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Evaluation of Antibacterial Activity of Essential Oils of Different Types *Rosmarinus officinalis* L.

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Abstract: In this work, we studied antibacterial activity of oils extract of *leaves Rosmarinus officinalis*. Rosemary is well known as a spice and widely used plant in ethno medicine worldwide. This study showed that essential oil extract of *Rosmarinus officinalis* from leaves (grow in Al-Jabal Al Akhdar and house) at 10%, 20% and 30% concentration were effective against both Gram-positive and Gram-negative bacteria., while the essential oil commercial was no effective against both Gram-positive and Gram-negative bacteria at all concentration. We suggest the oils of *Rosmarinus officinalis* (grow in Al-Jabal Al Akhdar and house) rich in phenolic constituent such as a-pinene and 1.8-cineole has the highest anti-oxidant activity against bacterial.

Key words: *Rosmarinus officinalis* L · *Essential oil* and Antibacterial Activity Rosemary

INTRODUCTION

Herbal medicines are widely used all over the world. They are often perceived as being natural and therefore harmless. Many herbal remedies individually or in combination with different formulations such as leaf, powder, pastes, decoction, infusion, etc. had been recommended to treat various diseases. Many, if not most of medicinal plants contain flavonoids; such compounds have been associated with several beneficial effects such as anti-oxidation which consider to be a fundamental property important for life [1].

Many plant products exert antioxidant effect by quenching various free radicals and the singlet form of molecular oxygen. Spices exhibit wide range of beneficial, pharmacological, antioxidant, anti-carcinogenic and anti-inflammatory, antimicrobial effects [2].

Rosemary is a powerful herb belonging to the family Lamiaceae that originates from the Mediterranean region. It is derived from the Latin word *ros* (dew) and *marinus* (sea) which means 'dew of the sea' [3]. Rosemary (*Rosmarinus officinalis* L.) is a spice and medicinal herb widely used around the world. Of the natural antioxidants, rosemary has been widely accepted as one of the spices with the highest antioxidant activity [4]. Rosemary leaves have many traditional uses based on their antibacterial and spasmolytic actions. Used orally for the treatment of dyspeptic complaints [5].

Essential oils are natural, concentrated, volatile aromatic compounds isolated from plants. These compounds possess a wide spectrum of pharmacological activities. The main advantage of natural agents is that they do not enhance the "antibiotic resistance", a phenomenon encountered with the long term use of synthetic antibiotics. One of the known plants that used in the folk medicine is *Rosmarinus officinalis* [6]. Many compounds have been isolated from rosemary, including flavones, diterpenes, steroids and triterpenes. Of these, the antioxidant activity of rosemary extracts has been primarily related to two phenolic diterpenes: carnosic acid and carnosol [6]. Rosemary essential oil is also used as an antibacterial, antifungal [7, 8] and anticancer agent [9]. Ouattara *et al.* [10] investigated the antibacterial activity of selected essential oils against some food spoilage organisms. They concluded that the essential oils of cinnamon, clove and rosemary were the most active. Similar results were obtained by Valero and Salmeron [11] for the antibacterial activity of rosemary essential oil

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against *Bacillus cereus* strains grown in carrot broth. The purpose of the present work is to determine the antimicrobial activity of essential oils of *Rosmarinus officinalis* grow in Al-Jabal Al Akhdar and house in Benghazi and commercial from a super market.

MATERIALS AND METHODS

Plant Material: The leaves of *Rosmarinus officinalis*, were collected from Al-Jabal Al Akhdar area in Benghazi, Libya 2013 (sample 1) and The leaves of *Rosmarinus officinalis* were collected from house in Benghazi, Libya 2013 (sample 2), but oil of *Rosmarinus officinalis* commercial from a super market, Benghazi, Libya 2013 (sample3).

