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EVALAUATION OF THE COMPONENTS AND ANTIMICROBIAL ACTIVITY OF VOLATILE OIL FROM *ZANTHOXYLUMLIMONELLA* FRUIT.

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ABSTRACT

Essential oils constitute a relatively common group of natural products present in aromatic medicinal plants. They are volatile liquids usually with pleasant and sometimes intensive odors (aroma).Essential oils are well known for its activity in lungs related diseases.They maintained the ventilation and drainage of the sinuses, had an anti-inflammatory effect on the trachea[5] and reduced asthma.The essential oil isolated from *Zanthoxylumlimonella*were proved a large number of compounds. Many compounds were detected and proved by previous workers, some of them yet to be identified and screened.Traditional usage of the plant indicates various uses even some are contradictory.In the present study showed that the oil is moderately active against gram-positive and significantly no action against gram-negative.

KEY WORDS:,**Gram-positive, *Zanthoxylumlimonella*,Gram-negative Essential oil.**

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INTRIDUCTION

Primitive people have used plants to cure a variety of human ailments. Even today, 85% of Indians use higher plants as effective anti-microbial for the treatment of various diseases (1). A large number of anti-microbial agents derived from traditional medicinal plants are available for treating various diseases caused by micro-organisms (2). They are used to eliminate the infecting micro-organisms. The therapeutically useful novel agents should inhibit the germs and exhibit greater selective toxicity towards the infecting germ than the host cells (3). The mode of action for plant-derived agent should target biochemical features of the invading pathogens that are not possessed by the normal host cell. Some of the factors important for anti-microbial treatment include methods such as the sensitivity of the infecting micro-organism to a particular agent (4). Side-effects of the plant-derived agent can be tested relative to direct toxicity upon animal cells because of their close association with human tissues or cells. So, we attempt to summarize information linked to plant extracts/chemical substances for the effective treatment of certain bacterial and fungal diseases. We also discuss the obvious necessity for new anti-microbial agents in various therapy regimens.

Recent Trends in the Evaluation of Anti-microbial

Anti-bacterial screening of traditional medicinal plants has been the source of innumerable therapeutic agents(.2). In the area of antibiotics, random screening as a tool of discovering new biologically active molecules has been most productive. Chemotaxonomic considerations and target-directed screening also play a crucial role. For a successful outcome the main requirement is access to a large number of compounds/extracts that must be well screened (5). Ethanol extracts of 78 traditional medicinal plants from India are used for treating infectious diseases and show bacterial and fungal activity at 1.6 mg/ml (6). The 50% ethanol extracts of 285 plant materials were screened for 61 biological activities and revealed effective anti-bacterial, and a wide range of pharmacological, activities (7). Anti-microbial and phytochemical studies revealed 45 Indian medicinal plants effective against multi-drug- resistant bacteria (8). These results suggest the presence of either good anti-bacterial potency or the high concentration of an active principle in the extract. Plant extracts were screened phytochemical and 20% of the species yielded positive reactions for alkaloids, 25% species contained steroids/triterpenoids and 45% of species possessed saponins (9). Those plants with anti-bacterial effects are rich in polyphenolic substances such as tannins, catechins, alkaloids, steroids and polyphenolic acids. The anti-bacterial activity also could be due to various chemical components and the presence of essential oils in adequate concentrations, which damage micro-organisms (10). The insolubility of essential oils and non-polar extracts make it very difficult for them to be used in an aqueous medium during the study of anti-microbial activity (4). A great number of factors can influence the results such as the extraction method, volume of media, culture composition and incubation temperature. However, the recent advanced method of bioautographic TLC assays makes it possible to localize anti-microbial activity on a chromatogram (11); while bioassay-guided fractions led to the isolation of compounds (12).

