# EVALUATION OF ROSEMARY ESSENTIAL OIL FOR ITS CHEMICAL CONSTITUENTS, ANTIMICROBIAL, ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES

BY

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## ABSTRACT

*Rosmarinus officinalis* L. was extracted by steam distillation for its essential oil. Rosemary essential oil yielded 0.35 % v/w. The essential oil was characterized by using GC/MS. Thirty-two components were identified in rosemary essential oil. The main components were α-pinene (25.21%), 1,8 cineole (17.87%), camphor (10.69 %),verbenone (4.40 %),camphene(4.32% ) and borneol (4.01%), respectively. The tested oil was examined for its antimicrobial activity against five bacterial and five fungal strains by using Disc –Diffusion technique .Results indicated that Gram-negative bacterial strains were susceptible towards the rosemary essential oil than Gram- positive ones. On the opposite side *saccharomyces cerevisiae* exhibited resistant to rosemary oil. Molds, represented in *Aspergillus niger* and *Aspergillus flavus* exhibited highly sensitivity. It was noticed that the potential effect depends on essential oil concentration. Radical-scavenging activity was determined by means of 1, 1-diphenyl-2-picryl hydrazyl (DPPH). The radical scavenging performance of rosemary essential oil recorded (82.33 %), at 30µl /ml compared with that of BHT (80.40%) at 0.2mg /ml. This study was also designed to examine the hypoglycaemic effect of oral administration of various doses of rosemary essential oil 0.1, 0.15 and 0.17 mg/kg body weight .The acute effect of rosemary essential oil indicated that as the orally administrated doses of rosemary essential oil increased for the diabetic tested rats, the blood glucose levels of the corresponded rats decreased. The subacute effect of rosemary essential oil was examined for 8 days .The diabetic rats administrated orally with different doses of rosemary essential oil had shown reduction in blood glucose levels on the $8^{th}$ day of the experiment.

**Keywords**: rosemary oil, antimicrobial, antioxidant, antidiabetic

# INTRODUCTION

*Rosmarinus officinalis* L.( Lamiaceae ) is a small evergreen plant which grows wild in most Mediterranean countries (Héthelyi *et al*.,1987) The main producers are Italy, Dalmatia, Spain, Greece, Turkey, Egypt, France, Portugal and North Africa (Svoboda and Deans, 1992). Essential oils of R. officinalis, known as rosemary oils, are obtained by steam distillation of the fresh leaves and twigs, and the yields range from 0.5 to 1.0 % (Tewari and Virmani, 1987). It is an almost colorless to pale yellow liquid with a characteristic, refreshing and pleasant odor. The major constituents of the rosemary essential oil reported in literature being α- pinene , 1,8-cineol and camphor; associated with variable amounts of camphene, limonene, borneol, verbenone, etc (Atti-Santos *et al*.,2005, Lo Presti *et al*., 2005, Rezzoug *et al*., 2005 and Katerinopoulos *et al*., 2005).

The rosemary oil is used as a seasoning for food stuffs, such as meat salami and sauces (Lo Presti *et al*., 2005), but due to its chemical active constituents properties, it is used as an antioxidant (for food preserving), antibacterial and antifungal agents against some spoilage organisms (Rezzoug *et al*., 2005). The oil is also used in traditional medicine as tonic pulmonary antiseptic, choleretic and colagoguic agents (Pintore *et al*., 2002). it has been reported to posses a number of therapeutic applications in folk medicines in curing or managing of a wide range of diseases such a diabetic mellitus (DM), respiratory disorders, stomach problems and inflammatory diseases (AL-Serreiti *et al*., 1999 and Kültür., 2007).

The present study was undertaken to investigate the chemical composition of the extracted rosemary essential oil and the antioxidants as well as the antimicrobial rosemary essential oil, and the possible hypoglycaemic effect on rats.

