

RESEARCH ARTICLE

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Application of wild Saudi plant extracts to control antibiotic resistant *Staphylococcus aureus*

ABSTRACT:

Wild plants are always the promising sources to explore novel therapeutic agents. Kingdom of Saudi Arabia (KSA) has a wide collection from wild and medicinal plants that contains hundreds of precious species. *Staphylococcus aureus* is from the most clinically dangerous bacterial pathogens that could acquire many resistance types toward numerous chemical antibiotics. Seven wild KSA plants, e.g. *Bassia eriophora*, *Blepharis ciliaris*, *Ducrosia anethifolia*, *Pulicaria crispera*, *Rumex vesicarius*, *Tamarix aphylla* and *Teucrium oliverianum*, were extracted with 70% ethanol solution and screened for their antibacterial activity against various *S. aureus* strains, including reference, methicillin susceptible (MSSA) and resistant (MRSA) strains. Most of screened extracts exhibited remarked antibacterial activity against the entire *S. aureus* strains; the most powerful extracts were those from *B. eriophora* and *T. oliverianum*, using different antimicrobial quantification assays. These extracts were applied onto cotton textiles as finishing agents, to produce anti-*Staphylococcus aureus* fabrics; treated textiles showed powerful antibacterial activity against all examined *S. aureus* strains, including MRSA isolates. Extract-treated textiles could maintain most of their antibacterial activities after two laundering cycles. The present work showed that the antibacterial potentiality of KSA wild plant extracts could be proposed for the control of resistant *S. aureus* strains and fabrication of anti-*S. aureus* textiles.

KEY WORDS:

Antibacterial, Natural derivatives, MRSA, Textile finishing

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INTRODUCTION:

Wild plants were always the promising source to explore novel therapeutic agents due to the diversity and potency of their chemical contents, compared to synthetic agents. Kingdom of Saudi Arabia (KSA) has a wide collection from wild and medicinal plants that contains hundreds of precious species; many of these plants were characterized as rich curing agents and health promoting to fight numerous infectious diseases (Migahid, 1996; Mandaville, 1990; Harvey, 2008).

It was appraised (Rahman *et al.*, 2004) that the KSA medicinal and aromatic flora has a wide diversity (more than 1200), which consist about half of world identified species.

The emergence of microbial pathogens, with single and multiple antibiotic-resistance, was resulted from the extensive and misuse of chemical and synthetic antibiotics (Kumar and Schweizer, 2005). This terrible statement enforced the urgent need for effective alternatives rather than standard therapy of antibiotics; these alternatives should have wide spectra of efficacy, high biosafety for patients, and multiple compounds with antibiotic potentiality to work against these antibiotic-resistant microorganisms (Treadway, 1998). It was indicated that medicinal plants, principally species that are employed in folk medicine could serve as these promising alternatives to synthetic antibiotics, as they have numerous antimicrobial agents and diverse action mechanisms (Abdallah and El-Ghazali, 2013); the main step for discovering innovative antimicrobial alternatives is assumed to be the evaluation of wild medicinal plants.

The resistance of microbial strains to antimicrobial agents is multifactorial and could involve many influencing parameters, e.g. environmental factors, relationship between antibacterial agent and bacterial cell, host characteristics and the form of antibacterial application (Adwan and Mhanna, 2008).

These factors and side-effects of commercial antibiotics have urged scientists to investigate more innovative microbicidal agents originated from uncovered sources, such as wild and folkloric plants, to be

potential alternative sources for antimicrobial chemotherapies; these natural products are cost effective and generate least of adverse effects, compared to synthetic drugs (Cowan, 1999; Tayel *et al.*, 2013a).

Staphylococcus aureus is a bacterial pathogen that is well-established to acquire many resistance types toward numerous chemical antibiotics; the resistant *S. aureus* strains to methicillin and/or to its derivatives, e.g. MRSA, was regarded to be from the most aggressive causative agents for nosocomial infections; some MRSA strains could resist most clinically commercial antibiotics, thus these strains become very difficult to be challenged (Brooks *et al.*, 2007).

