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Research Article

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Antibiotic Effect of Wild Olive Wood Tar Oil Growing in Albaha District, Saudi Arabia

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ABSTRACT

Screen Qutran oil and vapor of wild olive (Olea europaea subsp Cuspidate) extracted for their efficiency against some strains of microorganisms. Bacillus subtilis, MERSA (Methicillin-resistant Staphylococcus aureus), Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia. The tested materials were estimated using disc diffusion method. The efficacy of wood tar oil against activity of tested microorganisms was greater than that induced by both vapor and streptomycin. The inhibition rate of bacteria growth reached around 16.33 to 46.00mm. Pseudomonas aeruginosa was the greatest sensitive strain, after that Micrococcus luteus, whereas Staphylococcus aureus had been induced the greatest resistant strain of bacteria with growth area of inhibition 16.33mm. GC-MS analysis revealed that the Qutran contained mainly Colchifoleine, acetic acid, D-Cycloserine, Malonamic acid, Acetaldehyde and Kaempferol. All recognized agents possess antimicrobial activity.

Key words: *Antimicrobial activities, Olea europaea subsp., Wood tar oil Cuspidate, GC-MS analysis*

INTRODUCTION

In general, Wood tar oil is used as a preservative for utensils made from woods and objects against rot, as it is waterproof agent. Mid and light wood tar oils, in addition, are used for wood protection, shining and glossing agents. A diluted tar oil called “tar water” also has many applications like a flavor in candies and alcohol; the same as a flavor for food; as a perfume for saunas; as an anti-dandruff agent in shampoo and as a component of cosmetics. It is well known that bacterial infections lead to increase in the mortality rate among human populations and aquaculture organisms. Due to this and more indiscriminate use of antibiotic, pathogenic bacteria become more resistant to drugs. Likewise, because of severe unsatisfactory for these drugs, effective natural alternatives, therefore, needed with desirable side effects, e.g. extracts of those plant species known to be main source of extracting substances having antibacterial activity. There were many studies on plant extracts antimicrobial properties, e.g. [1, 2]. Moreover, no antimicrobial studies showed on oil of wood tar extracted from Olea europaea subsp. Cuspidata, especially those growing in Albaha district, Saudi Arabia. There was limited study in this respect done by [3]. The present investigation provides a recent advance for using oil of wood tar (Viscosity = 1.8689 millipoise) extracted from Olea europaea subsp. Cuspidata as bacteriostatic or inhibitor against a quantity of pathogenic bacteria.

MATERIAL AND METHODS

Collection and preparation of samples

Preparation of wood tar oil from native populations of plant Olea europaea subsp. Cuspidata was obtained during May, 2014 from the cool summit at 2242 M.A.S.L. at Albahah district, southwestern Saudi Arabia (19°59'14.12"N, 41°27'53.01"E). Species identification of the plant was carried out at Faculty of Sciences Herbarium (Serial No. 1597), King Abdul-Aziz University, Jeddah. This wood tar oil was tested for its antibacterial activity.

Extract preparation

Extraction of O. europaea subsp. Cuspidate Wood Tar oil (Viscosity = 1.8689 millipoise) was done by applying destruction distillation method.

Bacterial strains

Bacterial strains (Escherichia coli; Klebsiella pneumoniae; Pseudomonas aeruginosa; Bacillus subtilis; Methicillin-Resistant Staphylococcus aureus; Staphylococcus aureus and Micrococcus lutes) were achieved from King Abdul Aziz hospital, Jeddah city, Saudi Arabia. Different strains of bacteria were delivered by Microbiologics® USA. Then, cultured bacterial strains were tested on nutrient agar media. After that, bacterial strains were incubated at 37°c. Finally, the agar slants were maintained at 4°C.

Antibacterial action

The tested materials were calculated by using disc diffusion method as defined through [4]. Positive controller for strains of bacteria were used ciprofloxacin and streptomycin (10 mg/disc), cultures of bacteria prepared in triplicates. Then, cultures of bacteria were tested at 37°C for 24 h. As an antimicrobial standard, a concentration of 10 mg /ml of streptomycin and ciprofloxacin was used. Measurement of inhibition area is conducted to be determined against the bacterial growth [5].