## MATERIALS& METHODS

### Materials:-

Fresh plant leaves of rosemary ( *Rosmarinus officinalis* L. Lamiaceae) were collected from Horticulture Res. Instit., Agricultural Research Center, Giza, Egypt. The leaves were dried in a cool dark place at room temperature ($25^{o}$ C) for 4 days. The average moisture content for the dry plant material was 11%. The dried samples were ground prior to extraction of essential oil.

### Methods:-

**Extraction of essential oil**

The essential oil was extracted from the dried and ground rosemary leaves by steam distillation method for two hours as described by by Atti – Santos *et al*. (2005).The essential oil was dried over anhydrous sodium sulfate and stored in glass vials with sealed caps at -18 ± 0.5°C, till analysis.

**Identification of essential oil by GC /MS:**

GC-MS analysis were carried out according to the method described by Atti – Santos *et al*. (2005).The GC-MS analysis were performed in a HP 6890 GC using amass selective detector Hewlett Packard 6890 / MSD 5973,equipped with HP chemstation software and wiley 275 spectra data. A fused silica capillary colume HP-Innowax (30m x 0.25 mm), 0.25µm film thickness (Hewlett Packard, palo Alto,USA) was used.The temperature program was interface 280°C split ratio 1:100 carrier gas He (56 kpa )flow rate 1.0 ml /min ionization energy 70 ev;

mass range 40-350; volume injection 0.4µl diluted in hexane (1:10). Identification of the individual components was based on comparison of their GC retention indices (RI) on polar columns and comparison with mass spectra of components by GC /MS. Retention indices (RI)were determined relative to the retention times of series of n-alkanes with linear interpolation (Target compounds software of perkin –Elmer).

**Antimicrobial activity:-**

The antimicrobial activity of extracted essential oil was tested on five different bacterial strains, including *Escherichia coli* ATCC 6933 , *Escherichia coli* 0157:H7 ATCC 35150, *Bacillus subtilis* ATCC 33221, *Staphylococcus aureus* ATCC 25923 and *Salmonella Typhimurium*, ATCC 20231.Three strains of mold were used that included *Aspergillus niger* NRRL 2322, *Aspergillus flavus* EMCC 100 and *Fusarium tricinctum CBS* 514478.Two strains of yeast were used that included *Saccharomyces cerevisiae* NRRLY 2034 and *Candida lypolitica* NRRLY 1095.The previous strains were obtained from the Egyptian Microbial Culture Collection (EMCC), Faculty of Agriculture, Ain Shams University, Egypt. Test strains of bacterial were inoculated into Brucella broth (Difco-Manual 1998) and incubated at 35°C for 24 h. The cultures were kept refrigerated on tryptose soy agar slants during the experiment. Yeast and mold cultures were maintained on potato dextrose agar slant at 4°C. Bacterial suspensions were made in Brocella broth to a concentration of approximately $10^8$ CFU/ml .The disc diffusion method was employed for the determination of antimicrobial activities of rosemary essential oil according to Gachkar *et al* (2007). So ,0.1 ml from $10^8$ CFU/ml bacterial suspension was spread on the Muller Hinton Agar (MHA) plates using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates .The essential oil was

dissolved in 10 % aqueous dimethyl sulfoxide (DMSO) as follows 1:1, 1:5, 1:10 and 1:20 .Under aseptic conditions, empty sterilized discs (whatman no5 6 mm dia ) were impregnated with 50 µl of the previous dilutions of the essential oil and placed on the Muller Hinton Agar surface .The plates were incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters. All tests were performed in triplicate .

Fungal spores suspensions were prepared by using sterile triton x-100 that was added to the slant to prepare a spore suspension .Fungal spore suspensions were diluted to achieve a final concentration of $10^8$ CFU/ml .Sabouraud agar medium was used for the disc diffusion method .Different dilutions of essential oil was used as mentioned above for the antibacterial agent .The plates were incubated at 30°C for 48 hours.

