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ANTIBACTERIAL & ANTIFUNGAL POTENTIALITY *OF RICINUS COMMUNIS* & *COLEUS FORSKOHLII* ON SOME HUMAN PATHOGENIC MICROORGANISMS

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ABSTRACT

The objective of the present study was to explore the antibacterial and antifungal activity of two plant extracts (*Ricinus communis* & *Coleus forskohlii)* against some selected pathogen (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *candida albicans*) using microdilution method (MIC) at different concentrations (100-0.195mg/ml) and Disc diffusion method (200 mg/ml). The extracts of plants were prepared using Soxhlet apparatus for 8 hr. The results of this study proved of the water extract of *Ricinus communis* showed maximum zone of inhibition against *E. coli* and the lowest minimum inhibitory concentration against all tested microorganisms. *Coleus* showed powerful antimicrobial activity against Gram positive and Gram negative bacteria. The water extract of Coleus showed maximum zone of inhibition against k. pneumonia.

KEYWORDS: Antibacterial & antifungal, microdilution method (MIC), Disc diffusion method, Ricinus communis, Coleus forskohlii.

INTRODUCTION

The development of bacterial resistance necessitated the need to look for antimicrobial agents. The indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases is the main cause of these resistant strains (Ahamad, 1998; Kapoor *et al.*, 2015). The interest has increased in recent years in medicinal plants and herbs as the most important sources that possess health protective effects. The use of plant extracts

to protect against bacterial infections is a promising and effective approach due to low cost, environmental friendliness, and effectiveness against certain bacteria (Hardi *et al.*, 2013). With the advancement of science and technology, researchers recognized the importance of large numbers of these plants and use their as natural alternatives to synthetic compounds such Garlic (Palaksha *et al.*, 2010; Hosseini *et al.*, 2014).

In Palestine and other different countries there are many local medicinal plants described for treatment of many diseases. *Ricinus communis,* is a species of flowering plant in the spurge family, Euphorbiaceae (Momoh *et al.*, 2014). It can be used in treating diseases caused by Gram-positive and Gram-negative bacteria (Jeyaseelan & Jashothan, 2012; Momoh *et al.*, 2014). *C. forskohlii* (Family: Lamiaceae) is a perennial herb belongs to mint family of plants that mainly used in treatment of eczema and psoriasis (Senthilkumar *et al.*, 2014). *Coleus* species used for treatment of gastrointestinal, reproductive tract and skin infections (Tarh *et al.*, 2017). Most of studies indicate that the extract from coleus root, shoot and leaves have a potential broad spectrum antimicrobial activity (Malleswari *et al.*, 2013).

There is a need to develop alternative antimicrobial drugs to control some human pathogenic microorganisms so the present study is to authenticate the antibacterial and antifungal properties of crude extracts from *R. communis* and *C. forskohlii*.

2. MATRIAL AND MEETHODS

2.1. Chemicals

Mueller Hinton agar (MHA), Mueller Hinton Broth (MHB), Sabouraud Dextrose Agar (SDA), RPMI 1640 Media and Distilled water (D.W). all chemicals from Liofilchem company, Italy.

2.2. Used Plants

R. communis and C. forskohlii were the two plants used to prepare the extracts. The plants collected from different areas in Gaza strip.

2.3. Extraction

The parts used from these plants were leaves from C. forskohlii and seeds from R. communis. Leaves of Coleus prepared by air drying for three days. The dried leaves grind to powder. Seeds of R. communis prepared by removing the endocarp, sun draying for 5 days to reduce moisture, winnowing to separate the shell from the nibs(cartyledon), then they are crushed to powder.

Each preparation of the two plants exposed separately to extraction by using soxhelt extractor by using 150 ml of D.W for 8hr. The resulting extracts were evaporated using oven temperature 37 C^0 for 3 days. The extracts then dissolved in DMSO. 200 mg/ml of stock was obtained as standard concentration. Finally the extracts were sterilized using 0.22 µm membrane filter and stored at - 4 ^{0}C.

2.4. Antimicrobial susceptibility test

2.4.1. Microorgnisms

The microorganisms used for the susceptibility study are the bacteria (Staphylococcus aureus, Escherichia coli and klebsiella pneumonia) and the fungus (Candida albicans) which obtained from microbiology laboratories of Islamic university of Gaza.

2.4.2. Antimicrobial test

2.4.2.1. Agar disc-diffusion method

It was used to determine the antimicrobial activity of plant extracts. A suspension of pathogenic microorganisms was introduce to MHB and SDA and swirl gently to mix well, after solidification , the sterile filter paper discs approximately 6 mm in diameter saturated with stock extract of concentration 200 mg/ml from plant, then put on the surface agar plate. After incubation period of 24 h at 37 ^{0}C for bacteria and (24 - 48 hr) at 37 ^{0}C for fungi, the inhibition zone of microbial growth surrounding the disc is measured to evaluated the antimicrobial activity of plant extracts (Sharma, 2011; El Hindi *et al.*, 2017).