Bacteria Used: Bacteria were taken from the laboratory of microbiology in Banghazi medical center, which know as multi drag resistant bacteria. The bacteria used were *Escherichia coli* (MDR)ATCC, *Staphylococcus aureus* (MDR) ATCC, *Pseudomonas aeruginosa*(MDR) ATCC, *Klebsiella pneumonia* (MDR) and Acinetobacter sp (MDR). Other bacteria were not multi drag resistant such as *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa*. The organisms were isolated and identified by standard methods and identification confirmed by using phonex. The organisms were then subcultured and maintained on nutrient agar slants.

Sample Preparation

Extraction of *Essential oil*: The dry powdered leaves of *Rosmarinus officinalis* (sample 1 and sample 2) (500g) were subjected to hydrodistillation using Clevenger apparatus. The isolation of volatile oils was complete within 6 hours [12]. The oil samples were stored at 7°C in dark air-tight.

Antibacterial Activities of of Essential Oil Extract Rosemary Collected from Al-Jabal Al Akhdar, House and Commercial Rosemary Oil: In this study diluted essential oils were used. The diluted essential oils were prepared by using Dimethyl Sulfoxide (DMSO) to obtain 10%(v/v), 20%(v/v) and 30%(v/v) concentrations. DMSO was used as negative control. A screening assay using well diffusion [13]. Muller Hinton agar plates were inoculated by rubbing sterile cotton swabs after immerse 100µ l bacterial suspensions on plates (overnight cultures grown at 37°C on nutrient agar and adjusted to 0.5 McFarland in sterile saline) over the entire surface of the plate. After inoculation 9 mm diameter wells were cut into the surface of the agar using a sterile cork borer. Different concentrations (10, 20 and 30 %) were added to the wells. Plates were incubated at 37°C for 24 h. Control wells contained solvent DMSO. Zones of inhibition were measured by using ruler. The diameter of zones was recorded. Each assay was carried out in triplicate. The antibacterial assay plates were incubated at 37°C for 24hr. The effect of essential oils on the tested bacteria was compared with the sensitivity of the same bacteria to five antibiotics Colisti sulphate, Amicacin, Amoxycillin, gentamicin and sulphamethoxazole trimethoprim (60µg/ml) Ruberto and Barata [14] and Oyedeji *et al*., [15].

RESULTS

Antibacterial evaluation of essential oil extract rosemary collected from Al-Jabal Al Akhdar, house and commercial rosemary oil.

The antibacterial activity of essential oil extract from rosemary collected from Al-Jabal Al Akhdar, house and commercial rosemary oil were tested using different bacteria and the results are listed in Table (1). The effect of essential oils on the tested bacteria was compared with the sensitivity of the same bacteria to five antibiotics Colisti sulphate, Amicacin, Amoxycillin, gentamicin and sulphamethoxazole trimethoprim are shown in Table (2).

This results showed that two type oils extracts of rosemary (collected from Al-Jabal Al Akhdar and house) were effective against both Gram-positive and Gram-negative bacteria, while commercial oil rosemary no any activity against both Gram-positive and Gram-negative bacteria.

It was found that the essential oil from rosemary collected from Al-Jabal Al Akhdar was potentially active against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter sp and Klebsiella pneumonia with zones of inhibition ranging from 4 to 9 mm at 10 %, 7 to 12 mm at 20 % and 9 to 18mm at 30%. Staphylococcus aureus bacteria showed a better inhibitory effect on the essential oil from rosemary collected from Al-Jabal Al Akhdar at 10 % concentration (9mm) out of five bacterial strains tested, while at 20 % and 30 % concentrations (11mm and 14mm) as shown in Figures (5). Figure (2) show the essential oil from rosemary collected from Al-Jabal Al Akhdar inhibit the growth of *E. coli* bacteria strains produced a zone diameter of inhibition from 4 - 11 mm at 10 %, 20 % and

Table 1: Screening of Antibacterial activity of essential oil extracted of rosemary collected from Al-Jabal Al Akhdar, house and commercial rosemary oil

