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COMPARATIVE STUDY OF ANTIBACTERIAL ACTIVITY BETWEEN SELECTED INTERNATIONAL AND INDIAN ESSENTIAL OILS AGAINST SELECTED PATHOGENIC BACTERIA

Medical Microbiology

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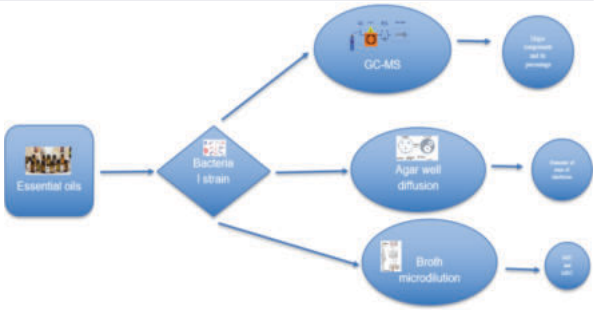
ABSTRACT

Plant extracts are used to make essential oils that include bioactive components with antioxidative and antibacterial activities that have been shown. This study looked at the effects of a number of commonly used essential oils in micellar and hydrophilic extract on some of the most frequent pathogenic microbes. The most common bioactive compounds found in these Essential oils were found to be Eugenol, Eucalyptol, cineole, and terpinene using GC-MS analysis. Essential oils of clove, lavender, tea tree, eucalyptus, and OnGuard(blended) were evaluated against different multi drug resistant pathogents families of Staphylococcaceae, Enterococcaceae, Enterobacteriaceae and Pseudomonadaceae from India and abroad. Using agar well diffusion and Microbroth dilution techniques, the minimum inhibitory concentration (MIC) and bactericidal concentration (MBC) of essential oils in aqueous and micellar solutions were determined.



Graphical Abstract:

On evaluation of all bacteria against essential oils, clove, Tea-Tree, and on guard (blended Essential oil) were the most active. Uv spectrophotometry revealed that the oil samples were approximately 90-95 percent pure with Tea Tree and lavender being ionically neutral. The absorbance ranged from the value of 3.0 -4.0 for the 200-400 nm wavelength. Zeta potential findings indicated a maximum value of -51.4 mV for on guard essential oil to 0 mV for Lavender India. The current findings could lead to the development of formulae including colloid or micelle suspensions of entire essential oils such as clove, OnGuard, or Tea-Tree oil, which could aid in antimicrobial activity.

KEYWORDS

MIC, MBC, GC-MS, ZOI, Antibaterial Activity, Essentail Oils

INTRODUCTION:

Essential oils are highly volatile liquids obtained from plants through various chemical extraction methods. Hydro distillation is one of the frequent ways to purify volatile oils along with ,hydro diffusion, and solvent extraction. Terpenes (mainly monoterpenes and sesquiterpenes), terpenoids (oxygenated chemicals like phenols, alcohols, aldehydes, ketones, or ethers), and aromatic hydrocarbons make up the majority of the composition. Some of these substances are water insoluble, whereas others are hydrophobic. Terpenes may have some water solubility despite their hydrophobic characteristics, depending on their structure and mixing temperature. Terpenoids have a higher water solubility than terpenes. Essential oils have formed a wide range of traditional medicines and blends in Asian countries. Because of their low toxicity, accessibility of availability, and commercial feasibility, essential oils have attracted interest from all around the world. As a result of this many manufacturing companies have risen in the essential oil market. Despite the fact that essential oils have antibacterial properties, little is known about their basic elements and mechanisms of action. Essential oils have already been used successfully in the treatment of a variety of conditions, including pain relief linked to chronic conditions or medical procedures, postsurgical dizziness reduction, and the autonomic response to pain for possible symptom relief in cancer patients.

Natural essential oils have the potential to be a formidable weapon against bacteria, viruses, and fungi, among other ailments. The goal of this research is to learn more about essential oils' antibacterial properties. A more effective antibacterial blend could be developed as a result of this research.

Furthermore, essential oils could be used as disinfectants and air purifiers as these can be dispersed as aerosols as well. In this present time these could prove to be an important tool in fighting the pandemic.

This research looked at the impact of some essential oils that are frequently utilized on very habitual multi drug resistant pathogens.