Staphylococcus aureus was characterized as one from the foremost causative agent for scalded-skin syndrome, food poisoning and toxic shock, through secretion of numerous dangerous toxins types (Lowy, 1998; Washington *et al.*, 2006);

Thereafter, this investigation was intended to assess the effectiveness, as potential antibacterial agents, of extracts from many KSA wild plant species against *S. aureus*, including their MRSA strains, to emphasize the most powerful species and to present potential practical applications for these antibacterial extracts.

MATERIAL AND METHODS:

Plant materials' extraction:

The examined plant materials, e.g. areal parts of wild Saudi plants, were collected from the central region desert, near Qassim, KSA. The plant species included *Bassia eriophora*, *Blepharis ciliaris*, *Ducrosia anethifolia*, *Pulicaria crispa*, *Rumex vesicarius*, *Tamarix aphylla* and *Teucrium oliverianum*. Collected plants were identified and authorized by the botany specialists, College of Science and Humanitarian Studies, Shaqra University, KSA, using a reference book for Saudi plants classification (Jubran and Hizon, 1999).

Plant materials were cleansed, washed with deionized water, dried with hot air at 40°C, then dried materials were ground using mechanical grinder and thieved to have average particle sizes of ~ 50 mesh. Weights of 250 g from individual plant powders were subsequently extracted using occasional shaking for 36 h at 120 xg, after immersion in 1200 ml of aqueous ethanol solution (70%, v/v). Filtration was then conducted to eliminate the residual plant particles and the extracts were subjected to vacuum evaporation (Büchi, Flawil, Switzerland) at 42°C; the resulted semi-dried extracts were then re-suspended in aqueous dimethyl sulfoxide solution (DMSO, 20%) to achieve final concentrations of 10% from each extract (Tayel *et al.*, 2012).

Isolation of *Staphylococcus aureus*:

Investigated *S. aureus* strains were isolated from infected skin ulcers in patients, with physician aid in Shaqra University hospital. Sterile swabs were used for samples collection then they were spread onto mannitol salt agar medium (MSA) and incubated at 37°C for 12 h; individual appeared colonies were then isolated, purified, microscopically examined and subjected to the biochemical tests for microbial identification (Barrow and Feltham, 1993). For microbial identity confirmation, isolated strains were examined using VITEK 2 highly automated System (BioMérieux Co., France), using *S. aureus* (ATCC 25923) as a reference strain for comparison. The identified strains were then challenged with standard antibiotic discs, after their spread onto Nutrient Agar (NA) plates, to evaluate their potential resistance to different antibiotic groups.

Evaluation of plants' antimicrobial potentiality:

Plant extracts were qualitatively and quantitatively evaluated for their antibacterial activities using tow assays, i.e. disc diffusion and minimal concentration for inhibition (MIC), respectively.

In the disc assay, the bacterial cell suspensions, in 0.9% saline solution, were swabbed uniformly onto solidified NA, then sterile filter paper discs (6 mm diameter) were put onto the surface of inoculated plates and impregnated with 25 µl from each plant extract. Plates thereafter were incubated for 24-36 h at 37°C and the diameter of appeared growth inhibition zones (ZOI) were precisely measured. DMSO solution was used as control for comparison.

The determination of extracts' MIC, toward bacterial strains, was conducted using the described microdilution method (Tayel *et al.*, 2010), using a concentration range from extracts between 25–300 µg/ml. The results were confirmed using triphenyl tetrazolium chloride (TTC) indicator.

