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Antibacterial activity of extracts from selected Arabian plants against major human pathogens including multidrug resistant strains

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Abstract
The present study extraction and antibacterial activity of essential oil from eleven plants, *Tamarix aphylla L, Rosmarinus officinalis L, Peganum harmala L, Hydnora Africana L, Khaya senegalensis L, Guiera Senegalensis L, Acacia Arabica L, Hibiscus sabdariffa L, Combretum aculeatum L, Punica granatum L* and *Eucalyptus oblique L* from Jazan (Saudi Arabia) have been determined. Essential oil from this plants shows good antibacterial activity between 7 – 17 mm. *S. aureus*, *MRSA*, *E. coli*, *B. cereus* and *S. aureus* shows good antibacterial activity against to ethanolic extracted essential oils. *Acacia arabica L, Combretum aculeatum L and Punica granatum L* shows good Minimum Inhibition concentrations (MIC) and minimum bacterial concentrations (MBC). The essential oils of present study could be used as natural source of antibacterial activity.

Keywords: Antibacterial activity, Essential oils

1. Introduction
The first antibacterial, penicillin, was discovered by Alexander Fleming in 1928 [1]; since then many antibiotics were discovered and introduced into clinical practice, saving lives of millions of people who would have been killed by infectious diseases [2] Since then antibacterial resistance has grown rapidly to become a major health problem worldwide. Bacterial resistance has developed over years against antibiotics used in clinical practice with gradual emergence of strains that are resistant to multiple antibiotics, a phenomenon referred to as multidrug resistance [4].

Resistance to antibacterial agents is a growing problem world-wide. Infections with multi-drug resistant bacteria are difficult and expensive to treat and result in significant mortality, morbidity and economic burden. Among strategies proposed to control this phenomenon is to search for alternative, novel antibacterial agents from natural sources. Since plants and herbs around the world represent a huge potential source for such compounds, the objective of this study was to assess the antibacterial activity of extracts from over 32 plants and herbs from Yemen, Sudan and Saudi Arabia on major human pathogens including multidrug resistant strains.

The Arab region is rich in natural resources, including plants and herbs. However, most of these remain unexplored for their biological activities including those used in traditional medicine. With the growing problem of bacterial resistance to major classes of antibiotics, which is associated with increases in morbidity and mortality, length of hospitalization, cost of health care and limited therapeutic options, the search for alternative treatments from natural resources becomes a pressing issue.

Multidrug-resistant (MDR) bacteria presents an increasing problem in medical care [5]. Cases reported with MDR bacteria increased in number and failure of routine antimicrobial therapy has extended to cover more patients even those who are not seriously ill or complicated [6]. Important MDR bacteria include methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum cephalosporin-resistant and carbapenem-resistant *Enterobacteriaceae*, vancomycin-resistant *S. aureus* (VRSA), vancomycin-resistant *Enterococci* (VRE), *Acinetobacter* species, and *Pseudomonas aeruginosa*. While MDR infections have been mainly acquired in healthcare settings, i.e. being healthcare-associated infections (HAIs), they are also increasingly being acquired in the community. MDR infections are more difficult and expensive to treat, and result in significant mortality, imposing high economic burden on healthcare systems [6].

In Saudi Arabia MDR bacteria represents a major problem (14-16), increased morbidity and mortality is retained to several species of Gram-positive cocci and *Enterobacteriaceae* which are major nosocomial or community pathogens [17, 18]. The availability of antibiotics on shelf for the public in Saudi Arabia contributes to the spread of MDR bacteria [19]. The most frequent clinically known resistant bacteria: are extended-spectrum Beta-lactamase (ESBL) -producing Gram-negative bacilli, vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and recently multidrug-resistant *Pseudomomas aeruginosa* (MDRP) [20].

Currently, as a result of arising of those resistant organisms options for treatment with antimicrobial agents are limited, and newly introduced drugs placed on the market are decreasing, and because it is quite important problem, many efforts are directed to limit those bacteria from spreading [21]. Infection control represents the first steps towards limiting those resistant bacteria and secondly comes the introduction of new antibiotics in the market.