BACTERIUM	ESSENTIAL OILS
Klebsiella pneumoniae	On - Guard
Acinetobacter baumanii	Clove
Enterococcus spp	lavender
Pseudomonas aeruginosa	clove
Staphylococcus aureus	Tea tree
E coli	On - Guard

Case Study:

Essential oils have been traditionally used as an antimicrobial agent. However, their mechanism of action has not been studied well. We hypothesize that these oils may have some lysing activity and even work at cellular level too on pathogens. Further these essential oils could provide an alternative to antibiotics thereby reducing antibiotic resistance due to excessive usage.

OBJECTIVE:

- To determine the antibacterial ability of aetheroleum using micro broth dilution method by evaluating MIC and MBC.
- To determine the sensitivity and susceptibility of bacteria to essential oils by microbroth dilution and agar well diffusion method.
- To determine the major components of the essential oils and its percentage by GC-MS.

METHODOLOGY AND MATERIALS:

The tea tree, on guard, clove, eucalyptus, and lavender essential oils utilized for this study were procured from a company organic harvest in India and US based company Doterra. Plant based essential oils were excerpted employing steam distillation method. This study was conducted at SRM Medical and Research Centre.

Study Design:

A Centurion GC machine 5800 accompanied by the shamrock 163 ion mass spectrometry using a funicular glide generation 3 manner amplifier has been used for analysing the percentage of components present in the oil using these equipments. The funicular column having dimensions (35m 0.5mm 0.75m) was involved. The calefaction of GC microwave has been scheduled to ascend from 60 to 300 levels Celsius at a price of 3 levels Celsius according to minute for 15 mins. At 16.255 psi, helium has been used as a carrier fuel (consistent strain mode). 5µl of the oil was interjected, a car-injection gadget (centurion 5800 collection Interjector) - turned into used. The make-up less mode became used to assess the pattern. The injector temperature become three hundred ranges Celsius, and the detector temperature became three hundred degrees Celsius. MS facts have been acquired inside the exposure indicator method using a test variety of 40–400 mass to charge ratio, a make-up calefaction of two hundred degrees Celcius, a calefaction of one hundred fifty C, and left for an interval of 4 minutes. The components inside the process had been determined with the aid of analysing their retention time and indices with true standards from the NIST11 databases.

Then the antibacterial susceptibility testing was done using a modified agar well diffusion method. The anchorites which were defiant to the antibiotic amikacin were identified. In this research, six highly virulent pathogenic strains were used. six organisms isolated from clinical sample was used which belonged to bacillus and coccus family of gram negative and gram-positive bacteria. The frequently secluded pathogens were represented by bacterial strains belonging to patient infections.

For antibacterial screening of essential oils, the Ouchterlony test in Mueller-Hinton agar plates was used. In peptone water, the isolated bacteria were inoculated for 20 minutes at 4 degrees Celsius in the refrigerator. Utilising formalised technique media was prepared using Mueller Hinton agar and distilled water and poured into Petri plates and media plates were obtained after 24 hours. Essential oils were primed in an organosulfur solvent DMSO(C2H6OS) at a concentration of 20µg/ml. In the inoculated media, four wells of 8mm diameter were bored. Each well got 20 µl volume of oils, along with an insertion of amikacin (disc) which acted as the positive control & a negative control of Millipore water. The dispersion of oils in the well lasted around 30 minutes at 25 degrees Celsius, and the incubation time was 24 hours at 37.2 degrees Celsius. Plates were observed after incubation to ensure a clear result. Plates were observed after incubation to see if there was a free area circling (zone of inhibition) the holes of well that pertained to antibacterial activity. The diameter of the zone of inhibition were identified as well as evaluated (mm).

ZOI for Various Concentration (in mm)

Essential oils/ Bacteria	On Guard			Tea Tree DT			Tea Tree I			Eucalyptus DT			Eucalyptus I			Lavender DT			Lavender I			Clove DT			Clove I		
	20µl	30µl	40µl	20µl	30µl	40µl	20µl	30µl	40µl	20µl	30µl	40µl	20µl	30µl	40µl	20µl	30µl	40µl	20µl	30µl	40µl	20µl	30µl	40µl	20µl	30µl	40µl
Klebsiella pneumoniae	20	22	24	27	30	33	7	9	10	15	16	17	16	17	19	5	6	8	4	5	9	16	17	18	17	19	20
Acinetobacter baumanii	23	24	25	25	30	35	21	23	24	19	20	22	15	18	19	12	17	18	12	15	17	15	17	18	16	17	19
Staphylococcus aureus	15	17	19	23	27	35	32	36	38	36	17	18	10	14	16	14	16	19	9	11	14	19	20	23	21	23	24
Escherichia coli	15	18	21	16	18	40	11	13	15	19	20	21	11	12	13	9	10	13	9	10	12	16	17	18	20	21	24

