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DETERMINE THE BEST CONDITIONS FOR EXTRACTING BIOATIVE COMPOUNDS FROM ROSMARINUS OFFICINALIS LEAVES

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ABSTRACT

The aim of this study is to study the effect of some factors on the efficiency of ultrasound assisted extraction of some compounds from Rosmarinus officinalis in order to determine the best extraction conditions, detection of Polyphenol compounds in extracts, and test the biological activity of alcoholic and water extracts of leaves of rosemary (Rosmarinus officinalis) against some Gram- negative pathogens (Escherichia. coli) and Gram-positive (Staphylococcus aureus) by agar well diffusion method. The results of extracts showed that the best conditions are: the solvent is ethyl alcohol 85%, temperature is 45 ^{0}C, Time is 40 min. alcoholic extract gave a higher extraction yield and Polyphenol content, and more biological activity. the highest biological activity was at concentration (100mg/ml), of alcoholic extract, the average inhibition diameter was 19 mm against Escherichia. coli, and 15 mm against Staphylococcus aureus, while the aqueous extract did not show any activity against the bacteria under study until the concentration 100 mg/ml.

KEYWORDS: Bioactive compounds, Rosmarinus officinalis, ultrasound.

1. INTRODUCTION

1.1. Rosmerinus officinalis

Recent years have witnessed a great deal of interest in medicinal plants, this is for its biological effectiveness, few side effects, and it's contains many effective compounds.

Plant extracts as well as essential oils are of considerable interest because of their antifungal activity and plant-derived poly phenols receive high attention because of their potential antimicrobial properties[1,2].Rosemary is a spice and medicinal herb widely used around the world. Rosemary essential oil is also used as an antibacterial and antifungal[3]. The main compounds responsible for the antimicrobial activity are α-pinene, camphor and 1,8-cineole[4]

The rosemary (*Rosmarinus officinalis L.*) it is consider as one of the most important medicinal plants belong to Labiate family. It is perennial plant, reach to about 1-2 meter in height with small and needle leaves. This plant is grown in houses, gardens in Jordan, Syria, and Plastain. The aqueous infusion of leaves for urinary tract infection Herbal cough, asthma and tonic for red blood cells it is used in anemia patients. The aqueous infusion also used as gargle for mouth and tonsillitis.

The leaves of this plant are contain about 2-3% of volatile oil that include many active compounds such as camphor, pinene and boraneol. The volatile oil it is used in medical ointments production especially for gout or rheumatism as muscles pain relife (Evans.W.,1998).

1.2. Ultrasound Technique

The yield and efficiency of extraction depend on many factors, solvent concentration and type, temperature, time, solid/solvent, and the method of extraction.

Ultrasound- assisted extraction is one of modern extraction methods that have been proven by many studies to enhance the yield and the efficiency of extraction through the acoustic gap and some mechanical effects as Cavitation and increase mass transmission.[5]

Cavitation is physical phenomenon that occur as a result of a sudden drop in pressure inside a liquid resulting from the propagation of sound waves and leads to the

appearance of bubbles, and when the pressure returns to its normal position, the bubbles burst causing a defect in the cell wall, which allows better solvent penetration and thus better material transfer, which enhances the extraction efficiency.[6]

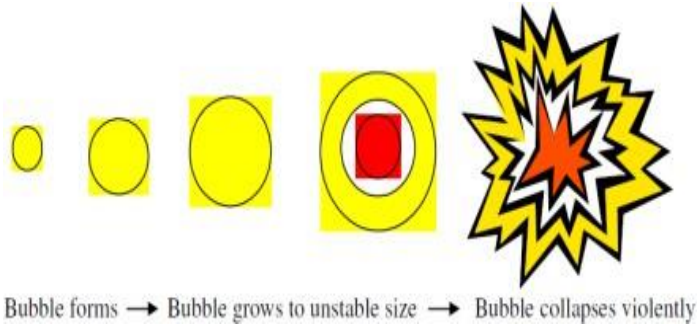

Figure 1: Cavitation phenomenon.

2. MATERIALS AND METHODS

2.1. Prepration of Plant Leaves

Rosemary leaves were obtained at September, and dried, Then ground separately to a fine powder in a mixer for ten seconds, the particle size distribution was determined with a vibratory sieve shaker. The ground particles were stored in freezer until use.

2.2 The Extraction

For each sample, 10 gr of dry plant powder was placed in a beaker and 100 ml of solvent was added to it, the beakers had but in the ultrasonic bath (PS-80A model, made in China, work at frequency 40 kHz). To determine the best extraction condition, we have to study the effect of; solvent type and concentration, extraction Temperature, and extraction time.

2.2.1 Concentration and type solvent effect:

Tow solvents had studied; Ethyl alcohol with five concentration, Water, Experiments was don at 45 ^{o}C and for 20 min.

2.2.2 Tempreture extraction effect

After determine the best solvents, The maximum extraction temperature was set 45^{o}C, in order to preserve the organic compounds from spoilage. Three temperature degree were studied which are (25- 35- 45) ^{o}C.

2.2.3 Time extraction effect

Five times were studied; (10- 20-30- 40- 50) min to choose the best time extraction.

2.3 Yild calculated

After extraction, The samples were filtered, The solvent was evaporated using rotary Vaporizer at 40 ^{o}C and for 20 min, Then, The samples were derided at 40 ^{o}C until the weight is stable.

