ANTIMICROBIAL ACTIVITY OF RUTA SP. FROM NORTH JORDAN USING DIFFERENT EXTRACTION METHODS

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Abstract- In recent years, there has been a growing interest in medicinal plants which considered an important source of a novel antimicrobial agents to combat microbial resistance. Several researchers have carried out antimicrobial activity of different plant extracts. Therefore, a greater attention has been paid to screening and evaluating extraction methods. Several extraction methods are well known and commonly used. In the present study, antimicrobial activity of Ruta graveolens extracts against two Gram-positive—Staphylococcus aureus, Bacillus subtilis; Gram-negative bacteria (Escherichia coli); and two fungal strains—Aspergillus niger, and Candida albicans was tested using three extraction methods. The results showed that there were a significant differences in zone of inhibition between the three extraction methods.

Keywords- Ruta graveolens, Medicinal Plants, Antimicrobial Activity, Plant Extracts.

I. INTRODUCTION

Since ancient times, medicinal plants have been used for the prevention and cure of human diseases and offer a new source of biologically active chemical compound to treat many infectious diseases. Reports show that most of the medicinal plants considered as bio-resources of drugs of traditional medicinal systems, food supplements, folk medicines, antimicrobial, antioxidant, and also have potential benefits for the society. More than 80% of the world population relies on medicinal plants for their primary health care needs according to World health organization (WHO) [1-3].

Ruta graveolens is a plant of the family Rutaceae, originally native to the Mediterranean region [4]. Ruta graveolens is an evergreen shrub with bluish-green leaves that emit a powerful odor and have a bitter taste (Figure 1). Ruta graveolens is characterized by a particularly wide spectrum of different plant constituents. In Jordan, it is used as a flavoring agent in foods and beverages and also used as food preservative. In traditional medicine, it is used for its antispasmodic, diuretic, sedative, and analgesic effects, and externally for its anti-rheumatic effect [5]. Only limited data on acute toxicity of Ruta graveolens were available [6].

Extraction methods involve separation of medicinally active fractions of plant tissue from inactive/inert components by using selective solvents and extraction technology. Solvents diffuse into the solid plant tissues and solubilize compounds of similar polarity [7]. Quality of plant extract depends on plant material, choice of solvents and the extraction methods. Because of the huge number of the plant species available for the study, it is important to have efficient extraction methods to evaluate antimicrobial activity of Ruta graveolens as antimicrobial agent [8,2]. The aim of the present study is to test antimicrobial activity of Ruta graveolens extracted in different methods against bacteria and fungi in order to determine the best extraction method.



Fig.1. Photograph of Ruta graveolens

II. MATERIALS AND METHODS

2.1. Plant Sampling

Flowering aerial parts of Ruta graveolens were collected during the period of March 2016 and were collected from plants growing near Al-mafraq city (687m, 32°37‘1805"N, 36°07‘8654"W) in the northern of Jordan.

2.2. Plant Extraction Preparation Methods

Plant sample was extracted with different extraction methods; in this experiment hydrodistillation was used to extract essential oils from plant, but we did not get any essential oils after 4 hours of steam distillation.

Method one: 50 g aerial parts of Ruta graveolens were chopped and subjected to 200 ml of acetone for 24h, then supernatant separated and Powdered sample was formed after acetone evaporated using rotary

evaporator. Dry extract was resuspended in 10 ml ethanol (99%). The crude extract was stored at 2-4°C until used for further investigation for potential of antimicrobial activity. Method two: 100 g of fresh aerial parts of Ruta graveolens were chopped and mixed with 150 ml ethanol (99%) and 150 ml vegetable oil incubated at room temperature with continues shaking for 72 h. Then extracts were separated by centrifugation (3000 rpm for 10 min), after standing for three hours, the two layers were separated by separatory funnel and were stored at 2-4°C until used for further investigation for potential of antimicrobial activity. Method three: 100 g of fresh aerial parts of Ruta graveolens were dried in shade and powdered in a mechanical grinder. The powder (50 g) of the plant materials were mixed with 75 ml ethanol (99%) and 75 ml vegetable oil incubated at room temperature with continues shaking for 72 h. Then extracts were separated by centrifugation (3000 rpm for 10 min), after standing for three hours, the two layers were separated by separatory funnel and were stored at 2-4°C until used for further investigation for potential of antimicrobial activity [9].