Antimicrobial cotton fabrics using plant extracts:

Scoured fabrics (100% cotton, Style S/400, 106 g/m² plain texture, TESTEX, Germany) had been used in this experiment. Cotton fabrics were immersed in each plant extract (1%), with stirring for 100 min at 60°C, strictly padded, then firmly pressed using two dips and nips. Treated fabrics were then dried with hot air at 70°C for 4 min and cured at 125°C for 8 min; the fabrics were then cut to equal pieces of 1 cm² and kept in sterile bags for subsequent sensitivity assays. The durability of treated cotton fabrics was evaluated through repetitive laundering cycles, according to the American Association of Textile Chemists and Colorists (AATCC,

2000), test method 61(2A)-1996. Using a home laundry machine, with neutral water at temperature of $38 \pm 3^\circ\text{C}$, fabric pieces were treated with 2 consecutive laundering cycles and after each of them, fabrics were squeezed and air-dried (Tayel *et al.*, 2011). The antimicrobial textiles, before and after laundering cycles, were evaluated for their antibacterial activity, against *S. aureus* strains, using the inhibition zone assay by positioning the textile pieces onto inoculated NA plates, incubation for 36 h at 37°C , then measuring the appeared inhibition zones' diameters.

RESULTS:

The biochemical assays of isolated bacterial strains verified the isolation of four different *S. aureus* strains; the subsequent antibiotics susceptibility assays specified the isolates as 2 methicillin sensitive and 2

methicillin resistant strains, i.e. MSSA1, MSSA2, MRSA1, and MRSA2, respectively.

The entire isolated *S. aureus* strains and the reference strain (ATCC-25923) were subjected to qualitative and quantitative antimicrobial tests, using crude plant extracts, to elucidate their potential antibacterial activities.

The antibacterial potentialities from the extracts of examined plants, against *S. aureus* strains, was summarized in table 1. Most examined plant extracts had remarkable antimicrobial activity against examined *S. aureus* strains, the bactericidal activity varied according to the tested extract and the exposed strain. It could be claimed, regarding the appeared ZOIs diameter and MICs value, that the most powerful extracts were those of *B. eriophora* and *T. oliverianum*, whereas the weakest antibacterial extracts were from *B. ciliaris* and *D. anethifolia*, respectively.

Table 1. Antimicrobial activity of examined plants extracts against *Staphylococcus aureus* strains, measured as zone of inhibition diameter (ZOI, mm*) and minimal concentrations for inhibition (MIC, $\mu\text{g/ml}$)

<i>Staphylococcus aureus</i> strains	Assay	Extracted Plants						
		<i>Bassia eriophora</i>	<i>Blepharis ciliaris</i>	<i>Ducrosia anethifolia</i>	<i>Pulicaria crispa</i>	<i>Rumex vesicarius</i>	<i>Tamarix aphylla</i>	<i>Teucrium oliverianum</i>
ATCC- 25923	ZOI	18.1 ± 0.6	8.9 ± 0.4	10.2 ± 0.5	13.9 ± 0.6	10.9 ± 0.6	13.5 ± 0.7	17.8 ± 1.2
	MIC	75	275	150	150	200	150	75
MSSA1	ZOI	18.8 ± 0.7	8.3 ± 0.3	9.6 ± 0.6	14.8 ± 0.4	11.2 ± 0.3	13.7 ± 0.7	17.4 ± 0.9
	MIC	50	300	175	150	200	175	75
MSSA2	ZOI	19.5 ± 1.1	7.9 ± 0.3	9.2 ± 0.4	15.2 ± 0.8	11.6 ± 0.5	14.3 ± 0.5	18.4 ± 0.8
	MIC	25	> 300	250	150	175	125	50
MRSA1	ZOI	17.6 ± 0.8	ND	8.7 ± 0.6	14.2 ± 0.6	ND	12.6 ± 0.4	17.2 ± 1.0
	MIC	75	> 300	225	175	> 300	150	75
MRSA2	ZOI	17.3 ± 0.6	ND	7.8 ± 0.4	12.1 ± 0.9	7.4 ± 0.2	13.5 ± 0.6	16.8 ± 0.7
	MIC	75	> 300	300	200	> 300	175	100

* The zone of inhibition diameter is the mean of triplicates and includes the diameter of assay disc (6 mm) ± standard deviation

Regarding the sensitivity of examined *S. aureus* strains, it could be generally noticed that the MRSA strains were more resistant to extracts' antibacterial activity. The MRSA1 strain had the highest resistance followed by MRSA2. MRSA1 showed the highest resistance against *B. eriophora* and *R. vesicarius*, e.g. no ZOI was appeared and the MIC values exceeded 300 $\mu\text{g/ml}$.

