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SELECTIVE WILD MEDICINAL PLANTS IN MADINAH CITY AGAINST PATHOGENIC BACTERIA

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ABSTRACT

Background: Bacterial resistant to antibiotic needs an alternatives agent due to huge uses globally. Wild plant extraction is considered a source to solve the problem. The aim of this study is evaluating and comparing the effectiveness of five wild plants extracted by ethanol on the selected pathogenic bacterial strains Escherichia coli (E. coli) and Bacillus cereus (B. cereus) as Gram-negative bacteria, Streptococcus pneumoniae (S. pneumoniae) and Staphylococcus aureus (S. aureus) as Gram-positive bacterium. **Methods:** Experiments achieved with five wild plants collected from Madinah, Ferula asafetida (F. asafetida), Xanthium strumarium (X. strumarium), Onopordum heteracanthum (O. heteracanthum) and Glinus lotoides (G. lotoides). All were in vitro and alcoholic plant extracts concentrations (100, 50, 25 mg / ml) prepared before antibacterial sensitivity accomplished using disc diffusion methods. Pathogens used in this study were E. coli, B. cereus, S. pneumoniae and S. aureus. **Results:** Several plant extracts showed inhibition zones in Petri dishes. The data demonstrated that Ferula asafetida affects all bacterial strains. On contrary, Ficus cordata and Ficus cordata showed the lowest effect. **Conclusion:** Among the studied plants, Ferula asafetida showed the highest potentially and efficiency as natural antibacterial agent, and thus could be a promising medicinal plant against bacteria.+

KEYWORDS: Gram-negative bacteria, Gram-positive bacteria, Antibiotic resistance, antibacterial, plant extract, Madinah, wild plants.

INTRODUCTION

Historically, plant products and their extractions have treated infectious diseases that are recently tested scientifically.[1] Early studies revealed an obvious ability of plant extracts as antimicrobial drugs, and scientific efforts proved effects of plant products on microbial pathogens. In 1943, Osborn reported 2300 plant species that illustrated antimicrobial activities.[2] In addition, Nickell was presented 157 plants as antimicrobial agents and Sehgal registered the highest plants along with their antimicrobial effects.[3,4] Furthermore, Farnsworth has surveyed antimicrobial activities of numerous plants. In India, scientists tested the biological activity of 880 plants.[5, 6, 7, 8] Moreover, Stoessl investigated 197 plant species that have antimicrobial activities.[9] Additionally, Gupta and Banerjee presented several plants with antimicrobial activity.[10] These findings might be highly important due to antibiotics indiscriminate uses, which result in bacterial resistance. **In addition, the undesirable side effects of antibiotics have obligated scientists to find new antimicrobial substances from different sources, e.g., medicinal plants or microorganisms. Antibiotic resistance is a serious threat to public health worldwide. Accordingly, researchers are attempting to conquer this challenge by producing antibiotics that are effective and new.[11]

For an example, a number of bacteria including S. aureus and E. coli can resist antibiotics.[12,13] Entero hemorrhagic E. coli has an ability to produce shiga (toxin)[14], Additionally, two types of intestinal diseases caused by B. cereus, there symptoms are diarrhea, nausea and vomiting. Moreover, B. cereus are implicated in respiratory, eye and wound infections.[15]

S. Pneumoniae, is existing in the respiratory tract as a normal micro-flora. It might convert to pathogenic in some conditions, for instance, when the host's immune system is weakened. Its most virulence factors are invasions including immunogenic cell wall components, various adhesives, pneumolysin and antiphagocytic capsule.[16]

Wild plants in nature produce a collection of chemical compounds with known health effects worldwide. Chemicals include flavonoids, polyphenols and alkaloids. Screening of natural plants in Saudi Arabia for antimicrobial activities reflect that plant extracts can be used as sources of promised antibiotics. Five plants were studied in this work, Ferula asafoetida (Falc.) H.Karst

(Apiaceae, vernacular local name: Kalak). According to Plants of the World online (Kew science), F. asafoetida is a native plant in Afghanistan to central Asia as well as Northwest and north of Pakistan. It is used in folk medicine as a digestive aid, in both pickles and food. Additionally, it has been prescribed for hysteria.[17] Xanthium strumarium L. (Asteraceae, vernacular local name: Shubbait), originally in the USA. The plant is largely known to treat rhinitis and headache, dye diseases and hepatic heat.[18]

Onopordum heteracanthum C.A. Mey. (Asteraceae, vernacular local name: Kuaar). Native in Arabian Peninsula, Iraq, Iran and Turkey. Studying of its cytotoxic effects revealed that it has a clear result against several types of cancer in vivo.[19]

Ficus cordata Thunb. (Moraceae, vernacular local name: Teen barry(. Mainly found in Oman and United Arab Emirates. Methanol extract has demonstrated that cordata possess an antimicrobial activity.[20]

Glinus lotoides L. (Molluginaceae, vernacular local name: Mughibrah). Native in Africa, Spain, Arabian Peninsula, Australia and India. Has a strong reputation to cure parasite infection, for instance, against tapeworm infestation in Ethiopia.[21]

Excessive use of antibiotics has led to appear pathogens that are multi-drug resistant. The medical world takes this global challenge and try to solve it.[22] The aim of this study is to evaluate and comparing the effectiveness of five wild plants extracted by ethanol on pathogenic bacterial strains.