2.3. Antimicrobial Activity

antimicrobial activity was determined by the wells diffusion method, For these assays, cultures of the following microorganisms were used: two Gram-positive (Staphylococcus aureus and Bacillus subtilis), one Gram-negative (Escherichia coli bacteria, and fungal strains (Aspergillus niger and Candida albicans).

Bacterial cultures were maintained on nutrient agar (NA) medium, while fungal cultures were maintained on potato dextrose agar (PDA). Suspensions of the tested microorganisms were spread on the solid Muller-Hinton medium and PDA plates. 100 µl of extracts were added to agar wells. A positive control containing microbial culture without extracts and a negative control containing only the medium were performed as well. At the end of the incubation time (24 h at 37 C for bacteria and 25 C for fungi), positive antibacterial and antifungal activities were established by the presence of a measurable inhibition zone and recorded in its width (mm) which includes the well diameter. Each test was performed in three replicates [10].

III. RESULTS AND DISCUSSION

The antimicrobial activities of Ruta graveolens using different extraction methods were given in figures 2, 3 and 4. The results showed that there was a significant difference in antimicrobial activity between extraction methods. In this research fresh and flowering aerial parts of Ruta graveolens were chopped and were subjected to hydrodistillation to get essential oils, but unfortunately we did not get any essential oils after 4 hours of steam distillation, despite other researcher collected pale yellowish oil with a yield of 0.27% [6]. This difference may be due to collection time because in Al-Shuneigat study they collected Ruta graveolens before flowering while in this study flowering parts were used to get essential oils.

All extracts showed antimicrobial activity against both Staphylococcus aureus and Bacillus subtilis, while all extracts did not show any activity against both Aspergillus niger and Candida albicans. The highest antibacterial activity of Ruta graveolens extract with inhibition zone diameter 41±2.8 mm was observed against Bacillus subtilis when using method one to extract Ruta graveolens. While Ruta graveolens extract using method three showed the lowest antibacterial activity with zone of inhibition 11.5± 0.7 mm against Staphylococcus aureus. Quality and activity of plant extract depend on several factors such as plant material, choice of solvents and the extraction methods [2].

Quantity and chemical composition of an extract may depend upon the length of the extraction period, type of the solvent used and its pH, extraction temperature and the ratio between plant sample and extraction solvent [11,2]. Different methods of extraction were used and the basic principle in all these extraction methods was to grind the plant material to very fine then extract active compounds with different solvents under different ratios and extraction conditions. Earlier studies reported that the ideal solvent to sample ratio used was 10:1 (v/w) solvent to dry weight ratio [7,2].

The most widely used extraction method was homogenization the plant tissue with solvent, shaken vigorously for a certain time or left for 24 h after which the extract is filtered or centrifuged. The supernatant then may be evaporated under reduced pressure and then the filtrate redissolved in the solvent to determine the concentration [12-16,2,17].

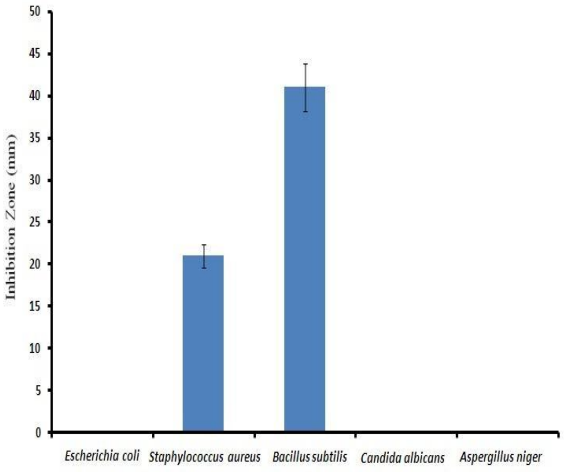


Fig.2. Antimicrobial activity (mm) of Ruta graveolens extracted with method one against five tested species.

