In vitro Scavenging Activity of Rosemary Extract and its Activity Against Some Pathogenic Microorganisms

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Abstract

Rosemary (*Rosmarinus officinalis L.*) is one of the most economically important species of the family Lamiaceae. Rosemary extract was examined by applying 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assays. The result proved that rosemary extract had a higher antioxidant activity by absorption at a wavelength of 517a nm by using three different concentrations (0.5, 1.0 and 3) mg/ml which performed the absorbance at (2. 314, 0. 211 and 0.296) nm in comparison with control (21.8, 92.2 and 90) nm respectively. Results obtained using chemical detection of the phytochemicals indicated the presence of flavonoids, phenols, saponins, Steroids and cardiac in rosemary water extract. Water extracts of *R. officinalis* leaves were investigated for their antimicrobial activity. Checker box method was used to estimation the minimum inhibitory concentrations (MIC) against Gram-positive bacteria and Gram-negative bacteria. The results showed the gradual concentration of the extract from the top to the bottom and the change of colors (pink to blue) according to the presence of bacterial growth. Rosemary extracts showed inhibitory effect for some species bacteria by estimation minimum inhibitory concentrations (MIC) against Gram-positive bacteria and Gram-negative bacteria. The study indicates that higher concentrations of the extract were required to inhibit the bacteria. Result of inspection by digging on the culture media was more effective than using the plates.

Key words: rosmary extract, antioxidant activity, DPPH, MIC

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Introduction

Rosmarinus officinalis, ordinarily famous as rosemary, is an ever green permanent sapling pertinence to the *Lamiaceae* family (Pérez, *et al*., 2007). Rosemary is distributed Southern Europe, the Mediterranean basin, North Africa and South Asia (Checklist, 2014) and it has been used for memory

enhancement (Minaiyan *et al*., 2011). *R. officinalis*is strong anti-bacterial, cytotoxic, anti-mutagenic, antioxidant and chemical protection properties. The importance of rosemary has been identified and contains strong compounds and antioxidants, as well as its effect on inhibiting the growth of microbes (Hussain *et al*., 2010). The antioxidant activity makes rosemary anticancer and antidiabetic mechanisms (Abdelaziz, *et al*., 2007).

Polyphenols are compounds that are able to modify cell growth and differentiation and thus interfere with tumor development and progression (Kar, *et al*., 2012). Since rosemary is rich in phenolic compounds and many studies have been targeted for anti-tumor activity, about 20% (Barni*et al*., 2012).Numerous studies on rosemary has shown that these natural antioxidant activities are system-dependent and that it is difficult to prognosis their effectiveness in different diets(Frankel,*et al* ., 1996).

Several studies have shown that rosemary have potent antimicrobial and antifungal activity. Rosemary's antimicrobial activity reckon on the chemical composition of the extract, (Jordán,*et al*., 2013).It can prevent the growth of some bacteria such as *E. coli,Staphylococcus aureus*, according to one of the rosemary studies, it has the ability to block drug strength to some bacteria by reducing the permeability of the membranes of these bacteria. Rosemary can also increase the sensitivity of some bacteria to standard antibiotics (Oluwatuyi *et al*., 2004 and Marinaş *et al*., 2012). The presence of medicinal chemicals containing flavonoids (mainly flavonoids, although flavonoids and flavonoids have also been detected (Perez-Fons, *et al*., 2010: Kontogianni ,*et al*., 2013). Rosemary also contains cardiac, sterol, phenol, and soap material. Flavonoids have been identified as one of the largest groups of the most common secondary receptors of the plant and this substance has antioxidant properties (Corradini*et al*., 2011:Al-Khazraji, *et al*., 2013).These are attributed to its anti-oxidant properties, these are natural elements that destroy free radicals and have benefits to ward off infection and infections, anti-mutations, and anti-cancer, in addition to their ability to modify The main cellular enzyme function(Panchel ,*et al*.,2016).

Sterolis a large group of natural alcoholic and phytochemical compounds produced by the plant (Katan, *et al*., 2003). Phytosterol and phytostanol are added to foods because they have the property of reducing cholesterol absorption in the intestine and thereby lowering cholesterol levels in the blood (Thanh, et *al*., 2006).

Phenol is a solid colorless or white when it is pure, usually sold and used as a liquid (Altanta, 1998). Phenol is used as a bactericide, as a disinfectant, as well as in medical products such as ear and nose drops, lozenges Throat and mouthwash (Altanta, 1998). Saponins are considered a diverse group of compounds distributed widely in the plant kingdom where their structure is characterized by containing triterpene or steroid aglycone and one or more sugar chains. (Price, *et al*., 1987; Oakenfull, 1986).