Bacteria/Essential oil	Conc.	Klebsiella pneumoniae	Acinetobacter baumanii	Staphylococcus aureus	Escherichia coli
On Guard	20µl	20	23	15	15
	30µl	22	24	17	18
	40µl	24	25	19	21
Tea Tree DT	20µl	27	25	23	26
	30µl	30	30	27	33
	40µl	33	35	35	40
Tea Tree I	20µl	7	21	32	11
	30µl	9	23	36	12
	40µl	10	24	38	15
Eucalyptus DT	20µl	15	19	16	19
	30µl	16	20	17	20
	40µl	17	22	18	21
Eucalyptus I	20µl	16	15	10	11
	30µl	17	18	14	12
	40µl	19	19	16	13
Lavender DT	20µl	5	12	14	9
	30µl	6	17	16	10
	40µl	8	18	19	13
Lavender I	20µl	4	12	9	9
	30µl	5	15	11	10
	40µl	9	17	14	12
Clove DT	20µl	16	15	19	16
	30µl	17	17	20	17
	40µl	18	18	23	18
Clove I	20µl	17	16	21	20
	30µl	19	17	23	23
	40µl	20	19	24	24

Bacteria/Essential oil	Conc.	Klebsiella pneumonia	Acinetobacter baumanii	Escherichia coli
On Guard	20µl	20	17	28
	30µl	27	20	32
	40µl	29	22	34
Tea Tree DT	20µl	20	16	10
	30µl	22	19	22
	40µl	28	24	23
Tea Tree I	20µl	21	16	19
	30µl	27	14	23
	40µl	31	20	25
Eucalyptus DT	20µl	25	19	30
	30µl	28	23	31
	40µl	30	29	32
Eucalyptus I	20µl	22	22	16
	30µl	23	24	18
	40µl	25	28	20
Lavender DT	20µl	11	19	9
	30µl	15	20	11
	40µl	19	23	15
Lavender I	20µl	14	15	15
	30µl	17	17	16
	40µl	23	19	18
Clove DT	20µl	21	19	32
	30µl	27	22	34
	40µl	28	28	35
Clove I	20µl	20	20	38
	30µl	22	21	39
	40µl	23	24	40

The technique which was one among the antimicrobial susceptibility testing known as broth micro dilution was applied in measuring the minimal inhibitory concentrations (MIC). The Minimum Inhibitory Concentration (MIC) is described as the minimum concentration of a bacteriostatic antimicrobial ingredient or agent (averts the evident progression of bacteria). Direct dilutions of aetheroleum in the fractions of 1,0.5,0.25,0.125 and 0.06 were executed in a 96-well microplate containing Mueller-Hinton broth in two stages. Each well received 200l of 0.5 mcf bacterial inoculum. undiluted oil acts as a standard reference. For a time period of 18 hours at the calefaction of 37.5 degrees celcius the media plates were incubated. After incubation based on the turbidity the MIC was identified. The minimal inhibitory concentrations are the least dilution of the aetherpleum which can reticence the growth of bacteria. Minimum bactericidal concentration (MBC) was also determined by subculture. The MBC is the least concentration of essential oil that can kill the bacteria.

UV spectroscopy which is a frequent and economical technique applied for evaluating the optical penetration or its convergence in the colloids. The mechanism behind is transmitting a ray of light around the oil, where all the components present in the oil penetrating or transfusing gleam of specific wavelengths. Gleaming origins were necessary in the functioning of spectroscopes. Gleam origins generally differ in nature along with utilizing a broad scope of wavelengths, such as viewable spectrum, Ultraviolet, Infrared, because of various components present in oil. spectrophotometers can perform computations and analysis in relation to digital displays. When all of the parameters have been established in the instrument, the results and analysis are displayed when the method is secured. A Ultra violet visible spectrophotometer utilizes light through the ultraviolet range (285–500 nanometer) and viewable (100–800 nanometer) are the different scopes of wavelength of the electromagnetic spectrum. Whereas An Infrared spectroscope, employs gleam in the heat radiation region (750 – 10000 nm). Red light has a wavelength of 750-800 nm, while blue light has a wavelength of 450-600 nm. if the wavelength is less than 300 nm it is Ultra violet and has greater energy. The most accurate way to measure and formulate essential oils is by weight, or by drops or volume. Access the Weight and spectrophotometric conversion to access a convenient measuring calculator that converts various metric and imperial units. 20 drops of the essential oil weighed exactly 1 micro litre which weighed 20 drops from the same bottle. The process was done for all the six essential oils which are loaded into the spectrophotometer.