3. MATERIAL AND METHODS

3.1. Plant material

The plant collections were F. asafetida, X. strumarium, O. heteracanthum, F. cordata, and G. lotoides. They were identified in Biology Department, Faculty of Science, Taibah University. Air dried technique was optimizing for all plants, and the drying period was 4- 5 days at room temperature. Then, all plants were sliced and ground. After that, they extracted by using a 70% ethanol – water mixture (70/30V/V) for 48h. Each extraction filtered and concentrated with reduced vacuum pressure at below 50 °C. The final production kept at 4°C for further works.

Phytochemical screening

Secondary metabolites existence such as flavonoids, tannins, saponins, quinone, alkaloids, coumarins, anthraquinones and terpenoids, demonstrated herein from previous studies.[23,24]

3.2. Bacterial isolates

Bacterial isolates provided by King Fahad Hospital in Al Madinah City. They are four different bacterial species: *S. aureus*, *B. cereus and S. pneumoniae* and *E. coli*.

3.3 Antibacterial activity

Three of Gram-positive bacteria and one Gram-negative bacteria used for assessment of antibacterial activities with plant extractions by using modified agar disc diffusion of three concentrations extracted from the five wild plants (25, 50 and 100 mg / ml) through the serial dilution technique by adding dimethyl sulfoxide (DMSO).[25, 26] Commercial antibiotics Clindamycin (DA2), Rifampicin (RD5), Erythromycin (E15) and Ampicillin (Amp10) were applied as positive controls. The bacterial isolates were distributed equally in plates that contain Müller Hinton agar. Then, discs of 6 mm size from filter paper were loaded with every extraction before placing on the plates. All plates were incubated at 37 ºC for 24h. Finally, the determination of antibacterial activities achieved by calculating the inhibition zone area.

RESULTS

Phytochemical screening of five aerial plant extracts

Phytochemical qualitative screening of five aerial plant extracts confirmed the influence of saponins, alkaloids, tannins, flavonoids and sterols and the absence of triterpenes, anthraquinones, quinones and cardenolides. All phytochemicals are summarized in Table 1.

Table 1: The Qualitative screening of the phytochemical of five aerial plant extracts.

Scientific name	Chemical constitutes
F. asafetida	Bisabolol, DPPH, Carvacrol, (E)-1-propenyl-sec-butyl-disulfide, Ferric, Flavonoid, Phenolic: Umbelliprenin – ferulicacid and (Z)-b-ocimene.[27]
X. strumarium	Flavonoids, Fatty acid, Iridoids, Monoterpenes, Neoclero-dane M diterpenoids, Polyphenols, Sesquiterpenes and Terpenoids.[28]
O. heteracanthum	Alkaloids, glycoside, Flavonoids, Phenols, Saponins and Tannins.[29]
F. cordata	Cardiac glycosides, Carbohydrates, Diterpenes, Flavanones, Lignin, Phenols, Proteins & amino acids, Reducing sugar, Saponin, Sterols and steroids, Tannins and Terpenoids.[30]
G. lotoides	Alkaloids, Cardiac glycoside, Flavonoids, Phenols, Proteins, Saponins, Tannins and Terpenoids (diterpenes, sesquiterpenes).[31]

Table 2: The Antibacterial activities of five aerial plant extracts examined against four pathogenic bacterial isolates.