The yield calculated from the relationship:

$$y = \frac{m_f}{m_i}$$

m_i : sample initial weight
m_f : sample weight after drying

2.4 Polyphenols content

The polyphenols content of extracts have been determined by Folein- c Ciocalteu method.[7] Folin-Ciocleu consists of Phosphotungstique acid and Phosphomolybdique acid, the detector reacts with phenols and produces blue mixture consists of(W_8O_{23}) and (MO_8O_3) wich is absorbed at 760 nm.

2.4.1. Standard curve drawing

A standard series if Gallic acid was prepared in concentration (0.01-0.1 mg\L), 2 ml of extension Folin-Ciocleu is added to 2ml of acid solution, the mixture is leaved for few minutes, then 2ml of(20% Na_2CO_3) is added to the mixture, the solutions are kept in the dark for 90 mint, then the absorption was read using spectrophotometr at 760nm.

2.4.2 Sample preparation

The same previous substances were added to the diluted solution 120 times from extract and the absorption was read. The polyphenols content of extracts are calculated from the relationship

$$C = \frac{AV}{W}.10^{-1}$$

C: The concentration of polyphenols.
A: The concentration of polyphenols from chart V: extract volume (ml)
W: sample wight (g)

2.5. Antibacterial assay

The well diffusion method was used to evaluate the efficiency of extracts against S. aureus and E. coli. A bacterial suspension was prepared from both S. aureus and E. coli with turbidity equivalent to 0.5 McFarland standard [approximately 1.5 × 108 colony-forming unit (CFU)/mL].[8,9,10] Mueller Hinton Agar (MHA) medium was prepared according to the manufacturer's instructions then it was distributed in Petri dishes. tow of

dishes containing MHA medium were cultured with 100 µL of S. aureus bacterial suspension while the other two dishes were cultured with 100 µL of E. coli bacterial suspension. To prepare a solution of extracts at a concentration of 50&100 mg/ml, the solution of the Rosemary extract was weighed aqueous extract has been dissolved in distillated water, alcoholic extract has been dissolved in DMSO, The solutions are inactive solvent against bacteria. Each Petri dishes containing MHA medium cultured with S. aureus and E. coli six wells were made in each dish. All dishes were incubated in the microbiological incubator at 37 °C for 24 hours.

3. RESULTS & DISCUSSION

3.1 Concentration and type solvent effect

The results in table (1) show that the extraction yield increases with the increasing polarity of solvent, This means that compounds contained in the plant are high polarity, However, Ethyl alcohol was better than water because it is an organic solvent.

3.2 Temperature and time effect

The results in table (2) show that the extraction percentage increases with the increasing temperature and time extraction,

$$D = D_o e^{-\frac{E}{RT}}$$

D diffusion coefficient (m^2/sec) D_O diffusion coefficient at maximum temperature (m2/sec), E activation energy (J/mol), T temperature(K).[11] and increasing the extraction time increases the contact time between the solvent and the plant, This contributes to an increase in the extraction yield.

3.3 Polyphenols content

The results in table (3) show that the polyphenols content in alcohol extract was higher than water extract, table show the results of the polyphenols content in the extracts expressed by Gallic acid concentration in dry leaves

3.4 Antibacterial assay

After 24 hours of incubating the dishes in the microbiological incubator, the antibacterial activity of the 50&100 mg/ml alcohol and water extracts solution was evaluated and compared with an antibiotic known for its tested antibacterial activity. The antibacterial activity of alcohol extract against strains of S. aureus and E. coli was good as the inhibition diameter reached to 15 mm for S. aureus, and 19 mm for E. coli, while the aqueous extract did not show any inhibitory activity against strains of S. aureus and E. coli until concentration 100 mg/ml.

4. CONCLUSION

The results confirm that the best conditions were, the solvent is Ethanol at 45 ^oC and for 40 min. The highest extraction yield was 18.85%, and the polyphenols contain (expressed by Gallic acid concentration in dry leaves) was $67.42 mg_{Gal\ acid}/g_{dry.L}$

The highest biological activity was at concentration (100mg/ml), of S. aureus and E. coli until concentration 100 mg/ml.

Table (1): The effect of solvent concentration and type on extraction yield.

Extraction yield%	Time (min)	Temperature	Solvent
5.85	20	45	Ethyl
6.92			Ethyl
9.22			Ethyl
17.90			Ethyl
17.15			Ethyl
13.00			Distillated

Table (2): The effect of temperature and time on extraction yield.

ethyl alcohol extract 85%	Temprature (^oC)	(Time) min
7.30	25	10
9.65	25	20
11.94	25	30
12.75	25	40
13.75	25	50
9.85	35	10
13.64	35	20
14.99	35	30
16.11	35	40
15.87	35	50
14.75	45	10
17.90	45	20
18.33	45	30
18.85	45	40

14.65	45	50

Table (3): The effect of Solvent type on the extraction efficiency.

Solution	Gallic acid concentration mgGal\g dry.L
Ethyl Alcohol 85%	67.42
Distillated Water	30.00

Table (4): inhibition diameter against Bactria.

inhibition diameter (mm)	Concentrationmg /ml	Sample	Bacteria type
11	50	Ethanol extract	Staphylococcus Aureeus(S.T)
16	100	Ethanol extract	
0	50	Water extract	
0	100	Water extract	
18	100	Gentamicien	
13	50	Ethanol extract	E.Coli
19	100	Ethanol extract	
0	50	Water extract	
0	100	Water extract	
19	100	Gentamicien	

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