The most effective and powerful plants extract, i.e. *B. eriophora* and *T. oliverianum*, were selected to be applied as textile finishing agents, to produce anti-*Staphylococcus aureus* textiles. Effectively, antibacterial textiles, against the entire examined *S. aureus* strains, could be achieved after the treatment with plant extracts (Table 2). The antibacterial action of

treated textiles was matching with their relevant ZOI and MIC values, toward each strain. The appeared ZOIs from the treatment with antibacterial textiles were very clear and indicative. MRSA1 was the strongest strain, toward the treated textiles, although it showed high susceptibility to both treated textiles.

The durability of extract-treated textiles, after several laundering cycles, are also presented in table 2. It could be observed that the entire treated textiles maintain the majority of their antibacterial activity after two laundry cycles. *B. eriophora* treated textiles were more effective toward all examined strains before washing; after the first laundering cycle, they maintain more than 95% of their activity toward the reference strain and MSSA1.

Table 2. Inhibition zones of treated cotton textile with plant extracts (10 %) against *Staphylococcus aureus* strains after laundering cycles

<i>Staphylococcus aureus</i> strains	<i>Bassia eriophora</i>			<i>Teucrium oliverianum</i>		
	C	1 st cycle	2 nd cycle	C	1 st cycle	2 nd cycle
ATCC- 25923	27.9 ± 1.2 ^a	26.6 ± 0.9 ^a	22.2 ± 0.5 ^b	26.1 ± 0.9 ^a	24.3 ± 0.8 ^b	20.6 ± 0.5 ^c
MSSA1	29.2 ± 1.0 ^a	27.8 ± 1.1 ^a	24.9 ± 0.8 ^b	28.2 ± 1.2 ^a	25.9 ± 0.9 ^b	22.8 ± 0.4 ^c
MSSA2	32.6 ± 1.1 ^a	30.4 ± 1.2 ^b	26.6 ± 0.7 ^c	31.1 ± 1.3 ^a	27.4 ± 0.6 ^b	24.6 ± 0.9 ^c
MRSA1	24.6 ± 1.1 ^a	21.3 ± 0.6 ^b	18.1 ± 0.6 ^c	20.8 ± 0.9 ^a	19.8 ± 0.7 ^b	15.2 ± 0.6 ^c
MRSA2	22.3 ± 0.8 ^a	20.8 ± 0.5 ^b	17.7 ± 0.7 ^c	20.4 ± 0.5 ^a	19.3 ± 0.6 ^b	14.5 ± 0.4 ^c

* The zones of inhibition diameters are the means of triplicates ± SD and include the width of textile pieces (10 mm)

** For each plant, different superscript letters in the same raw indicate significant difference at CI=95%

On the other hand, *T. oliverianum* treated textiles maintained more percentages from their antibacterial activity after the 1st washing cycle toward antibiotic resistant strains. Figure 1 exemplify the appeared ZOs after challenging the MRSA2 (the most resistant

strain); the appeared clear zones of growth inhibition indicate the efficacy of extracts-treated textiles and the continuance of ZOI appearance after two laundering cycles indicate the high durability of antibacterial textiles.