Essential oils

Essential oils constitute a relatively common group of natural products present in aromatic medicinal plants. They are volatile liquids usually with pleasant and sometimes intensive odors (aroma). They also are referred to as volatile oils, ethereal oils, or essences of many plants. The chemical composition is quite different from one plant to another and the main chemical component determines the aroma and its biological activities. Therapeutically, they exert a wide spectrum of biological activities such as: antiseptic (to avoid infections),

stimulant, carminative, diuretic, anthelmintic, analgesic and many others according to the chemical composition. Essential oils produced by plants have been traditionally used for respiratory tract infections, and are used nowadays as ethical medicines for colds.[1,2] In the medicinal field, inhalation therapy of essential oils has been used to treat acute and chronic bronchitis and acute sinusitis. Inhalation of vapours of essential oils augmented the output of respiratory tract fluid,[3] maintained the ventilation and drainage of the sinuses,[4] had an anti-inflammatory effect on the trachea[5] and reduced asthma.[6] Essential oils are known to possess antimicrobial activity, which has been evaluated mainly in liquid medium. Systematic evaluation of the vapour activity was first reported by Maruzzella*et al.*[7,8] and Kienholz[9] in 1959, using the inverted Petri dish technique. The technique, in which a volatile compound placed in a cup or a paper disc was exposed to the inverted agar medium plate inoculated with test strains at about 5 mm distance, was convenient and has been used by subsequent researchers.[10,11] Under these conditions, the air space was too small to measure the vapour concentration of essential oil. We employed an airtight box of 1 L air capacity for the measurement of vapour activity.[12,13] Although evaluation of cinnamon bark oil against respiratory tract mycoses has been reported.[14]

Zanthoxylum limonella

Plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (antibiotic activity) or modulating the development of vegetables (allelopathic activity)[15,16]. Apart from using in a folk medicine, the use of plant natural products as the biopesticides in crop production have been more attention for the reduction of chemical pesticides application[17,18] which including the control of weeds[19,20]. The field of this research involved the biological activities screening of Zanthoxylum limonella Alston fruits extracts. The genus Zanthoxylum belongs to the family Rutaceae[21,22]. The genus contains over 150 species and most of the species are commercially and medicinally important. Z. limonella is the one of a species that has been found in India.However the activity against microbes reported varying from place to place and many are cotraversial especially against gram -negative.

METERIALS AND METHODS

Plant material

Fresh fruits of *Zanthoxylum limonella* were collected from different parts of India Mumbai,Delhi and Kerala and was authenticated by NISCAIR delhi.

Extraction of essential oil

The fruits (300g) were steam distilled by using water for 3 hours without powdering or maceration . The distillate was collected and extracted with diethyl ether and dried over unhydrous sodium sulphate.The solvent on evaporation yielded 5.5g of oil based on fresh weight.

Antibacterial activity

Zanthoxylum limonella fruit essential oil was examined for antibacterial activity against Gram-positive bacteria Bacillus cereus (MTCC430), Staphylococcus aureus (MTCC 96) and gram-negetive such as Escherichia coli (MTCC 443) Salmonella typhimurium (MTCC 98).The antibiotic Gentamycin considered as standard as it inhibit cell wall biosynthesis.The agar diffusion methodis used for the antimicrobial evaluation.Wells of 8mm(0.8cm)diameter were dug on the inoculated nutrient agar medium with a sterile cork borer and 50micro liter of oilim DMSO(Dimethyl sulphoxide)[24,25]were added in each well.The diameter of the Zone of inhibition was measured in mm.

GC-MS analysis

GC-MS was performed with GC Clarus 500 PerkinElmer equipment. Compounds were separated onElite-1 capillary column (100%Dimethylpolysiloxane). Samples were injected with a split ratio of 10:1 with a flow rate

of helium 1 ml/min. (carrier gas). Mass detector - Turbo Mass gold- Perkin Elmer Software- Turbomass 5.1 was used as a detector. Other conditions are oven temperature up to 1100 - 2 min. hold; up to 2800 at the rate of 5deg/min-9 minutes hold. Injector temperature was maintained at 2500C.The constituents were identified after comparison with those available in the Computer Library (NIST ver.2.1) attached to the GC-MS instrument and reported

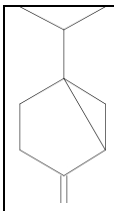
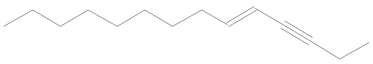
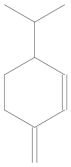
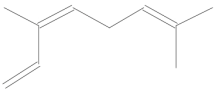
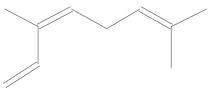
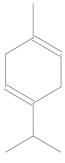
RESULTS AND DISCUSSION

