Streptomycin	10µg
9	Tobramycin	10µg
10	Trimethaoprim	5 µg

Antibiotic susceptibility test was done using disc diffusion method (Bauer *et al.*, 1966).

Disc diffusion method: In this method the microorganisms were swabbed on the agar plate under sterile condition and antibiotic discs were placed on the surface of the inoculated agar plate. The plates were incubated at 37 ^{O}C for 24 h after completing incubation the zone of inhibition were measured and recorded in mm then the values was compared with national committee for clinical laboratory standards (Thornsberry *et al.*, 1985) to determine the sensitivity resistance pattern of the test microorganism.

Statistical analysis: The data obtained was subjected to analysis of varience (two way classification) at 5% level of significance and interpreted accordingly.

Results and Discussion

Antibacterial activity of *Ocimum sanctum* extract against selected enteric pathogenic bacteria: The *Ocimum sanctum* extracts obtained from different solvents *viz*. hexane, petroleum ether, chloroform, ethyl acetate, acetone and methanol showed a varying degree of activity against selected gram negative pathogens (Table-3)

Table-3: Antibacterial activity of *Ocimum sanctum* extract against selected enteric pathogenic bacteria.

Test Organisms	Extracts						Results
	Zone of inhibition(mm)*						
	Hexane	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	
E.coli	0.00	0.00	0.00	11.33	13.00	0.00	S,p<005
S.dysenteriae	0.00	0.00	0.00	13.66	15.33	9.33	S,p<0.05
S.typhi	0.00	0.00	0.00	13.66	12.33	0.00	S,p<0.05
V.cholerae	0.00	0.00	0.00	8.33	9.66	8.33	S,p<0.05
P.aeruginosa	0.00	0.00	0.00	0.00	0.00	0.00	

Results	S, p<0.05	S, p<0.05	S, p<0.05

*includes well size of 5mm diameter Due to organisms: F_{cal}=12.72>$F_{tab(5\%)}$=2.4683; CD=1.577 Due to solvents: F_{cal}=47.81>$F_{tab(5\%)}$=2.1482; CD=1.866 *S means significant

While categorizing each extract against selected pathogenic bacteria, it was found that acetone showed highest antibacterial activity (15.33mm) against *S.dysenteriae* and lowest (9.66mm) against *V. cholera*. Ethylacetate extract showed maximum antibacterial activity (13.66mm) against *S. dysenteriae*, *S. typhi* and minimum (8.33mm) against *V. cholerae* while no antibacterial activity against *P. aeruginosa* (p<0.05). Methanol extract showed maximum antibacterial activity (9.33 mm) against *S. dysenteriae* and minimum activity (8.33mm) against *V. cholerae*. Using hexane, petroleum ether and chloroform extract zero zone of inhibition was found. With respect to individual pathogen, it was found that acetone showed significantly highest antibacterial activity than ethyl acetate, methanol, hexane, petroleum ether and chloroform extract. However, significantly lower antibacterial activity were observed with ethyl acetate and methanol extract respectively.

In a similar study of Perez *et al*. (1990) as adopted earlier Ahmad and Beg (2001) was used "0.1ml of diluted inoculums (10^5CFU/ml) of test organism was spread on agar plates. Well of 8mm diameter were punched into the agar medium and filled with 100µl (150mg/ml) of plant extract, solvent blanks and antibiotic (Chloramphenicol, 100µg/ml conc.) to which the test bacteria were sensitive". "It was also indicated by results that the best solvent for extracting antibacterial substances from the leaves of tulsi plant is methanol" (Tyagi M *et al*., 2021).

Antibiotic susceptibility test of the test organisms: The selected pathogenic bacteria were tested for antibiotic susceptibility pattern by disc diffusion method. All the organisms *viz*. *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Vibrio cholerae* and *Pseudomonas aeruginosa* were found to be resistant towards Kanamycin and Piperacillin (Table-2). It was observed that *Escherichia coli* showed intermediate activity towards Chloramphenicol, Ciprofloxacin and Ofloxacin while it was found to be resistant towards other antibiotics i.e. Gentamicin, Kanamycin, Norfloxacin, Piperacillin, Streptomycin, Tobramycin and Trimethoprim. *Shigella dysenteriae* showed intermediate activity only towards Chloramphenicol and was found to be resistant to all other antibiotics *viz*. Ciprofloxacin, Gentamicin, Kanamycin Norfloxacin, Ofloaxacin, Piperacillin, Streptomycin, Tobramycin and Trimethoprim. Similarly *Salmonella typhi* was found to be sensitive against Ciprofloxacin, Norfloxacin and Trimethoprim while it showed intermediate activity towards Chloramphenicol, Gentamicin, Ofloxacin, Streptomycin and Tobramycin and showed resistance towards Kanamycin and Piperacillin. *Vibrio cholera* was the only microorganism which showed resistan ceto all the antibiotics used. *Pseudomonas aeruginosa* was sensitive to Tobramycin showed intermediate activity towards Ciprofloxacin, Gentamicin, Norfloxacin and Streptomycin while it showed resistance to all other antibiotics.