A dynamic light dispersion device (BI-ZTU autotitrater nanobrook zeta potential analyser) was used to evaluate the zeta potentials of EOs.The process was carried out in the temperature of 40 degrees celcius.20 µl of EOs have been mixed thoroughly with Millipore water(2ml) by vortexing. The electrophoretic mobility was evaluated with the help of Marian equation.

OIL(MICROLITRE)	MILLIPORE WATER(MICROLITRE)	VOLUME RATIO
200	200	1:1
200	400	1:2
200	800	1:4
200	1600	1:8
200	3200	1:16

Laser analysis was conducted using a white light laser, the essential

oils was taken in cuvette and fixed between the sensors which passed through a white light. The absorption and transmission were measured at the range of 100 – 1000 nm.

Then the antibacterial susceptibility testing was done using a modified agar well diffusion method. The anchorites which were defiant to the antibiotic amikacin were identified. In this research, four multidrug resistant carbapenem resistant Enterobacteriaceae pathogens were used. four organisms isolated from clinical sample was used which belonged to bacillus and coccus family of gram negative and gram-positive bacteria. The frequently secluded pathogens were represented by bacterial strains belonging to patient infections.

For antibacterial screening of essential oils, the Ouchterlony test in Mueller-Hinton agar plates was used. In peptone water, the isolated bacteria were inoculated for 20 minutes at 4 degrees Celsius in the refrigerator. Utilising formalised technique media was prepared using Mueller Hinton agar and distilled water and poured into Petri plates and media plates were obtained after 24 hours. Essential oils were primed in an organosulfur solvent DMSO(C2H6OS) at various concentration (20µg/µl , 30µg/µl , 40µg/µl). In the inoculated media, four wells of 8mm diameter were bored. Each well got 20 µl volume of oils, along with an insertion of amikacin (disc) which acted as the positive control & a negative control of Millipore water. The dispersion of oils in the well lasted around 30 minutes at 25 degrees Celsius, and the incubation time was 24 hours at 37.2 degrees Celsius. Plates were observed after incubation to ensure a clear result. Plates were observed after incubation to see if there was a free area circling (zone of inhibition) the holes of well that pertained to antibacterial activity. The diameter of the zone of inhibition were identified as well as evaluated (mm).

RESULTS:

Phytochemical constituents are chemical compounds present in trace quantities in herbs, crops, pulses, lipids and grain products. These compounds play a role in enhancing health in the body. They were intensively investigated in the remission of diseases like asthma, cardiovascular diseases, cancer, etc. Composition and structure distinguish various distinct groups such as alcohols, ketonic groups, phenolic acids, tannins, quercetin, saponins, terpenoids. The data regarding the major subgroup of the phytochemical constituents of each essential oil studied are shown forth in the table below. (Table 1)

MINIMUM INHIBITORY CONCENTRATION (µg/ml)									
ESSENTIAL OILS	CLOVE I	CLOVE DT	LAVIS	LAV DI	TEA TREE I	TEA TREE DT	EUCALYPTUS I	EUCALYPTUS DT	ON GUARD
BACTERIUM									
Klebsiella pneumoniae	0.06	0.11	0.17	0.29	0.29	0.09	0.18	0.3	0.06
Acinetobacter baumanii	0.11	0.06	0.29	0.29	0.09	0.09	0.3	0.3	0.12
Pseudomonas aeruginosa	0.11	0.06	0.17	0.17	0.09	0.09	0.3	0.18	0.12
Enterococcus spp	0.6	0.06	0.17	0.29	0.09	0.29	0.18	0.3	0.06
Staphylococcus aureus	0.6	0.11	0.29	0.29	0.29	0.09	0.3	0.18	0.12
E coli	0.11	0.11	0.17	0.29	0.29	0.09	0.3	0.3	0.06