			Zone of Inhibition (mm) ± Standard deviation				
S/No.	Treatment	Concentration (%)	EC	PA	SA	KP	AS
1.	C		-	-	-	-	-
2.	Essential oil extracted of rosemary collected from Al-Jabal Al Akhdar	10	4±0.31	0.015±	9±0.15	7±0.07	8±0.11
		20	7±0.26	7±0.24	11±0.24	12±0.09	11±0.21
		30	11±0.22	9±0.12	14±0.07	18±0.27	14±0.091
3.	Essential oil extracted from rosemary collected from house	10	9±0.31	1±	9±0.01	5±0.03	-
		20	14±0.26	8±0.33	11±0.04	7±0.15	2±0.35
		30	20±0.22	9±0.92	14±0.21	9±0.21	2±0.21
	Commercial rosemary oil	10	-	-	-	-	-
		20	-	-	-	-	-
		30	-	-	-	-	-

Values are expressed as Mean (X)+ SD, n=3 Abbr. EC= *Escherichia coli,* PA = *Pseudomonas aeruginosa,* KP = *Klebsiella pneumonia,* SA= *Staphylococcus aureus,* AS= Acinetobacter sp, C: Control (DMSO).

Table 2: Antibiotic activity of different type of bacteria

	Zone of Inhibition (mm) ± Standard deviation				
Antibiotic	EC	SA	PA	AS	KP
Colisti sulphate	-	2±0.01	3±0.01	6±0.03	4±0.01
Amicacin	15±0.02	13±0.02	9±0.01	-	12±0.04
Amoxycillin	-	3±0.01	-	-	2±0.01
Gentamycin	10±0.03	6±0.01	5±0.02	3±0.03	1±0.01
Sulphmethoxazole	3±0.12	19±0.03	-	4±0.08	-

Values are expressed as Mean (X)+SD, n=3. EC= *Escherichia coli,* PA = *Pseudomonas aeruginosa,* KP = Klebsiella pneumonia, SA= *Staphylococcus aureus,* AS= A*cinetobacter sp*

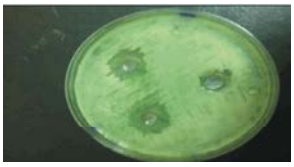

Fig. 1: Antibacterial activity of essential oils extracted of leaves grow in Al-Jabal Al Akhdar against *Acinetobacter sp* bacteria at 10 %, 20 % and 30 % concentrations

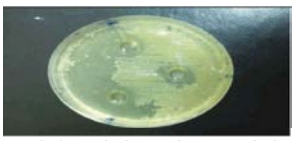

Fig. 2: Antibacterial activity of essential oils extracted of leaves grow in Al-Jabal Al Akhdar against *E. coli* bacteria at 10 %, 20 % and 30 % concentrations

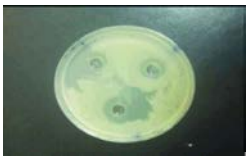

Fig. 3: Antibacterial activity of essential oils extracted of leaves grow in Al-Jabal Al Akhdar against *Klebsella pneumonia* bacteria at 10 %, 20 % and 30 % concentrations

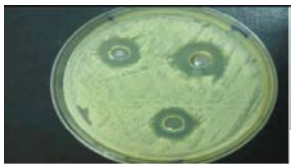

Fig. 4: Antibacterial activity of essential oils extracted of leaves grow in Al-Jabal Al Akhdar against *P. aeruginosa* bacteria at 10 %, 20 % and 30 % concentrations

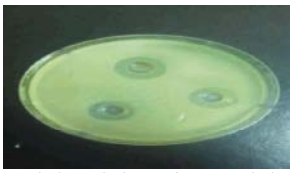

Fig. 5: Antibacterial activity of essential oils extracted of leaves grow in Al-Jabal Al Akhdar against *Staphylococcus.arues* bacteria at 10 %, 20 % and 30 % concentrations