Bacterial Species / Positive control	*E. coli*			*S. pneumoniae*			*S. aureus*			*B. cereus*		
AMPICILLIN(Amp10)	2.71 ± 0.02			2.81 ± 0.02			2.75 ± 0.04			2.8 ± 0.04		
ERYTHROMYCIN (E15)	2.51 ± 0.02			2.48 ± 0.02			2.51 ± 0.02			2.46± 0.02		
RIFAMPICIN(RD5)	2.78 ± 0.02			2.73 ± 0.02			2.71 ± 0.02			2.81± 0.02		
Plant												
Doses (mg/ml)	25	50	100	25	50	100	25	50	100	25	50	100
F. asafetida	1.85 ±0.04	2.15 ± 0.04	2.41 ± 0.02	--	1.51 ± 0.06	--	1.84 ±0.18	2.21 ±0.02	2.35 ±0.04	2.98 ±0.02	3.11 ±0.02	3.23 ±0.02
X. strumarium	--	--	--	2.2 ±0.04	2.35 ± 0.04	2.5 ± 0.04	2.06 ±0.04	2.31 ±0.02	2.53 ±0.02	--	1.91±0.02	1.98 ±0.02
O. heteracanthum	--	1.58 ±0.02	1.95±0.04	--	--	--	--	--	--	2.7±0.02	2.93±0.04	3 ± 0
F. cordata	--	--	1.55 ± 0.04	--	--	--	--	--	--	--	2.9 ± 0.04	3.06 ±0.04
G. lotoides	--	--	--	--	--	1.6 ± 0.06	--	--	--	--	2.41 ±0.02	2.53 ±0.04

Three of the Gram-positive bacteria and one from Gram-negative bacteria used in the assessment of antibacterial activities for 5 plant extractions at different concentrations (25, 50 and 100 mg/ml) are shown in Table 2. The results in Table 2 reflect that the F. asafetida affected all pathogenic bacterial strain examined in this study, whereas G. lotoides was the lowest. Concentrations of F. asafetida were effective on E. coli and the effect was concentration dependent. However, it affected S. pneumoniae at dose 50mg/ml. Interestingly, F. asafetida affected S. aureus and B. cereus in all concentrations. X. strumarium ethanolic extract shoed no effect on E. coli but on S. aureus and S. pneumoniae at all concentrations, with a concentration dependent. X. strumarium effects on B. cereus was in the two doses 50 and 100 mg/ml. O. heteracanthum effects was on E. coli and B. cereus at both doses 50 and 100 mg/ml and all concentrations respectively. Furthermore, no effect either at S. pneumoniae nor S. aureus. F. cordata affects B. cereus at 50, 100 mg/ ml. Moreover, its effect on E. coli was at dose 100 mg/ml. On the other side, there were no effects on S. pneumoniae or S. aureus. Similar to F. cordata, G. lotoides showed an effect on B. cereus at dose 50, 100 mg/ ml. In addition, it effects S. pneumoniae at dose 100 mg/ml, but there were no effects on neither E. coli nor S. aureus.

DISCUSSION

From a medical viewpoint, there is a serious global concern came from antibiotic bacterial resistance. Accordingly, researchers hardly attempt to conquer this challenge by producing and synthesising new chemical compounds that are effective on clinical bacteria. In this work, five wild plants were extracted and tested the antibacterial effectiveness. The secondary metabolites of plant productions are variety and wide, such as alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins.[32] Furthermore, the origin of these biomolecules, plant-derived-antimicrobial- substances, are wild plants.[33] Several natural products could effectively treat the bacterial infections.[34] Our data shown that the Gram-negative bacteria was more sensitive than Gram-positive bacteria (Tables 2). This could refer to that the bacterial cell wall structure contains different layers of peptidoglycan between Gram-positive and Gram- negative. Moreover, there are 70-100 layers of peptidoglycan in Gram-positive bacterial cell wall and contained two polysaccharide types, N-acetyl-glucosamine and N-acetyl- muramic acid by peptide side chains and cross bridges. Thus, the previous construction could explain the differences of bacterial sensitivity against plant extracts.

There is a resistance ability in Gram-negative bacteria against some antibiotics such as penicillin, secretion of lactamase enzyme that present between the thin outer membrane and the cytoplasmic membrane in the periplasmic space might be one of the reasons.[35] Scientists seek for controlling microorganisms' multidrug resistance by exploring natural antimicrobial agents.[36] In this work, three Gram-positive bacteria and one Gram- negative bacteria performed to assess antibacterial activities for ethanolic arial part of plant extractions. Flavonoids were detected in all plant extractions. These chemical compounds have antimicrobial activates. Furthermore, they can inhibit nucleic acid structural, synthesis and proteins.[37, 38] F. asafetida is the highest effective plant on all bacterial strains used, and that could be due to exist of umbelliprenin and ferulic acid, as phenolic compounds with antimicrobial activities.[26]

CONCLUSION

The present study aimed to address the antimicrobial activity of ethanolic aerial plant extracts from Madinah flora in Saudi Arabia in order to evaluate them against four different clinical bacterial species, S. aureus, B. cereus, S. pneumoniae and E. coli. Findings show that the ethanolic extracts were promising as antibacterial agents. F. asafetida was the highest effective plant on all bacterial strains tested in this study. Indeed, identification of active compounds could be valuable. Hence, further work is required to investigate both active possible compounds and bio mechanism that inhibited those bacterial growth.

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