Cardiac glycosides are a chemical, a class of organic compounds that increases the strength of the cardiac output and increases the rate of contractions by working on a cellular ATPase sodium pump (Patel, 2016).Cardiac glycosides are used in major medical therapies that are useful in treating congestive heart failure, arrhythmias and their ability to increase muscle contraction while lowering the heart rate (Ambrosy *et al*., 2014). The aim of this study was to evaluate in vitro scavenging activity of rosemary extract and its activity against some pathogenic microorganisms

Methods and materials

Qualitative phytochemical analysis of extract

Rosemaryextract were tested to find out the bioactive compounds such as alkaloids, glycosids, steroids, flavonoids, tannins, phenolic compounds and saponins was extracted following standard procedures (Harborne, 1998).

Detection of Antioxidants activity from Rosemary

2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect was determined according to the method of Mättaus (2002).The radical scavenging activity was expressed as % of inhibition according to the following formula (Brand-Williams, *et al*.,1996).

$$\text{Scavenging activity (\%)} = (1 - \text{Absorbance of sample} / \text{Absorbance of control}) \times$$

Preparation of the plant extract

The procedure was done as described by Erkan with some modification (Erkan, *et al*., 2008).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) of plant extracts were determined according standard procedures (Jadaun and Eloff, *et al*., 2007).

Inhibitory efficacy test of extracts against bacteria

Well Diffusion Assay was carried out according to Gupta ,as it was taken Pure single colonies from the surface of bacterial contaminated bacteria with a sterile bacterial loop and placed in a tube Test containing 4 ml of broth nutrient medium and shake well "so that the opacity of the MacFarland tube approach. 10 × 1 (No. MacFarland tube (8-1) Cell. Ml (Use this number for all bacteria used) at length480 nm wave using Spectrophotometer device. Plant Petri dishes containing 20ml of medium Agar Hinton Mueller with bacterial trap and 1.0 ml by micropipette and cotton swab (Swab Cotton) sterile after removing excess quantities of bacterial leeches by pressing the cotton swab firmly Inside the test tube walls, use two plates for each bacterial isolation and leave the plates to dry for 15-30

minutes. Then, digging in the agar using a cork drill and a diameter of 5.0 cm, after that, adds 1.0 ml of plant extracts. 0 in each hole using a micropipette, then incubated at 37 ° C for 24 hours. Then the diameter of the inhibition zone around it was measured in millimeters by the usual ruler (inhibition zone: the growth-free region of the bacterium) as sterilized distilled water was used as a control sample. Experimental microorganisms were selected To detect the inhibitory efficacy of plant extracts, shows the isolates selected in this study and their sources (Perez, *et al*., 1990).

Results and Discussion

Phytochemicals of rosemary extract

Rosemary contains chemical constitutions that may be useful various herbal preparations as anti-inflammatory, analgesic, antipyretic, cardiac tonic, and Anti-asthma**.** Results obtained using chemical detection indicated the presence of flavonoids, phenols, saponins, Steroids and cardiac in rosemary water extract (Table 1).

Table (1): Phytochemical compound detected in water extracts of *R. officinalis* leaves.

Typephytochemical compound	Alkaloid	Flavonoids	Phenols	Saponins	Steroids	Tannins	Cardiac
Result	*-ve*	+ve	+ve	+ve	+ve	-ve	-ve

+ve : indicates the presence of Phytochemical compound.
-ve: indicates the absence of Phytochemical compound.

Hossnzadeh and Nourbakhsh, (2003) detected the presence of flavonoids, alkaloids and saponins in *R. officinalis* water extracts,phenols were isolated and identified from *R. officinalis* by Almela et al. (2006).

DPPH Assay

Table (2) shows the absorbance of the three concentrations of the extract containing DPPH. The result is according the following equation:

$$SA = 1- (AS/AC)\times 100$$

AC = Absorbance control = 2.959

Table (2): Concentrationsand absorbance of rosemary extract.

Concentration	AS(nm.)	SA (nm.)
0.5 mg/ml	2.314	21.8

1 mg/ml	0.211	92.2
3 mg/ml	0.296	90

AS = Absorbance solution, which is the absorbance of the concentration at 517nm., SA = standard absorbance.

In fact, the antioxidant activity of the various extracts (Fig. 1) is directly related to the concentration of the active ingredients, which, in this current study, were high in rosemary extract so we can assume that the natural extracts have higher antioxidant activity than the synthetic materials (Wojdyło,*et al*,.2007).

(Fig. 1): Antioxidant activity of the various phytochemicals of rosemary extracts

Minimum Inhibitory Concentration (MIC) of the rosemary extract

The result is that at a concentration of 100, indicated that the extract prevented bacterial growth, and a dark blue-blue color appeared by ELISA technique.Taking into account the concentration of the extract we considered 100 and its dark color.Rosemary extracts showed a high radical scavenging activityand leverage as antimicrobial agent by antioxidant that have a well liaison between them and phenols.