30 % concentrations. Antibacterial activity of essential oil from rosemary collected from Al-Jabal Al Akhdar against *Klebsella pneumonia* bacteria at 10 %, 20 % and 30 % concentrations (7mm, 12mm and 18mm) respectively are shown in Figure (3). The essential oil from rosemary collected from Al-Jabal Al Akhdar showed an inhibitory

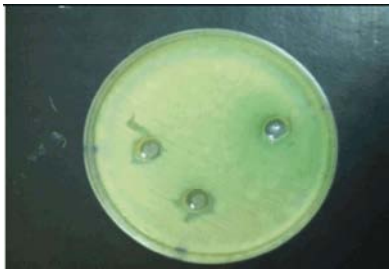

Fig. 6: Antibacterial activity of essential oils extracted of leaves grow in house in Benghazi from *Rosmarinus officinalis* against Acinetobacter sp bacteria at 10%, 20% and 30% concentrations

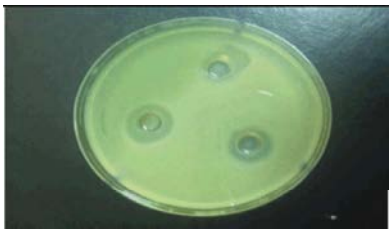

Fig. 7: Antibacterial activity of essential oils extracted of leaves grow in house in Benghazi from *Rosmarinus officinalis* against *Klebsella. pneumonia* bacteria at 10%, 20% and 30% concentrations

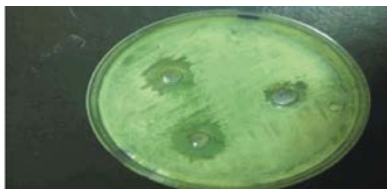

Fig. 8: Antibacterial activity of essential oils extracted of leaves grow in house in Benghazi from *Rosmarinus officinalis* against *P.aeruginosa* bacteria at 10%, 20% and 30% concentrations

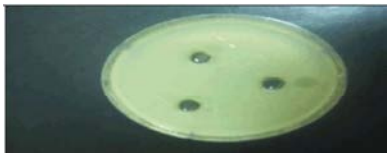

Fig. 9: Antibacterial activity of commercial rosemary oil against Acinetobacter sp bacteria at 10%, 20% and 30% concentration

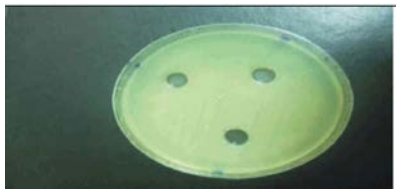

Fig. 10: Antibacterial activity of commercial rosemary oil against *E.coli* bacteria at 10%, 20% and 30% concentration

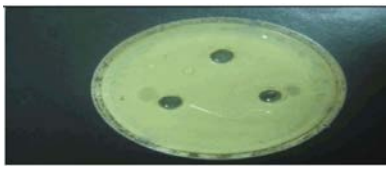

Fig. 11: Antibacterial activity of commercial rosemary oil against *Klebsella. pneumonia* bacteria at 10%, 20% and 30% concentration

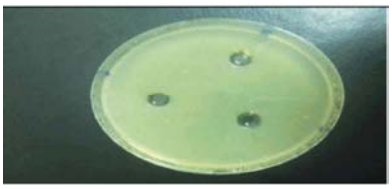

Fig. 12: Antibacterial activity of commercial rosemary oil against Pseudomonas aeruginosa bacteria at 10%, 20% and 30% concentration