Gas chromatography-Mass spectrometry analysis

Extraction of the volatile constituents:

A volume of 200ml of Wood Tar oil extracted from O. europaea subsp. Cuspidate was extracted with ether [6]. The ether extract was subjected to many processes: filtration, reduction of volume in rotary evaporator, and the ether detachability at 30°C after dehydration over anhydrous sodium sulfate. The obtained amount was run in the GC/MS spectrometer.

Investigation of the volatile constituents

The volatiles were analyzed by using GC/MS instrument. Then, it was identified constituents by matching their retention times and mass fragmentation forms using the libraries database [Wiley (Wiley Int.USA)] and the published data [7]. Computerized peak region dimensions were used to determinate quantitates.

Statistical studies

By using SPSS software, the means of parameters and standard error (M±SE) were determined to verify the significant variations among the control and the treated strains of bacteria. Each trial has three replications and three determinations were calculated.

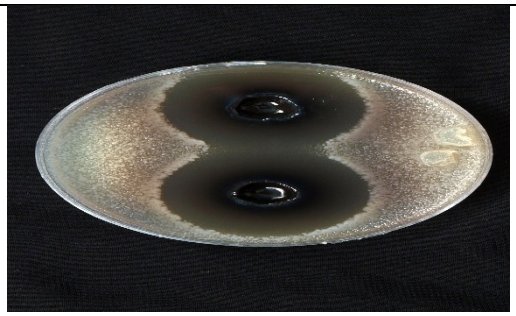
RESULTS, DISCCUSIONS,CONCLUSION

Table [1] and Figs. [1-7] showed the current data of the antimicrobial influence of the wood tar oil extracted from O. europaea subsp. Cuspidate, on four Gram + ve bacteria and three Gram – ve. using disc diffusion routine at concentrations 0f 200 mg/ml. The former includes B.subtilis, M.RSA, S.aureus and M.lutues, and the latter includes E.coli, K. pneumonia and P. aeruginosa. The extract of wood tar oil revealed greater effect on tested strains of bacteria than Streptomycin antibiotic and vapor of wood tar oil. Pseudomonas aeruginosa was greater susceptible bacteria than Micrococcus luteus strain. Mean inhibition zones diameter of the oil ether extract demonstrated on strains of tested bacteria were 46.00mm and 45.33mm, respectively (Table 1). Whereas, Bacillus subtilis being the greatest resistant bacterial strain showed 40.33mm inhibition area. Oil extracted from Olea europaea subsp. Cuspidate showed additional active advance using streptomycin antibiotic and tar oil vapor against all verified bacterial strains. The current study confirms the data of [8], who reported that the tar oil of Olea europea sylvestris possesses high anti-

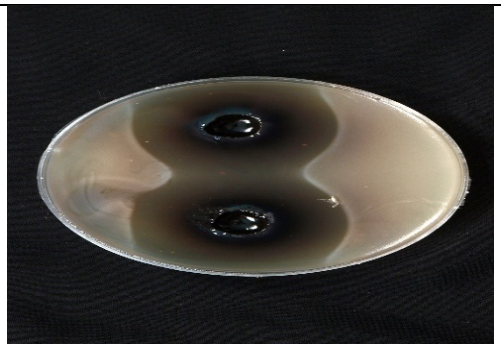
bacterial efficiency toward many of the studied bacterial strains. The rate of growth inhibition diameters alternated between 20 until 34mm. Klebsiella pneumoniae strain revealed maximum area of non-growth in 34mm diameter. [9] demonstrated the significant values of antibacterial activities of coal tar oil by using the method of agar well diffusion. The bacterial growth inhibition area was definite on different strains of microbes that showed a positive effect in the Gram stain test that higher zones of inhibition matched to the microorganisms and depicted a negative effect during the Gram stain check. Close studies were carried out to demonstrate the antibacterial influence of coal tar gel of organic solvents and they showed that it has an efficacy against pathogenic strains [10]. Pine tar was applied effectively to cure psoriasis skin diseases [11-13]. Also, valuable results were verified with wound therapeutic [14]. Investigated antibacterial actions of Qutran by using three altered methods such as the counts of colony forming unit, agar and disk diffusion assay [15, 16] wholly recognized the different Qutran extract having definitely a high drug efficacy against wide strains of bacterial diseases, where E. coli and P. aeruginosa strains showed a lesser amount of sensitiveness to the bactericidal dose of Qutran.