DIAMETER IN CM									
ESSENTIAL OILS	CLOVE I	CLOVE DT	LAV I	LAV DI	TEA TREE I	TEA TREE DT	EUCALYPTUS I	EUCALYPTUS DT	ON GUARD
BACTERIUM									
Klebsiella pneumoniae	1.7	1.5	0.5	0.6	0.8	0.8	1.6	0.9	1.2
Acinetobacter baumanii	3.5	3.4	1.2	1.3	2.0	2.3	1.7	1.8	3.2
Pseudomonas aeruginosa	2.3	1	1.1	2.1	1.5	1.3	0.7	1.3	1.9
Enterococcus spp	3.5	3.4	1.0	1.1	0.9	0.7	0.5	0.6	0.9
Staphylococcus aureus	2.2	1.8	1.0	1.3	3.4	3.3	1.1	1.9	1.6
E coli	2.1	1.7	0.9	1.0	1.2	1.0	1.1	1.0	1.3

In addition, essential oils were assed for their antibacterial activity against six bacterial strains belonging to Escherichia, Pseudomonas, Acinetobacter, Staphylococcus, Enterococcus and Klebsiella which produced high levels of antibacterial activity. The study exhibited that On-guard essential oil is richest in Limonene (43.86%), while significant concentrations of Eugenol (40.84%), Eugenol-acetate (4.05%), Caryophyllene (2.38%) were found. Clove essential oil, as well as Tea tree contains a lot of favourable properties, because compounds such as eugenol and terpinen-4-ol, have high antiseptic and antifungal properties are present in clove and tea tree essential oil. Hence, they are widely used in treating various skin infections caused by bacteria and fungi. The phytochemical compounds β-linalool (8.41%), linalyl acetate (9.66%), and -alpha terpinyl acetate (11.09%) were abundant in lavender essential oil., which plays an important role in treating epilepsy and also has sedative effects. (Table2)

The phytochemical constituents of essential oil compounds were observed. All the essential oil compounds used in the study exhibited a huge range of antibacterial an activity that can be chiefly ascribed to terpinene (tea tree), limonene (On-guard), eucalyptol (Eucalyptus), eugenol (Clove) and linalool Essential oils are considered to be high in active biomolecules that have a wide range of therapeutic potential. Our study examined the antibacterial potential of six distinct essential oils, each with a different primary bioactive component, to pathogenic organisms. Kirby-Bauer standard disc diffusion study was conducted to assess antibacterial activity of various oils in our research when particularly in comparison to Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, K. klebsiella (Lavender) present in them. pneumonia, Enterococcus. Spp, A. baumanii, the pathogenic bacteria tested have been linked to the onset of a spectrum of ailments. According to our findings, all essential oils being used in our research had varying levels of antimicrobial activity when compared to harmful pathogens.

	DIAMETER (IN CM)					
ESSENTIAL OILS	CLOVE	ON-GUARD	LAVENDER D	LAVENDER C	TEA TREE	EUCALYPTUS
BACTERIUM						
Klebsiella pneumoniae	1.1	2.2	1.4	1	0.8	0.9
Acinetobacter baumanii	3.5	3.2	1.2	1.4	1.2	1.2
Enterococcus spp	0.6	0.8	1.5	1	0.5	0.8
Pseudomonas aeruginosa	1.4	1	1.5	1	1	0.8
Staphylococcus aureus	3	2.8	1.1	1.2	2	2.4
E. coli	1.5	1.7	0.9	1	1	0.9

Observed outcomes highlighted that the On-guard and Clove oils were the vast prominence among the many analysed essential oil compounds. Observed (ZOI) against E. coli was 19mm(maximum) in On-guard, K. pneumoniae was 23 mm(maximum) in On-guard, 30 mm(maximum) against S. aureus in Clove, 15 mm(maximum) against E. spp in lavender, 35 mm(maximum) for A. baumanii in clove, 21 mm(maximum) for P. aeruginoThe antibacterial ability of the essential oil compounds was examined through calculating the (MIC) minimum inhibitory concentration. From the analysed essential oil compounds in our research, On-guard is identified in displaying high antibacterial properties. Observance of the MIC with K. pneumoniae was 0.05 µg/µL, 0.11 µg/µL of A. baumanii for On-guard essential oil was the lowest and 0.29 of A. baumanii µg/µL and 0.17 µg/µL of P. aeruginosa in lavender essential oil.

