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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF MEDICINAL FLORA GROWING IN THE CENTRAL REGION OF SAUDI ARABIA

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Abstract

Background: This study was designed to the prospect of applying n-hexane extracts whole parts plants of *Calligonum comosum* L. , Zygophyllum *coccineum* L. , *Heliotropium digynum* L. , *and Rhazya stricta* L. to overcome the multi-drug resistant human pathogenic bacteria isolates; *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae*.

Methodology: The medicinal plants were collected from Riyadh Region, Kingdom of Saudi Arabia and were extracted with n-hexane. Four Multi-drug resistant human pathogenic bacteria isolates i.e. *E.coli, P. aeruginosa, S. aureus and K. pneumonia* were selected for this study. The efficacy of n-hexane extracts was tested by using agar well diffusion assay and the zones of growth inhibition were measured in millimeter (mm).

Results: The n-hexane extracts showed varied inhibition zones against tested multi- drug clinical isolate human pathogens. The results determined that, all the plant extracts showed antibacterial activity against four tested bacterial pathogens. The n-hexane extract of *C. comosum* showed stronger and broad spectrum activity against the tested isolates as related to the other extracts that indicate moderate action. Data showed the highest and lowest zone inhibition effects against multi drug resistant clinically Human Pathogens at concentration of 100mg/ml.

Conclusions: It is concluded that the n-hexane extracts of *C. comosum, Z. coccineum, H. digynum, and R. stricta* have antimicrobial potential activity against common human bacterial pathogens.

Introduction

Medicinal and aromatic plants are potential source of raw materials used for manufacture of drugs and perfumery products. More than a quarter of all the medicines used in the world today contain natural compounds derived from plants that often serve as lead molecules whose activities can be enhanced by manipulation through combinations with chemicals and by synthetic chemistry that can be exploited in the field of new drugs research and development. The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment [1,2].

Many human diseases are known to have been treated with herbal medicine throughout the history of human beings. The increasing evolution of multi drug resistant bacteria and the recent appearance of strains with reduced susceptibility to antibiotics lead to the emergence of untreatable bacterial diseases [3,4].

Antibiotic resistance is a form of drug resistant whereby some sub-populations of a microorganism, usually a bacterial species, are able to survive after exposure to one or more antibiotics. In other word, the term "antibiotic resistance" is refers to the ability of a microorganism to withstand the effect of an antibiotic. The use of antibiotics is

limited because bacteria have evolved defenses against certain antibiotics. One of the main mechanisms of defense is inactivation of the antibiotic. This is the usual defense against penicillin and chloramphenicol, among others. Another form of defense involves a mutation that changes the bacterial enzyme affected by the drug in such a way that the antibiotic can no longer inhibit it. This is the main mechanism of resistance to the compounds that inhibit protein synthesis, such as the tetracycline [5,6,7].

To date, limited information is available on medicinal plants with respect to its potential in the treatment of human pathogens. Therefore, the present study was conducted and the principal aim was to analyze and evaluate the antibacterial activity in the leaves, stem and roots extracts of medicinal plants against some human pathogenic bacteria

The present study was aimed to evaluate the antimicrobial activity of Saudi medicinal plants extract against multi-drug resistant human pathogenic bacteria.

Materials and Methods:-

Collection of Plant materials:-

The healthy plants; *Calligonum comosum* L.(*C. comosum* L.) , Zygophyllum *coccineum* L. *(Z. coccineum* L.) *Heliotropium digynum* L.(*H. digynum* L.) *and Rhazya stricta* L. *(R. stricta* L.) were obtained from Riyadh Region, Kingdom of Saudi Arabia.

Preparation of extracts:-

All plant parts were washed with distilled water dried in shade, grinded to fine powder and stored in airtight containers at room temperature in dark until used. The powdered samples were subjected to extraction by the following method of [8]. The ground samples were used for the preparation of the n-hexane extracts.

Extraction of Hexane:-

The n-hexane extracts of the plants were obtained according to the method described by [9], with slight modifications. 100g of each dried powder of each plant samples was mixed with 400 ml of n-hexane. The mixture was gently stirred, tightly covered with cotton wools and foiled, then allowed to stand for 3 days at room temperature. Each extract was decanted and filtered through muslin cloth. The filtrates obtained were placed in a water bath and allowed to stand to evaporate the solvent. The residues obtained were used for testing the antibacterial activity of the plants extracts.