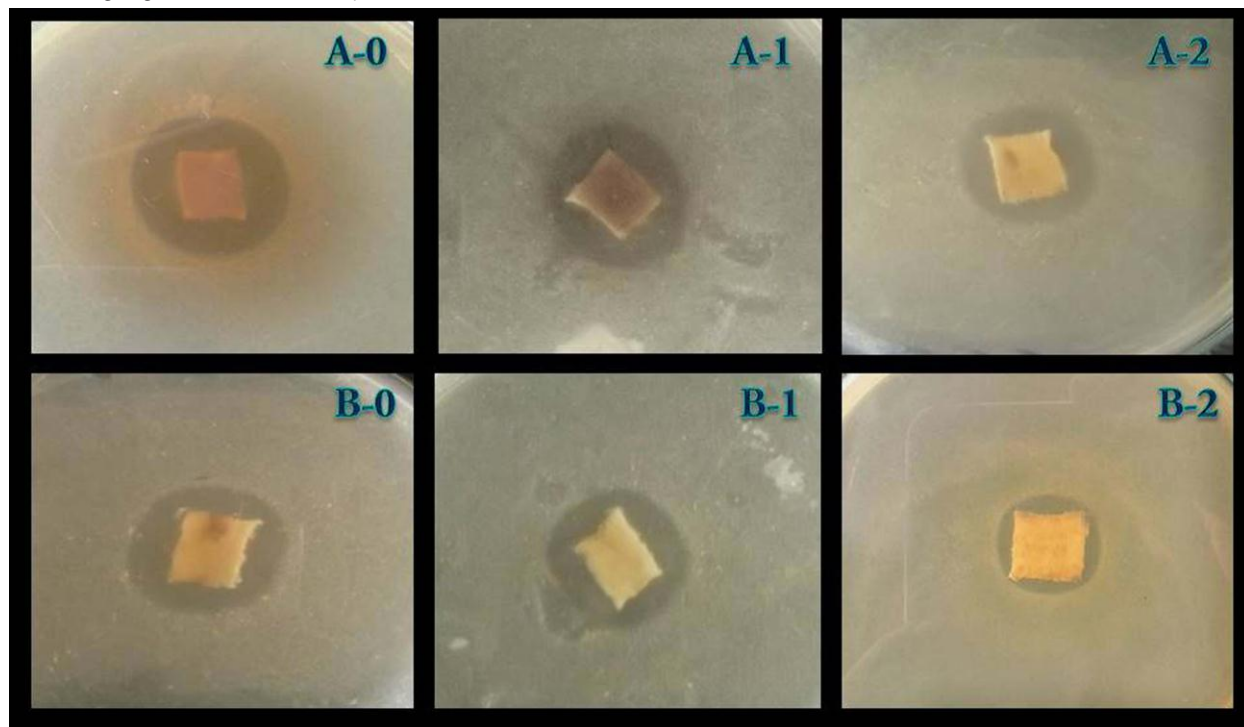


Fig. 1. Antibacterial activity of treated textiles with plant extracts of *Bassia eriophora* (A) and *Teucrium oliverianum* (B) against antibiotic resistant *Staphylococcus aureus* (MRSA2), after (1) and (2) laundering cycles, compared with unwashed samples (0).

DISCUSSION:

The base for selecting and applying plant species, in current investigation, was their reported bioactivities and applications in folkloric medicine in Arabian regions. The diversity of plants antibacterial potentialities is basically attributed to their contents form bioactive phytochemicals.

Bassia eriophora was applied as an herbal remedy in Arabian folkloric medicine to treat many rheumatic and renal disorders (Al-Yahya *et al.*, 1990); the recurrent usage in wound healing and skin protection indicate the safety of this plant usage.

The preliminary exploration of *B. eriophora* phytochemicals, in alcoholic extract, revealed their containment of glycosides, phytosterols, alkaloids, terpenes, flavonoids, phenolic compounds, tannins and saponins (Yusufoglu, 2015).

No sufficient data could be achieved regarding the phytochemical analysis, antioxidant and antimicrobial activity of *B. eriophora* extract; however, it was recommended that further investigations are required to confirm these attributes (Khalil *et al.*, 2017).

Secondly, the extract of *B. ciliaris* was characterized in literature to have remarkable antimicrobial properties against many species from bacterial pathogens (El-Shanawany *et al.*, 2013; Abdallah and El-Ghazali, 2013), they suggested that this plant after extraction and purification could be a source to generate new effective drugs and antibacterial agents, especially against antibiotic resistant pathogens.