Many trials were aimed to introduce new antimicrobial agents from natural extracts [22]. Extracts of Echinacea purpurea (EP, purple coneflower) revealed a selective antiviral and antimicrobial activities [22]. Other trials were to test new anti-tuberculous agents extracted from garlic [23]. Many other trials were done to test Cameroonian extracts against MDR Phenotypes [24, 25]. Cranberries are known for its active action against urinary tract infections [26].

Several studies in Saudi Arabia have searched the antimicrobial effect of some plant extracts on clinical isolates [27]. Some of which were examining extracts from wild plants grown on the Saudi lands but results were moderate or totally unfortunate when tested on fungal [28] and bacterial pathogens [29]. This study aimed to Screen ethanolic extracts from selected Saudi, Sudanese, and Yemeni plants for antibacterial activity against major human pathogens including multidrug resistant strains. To determine minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of active extracts, and then to make a shortlist of plants that should be considered for further testing and phytochemical analysis to identify active components.

2. Materials and Methods

2.1 Plant material and extraction of essential oil

The plants were collected around the Jazan and Yemen border, the plants were identified by the senior plant taxonomist and the specimens were deposited in College of Pharmacy, jazan University. The plants were dried under shade at room temperature and grind by the mixer. The essential oil was extracted with 80% ethanol by using Sohxlet apparatus about 72 hrs, not exceeding the solvent boiling point. And the extract was filtered with Whatman no 1 filter paper and then concentrated with rotary evaporator. The essential oil was placed at refrigerator at 4 °C temperature until for further analysis.

3. Antibacterial activity

3.1 Source of microbial strains

The present study investigation of antibacterial activity of essential oil against to human pathogenic micro-organisms. The following micro-organisms *Staphylococcus aureus, MRSA, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, Salmonella typhimurium, Staphylococcus epidermidis, Streptococcus pneumonia, Klebsiella pneumonia and Acinetobacter baumannii* were used. The disc diffusion and microdilution method were used for the determination antibacterial activity of the oils and the components of against bacteria.

3.2 Determination of disc diffusion method

The antibacterial activity of volatile oils were investigated by agar disc diffusion method using 100.0µL of tested microorganisms contains 10^6 cfu/mL of bacteria strains spread Sabouraud Dextrose agar (SDA) medium. The extracted essential oil 20.0µL was impregnated on disc were separately and placed on the tested micro-organisms. The plates were incubated at 37 °C for 24h for bacteria strains. Each test was carried out triplet.

3.3 Microdilution test

The plant extracts will be reconstituted in DMSO at 20 mg/ml and tested against the panel of selected bacterial strains using agar well diffusion method. For extracts showing activity, minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) will be determined using the agar-dilution and micro broth-dilution methods, respectively, according to the standards of Clinical Laboratory Standards Institute (CLSI).

4. Results and Discussion

The ethanolic extracts of essential oil from border of Jazan and Yeman. This essential oil was investigated antibacterial activity according to the standard reference (CLSI) strains. The essential oils show highest antibacterial activity against to the bacterial strains. The results are given in Table.1. According to the Table.1 shows the significant results as bearing the antibacterial potential. The potential was measured in terms of zone of inhibition in mm ranging from 7 mm - 17 mm. The essential oils of *Tamarix aphylla L, Hydnora Africana L, Khaya senegalensis L, Guiera Senegalensis L, Combretum aculeatum L, Punica granatum L* and *Eucalyptus oblique L* shows no antibacterial activity with disc diffusion method. these essential oils did not affect *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus epidermidis, Streptococcus pneumonia* and *Klebsiella pneumonia,* while inhibition zones for other bacterial species are 8 to 13 mm, 9.67 to 11.33mm, 8.67 to 11mm, 9 to 11.3mm, 8 to 9 mm, 7.67 to 14.67mm, 9.67 to 13mm and 9 to 10.67 mm respectively. the oil *Hydnora africana L, Khaya senegalensis L, Guiera Senegalensis L, Punica granatum L* and *Eucalyptus oblique L* did not show any inhibition zone against to *Acinetobacter baumannii*.