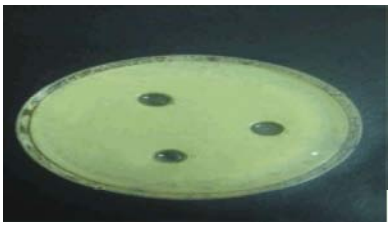

Fig. 13: Antibacterial activity of commercial rosemary oil against *Staphylococcus.arues* bacteria at 10%, 20% and 30% concentration

effect on *P. aeruginosa* bacterial strains with the inhibition zone ranging from 5 to 9 mm at 10 %, 20 % and 30 % concentrations as can be seen in Figure (4). The results showed that the essential oil from rosemary collected from Al-Jabal Al Akhdar has on inhibition of Acinetobacter sp bacteria when the concentration 10 % (8mm), while 20 % and 30 % concentrations have the effect (11 and 14mm respectively) as shown in Figure (1).

The results showed an inhibition slightly active against *Acinetobacter* sp at 20% and 30% concentrations of essential oil extracted from rosemary collected from house, while no inhibition was observed against *Acinetobacter* sp at 10% contraction as shown in Figures (6).

Figure (7) show the essential oil from rosemary collected from house inhibit the growth of *Klebsella pneumonia* bacteria strains produced a zone diameter of inhibition from 5-9 mm at 10%, 20% and 30% concentrations. Antibacterial activity of essential oil from rosemary collected from house against Pseudomonas aeruginosa bacteria at 10%, 20% and 30% concentration (1mm, 8mm and 9mm) respectively are Figure (8).

The commercial rosemary oil was not suitable for testing the antimicrobial activity against *Escherichia coli*, Staphylococcus aureus, Pseudomonas aeruginosa, *Acinetobacter* sp and *Klebsiella pneumonia* at all contractions as shown in Table (1) and Figures (9, 10, 11, 12 and 13).

DISCUSSION

There is an increasing interest in phytochemicals as new sources of natural antioxidant and antimicrobial agents. The use of synthetic antioxidants in the food industry is severely restricted as to both application and level [16]. Currently, there is a strong debate about the safety aspects of chemical preservatives, since they are considered responsible for many carcinogenic and teratogenic attributes, as well as residual toxicity [17].

Plant-derived polyphenols receive considerable attention because of their potential antioxidant and antimicrobial properties [17]. Phenolic compounds exhibit a considerable free-radical scavenging (antioxidant) activity, which is determined by their reactivity as hydrogen- or electron- donating agents, the stability of the resulting antioxidant-derived radical, their reactivity with other antioxidants and, finally, their metal chelating properties [18].

reported that rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria. High percent of the antimicrobial activity they attributed to carnosic acid and carnosol. It is clear that rosemary extracts have bioactive properties, but their antimicrobial activities have not been deeply characterized [19].

Many compounds have been isolated from rosemary, including flavones, diterpenes, steroids and triterpenes. Of these, the antioxidant activity of rosemary extracts has been primarily related to two phenolic diterpenes: carnosic acid and carnosol [8]. The main compounds responsible for the antimicrobial activity are a-pinene, bornyl acetate, camphor and 1, 8-cineole [20, 21, 22].

The activity of rosemary has been ascribed to the diterpene content, mainly carnosic acid and carnosol [23], as well as to the essential oil constituents [24]. Carnosic acid provides protection from the liver carcinogen aflatoxin [25].

Rosemary extracts are widely used in the food. Their major bioactive components have shown antioxidant, antimicrobial, anti-inflammatory, antitumorigenic and chemopreventive activities [24].

CONCLUSION

This results showed that two type oils extracts of rosemary (collected from Al-Jabal Al Akhdar and house) were effective against both Gram-positive and Gram-negative bacteria, while commercial oil rosemary no any activity against both Gram-positive and Gram-negative bacteria. We suggest the two type oils extracts of rosemary (collected from Al-Jabal Al Akhdar and house) more rich in phenolic constituent such as α-pinene, β-pinene, 1, 8-cineol, camphene, limonene and camphor than commercial oil. The phenolic constituent has the highest antioxidant activity against bacterial.

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