Table-4: Antibiotic susceptibility of selected pathogenic bacteria.

Organisms	Antibiotics									
	C	**Cf**	**G**	**K**	**Nx**	**Of**	**Pc**	**S**	**Tb**	**Tr**
E.coli	++	++	–	–	–	++	–	–	–	–
S.dysenteriae	++	–	–	–	–	–	–	–	–	–
S.typhi	++	+++	++	-	+++	++	-	++	++	+++
V.cholerae	–	–	–	–	–	–	–	–	–	–
P.aeruginosa	–	++	++	–	++	–	–	++	+++	–

(-):resistant, (++):intermediate, (+++):sensitive
C-Chloramphenicol, **Cf**-Ciprofloxacin, **G**-Gentamicin, **K**-Kanamycin, **Nx**-Norfloxacin, **Of**-Oflaxacin, **Pc**-Piperacillin, **S**-Streptomycin, **Tb**-Tobramycin,**Tr**-Trimethoprim

Effect of Acetone extract of *Ocimum sanctum* in comparison with antibiotics against selected pathogenic bacteria: A comparative study was done between the antibiotics and acetone extract and it was observed that both showed an equal pattern of sensitivity against the test pathogens(Table-5).

Table-5: Effect of Acetone extract of *Ocimum sanctum* in comparison with antibiotics against selected pathogenic bacteria.

Organisms	Antibiotics										Extract
	C	Cf	G	K	Nx	Of	Pc	S	Tb	Tr	Acetone
E.coli	++	++	–	–	–	++	–	–	–	–	+++
S.dysenteriae	++	–	–	–	–	–	–	–	–	–	+++
S.typhi	++	+++	++	-	+++	++	-	++	++	+++	+++
V.cholerae	–	–	–	–	–	–	–	–	–	–	+++
P.aeruginosa	–	++	++	–	++	–	–	++	+++	–	–

(+++):Sensitivity, (++):intermediate, (-)-resistant
C-Chloramphenicol, **Cf**-Ciprofloxacin, **G**-Gentamicin, **K**-Kanamycin, **Nx**-Norfloxacin, **Of**-Oflaxacin, **Pc**-Piperacillin, **S**-Streptomycin, **Tb**-Tobramycin, **Tr**-Trimethoprim.

Identification of the major and minor compounds in the acetone extract of *Ocimum sanctum*: For the identification of major and minor compounds in the acetone extract of *Ocimum sanctum* various solvent combinations hexane, petroleum ether, chloroform, ethylacetate, methanol and acetone were used. On the basis of spots and retention factor (R_f) value four major and thirteen minor compounds were identified at different combinations of acetone:hexane, acetone:petroleum ether, acetone:chloroform, acetone:ethylacetate and acetone:methanol in the acetone extract (Table-6).

Table-6: Major and minor compounds detected in the Acetone extract of *Ocimum sanctum*

Extract	Major compound	Mobile phase	R_f value	Minor compound	Mobilephase	R_f value
Acetone	1	EthylAcetate:Petroleum(7:3)	0.91	1	Methanol:Ethylacetate(9:1)	0.73
	2	EthylAcetate:Chloroform(9:1)	0.90	2	Methanol:Acetone(8:2)	0.72
	3	Acetone:Methanol(5:5)	0.73	3	Methanol:Acetone(9:1)	0.70
	4	Acetone:Methanol(3:7)	0.72	4	Acetone:Ethylactate(1:9)	0.67
				5	Methanol:Ethylactate(7:3)	0.66
				6	Methanol:Acetone(6:4)	0.66
				7	Methanol:Acetone(4:6)	0.63
				8	Chloroform:Ethylacetate(2:8)	0.61
				9	Petroleumether:Acetone(4:6)	0.42
				10	Hexane:Ethylacetate(2:8)	0.42
				11	Petroleumether:Acetone(2:8)	0.38
				12	Acetone:ethylacetate(6:4)	0.33
				13	Hexane:Acetone(9:1)	0.15

Isolation of different compounds in the acetone extract of *Ocimum sanctum*: Isolation of various compounds in the acetone extract of *Ocimum sanctum* was determined by eluting in the step wise gradient solvent system by TLC technique with 10-90% Acetone with hexane (*v/v*), 80-20% acetone with petroleum ether (*v/v*), 20-80% hexane with ethyl acetate (*v/v*), 60-40% acetone with ethyl acetate (*v/v*)and 40-60% petroleum ether with acetone (*v/v*). Increasing in polarity in acetone extract, a total of 37 fractions were obtained. The fractions with similar R_f values were pooled together and labeled accordingly (Table-7).