Gas chromatography-Mass spectrometry analysis

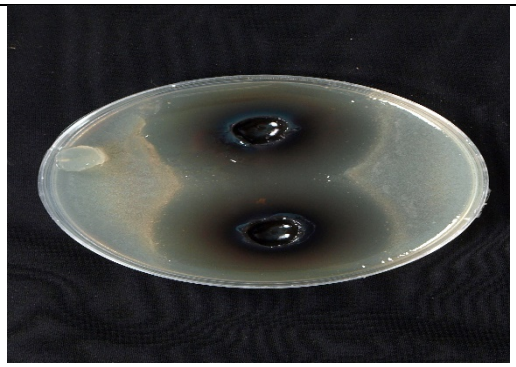
By using Gas chromatography-Mass spectrometry apparatus to analyze O. europaea subsp. Cuspidate, ether extract showed the mass spectra of the constituents on contrast with the NIST library and eleven peaks were obtained; the entire phytoconstituents were characterized and identified (Table 2). The retention times (RT) were measured by minutes. The main constituents were found to Colchifoleine, acetic acid, D-Cycloserine, Malonamic acid, Acetaldehyde and Kaempferol. Colchifoleine is well known as antioxidant and antibacterial [17]. Flavonoids are considered the essential portion of volatiles from plants. [18] Colchifoleine is commonly used as medicine and antiseptic qualities of prophylaxis to prevent plague disease. Acetic acid is used as antiseptic qualities because it has wide microbiological spectrum. Acetic acid revealed greatest effect as a bactericidal agent. At present time, acetic acid medical uses are in progress. Acetic acid established as a high efficiency bactericidal agent and, for that reason, acetic acid appears to be appropriate antiseptic qualities agent [19]. D-cycloserine ED50 (50% effective doses) shown against the Staphylococcus aureus ddl, a mutant-mediated, which infected larvae of silkworm [20]. [21] evaluated the malic acid antimicrobial effect beside different strains of bacteria in fruits extracts such as Listeria monocytogenes, Salmonella enteritidis and Escherichia coli. [22] reported the manufacturing of metabolites such as hydrogen peroxide, diacetyl, acetaldehyde, organic acids (lactic and acetic acid), acetone, ethanol, carbon dioxide, retiring, reutericyclin and bacteriocins from lactic acid microbes initiated the antibacterial efficacy of commercially important lactic acid bacteria. Because some strains of bacteria are resistant to antibiotics, there is eventual consuming acetaldehyde derived from lactic acid bacteria, mainly used as preservatives of food and alternates antibacterial effect. 4-methylcatechol is a phenolic compound confirmed as antibacterial effect beside some pathogenic strains such as Staphylococcus aureus, Bacillus cereus, Salmonella typhimurium and Escherichia coli. Moreover, [4]-methyl catechol is derived from green pepper, from other aspects, it is lacked in black pepper. And the separation and structure clarification of Kaempferol as antibacterial and/or antioxidant agents from Bryophyllum pinnatum is revealed out.



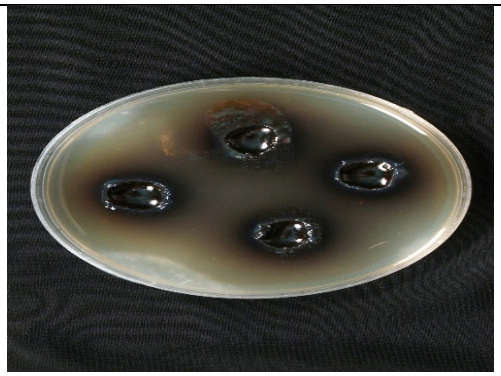
1- B. subtilius treated with wood tar oil of Oleaeuropaea subsp. Cuspidata