The antimicrobial activity from the essential oil of *D. anethifolia* and from its main component, (decanal) was reported against MRSA and MSSA strains (Mahboubi and Feizabadi, 2009)

The main phytochemical components of *D. anethifolia* were identified to contain many monoterpene hydrocarbons (myrcene, limonene and α -pinene), dodecanol and decanal were the chief constituents that contain oxygen; pangelin was also contained in this plant extract and found to act as the potent antimycobacterial coumarin. All these compounds, including the aliphatic components containing oxygen, exhibited remarkable biocidal activity against many dermatophytes species (Mahboubi and Feizabadi, 2009).

The antibacterial activity of *P. crispa* was investigated among the screening of selected Saudi plants' bioactivity (Kuate *et al.*, 2013), they reported that this plant had a moderate to weak antibacterial activity against *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. These findings agree with those obtained in current study as *P. crispa* extract had a middling antibacterial activity against examined *S. aureus* strains. It was reported that *P. crispa* was utilized in folkloric therapy for treatment of numerous disorders (Chhetri *et al.*, 2015); these diseases and ailments included gastrointestinal and heart diseases, because of the antioxidative nature of plant (Al-Hajj *et al.*, 2014).

The chemical analysis of the *Pulicaria* spp. essential oils revealed that they have high contents from phenolic compounds, sesquiterpene and monoterpene hydrocarbons (Mossa *et al.*, 1987). Chhetri *et al.* (2015) reported that *Pulicaria* species were successful for acting as antimicrobial, antioxidant, galactagogues, antiepileptics, and antifungal agents due to their contents from bioactive phytochemicals.

The content from monoterpenes, sesquiterpenes and diterpenes in *P. crispa* extract was suggested to be the responsible for its antioxidant and antibacterial activities (Stavri *et al.*, 2008; Nazzaro *et al.*, 2013).

Regarding *R. vesicarius*, the therapeutic significance of the plant could reflect its containment of biochemical compounds, e.g. anthraquinones (quercetin, chrysophanol and

emodin), flavonoids (orientin, isorientin, vitexin and isovitexin), carotenoids, tannins, quinines, triterpenoids, saponins, phenols and organic acids (Mostafa *et al.*, 2011; Prasad and Ramakrishnan, 2012), such anthraquinones and flavonoids are suggested to be powerful antibacterial agents toward numerous pathogenic species.

Panduraju *et al.* (2009) reported that the methanolic and aqueous extracts of *R. vesicarius* leaves were effective against both Gram- negative and positive bacteria.

Tamarix aphylla was recurrently used in traditional folk medicine to treat several ailments, e.g. eczema, hepatitis and skin diseases (Yusufoglu and Alqasoumi, 2011). The methanolic extract of *T. aphylla* was proved to have antioxidant and human health protective effects; six types of bioactive tannins were elucidated in *T. aphylla* galls extract (Orabi *et al.*, 2015). The polyphenols composition in *T. aphylla* leaves and stems (flavonoids and phenolic acids) were studied and suggested as potential sources for antimicrobial therapy (Mahfoudhi *et al.*, 2014). The leaves extract of *T. aphylla* possessed substantial microbicidal activity toward many human pathogens including *S. aureus* and *Candida* sp. (Alrumman, 2016).

Additionally, Iqbal *et al.* (2015) proposed that *Tamarix* species, especially *T. aphylla*, which have antiparasitic, antimicrobial and disease preventive possessions through its use as ethnomedicinal plant, contains many bioactive compounds that present alternative sources for drug therapy and control of infectious diseases.

The *Teucrium* genus contains more than 340 species; research investigations indicated the antibacterial, antifungal, insecticidal, antispasmodic, anti-inflammatory, anti-ulcer, anti-oxidant, analgesic and anthelmintic activities of the many species from genus (Fatima, 2016). The preliminary screening for phytochemicals content, in *T. oliverianum*, revealed the presence of tannins, saponins, flavonoids, alkaloids, sterols, coumarins, cardiac glycosides and volatile oil (Ajabnoor *et al.*, 1984; Fatima, 2016), most of these compounds have powerful bioactivities.