**Antioxidant activity DPPH assay**

The free radical – scavenging activity DPPH assay of rosemary essential oil was measured according to the method described by Choi *et al.* (2000).Different volumes of the rosemary essential oil were taken that represented 10, 25 and 30 µl. Each volume was mixed with 900 µl of 100 mM Tris-HCl buffer (pH =7.4), then mixed with 40 µl of ethanol. Tween 20 (Sigma, Aldrich) was prepared at the concentration of 0.5%, then, volumes of the prepared tween were added to the previous mixture at 30 to 50 µl (depending on the volume of used essential oil). Finally, 1 µl of 0.5 mM DPPH (Sigma –Aldrich) in ethanol was added .Tween 20 was used as on oil –in –water emulsifier. The mixture was shaken vigorously

and then immediately placed in Jenway 6300 spectrophotometer to measure the decrease in absorbance at 520 nm. Monitoring was continued for 70 min until the reaction reached a plateau. The control samples were prepared using water instead of essential oils (blank samples). The radical scavenging activities of samples were expressed as (%) inhibition of DPPH. They were calculated according to the following formula of Yen and Duh (1994).

$Ip = [( A_B - A_A) / A_B] X 100$

Where

Ip: Inhibition percentage

$A_B$ : Absorbance values of a blank samples checked after 70 min

$A_A$ : Absorbance values of essential oils checked after 70 min

**Antidiabetic effect of rosemary essential oil :**

**Experimental design**

Sixty six male wistar albino rats, weighing about 160-180 g were used throughout this study. The rats were obtained from the Research Institute of Ophthalmology, Giza, Egypt. The rats were housed in clean cages with temperature (23-25 $^{o}$C), 12-h light: 12h dark cycle and relative air humidity 60 %. The animals were fed on a basal diet for 14 days as an adaptation period .The composition of basal diet was prepared according to AOAC (2000). Salt mixture and vitamin mixture were prepared as described in A.O.A.C (1995 and 2000), respectively.

**Induction of diabetes:**

Sixty rats made diabetic by a single intravenously (marginal ear vein) injection of alloxan monohydrate (Sigma chemicals) 5% (w /v) in normal saline at a dose of 120 mg /kg body weight .Then 5 days later, blood

samples collected and glucose level were determined to confirm the development of diabetes .The rats with blood glucose level >300 mg /dl were consider to be diabetic and were used in the experiment.

**Acute and subacute effect of rosemary essential oil extract in alloxan –induced diabetic rats:**

Sixty - six rats were divided into six groups of six animals in each group .Group 1: Healthy control rats received only tween 80 in distilled water, 10 %( v/v) orally in a volume of 10 ml /kg .Group 2: Diabetic control. Group 3: Diabetic rats treated with insulin injection (20 units /kg /day )for one week .Group 4,5 and 6 :Diabetic rats given rosemary essential oil suspended in tween 80 in distilled water ,10%(v/v)were administered at the doses of 0.1 ,0.15 and 0.17 mg /kg (equivalent to 10 ,25 and 30µl for the DPPH assay) orally in a volume of 10ml /kg of body weight as recommended by Sacchetti *et al*.( 2005). Blood samples were collected from the eye plexuses by a fin capillary glass tubes prior to and at 1, 2 and 6 h after dosing for the acute effect procedure.

The subacute action of rosemary essential oil and the insulin were tested during a longer duration of treatments (8 days) .Thirty rats were also used and also the same concentration of insulin and essential oil were also used. .Blood samples were collected from eye plexuses by a fin capillary glass tubes at 1 st, 3 rd, 5 th, and 8 th days after each treatment .The samples were centrifuged for 10 min. at 3000 rpm and the serum was collected. Blood glucose level was measured .

**Determination of blood glucose concentration**

Blood glucose concentration was determined in serum by commercially available glucose kit (Spinereact,S.A.,Spain ) based on

Trinder s(1969) glucose oxidase method. The glucose levels were expressed as mg /dl.

**Statistical analysis:-**

The collected data of antimicrobial and biological examination were statistically analyzed by the least significant differences (L.S.D) at the 5 % level of probability procedure according to Snedecor and Cochran (1980).