Table-7: R_f values of acetone extract of *Ocimum sanctum* observed using different mobile phase system.

S.N.	Solvent system(Mobilephase)	R_f value	Fractionno.
1.	Hexane:Acetone(9:1)	0.15	1,7
2.	Petroleumether:Acetone (2:8)	0.38	11,12,13
3.	HexaneEthylacetate (2:8)	0.42	9,18
4.	Acetone:Ethylacetate(6:4)	0.33	20,21
5.	Petroleumether:Acetone (4:6)	0.42	23
6.	Chloroform:ethylacetate(2:8)	0.61	25
7.	Methanol:acetone(4:6)	0.63	28
8.	Methanol:Ethylacetate(7:3)	0.66	16,29,
9.	Methanol:Ethylacetate(9:1)	0.73	34
10.	Chloroform:Ethylacetate (1:9)	0.90	35
11.	Petroleumether:Ethylacetate(3:7)	0.91	36

Antibacterial activity of different fractions obtained from column chromatography: A varying degree of antibacterial activity of different extracts of *Ocimum sanctum* was done in the present study against enteric pathogenic bacteria. A high degree of antibacterial activity for the

fraction number 1, 7, 13, 21 was obtained from the acetone extract of *Ocimum sanctum* against the test pathogens.

Table-8: Antibacterial activity of different fractions obtained from acetone extract of *Ocimum sanctum.*

Extract	FractionNo.	Antibacterialactivity
Acetone	1,7,13,21	+++
	12	++
	2,3,4,5,6,8,9,10,11,14,15,16,17,18,19,20,22, 23,24,25, 26,27,28,29,30,31,32,33,34,35,36,37	–

*+++ = maximum antibacterial activity, ++ = moderate activity, – = no activity

Moderate antibacterial activity of one (fraction no. 12) was observed in the acetone extracts where no antibacterial activity was observed from there twenty three fractions (Table-8). Further, the fraction number13 with maximum antibacterial activity showed same zone of inhibition against *Escherichia coli, Shigella dysenteriae, Salmonella typhi* and *Vibrio cholera* but showed no zone of inhibition against *Pseudomonas aeruginosa.*

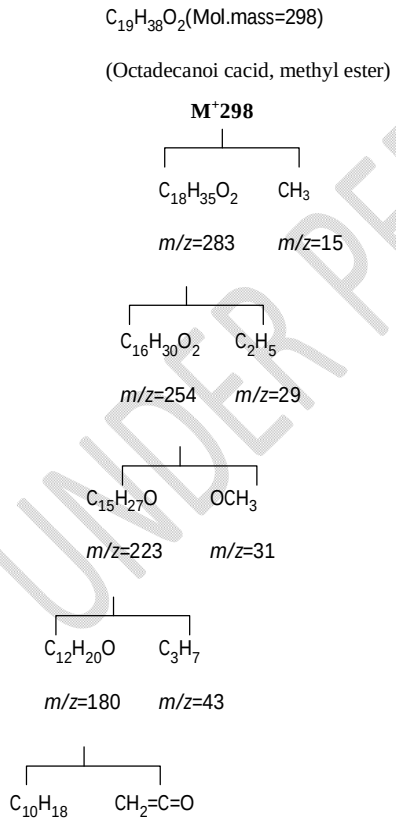
Table-9: Antibacterial compounds isolated from the acetone extract of *Ocimum sanctum.*

	TestOrganisms	Antibacterialcompou ndsAcetoneextract
	Escherichiacoli	7,13,21
	Salmonellatyphi	3,11,12,13,21
	Shigelladysenteriae	1,13,12,13,21
	Vibriocholera	7,13,11,21
	Pseudomonasaeruginosa	–

Identification of compound in the acetone extract of *Ocimum sanctum*: For the identification of the fraction number 1, 7, 13, 21 showing maximum antibacterial activity, the fraction was subjected to Gas Chromatography Mass Spectrometry (GC-MS) by diluting them in Acetone having a dilution factor of 10^{-6}. The range of spectrogram were observed to be from 40-299, hence the peaks having m/z ratio less than 40 were not visible. Moreover smaller molecules like $-CH_3$, $-C_2H_5$ and $-C_3H_7$ are so facile that the molecular ion peak was observed in the spectrum.