Detection of efficacy against bacteria using the wells method

The sample which used in this experiment is rosemary water extract leaves. The results presented in (Table 3) indicate that the high concentrations of rosemary leaf water extract 100 mg / ml have inhibitory effects against *S. aureus* with a diameter of the inhibition zone 10mm. While *Pseudomonas* had no response against the rosemary officinalis water extract.

In *Streptococcus*, a high concentration of rosemary water extract showed 100 mg / mm in diameter of the inhibition zone 12 mm. No inhibition of a lower concentration was observed. In *Klebsiella*, a high concentration of rosemary extract showed 100 mg / ml in diameter of the inhibition zone 14 mm and no inhibition was observed with lower concentrations. While in *E. coli*, show a high concentration of rosemary extract 100 mg / ml, the diameter of the inhibition zone 10 mm, and also show the diameter of the inhibition zone at a concentration of 50 mg / ml.In all the above mentioned species, all inhibition were not observed at the low concentrations of the rosemary extract.

Table (3): Diameter of inhibition zone caused by *R. officinalis* leaves water extract at various concentrations against G^{+ve}, G^{-ve} bacteria.

Conc. mg/ml	Diameter of inhibition zone (mm)				
	S.aureus	*Strepto.*	*Klebsiella*	*E.coli*	*Pseudo.*
Control	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
0.78	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
1.56	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
3.12	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
6.25	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
12.5	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
25	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
50	**0.00**	**0.00**	**0.00**	**9mm**	**0.00**
100	**10mm**	**12mm**	**14mm**	**10mm**	**0.00**

Previously prepared and described various rosemary extracts that contain high antioxidants and antibacterial (Moreno,*et al*., 2006).The methanol extract was more effective as an antioxidant and bacteriostatic and in this study the methanol extract was showing an antioxidant content by using the dpph assay. Several laboratory studies have addressed the antioxidant properties of poly phenol of rosmary (Erkan, Ayranci, & Ayranci, 2008; Hras *et al*., 2000).It was found, through the use of several

chemical reagents, for the rosemary plant extract that it contains many active ingredients such as Flavonoids ,Phenols,Saponins,Steroids and cardiac. And this result is consistent with what was mentioned by a number of researchers who confirmed that the rosemary plant contains these compounds (Zhao,*et al* .,1993).The results showed that after the process of incubating the pallet, the color begins with graduation and work on the relationship between the concentration of the extract and the concentration of bacteria. At the first concentration (which we considered 100%), the color is dark based on the concentration of the extract. As a sign of this concentration, the bacteria were not allowed to grow.While the color began to graduate from dark to light to indicate the lower concentration in relation to the extract was not on bacteria, it grew, places in the second concentration (50%) note that the color of the dye was lower than before due to the bacteria acquiring that the color becomes pink in the eighth concentration and this is evidence of not The effect of aqueous extract on bacteria. Our results suggested that the antimicrobial rosemary extracts efficacy was associated with their phenolic composition (Silvia, *et al*., 2006).These results were close to those of many researchers who confirmed the effectiveness of rosemary inhibiting many microorganisms (Katayama and Nagai, 1993). The effective effect of the extract may be on the growth of bacteria due to these extracts that contain a greater amount of phenol and acids (Penalve *et al*., 2005). The variation in the effectiveness of the extracts and the lack of effect of some extracts may be due to the nature of the extracts for each extraction method and the quality of the solvent used in the process Extraction as well as the type of bacteria selected (Mahasnesh *et al.*, 1996). They explained (Shelef, 1984) that the aqueous extract of rosemary plant does not possess efficacy against some types of bacteria. It is noted that the inhibitory effect of bacterial growth increases with increasing concentrations, and this is consistent with many studies that confirm that the effectiveness of most of the compounds inhibiting microbial growth increases with increasing dose. Used in testing (Suhad, *et al*., 2013). The inhibitory ability was apparent against *S. aureus* while it did not show any inhibition activity against negative bacteria of pseudomonas dye; these results are consistent with the results (Babayi *et al*., 2004). This proved that rosemary extract at 100 mg / mL showed inhibitory effects against S. aureus.There are no inhibitory effects against pseudomonas. The negative bacteria resistance to gram dye could be the permeability barrier provided by the ornament wall (Adwand and Abu- Hasan, 1998).The recommendationsis study the in vivo activity of these phytochemical such as anti-cancer activity, antibacterial, and antifungal activities of the plant's extract which make rosemary an effective food preservative with fewer side effects than artificial additives and study the genes which responsible of expression bout the production of these phytochemicals and the routes for stimulation the productivity.

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