Multi-Drug Resistant Human Pathogenic Bacteria:-

The multi-drug resistant human pathogenic bacteria (*E.coli*, *P.aeruginosa*, *S. aureus and K.pneumoniae*) were collected from Al-Quwayiyah General Hospital, Al-Quwayiyah Governorate, Kingdom of Saudi Arabia. The bacterial strains were identified on the basis of cultural and morphological characteristics and the sensitivity test were conducted to test their resistance to so many antibiotics drugs. *E.coli* was resistant to the Ampicillin, Ciprofloxacin, Nitrofloxacin, Clotrimazole, and Cefodoaxime. *P. aeruginosa* was resistant to the Nalidixic acid, Trimethoprim sulphamethaoxazole, Clotrimazole, Cefotaxime. *K, pneumoniae* was resistant to the pefloxacin, tarivid, streptomycin, chloranphenicol, sparfloxacin, amoxicillin, augmentin, and septrin. *S. aureus* was resistant to penicillin, amoxicillin, chloramphenicol, cloxacillin, erythromycin, streptomycin, and tetracycline.

Maintenance of the Bacterial Culture:-

The isolates were maintained by sub-culturing them into a new prepared nutrient agar slant and stored in an incubator at 37^{o}C.

Preparation of Inoculums:-

The Bacterial inoculums were prepared by sub-culturing of the test organisms from nutrient agar slants on another prepared nutrient agar plates and incubated at 37^{o}C for 24 hours. The pure cultures on the nutrient agar plates were used as the inoculums.

Antibacterial Efficacy Test:-

The agar well diffusion method described by [10] was used to determine the antibacterial activity of the plant extracts. The Mueller-Hinton agar media were prepared according to the method described by [11]. The prepared agar plates

were inoculated with test organisms (different bacterial inoculums). Five wells (holes) were made into the set agar in Petri-dishes containing the inoculums using a sterile syringe of 10mm diameter. The different concentration (50mg/ml, 75mg/ml, and 100mg/ml) of the extract were prepared. A 0.25ml (5 drops) volume of each prepared concentration (50mg/ml, 75mg/ml, and 100mg/ml) of the extract was dispensed into the different agar wells in the media. Reference drugs; Gentamycin (50mg/ml) was serve as positive control, while the solvent (hexane) was used as negative control. The cultures were incubated at 37OC for 24 hours. The zones of inhibition around the wells were measured as an indication of the antibacterial activity of the plant extract [12].

Measurement of inhibition Zone:-

The zone of inhibition is the diameter over the growth of microorganism inhibited due to the presence of an antimicrobial agent [13]. After the incubation period, the plates were observed for zones of inhibition (indicated by clear zones) around the wells. The antibacterial activities of the extracts were assessed by measuring the diameter of the zone of inhibition in (mm) around the wells using a transparent measuring ruler. The actual zone of inhibition was calculated by subtracting the diameter of the well from the measured diameter (including the well diameter).

Results and Discussion:-

Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found to have antimicrobial properties as anticancer agents, anti-diarrheal as well as antifungal activities [14, 15]. Medicinal plants play a crucial role in the search for alternative antimicrobial components. According to the World Health Organization, it is estimated that around 80% of the earth's population use some form of herbal medicine in their health care, whereas natural products are a preferable option than synthetic ones [16]. Medicinal and healing properties of herbs are closely related to their chemical components which are classified into some major groups like alkaloids, acids, essential oils, steroids, saponins, tannins etc. and getting this into herbal remedy depends upon the solubility of these compounds in various solvents [17]. Laboratories of the world have found literally thousands of phytochemicals having inhibitory effects on microorganisms [18]. The results of antibacterial activity in hexane extract of medicinal plants against multi drug resistant human pathogens were shown in **(Table 1 to Table 4).** The present investigation showed that the tested plant extract possess potential antibacterial activity against *E.coli, P. aeruginosa, S.aureus, K. pneumoniae.*

Table 1: Antimicrobial activity of n-hexane extract of C. *comosum* against human pathogenic bacteria

Multi Drug Resistant Human Pathogen Bacteria	Zone of Inhibition (mm)			
	50mg/ml	**75mg/ml**	**100mg/ml**	**Gentamycin 50mg/ml**
E.coli	1.5± 0.00	2 ± 0.00	4.5± 2.82	6.5 ± 0.00
P.aeruginosa	3 ± 1.41	2 ± 0.00	3.5± 0.70	10.5 ± 0.70
S. aureus	3±0.70	3 ± 0.70	2.5±2.00	22 ± 1.41
K. pneumoniae	2.5±1.41	4 ± 1.41	7.5±2.82	21.5 ± 2.12

Values are presented as mean ± standard deviation of triplicates. Gentamycin was used as positive control.