2.4.2.2. Minimum inhibitory concentration

Minimum Inhibitory concentration used by broth microdilution method (96 - well plates) to determine the activity of extracts against microorganisms. Extracts were diluted many times through a sterile diluent (MHB) to obtained range of concentration ranges from (100 to 0.1953) mg/ml. Then added 10 µl of suspension of bacteria overnight growth microorganisms to each well except a positive control. The 96-well plates were incubated at 37 ^{0}C for 24 hr. After 18 hr 50 µl of 0.01% solution of 2,3,5 triphenyl tetrazoluim chloride (TTC) added as indicator. The plates then incubated for 1hr and the TCC will be reduced by active bacteria to red color, but they remain of color in the wells after incubation 1 hr with TTC, indicates the inhibition of bacterial growth (Gupta *et al.*, 2006; Adwan *et al.*, 2008; El Hindi *et al.*, 2017).

Antifungal Assay

100 µl of *C. albicans* added as a suspension to each well except positive control. The plate was incubated for 24 hr at 37 ^{0}C to form of biofilm. After 24 hr the media was removed and washed the wells washed with phosphate buffer saline. The extracts were diluted many times in a sterile diluted RPMI 1640 media and the plate incubated the for 48 hr at 37 ^{0} C. The MIC was determined at the lowest concentration that inhibited visible growth of the *Candida* (Pierce *et al.*, 2010; El kichaoi *et al.*, 2015).

RESULTS

Bioactivity of plant extract by disc diffusion method

The results in Table 2 showed the antimicrobial activity by disc diffusion method against tested microorganisms. For *S. aureus*, the water extract of *R. communis* showed the effect with 9 mm zone of inhibition but the extract of *C. forskohlii* showed 7 mm. For *E. coli*, the water extract of *R. communis* showed the highest effect with 10 mm zone of inhibition but the *coleus* showed the effect with 8 mm. No antimicrobial activity was observed by *R. communis* on *K. pneumonia*, while *C. forskohlii* showed good effect with inhibition zone 10 mm. For *C. albicans* No antimicrobial activity was observed by the extract of *C. forskohlii* and *R. communis*.

Table 2: Disc diffusion method (mm) of *R. communis* and *C. forskohlii* against pathogenic microorganisms.

A.A.A	**Disc diffusion method (mm)**	
Samples M/Os	R. communis	C. forskohlii
S. aureus	9	7
E. coli	10	8
K.neumoniae	-	10
C. albicans	-	-

Bioactivity of plant extract by microdilution method

The MIC results in table 3 showed that all tested plant extracts were showed antimicrobial activity against *E. coli*, *S. aureus*, *k. pneumonia*, and *C. albicans* with MIC values ranging from 6.25 to 50 mg/ml. For *S. aureus* the MIC of the water extract of *C. forskohlii* with the best MIC 6.25 mg/ml and the same result for *E. coli*, *k. pneumonia* and *C. albicans.* The MIC of *R. communis* extract showed the best result on *k. pneumonia* with MIC 12.5 mg/ml but on against *E. coli* and *S. aureus* the MIC 25 mg/ml. For the *C. albicans* the MIC is 50 mg/ml.

Table 3: MIC (mg/ml) of *R. communis* and *C. forskohlii* against pathogenic microorganisms.

A.A.A	**MIC (mg/ml)**	
Samples M/Os	*R. communis*	*C. forskohlii*
S. aureus	25	6.25
E. coli	25	6.25
K. pneumoniae	12.5	6.25
C. albicans	50	6.25

DISCUSSION

Plants play an important role in human health because they produce wide array of bioactive molecules which have medicinal value including anti-microbial properties (DESHPANDE *et al.*, 2017). Despite the efforts in production a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased (Chandrakar *et al.* 2013).

Therefore attention directed to return to nature through the use of these medicinal plants in the treatment of many diseases as sources of bioactive substances and less harmful to health. This creates a need of new effective and safe antimicrobial therapeutic agents. In this study crude extracts from plants (*R. communis* & *C. forskohlii*) have been tested to identify the source of the therapeutic effects. The result of this study proved that crude extracts from these plants have antimicrobial activity against tested microorganisms (*S. aureus, E. coli, k. pneumonia* and the fungus *C. albicans*). *R. communis* showed antimicrobial activity against Gram- negative other than Gram positive -bacteria which could be due to the difference in the structure of the bacterial cell wall (Elbashiti *et al*., 2011; El kichaoi *et al.*, 2015; El kichaoi *et al.*, 2017). *C. forskohlii* showed powerful antimicrobial activity against Gram-positive and Gram-negative bacteria (Malleswari *et al.*, 2013;; Saklani *et al.*, 2017).

Also *C.* forskohlii showed powerful antimicrobial activity against *C. albicans* and this result were consistent with previous in vitro study which reported that *C. albicans* was susceptible to coleus species extracts (Tarh *et al.*, 2017). It showed a decrease in MIC to test samples which mean that the *coleus* contains bioactive antimicrobial agents that might inhibit bacteria and fungi by different mechanisms.

CONCLUSION

This study revealed that the *R.* communis and C. forskohlii contain potential antimicrobial components that may be of great importance for the development of pharmaceutical industries against various diseases. But in vivo studies on these medicinal plants are

necessary and must be investigated to determine toxicity of active constituents, their side effects, serum- attainable levels, pharmacokinetic properties and diffusion in different body sites.

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