GCMS of fraction number 13: The molecular ion identified by the final peak in the mass spectrogram at m/z =298 undergoes fragmentation with the removal of $-CH_3$ molecule (mol.mass=15) giving rise to the formation of a $C_{18}H_{35}O_2$ molecule (mol.mass=283) due to which a peak at m/z=283 was observed (Fig.-3). In the second step $C_{18}H_{35}O_2$ again gets fragmented into $C_{16}H_{30}O_2$ molecule (mol.mass=254) and a $-C_2H_5$ molecule (mol. mass= 29) giving rise to the large peak at m/z=254 in mass spectrogram. Further the cleavage of the

$C_{16}H_{30}O_2$ molecule takes place giving rise to the removal of an -OCH_3 molecule (mol. mass = 31) and in the formation of a $C_{15}H_{27}O$ molecule (mol. mass = 223) showing the peak at m/z=223. In the fourth step $C_{15}H_{27}O$ molecule gets cleaved into $C_{12}H_{20}O$ molecule (mol.mass=180) and a C_3H_7 molecule (mol.mass=42) giving rise to the peak at m/z=180 in mass spectrogram.In the next step $C_{12}H_{20}O$ again gets fragmented into $C_{10}H_{18}$ molecule (mol. mass=138) and a CH_2=C=O molecule (mol.mass=42) giving rise to the small peak at m/z=138 in mass spectrogram. Finally the $C_{10}H_{18}$ molecule gets cleaved into the C_6H_9 molecule (mol.mass =81) due to the removal of a C_4H_9 molecule (mol. mass =57). Therefore a peak at m/z = 81 was observed in the mass spectral data (Fig.-1). The comparison of the fragmentation pattern of the fraction number 13 with that of Octadecanoic acid having almost the same mol. wt. (mol. mass=298) (Fig.-2) the compound was identified as eugenol as also reported previously (Nakamura *et al.*, 1999; Janine de Aquino Lemos *et al.*, 2005). The compound eugenol has been demonstrated to have both antibacterial (Naamura *et al.*, 1999) and antifungal (Janine de Aquino Lemos *et al.*, 2005) activities.



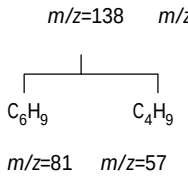


Figure-1: Schematic representation of the fragments obtained in the mass Spectrum of fraction number13.

However, in similar study the eugenol compounds have antifungal activity was isolated and identified. The antifungal activity of eugenol was evaluated against aspecies of *Alternari* from tomato and *Pennicilium chrysogenum*. The unmatched data of fraction could be due to the effects of the solvent used during the analysis of the sample. Further, slight variations in the peaks are observed and are within the acceptable limits. The higher range of mismatch in the sample due to various handling and processing techniques.

Figure-2: Mass Spectrum of Stearic acid, Methylester or Octadecanoic acid, Methylester.

Figure-3: Structure of Stearic acid, Methyl ester or Octadecanoic acid, Methylester.

The leaves of *Ocimum sanctum* were dried and powdered. The powder was thus subjected to solvent extraction *viz*. hexane, petroleum ether, chloroform, ethyl acetate, acetone and methanol. Further, the antibacterial activities of these extracts were performed by agar well diffusion method against the five selected enteric pathogen. Moreover, the antibacterial susceptibility pattern was determined by disc diffusion method against the selected test organisms, thereafter a comparative study was done between the extract and the selected antibiotics. The extract showing maximum activity by agar well diffusion method was further subjected to thin layer and column chromatography and the fractions thus obtained were evaluated by spot assay technique to determine the antibacterial activity. Fraction number 13 was identified as Octadecanoic acid, Methyl ester or Stearic acid, Methyl ester (Figure 2&3).

Conclusion

Many microorganisms, which cause damage to human health, exhibit drug resistance due to inadequate use of antibiotics. Thus there is need for the discovery of new substances from natural sources including plants a high proportion of health problem in developing countries like India has become a global concern. Resistance, too many antibiotics has created immense clinical problem in the treatment of many major diseases. Thus this situation has compelled for the search of new antimicrobial compounds that could be used as an alternative remedy for the treatment of infectious diseases. Hence, as per the result observed in the present study *Ocimum* sanctum has shown promising efficacy. Since the compound extracted is reported to have a number of advantages on human health, it can be concluded that this particular medicinal plant could be recommended for treatment over various human diseases and can also be used in the medicinal purposes.

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