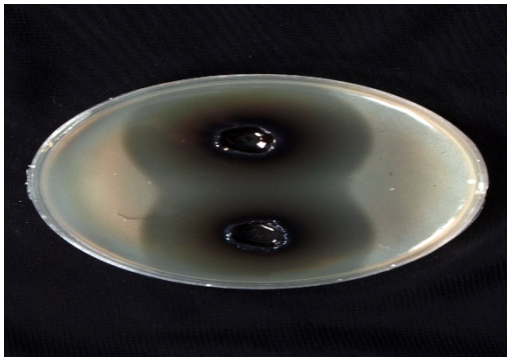
2- S. aureus treated with wood tar oil of Oleaeuropaea subsp. Cuspidata



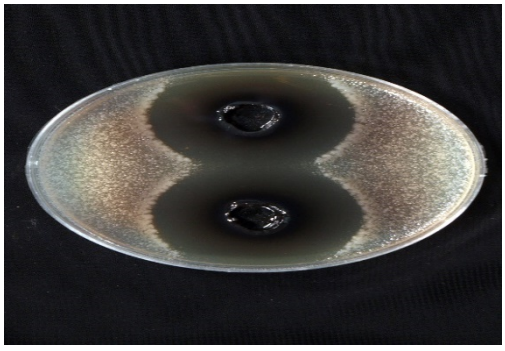
3- Micrococcusluteus treated with wood tar oil of O. europaea subsp. Cuspidata



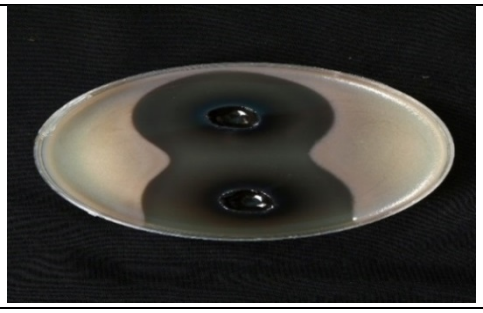
4- P.aeruginosa treated with wood tar oil of O. europaea subsp. Cuspidata



5- E.coli treated with wood tar oil of O. europaea subsp. Cuspidata



6- MRSA treated with wood tar oil of O. europaea subsp. Cuspidata



7- K.penumonia treated with wood tar oil of O. europaea subsp. Cuspidata

Figures (1-7): The effect of Oleaeuropaea subsp. Cuspidata wood tar oil on the tested bacterial strains

Table1. Antimicrobial activity of wood tar oil and its Vapor of Oleaeuropaea subsp. Cuspidata

Mean diameter of inhibition ±Standard error mean (SEM)			
Bacterial strains	Wood tar oil	Vapor	Antibiotic Streptomycin
Bacillus subtilis(ATCC11774)	40.33±0.33	21.67±0.33	25.00±00.00
MRSA(ATCC977)	42.00±0.33	29.33±0.33	23.67±0.33
Staphylococcus aureus(ATCC29213)	42.00±58.00	22.33±0.33	19.00±00.00
Micrococcus luteus (ATCC4698)	45.33±00.33	20.67±0.33	22.67±0.33
Escherichia coli (ATCC8739)	41.33±00.33	23.33±0.33	23.00±00.00
Klebsiellapneumoniae(ATCC700603)	41.67±00.33	24.33±0.33	25.00±00.00
Pseudomonas aeruginosa(ATCC27853)	46.00±0.00	27.33±0.33	22.00±00.00

Table 2: Phytocomponents identified wood tar oil of Oleaeuropaea by GC-MS**.**

Rt	**Name of the Compound**	**Molecular Formula**	**Molecular Weight**	**Area (%)**	**Activity**
8.18	Colchifoleine	$C_{21}H_{23}NO_7$	401	1.06	**Antimicrobial and antioxidant**
5.59	Acetic acid	$C_{31}H_{46}O_4$	482	1.53%	Antimicrobial and antioxidant activities
8.93	D-Cycloserine	$C_3H_6N_2O_2$	102	1.00	**Antibacterial Agents**
8.93	Malonamic acid	C3H5NO3	103	1.00	**Antibacterial Effect Against Listeria monocytogenes, Salmonella enteritidis and Escherichia coli**
62 .12	Acetaldehyde	C_2H_4O	44	3.01	**Antimicrobial Activity**
86 .22	4 Methyl CATECHOL	$C_7H_8O_2$	124	2.84	**Antimicrobial Activity**
26.82	**Kaempfero**l	$C_8H_8O_4$	168	1.37	**Antimicrobial and antioxidant activity**

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