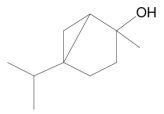
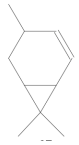
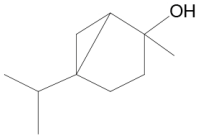
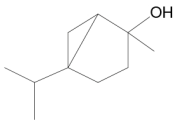
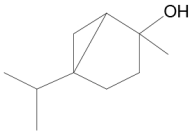
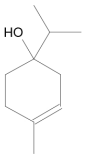
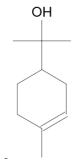
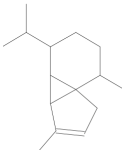
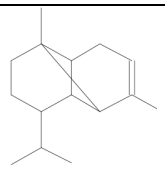
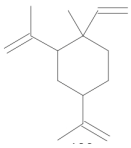
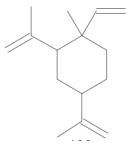
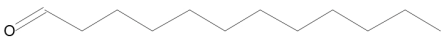
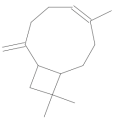
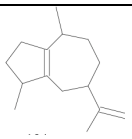
Compound detected in GC-MS

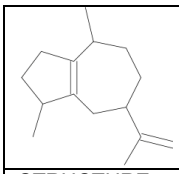
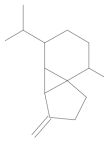
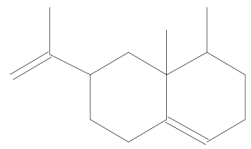
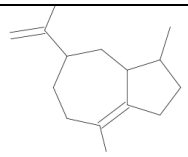
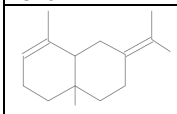
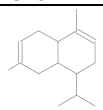
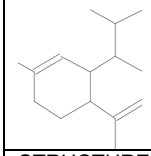
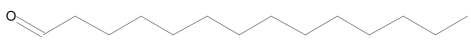
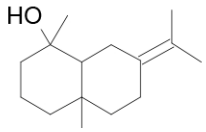
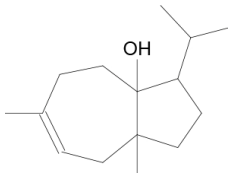
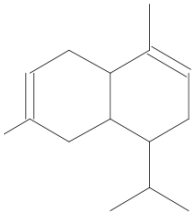
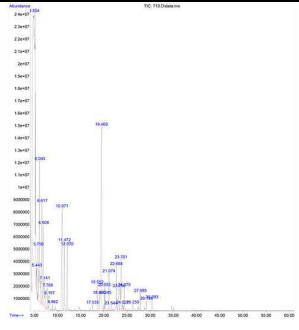
Sl.no	Compound	Mol formula	RT	Area
1	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	C10H16	4.857	48.39
2	5-Tetradecen-3-yne, (E)-	C14H24;	5.444	0.65
3	.beta.-Phellandrene	C10H16	5.757	1.52
4	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	C10H16	6.091	6.05
5	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	C10H16	6.616	3.46
6	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	C10H16	6.907	2.98
7	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-	C10H18O	7.140	1.47
8	(+)-4-Carene	C10H16	7.770	0.86
9	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-	C10H18O	8.159	0.94
10	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-	C10H18O	8.861	0.22
11	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-	C10H18O	10.971	5.25
12	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	C10H18O	11.470	2.88
13	3-Cyclohexene-1-methanol, .alpha.,.alpha.4-trimethyl-	C10H18O	12.071	2.33
14	.alpha.-Cubebene	C15H24	17.534	0.19
15	Copaene	C15H24	18.553	0.90
16	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	C15H24	18.989	**0.74**
17	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	C15H24	19.471	11.67
18	Dodecanal	C12H24O	20.092	0.68
19	Caryophyllene	C15H24	20.245	0.36
20	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,4.alpha.,7.alpha.)]-	C15H24	21.073	0.92

21	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,4.alpha.,7.alpha.)]-	C15H24	21.543	0.14
22	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha	C15H24	22.684	1.87
23	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.alpha.)]-	C15H24	23.255	1.06
24	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	C15H24	23.699	2.16
25	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)-	C15H24	24.021	0.24
26	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1.alpha.,4a.beta.,8a.alpha.)]-	C15H24	24.380	0.58
27	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,[1R-(1.alpha.,4a.alpha.,8a.alpha.)]-	C15H24	26.249	0.25
28	Tetradecanal	C14H28O	27.886	0.48
29	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1.alpha.,4a.beta.,8a.alpha.)]-	C15H26O	29.197	0.40
30	Carotol	C15H26O	30.393	0.35

STRUCTURES

	
ST RUCTURE 1 : Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- C10H16.	STRUCTURE 2 : 5-Tetradecen-3-yne, (E)- C14H24.
	