The abovementioned studies could give explanations for the antibacterial activity of examined plants, in this study. The wide diversity of bioactive phytochemicals in their extracts has an important role in their bactericidal activities because it is very difficult for any microbe to generate resistance against numerous antimicrobial agents together, rather than the application of single or few synthetic antimicrobial drugs.

The durability tests, for the treated textiles with plant extracts, indicated that extracts could form strong bonds with textile material (cotton cellulose), so that they

preserved most of their antibacterial activity even after two laundering cycles, matching suggestion was introduced from many relevant studies (Ramachandran *et al.*, 2004; Tayel *et al.*, 2013b) that suggested that antibacterial agents could covalently fixed and interacted onto cotton textiles.

However, the observed slight decrease in treated textiles activity after laundering may suggest that extracts were not chemically linked with textile fibers, and this facilitated the release of extracts from linked textiles, in the antimicrobial assay, and performance of their bactericidal action (Gupta and Laha, 2007).

The potential recommended applications of the antibacterial treated textiles may include the fabrication of hygienic bed sheets, surgery and health care clothes, disposable burn and wound covers, surgical gloves or other health related clothes.

Results obtained from current investigation indicated the antibacterial activity of Saudi wild plants against *S. aureus* strains, including MRSA isolates, and recommended the application of powerful plant extracts, e.g. *Bassia eriophora* and *Teucrium oliverianum*, as finishing agents for treating cotton fabrics and fabrication of antimicrobial textiles.

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تطبيق استخدام مستخلصات النباتات السعودية البرية للقضاء على البكتريا العنقودية المقاومة للمضادات الحيوية

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المختبرية نشاطا ملحوظا مضادا لجميع سلالات البكتريا العنقودية المختبرية، وقد كانت أقوى المستخلصات فعالية هي المستخلصة من *B. eriophora* و *T. oliverianum* باستخدام مختلف طرق التقدير. وتطبيق هذه المستخلصات لتعديل وتدعيم المنسوجات القطنية لإنتاج منسوجات مقاومة للبكتريا العنقودية، فقد أظهرت المنسوجات المعاملة أيضا نشاطا قويا مضادا لجميع السلالات البكتيرية المختبرية والتي تشمل سلالات MRSA. وقد احتفظت المنسوجات المعاملة بالمستخلصات النباتية بفعاليتها المضادة للبكتريا بعد دورتين من دورات الغسل الألي لها. يمكن التوصية بتطبيق المستخلصات النباتية للنباتات البرية السعودية من أجل القضاء على البكتريا العنقودية المقاومة للمضادات الحيوية والحصول على منسوجات مضادة لهذه البكتريا الممرضة.

تعد النباتات البرية دائما المصدر المأمول لاستكشاف مواد علاجية جديدة، وتذخر المملكة العربية السعودية بمجموعة كبيرة من النباتات البرية والطبية والتي تحتوي المئات من الأجناس الثمينة. تعتبر البكتريا العنقودية *Staphylococcus aureus* من أخطر أنواع الميكروبات الممرضة طبيًا والتي يمكنها اكتساب المناعة ضد العديد من المضادات الحيوية الكيميائية. تم استخلاص المواد الفعالة من سبعة أجناس من النباتات السعودية بواسطة محلول 70% من الإيثانول لتقدير فعاليتها كمضادات لسلالات البكتريا العنقودية سواء الحساسية (MSSA) أو المقاومة للميثيسيلين (MRSA) وهي *Bassia eriophora*, *Blepharis ciliaris*, *Ducrosia anethifolia*, *Pulicaria crispa*, *Rumex vesicarius*, *Tamarix aphylla* and *Teucrium oliverianum*. أثبتت أغلب المستخلصات النباتية