DISCUSSION:

On-guard and Clove essential oil expressed high antibacterial activity against all clinical pathogens. The remaining essential oils used throughout our research expressed scarce antibacterial performance compared to test the clinical pathogens focusing on MIC results. The outcomes were consistent with research found on antibacterial activity. Further restrictive standards for activity were formed, which clearly detects that if the MIC values of 100 g/ml are obtained, Properties are characterised like much and the readings are estimated between 150 and 550 mg/ml, it is found to be active and when the value obtained are between 500 and 1000 g/mL, Low activity is termed as those whose MICs higher than 2000g/ml consider to be dormant.

Antibacterial activities of the essential oil compounds were analysed

and tested, The MICs for six different bacterial strains were identified using well diffusion and the broth dilution method. The highest antibacterial effect was expressed by On-guard and clove oil. The characteristics and mode of action for On-guard and clove essential oil's antibacterial activities in six bacterial strains have been documented. Antibacterial dynamic patterns confirmed that as the essential oil concentration was increased, simultaneously did the cell rate for apoptosis and period of the growing interphase by observing the increase in size of the diameter of zone of inhibition. On-guard and clove essential oil displayed a broad spectrum of antibacterial properties. The Its effect on K. pneumoniae (gram-negative bacteria) and on E. spp (gram-positive bacteria) were studied utilizing a range of methods.

Even though the amount of essential oil compounds indicates strong antipathogenic activity, The limited antimicrobial properties of few essential oils don't form an overall view in the use for essential oils in the mitigation of infections. Hence, more research is needed to explore their performance in hindering the bacterial activity, fungal activity, pathogenic activity, and other infectious agents.

The MS-spectra calculates the mass to charge ratio of ions present in the Eos'. The LAV(A1) indicates the absorption ratio between 0.100Abs in the range of 530nm at 23.6C due to the chemical substances present between the wavelengths. The ISO Standard 11024 sheds light ont the chemical makeup of almost seven compounds like cineole, (Z)-beta-ocimene, Amyl vinyl carbinol acetate, camphor, linalyl-3- acetate and (R)-Lavandulyl acetate.

The MS-Spectra for the LAV-DT(A2) indicates the absorption ratio increase at 0.111Abs in the range of 660nm at 23.8C. This indicates a smaller number of chemical substances present in LAV-DT than LAV(A1). The ISO Standard 11024 sheds light ont the chemical makeup of almost seven compounds like cineole, (Z)-beta-ocimene, alpha-linalool, Amyl vinyl carbinol acetate, linalyl acetate and (R)-Lavandulyl acetate.

Tea Tree is an optimized EO which is used for excessive quantitative analysis. The MS-Spectra for the Teatree(B1) indicates the absorption ratio increase at 0.012Abs in the range of 590 nm at 23.8C. Due to the presence of chemical substances with isoelectric charge. The ISO Standard 11024 sheds light ont the chemical makeup of almost six compounds like (+)-4-Terpineol, γ-terpinene, , (Z)-beta-ocimene, p-Mentha-1,3-diene, alpha-TERPINEOL, Dolcymene, and beta-pinene The MS-Spectra for the Teatree(B1) indicates the absorption ratio increase at 0.136Abs in the range of 480 nm at 23.8C. Due to less amounts of chemical substances present that affect the mass to charge ratio. The ISO Standard 11024 sheds light ont the chemical makeup of almost seven compounds like (+)-4-Terpineol, γ-terpinene, , (Z)-beta-ocimene, p-Mentha-1,3-diene, alpha-TERPINEOL, Dolcymene, and beta-pinene Eucalyptus is a Fourier transformed EO that undergoes infrared analysis. The MS-Spectra for the Eucalyptus(C1) indicates the absorption ratio increase at 0.121Abs in the range of 430 nm at 23.9C. Mass-charge ratio affected by isoelectric substances. The ISO Standard 11024 sheds light ont the chemical makeup of almost seven compounds like Eucalyptol(Z)-beta-ocimene, p-Mentha-1,3-diene, alpha-TERPINEOL, Dolcymene, and beta-pinene.