STRUCTURE 3 : .beta.-Phellandrene C10H16.	STRUCTURE 4 : 1,3,6-Octatriene, 3,7-dimethyl-, (Z)- C10H16.
	
STRUCTURE 5 1,3,6-Octatriene, 3,7-dimethyl-, (Z)- C10H16.	STRUCTURE 6 : 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- C10H16.

	
STRUCTURE 7 :Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-C10H18O	ST RUCTURE 8 : (+)-4-Carene C10H16.
	
STRUCTURE 9 :Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)- C10H18O.	STRUCTURE 10 :Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)- C10H18O.
	
STRUCTURE 11 Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)- C10H18O.	STRUCTURE 12 -Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- C10H18O.
	
STRUCTURE 13 : 3-Cyclohexene-1-methanol, .alpha.,.alpha.4-trimethyl- C10H18O.	STRUCTURE 14 : .alpha.-Cubebene C15H24.
	
STRUCTURE 15 : Copaene C15H24.	STRUCTURE 16 : Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]- C15H24.
	
STRUCTURE 17 : Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]- C15H24.	STRUCTURE 18 : Dodecanal C12H24O.
	
STRUCTURE 19 : Caryophyllene C15H24.	STRUCTURE 20 : Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,4.alpha.,7.alpha.)]- C15H24.

	
STRUCTURE 21Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,4.alpha.,7.alpha.)]- C15H24.	STRUCTURE 22;1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha C15H24.
	
STRUCTURE 23 : Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.alpha.)]- C15H24.	STRUCTURE 25 : Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]- C15H24.
	
STRUCTURE 26 : Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)- C15H24.	STRUCTURE 27 : Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1.alpha.,4a.beta.,8a.alpha.)]- C15H24.
	
STRUCTURE 28 : Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,[1R-(1.alpha.,4a.alpha.,8a.alpha.)]- C15H24..	STRUCTURE 29 : Tetradecanal C14H28O.
	
STRUCTURE 30 : 1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1.alpha.,4a.beta.,8a.alpha.)]- C15H26O	STRUCTURE 31 : Carotol C15H26O.
	
STRUCTURE 32 : Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1.alpha.,4a.beta.,8a.alpha.)]-C15H24	**TIC: 710.D\data.ms**

The results pertaining to the GC-MS analysis are given in Table 1. thirty compounds were detected in essential oil of *Zanthoxylumlimonella fruit*. The results revealed that Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-)was found as major component followed by Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-,,1,3,6-Octatriene, 3,7-dimethyl-, (Z)- werefound as the major component in the essential oil*Zanthoxylumlimonella*s. In thepresent study, the GC-MS analysis of *Zanthoxylumlimonella*es revealed the presence ofphellandrene,carotol,copaene,alphacubebene ,caryophyllene,careen,Dodecanoic cid, Tetradecanoic acid, n-Hexadecanoic acid, 9, 12, Octadecanoic acid (Z, Z) -, Oleic acid, Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester, 1,2-Benzenedicarboxylic acid, diisooctylester. Among the identified phytochemicals,Dodecanoic acid, Tetradecanoic acid and n-Hexadecanoic acid have the property of antioxidantand antimicrobial activities . n-Hexadecanoicacidhas the property of larvicidal effect[27,28]. 9, 12,Octadecadienoic acid (Z,Z) – has the property of anti- inflammatory and antiarthritic as reported by earlier worker .