Rosmarinus officinalis L oil 8 to 15mm and it was not show inhibition zone against *Escherichia coli* and *Streptococcus pneumoniae*. The oil of *Acacia arabica L* 9 to 17mm and it did not show inhibition zone against *Staphylococcus epidermidis, Streptococcus pneumoniae* and *Klebsiella pneumonia*. The oil *Peganum harmala L* 7 to 15mm shows inhibition zone against all strains.

The results of minimum inhibition and minimum bacterial concentrations are investigated and results are given in Table.2. The essential oil of *Acacia arabica L, Combretum aculeatum L, Punica granatum L* and *Hydnora Africana L* showed highest MIC (<0.39 – 1.56 µg/mL) and MBC (<0.39 – 1.56 µg/mL), <0.39 – 0.78 µg/mL and 0.78 – 1.56 µg/mL, <0.39 – 0.78, <0.39 and 3.13 µg/mL and 0.78 – 1.56 µg/mL and 1.56 – 1.56 µg/mL minimum inhibition and minimum bacterial concentration with the microdilution method respectively. Oils from *Tamarix aphylla L,* shows MIC at 0.78 – 12.5 µg/mL and MBC at 1.56 – 12.5 lowest antibacterial

activity. MIC and MBC for Rosmarinus *officinalis L and Peganum harmala L* 1.56 - 50 µg/mL and 0.78 – 3.13 µg/mL are similar respectively. other oils of MIC and MBC shows lowest antibacterial activity, oils from *Khaya senegalensis L, Guiera Senegalensis L, Hibiscus sabdariffa L* and *Eucalyptus oblique L* shows 1.56 – 3.13 and 3.13 – 6.25 µg/mL, 0.78 – 3.13 and 3.13 – 12.5 µg/mL, 1.56 and 6.25 – 12.5 µg/mL and 1.56 – 3.13 and 1,56 – 12.5 µg/mL lowest MIC and MBC values respectively.

From the MIC and MBC assays it was found that the Gram-negative species are truly considered as more resistant to crude plant extracts than Gram positive species. The potent plant extracts are supposed to be targeted as the best approach to investigate by preparing fractional extracts rather than the crude extract as a whole. Multi drug resistant strains are the big challenges in medical practice and role of natural products can never be underestimated because of their less side effects. The pure components of the mentioned medicinal plants must be focused to avoid any major interactions at molecular level.

5. Conclusion

The present study was investigated antibacterial activity against micro-organisms. Further investigations are required to know the chemical composition and antifungal activity of the essential oil and also it should be extended to know the antioxidant activity studies. Further work is necessary to explore suitable concentrations to which may be used extension of self-life, food ingredients and natural therapy and pharmaceuticals for human management.

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Table 1: Disc diffusion results for active plants in terms of zone of inhibition

Type of strain	Micro-organism	*T. a*	*R. S*	*P. h*	*H. a*	*K. s*	*G. s*	*A. a*	*H. s*	*C. a*	*P. g*	*E. o*
Standard strain	*Staphylococcus aureus*	13	15	13	11.33	11	11	14	8	14.67	13	10.67
	MRSA	11.3	12	15	9.67	8.67	11.3	13.67	7.67	11.33	12.67	9
	Escherichia coli	0	0	11.3	0	0	0	9.67	0	0	0	0
	Pseudomonas aeruginosa	0	9	7	0	0	0	9	8	0	0	0
Clinical isolate strains	*Bacillus cereus*	9.67	8.67	14	10.33	9.67	9	16.33	0	7.67	9.67	7.67
	Staphylococcus aureus	9.33	12.67	14	10.67	9.67	9.33	17	7.67	11.67	10	9
	Salmonella typhimurium	0	7.33	13	0	0	0	12	0	0	0	0
	Staphylococcus epidermidis	0	8	11.7	0	0	0	0	0	0	0	0
	Streptococcus pneumoniae	0	0	10.3	0	0	0	0	0	0	0	0
	Klebsiella pneumonia	0	8	11.3	0	0	0	0	0	0	0	0
	Acinetobacter baumannii	8	12	15	0	0	0	13.67	9	12	0	0