The MS-Spectra for the Eucalyptu-DT(C2) indicates the absorption ratio increase at 3.052Abs in the range of 560 nm at 23.9C. Due to the Mass-charge ratio not possessing a lot of isoelectric substances. The ISO Standard 11024 sheds light ont the chemical makeup of almost seven compounds like (Z)-beta-ocimene, p-Mentha-1,3-diene, alpha-TERPINEOL, Dolcymene, and beta-pinene Clove is a DPPH radical essential oil which possesses a natural chief chemical constituency. The MS-Spectra for the Clove(D2) indicates the absorption ratio increase at 0.130Abs in the range of 450 nm at 23.9C. Due to the Mass-charge ratio possessing isoelectric substances. The ISO Standard 11024 sheds light ont the chemical makeup of almost four compounds like 4-allyl-2-ethoxyphenol, (-)-trans-Caryophyllene, 3,7,10-Humulatriene, and 4-Allyl-2-methoxyphenyl acetate.

The MS-Spectra for the Clove-DT(E1) indicates the absorption ratio increase at 3.351Abs in the range of 770 nm at 23.9C. Due to the Mass-charge ratio not possessing isoelectric substances. The ISO Standard 11024 sheds light ont the chemical makeup of almost four compounds like 4-allyl-2-ethoxyphenol, (-)-trans-Caryophyllene, 3,7,10-Humulatriene and 4-Allyl-2-methoxyphenyl acetate.

On guard is a highly concentrated blended mixture of essential oils that is utilized under multiple analysis techniques. The MS-Spectra for the on guard(E2) indicates the absorption ratio of 0.133Abs in the range of 670 nm at 23.9C. It is extracted and blended from different aromatic organic substances from the plants.

Most of the essential oils have a negative zeta potential value because of their surface charge which interacts with the essential oil. The higher the zeta potential value the antimicrobial activity is high. Here the on guard essential oil showed higher zeta potential which showed good antibacterial activity against pathogens in agar well diffusion method. The lavender essential had zero as the zeta potential value which showed moderate antibacterial activity against pathogens in agar well diffusion method.

The laser analysis proved that the lavender essential oil (which consists of linalool) showed both absorbance and transmission peaks in the negative range.

As the concentration of essential oil was increased the diameter size of the zone of inhibition also increased proving concentration is directly proportional to antibacterial activity.

Also, the core antibiotic disk proved to be ineffective in producing antibacterial activity, whereas the essential oils prove to be effective in acting against the CRE pathogens.

CONCLUSION:

The antibacterial activities of the essential oils were analysed, tested and the MICs for six different bacterial strains were identified using well diffusion and the broth dilution method. The highest antibacterial effect was expressed by On-guard and Clove oil. The characteristics and mode of action for On-guard and Clove essential oil's antibacterial activities in six bacterial strains have been documented.

From the estimated results, It was found that On-guard oil is potent with Limonene (43.86%), while significant concentrations of Eugenol (40.84%), Eugenol-acetate (4.05%), Caryophyllene (2.38%) were found. Clove essential oil contains a lot of favourable properties, because of compounds such as Eugenol (58.71%), Eugenol acetate (29.15%), it also consists of Caryophyllene (8.04%) and p-Allylphenol (0.56%) in a very minimal amount. Tea tree essential oil majorly consists of (+)-Terpinen-4-ol (38.94%), Crithmene (20.41%) and Terpilene (10.86%) and Eucalyptol (5.31%) in a minute amount which is also a major component of Eucalyptus oil. Eucalyptus essential oil majorly consists of Eucalyptol (74.29%), L-α-Terpineol (11.84%) and β- Pinene (1.73%) and β- Phellandrene (1.1%) in a very minute quantity. Lavender oil was found to be potent in Linalyl alcohol(32.43%), and Linalyl anthranilate (25.77%), it also consists of Caryophyllene (4.22%) and Linalyl acetate (4.42%) in a minute amount, which plays an important role in treating epilepsy and also has sedative effects. The commercially available oils used studied in this project indiated a wide range of antibacterial activity, attributed to (+)-4-Terpineol, Limonene, Eucalyptol, Eugenol and Linalool present in them.

The antibacterial ability of the commercially available was examined by calculating the minimum inhibitory concentration. Among the analysed essential oils in our research, On-guard was identified to display high antibacterial activity. The observed MIC of Klebsiella pneumoniae was 0.06 µg/µL, 12 g/ L of Acinetobacter baumanii for On-guard essential oil was the lowest and 0. 17 g/ L of Pseudomonas aeruginosa in lavender essential oil. On-guard and Clove essential oil expressed high antibacterial activity against all clinical pathogens.

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