Antimicrobial

The anti bacterial study of the essential oil of Zanthixylumlimonella for the organism Bacillus cereus (MTCC430), Staphylococcus aureus (MTCC 96), Escherichia coli (MTCC 443) Salmonella typhimurium (MTCC 98)were observed as,The Gram positive organisms: Bacillus cereus was inhibited for the volatile oil concentrations of 1 and 1:2 with a zone of 0.15 cm and 0.04 cm respectively. Staphylococcus aureus has given a zone of inhibition of 0.1cm and 0.05cm for the concentration of volatile oil 1 and 1:2.The volatile oil sample showed no activity against Gram negative pathogens tested.The standard used gentamycin (100µg/ml) has been giving a zone of inhibition of 1.3cm. Even though there are many controversial statements the work clearly indicates action of essential oils very moderate against gram-positive and absolute nil against gram-negetive bacteria.

OBSERVATIONS

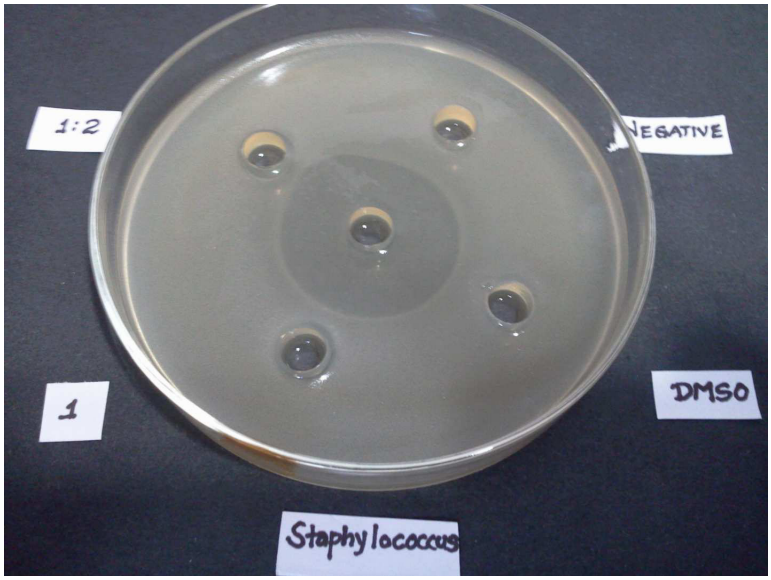


Figure1
Staphylococcus aurous

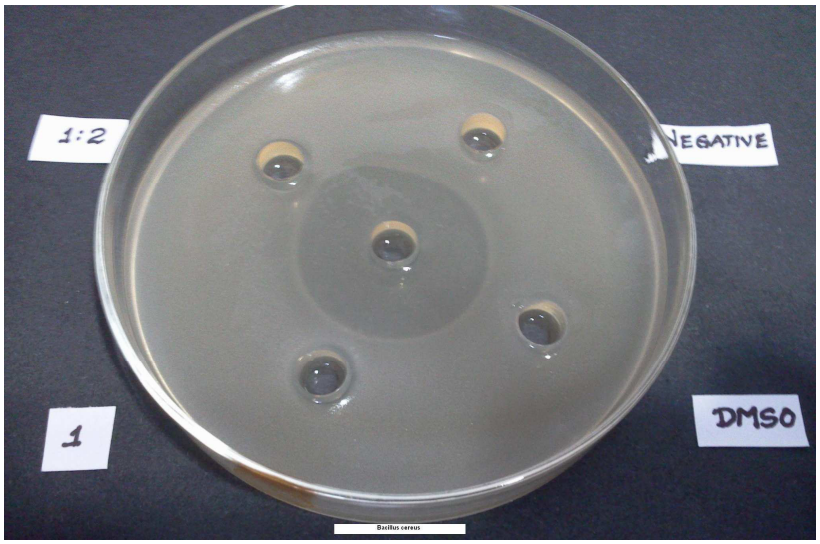


Figure 2
Bacillus cereus

CONCLUSION

The essential oil isolated from Zanthoxylum l*imonella*were proved a large number of compounds. Many compounds were detected and proved by previous workers, some of them yet to be identified and screened.Treditional usage of the plant indicates various uses even some are contradictory. Some parts of India the plat fruit is recognized as effective for tooth ache and for topical application and in many reports it was showed that it is a potential antibacterial. But in the present study showed that the oil is moderately active against gram-positive and significantly no action against gram-negative**.**

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