T. a: *Tamarix aphylla L*; *R. o*: *Rosmarinus officinalis L*; *P. h*: *Peganum harmala L*; *H. a*: *Hydnora africana L*; *K. s*: *Khaya senegalensis L*; *G. s*: *Guiera Senegalensis L*; *A. a*: *Acacia arabica L*; *H. s*: *Hibiscus sabdariffa L*; *C. a*: *Combretum aculeatum L*; *P. g*: *Punica granatum L*; and *E. o*: *Eucalyptus oblique L*;

Table 2: Antibacterial activity of essential oil (MIC and MBC in µg/mL)

Micro-organism		*T. a*	*R. S*	*P. h*	*H. a*	*K. s*	*G. s*	*A. a*	*H. s*	*C. a*	*P. g*	*E. o*
Staphylococcus aureus	MIC	0.78	1.56	0.78	0.78	1.56	0.78	<0.39	1.56	<0.39	<0.39	1.56
	MBC	3.13	1.56	1.56	1.56	3.13	6.25	≤0.39	12.5	1.56	1.56	1.56
MRSA	MIC	1.56	1.56	0.78	0.78	1.56	3.13	<0.39	1.56	<0.39	<0.39	3.13
	MBC	6.25	3.13	0.78	1.56	3.13	6.25	≤0.39	12.5	1.56	1.56	12.5
Escherichia coli	MIC	-	-	0.78	-	-	-	1.56	-	-	-	-
	MBC	-	-	1.56	-	-	-	1.56	-	-	-	-
Pseudomonas aeruginosa	MIC	-	50	3.13	-	-	-	<0.39	1.56	-	-	-
	MBC	-	50	3.13	-	-	-	0.78	6.25	-	-	-
Bacillus cereus	MIC	1.56	6.25	1.56	0.78	3.13	1.56	<0.39	-	<0.39	<0.39	3.13
	MBC	1.56	6.25	3.13	1.56	6.25	3.13	≤0.39	-	0.78	≤0.39	6.25
Staphylococcus aureus	MIC	3.13	3.13	0.78	0.78	3.13	1.56	<0.39	1.56	0.78	0.78	3.13
	MBC	3.13	3.13	1.56	1.56	3.13	12.5	0.78	12.5	1.56	3.13	6.25
Salmonella typhimurium	MIC	-	>50	0.78	-	-	-	0.78	-	-	-	-
	MBC	-	>50	3.13	-	-	-	1.56	-	-	-	-
Staphylococcus epidermidis	MIC	-	>50	0.78	-	-	-	-	-	-	-	-
	MBC	-	>50	1.56	-	-	-	-	-	-	-	-
Streptococcus pneumoniae	MIC	-	-	3.13	-	-	-	-	-	-	-	-
	MBC	-	-	3.13	-	-	-	-	-	-	-	-
Klebsiella pneumonia	MIC	-	>50	0.78	-	-	-	-	-	-	-	-
	MBC	-	>50	3.13	-	-	-	-	-	-	-	-
Acinetobacter baumannii	MIC	12.5	3.13	<0.39	-	-	-	<0.39	1.56	0.78	-	-
	MBC	12.5	3.13	0.78	-	-	-	1.56	3.13	0.78	-	-

T. a: *Tamarix aphylla L*; *R. o*: *Rosmarinus officinalis L*; *P. h*: *Peganum harmala L*; *H. a*: *Hydnora Africana L*; *K. s*: *Khaya senegalensis L*; *G. s*: *Guiera Senegalensis L*; *A. a*: *Acacia arabica L*; *H. s*: *Hibiscus sabdariffa L*; *C. a*: *Combretum aculeatum L*; *P. g*: *Punica granatum L*; and *E. o*: *Eucalyptus oblique L*; *Acacia arabica L, Combretum aculeatum L and Punica granatum L*

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