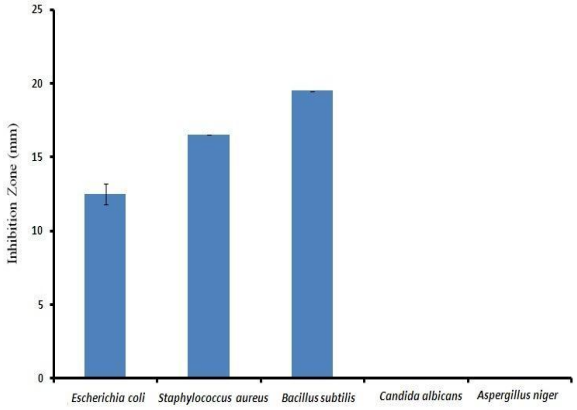


Fig.3. Antimicrobial activity (mm) of Ruta graveolens extracted with method two against five tested species.

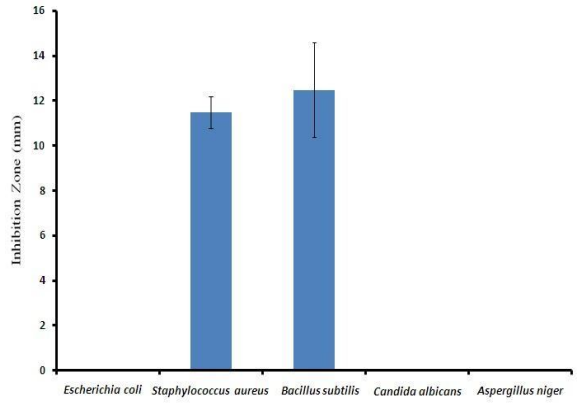


Fig.4. Antimicrobial activity (mm) of Ruta graveolens extracted with method three against five tested species.

The results showed that the extracts were found to be more effective against bacteria particularly Gram-positive bacteria compared with fungi, which may be due to different phyto-plant constituents. Ruta graveolens is characterized by a particularly wide spectrum of different plant constituents. These phyto-plant constituents belong to four major classes of substances which may present in different plant parts such as: Alkaloids, coumarins, flavonoids and essential oil [18].

Many studies reported that Ruta graveolens extracts or essential oils have antimicrobial activity, one of these studies is [6], which showed that Ruta graveolens have antibacterial activity and its activity against Gram-positive bacteria more active than Gram-negative bacteria which is consistent with the results of this study, which may be due to that the cell wall structures of the Gram-positive and the Gram-negative bacteria are different, being more complex in the latter[19-23, 6].

CONCLUSIONS

Plants contain different phyto-chemical constituents which considered valuable sources of active molecules with antimicrobial property. From the above discussion it is cleared that Ruta graveolens extracts may be act as promising natural anti-microbial drugs due to their activity against gram positive and gram negative bacteria and the activity is different according to extraction method. According to the previous results this study highly recommends to isolate pure chemical compound from these essential oils and test their potential antimicrobial activity. However, further studies are needed, such as testing different organic solvents and examine different concentrations, to determine and evaluate the potential of the crude extracts as natural anti-microbial drugs.

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REFERENCES

[1]. Vashist H. and Jindal A. "Antimicrobial activities of medicinal plants- review". Inter. J. Res. Pharm. Biomed. Sci, Vol. 3(1): pp. 222-224, 2012.

[2]. K. Das, R. K. S. Tiwari and D. K. Shrivastava. " Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends- Review". Journal of Medicinal Plants Research, Vol. 4(2), pp. 104-111, 2010.

[3]. Viswanad V, Aleykutty NA, Jayakar B, Zacharia SM, Thomas L. "Development and evaluation of antimicrobial herbal formulations containing the methanolic extract of Samadera indica for skin diseases". J Adv Pharm Technol Res. Vol. 3(2): pp.106-11, 2012.

[4]. Asgarpanah J and Khoshkam R. "Phytochemistry and pharmacological properties of Ruta graveolens L". J Medicinal Plants Res., Vol. 6(23): pp. 3942-3949, 2012.