Table 2: Antimicrobial activity of n-hexane extract of *R. stricta* against human pathogenic bacteria

Multi- Drug Resistant Human Pathogen Bacteria	Zone of Inhibition (mm)			
	50mg/ml	**75mg/ml**	**100mg/ml**	**Gentamycin 50mg/ml**
E.coli	1± 0.00	2 ± 0.70	2.5 ± 1.41	21 ± 0.70
P.aeruginosa	1± 0.00	-	-	23 ± 2.82
S. aureus	-	-	3.5 ± 0.70	22.5 ± 0.70
K. pneumoniae	-	2.5 ± 1.41	5.5 ± 2.12	10.5 ± 2.82

Values are presented as mean ± standard deviation of triplicates. Gentamycin was used as positive control.

Table 3: Antimicrobial activity of n-hexane extract of *Z. coccineum* against human pathogenic bacteria

Multi -Drug Resistant Human Pathogen Bacteria	Zone of Inhibition (mm)			
	50mg/ml	**75mg/ml**	**100mg/ml**	**Gentamycin 50mg/ml**
E.coli	-	1.5±1.41	4.5±0.70	10±1.41
P.aeruginosa	1±0.00	3.5±0.70	5.5±1.41	21±0.70
S. aureus	-	2.5±1.41	7.5±2.82	21.5±1.41
K. pneumoniae	1.5±1.41	5±2.82	6±0.70	23±2.82

Values are presented as mean ± standard deviation of triplicates. Gentamycin was used as positive control.

Table 4: Antimicrobial activity of n-hexane extract of *H. digynum* against human pathogenic bacteria

Multi- Drug Resistant Human Pathogen Bacteria	Zone of Inhibition (mm)			
	50mg/ml	**75mg/ml**	**100mg/ml**	**Gentamycin 50mg/ml**
E.coli	-	2.5±0.70	-	19.5±1.41
P.aeruginosa	1.5±0.70	-	5.5 -2.82	10.5±2.82
S. aureus	1±0.00	-	3.5±0.70	8±0.70
K. pneumoniae	-	4.5±1.41	4.5±2.12	23±1.41

Values are presented as mean ± standard deviation of triplicates. Gentamycin was used as positive control.

Plants remain one of the main sources of natural products for new therapies particularly in poor countries, because most of them are less cost, and can affect a wide range of antibiotic resistant microorganisms, and another interesting reason is that, the herbal medicines have fewer adverse effects compared to the conventional ones. Antibacterial activities of the plants extracts were tested using well diffusion method.

The n-hexane extract of the *C.comosum* showed the highest zone of inhibition on *K. pneumoniae* (7.5±2.82 mm) at 100mg/ml and the lowest on *E.coli* (1.5± 0.00 mm) at 50mg/ml as recorded in the (**Table 1**). The hexane extract of *R.stricta* showed highest zone of inhibition on *K.pneumoniae* (5.5±2.12 mm) at 100mg/ml and the lowest on *E.coli* and *P.auroginosa* (1± 0.00 mm) at 100mg/ml as seen in (**Table 2**). The hexane extract of the *Z.coccineum*, showed the highest zone of activity on *S. aureus* (7.5±2.82) at 100mg/ml and the lowest on *Pseudomonas auroginosa* (1.0±0.000 mm) at 50mg/ml as shown in (**Table 3**). The hexane extract of the *H.digynum*, showed highest zone of activity on *P. auroginosa* (5.5±2.82) at 100mg/ml and the lowest on *S. aureus* (1.0±0.000mm) at 50mg/ml as shown in (**Table 4**). Who reported that, the hexane extract of the plant has no antibacterial activity on the tested bacteria. According to the studies reported earlier by **[9],** the latex of the plant and other solvent extract of the plant have some antibacterial activities on some of the tested bacteria.

Conclusion:-

In conclusion of this study would lead to the establishment of some valuable compound that has to be used to formulate new, different and more potent antimicrobial drugs of natural origin. From the above results, it is concluded that some medicinal plants showed antibacterial activity. Medicinal plants are important to human beings in preservations our health. On comparing with the literature and present work indicates that, most diverse results can be obtained. The n-hexane solvent extraction was suitable for verifying the antibacterial properties of these medicinal plants. These plants have very promising antimicrobial activities and thus can be used traditionally to cure various infectious diseases cause by these resistant bacteria, and could serve as useful source of new antibacterial agents.

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