[5]. Khouri NA and EL-Akawi Z. "Antiandrogenic activity of Ruta graveolens L in male Albino rats with emphasis on sexual and aggressive behavior". Neuroendocrinology Letters, Vol. 26(6): pp. 823-829, 2005.

[6]. Jehad M.Al-Shuneigat, Ibrahim N.Al-Tarawneh, Mahmoud A.Al-Qudah, Sameeh A.Al-Sarayreh,Yousef M. Al-Saraireh and Khalid Y. Alsharafa. "The Chemical Composition and the Antibacterial Properties of Ruta graveolens L. Essential Oil Grown in Northern Jordan". Jordan Journal of Biological Sciences, Vol. 8(2): pp. 139 – 143, 2015.

[7]. Green RJ. "Antioxidant Activity of Peanut Plant Tissues". Master's Thesis. North Carolina State University. USA, 2004.

[8]. Cowan M. M. "Plant Products as Antimicrobial Agents". Clin Microbiol Rev. Vol. 12(4): pp. 564–582, 1999.

[9]. Al-fawwaz A.T. and Alsohaili S.A. "Antimicrobial Activity of Varthemia iphinoides and Majorana syriaca Essential Oils from Jordan and Their Potential Use as Natural Food Preservatives". Journal of Natural Sciences Research, Vol. 5(22): pp.155-161, 2015.

[10]. Alsohaili, S.A. and Al-fawwaz, A.T. "Composition and antimicrobial activity of Achillea fragrantissima essential oil using food model media". European Scientific Journal, Vol. 10(30): pp.156-165, 2014.

[11]. Amita Pandey, Shalini Tripathi. "Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug". Journal of Pharmacognosy and Phytochemistry. Vol. 2(5): pp.115-119, 2014.

[12]. Cichewicz RH, Thorpe PA. "The antimicrobial properties of chilli peppers (Capsicum species) and their uses in Mayan medicine". J. Ethnopharmacol. Vol. 52: pp. 61-70, 1996.

[13]. Taylor RSL, Edel F, Manandhar NP, Towers GHN. "Antimicrobial activities of southern Nepalese medicinal plants". J. Ethnopharmacol. 50: pp. 97-102, 1996.
[14]. Meyer JJM, Dilika F. "Antibacterial activity of Helichrysum pedunctulatum used in circumcision rites". J. Ethnopharmacol. Vol. 53(1): pp. 51-54, 1996.
[15]. Basri DF, Fan SH. "The potential of aqueous and acetone extracts of galls of Quercus infectoria as antibacterial agents". Indian J. Pharmacol. Vol. 37(1): pp. 26-29, 2005.
[16]. Parekh J, Jadeja D, Chanda S. "Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity". Turk. J. Biol. Vol. 29: pp. 203-210, 2005.
[17]. Ncube N. S., Afolayan A. J. and Okoh A. I. "Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends". African Journal of Biotechnology. Vol. 7(12): 1797-1806, 2008.
[18]. Gurjar M. S., Shahid Ali, Masood Akhtar, Kangabam Suraj Singh. " Efficacy of plant extracts in plant disease management". Agricultural Sciences. Vol. 3(3): 425-433, 2012.
[19]. Nazzaro F, Fratianni F, De Martino L, Coppola R and De Feo V. "Effect of essential oils on pathogenic bacteria". Pharmaceuticals, Vol. 6: pp. 1451-1474, 2013.
[20]. Saad NY, Muller CD and Lobstein A. "Major bioactivities and mechanism of action of essential oils and their components". Flavour Fragr J, Vol. 28: pp. 269–279, 2013.
[21]. Hyldgaard M, Mygind T and Meyer RL. "Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components". Frontiers in Microbiol., Vol. 3(12): pp. 1-24, 2012.
[22]. Kavanaugh NL and Ribbeck K. "Selected antimicrobial essential oils eradicate Pseudomonas spp. and Staphylococcus aureus biofilms". Appl Environ Microbiol, Vol. 78(11): pp. 4057-4061, 2012.
[23]. Gao Y, Van Belkum MJ and Stiles ME. "The outer membrane of Gram negative bacteria inhibits antibacterial activity of brochocin-C". Appl Environ Microbiol, Vol. 65: pp. 4329-4333, 1999.

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