## Results & discussion

The essential oil obtained from the dried rosemary leaves by steam distillation had an average yield of 0.35 % v/w. This result is in within the range that reported by Pintor, *et al.* (2002) and also by Atti – Santos, *et al*. (2005).

**Fractionation and identification of *Rosmarinus officinalis* essential oil by GC / MS:**

Results obtained by GC-MS analysis of the essential oils of *Rosmarinus officinalis* L. are presented in table ( 1 ). Thirty two were identified in the essential oil of *Rosmarinus officinalis* .The main compounds were α-pinene (25.21 % ),1,8 cineole (17.87 % ), camphor (10.69 % ), verbenone (4.40 %), camphene (4.32%) and borneol (4.01%).Another compounds were found in less amounts as linalool (3.54%),borenyl acetate (3.02%) and α-terpineol (2.40%).Santoyo, *et al*. (2005) reported that the presence of α-pinene, 1,8 cineole, camphor, verbenone and borneol ,constituting about 80% of the total *Rosmarinus officinalis* essential oil .Moreover ,the major components α-pinene, borneol ,camphene ,camphor, verbenone and borenyl acetate were also reported to be in *Rosmarinus officinalis* L. essential oil Angioni *et al*.

(2004).The differences in chemical compositions of the *Rosmarinus officinalis* L. essential oil could be attributed to climatic effects on the plant(Gachkar *et al*, 2007).

**Table (1): Chemical composition of *Rosmarinus officinalis* L. essential oil**

| No | Compound | RI | % |
|---|---|---|---|
| 1 | Tricyclene | 921 | 0.36 |
| 2 | α-Thujene | 923 | 0.40 |
| 3 | α-Pinene | 934 | 25.21 |
| 4 | Camphene | 945 | 4.32 |
| 5 | Verbenene | 947 | 1.20 |
| 6 | 3-octanone | 966 | 1.61 |
| 7 | β-Pinene | 971 | 2.17 |
| 8 | Sabinene | 972 | 0.50 |
| 9 | Myrcene | 982 | 1.97 |
| 10 | α -phylandrene | 977 | 0.43 |
| 11 | α -Terpinene | 1009 | 0.49 |
| 12 | ρ-Cymene | 1012 | 2.01 |
| 13 | Ocymene | 1013 | 0.72 |
| 14 | Limonene | 1021 | 2.10 |
| 15 | 1,8 Cineole | 1024 | 17.87 |
| 16 | γ-Terpinene | 1048 | 0.30 |
| 17 | Terpinolene | 1079 | 0.85 |
| 18 | Linalool | 1089 | 3.54 |
| 19 | Mycenol | 1104 | 0.75 |
| 20 | Cis-verbenol | 1124 | 0.60 |
| 21 | Camphore | 1127 | 10.69 |
| 22 | Borneol | 1155 | 4.01 |
| 23 | Terpinene – 4-ol | 1166 | 1.44 |
| 24 | α – Terpineol | 1174 | 2.40 |
| 25 | Myrtenol | 1181 | 0.40 |
| 26 | Verbenone | 1183 | 4.40 |
| 27 | Citronellol | 1208 | 0.22 |
| 28 | Geraniol | 1234 | 0.30 |

| | | | |
|---|---|---|---|
| 29 | Borenyl acetate | 1272 | 3.02 |
| 30 | Neryl acetate | 1341 | 1.94 |
| 31 | Geranyl acetate | 1359 | 0.60 |
| 32 | β-caryophyllene | 1424 | 1.12 |

RI: Retention indices

**Antimicrobial activity:**

The Antimicrobial activity of the different dilutions of the essential oil from the dried rosemary leaves against bacteria, yeasts and molds by the disc diffusion method, statistically analyzed and data was recorded in Table ( 2 ).The essential oil showed inhibition zones against all microorganisms tested. Data obtained from disc diffusion method indicated that the highest activity was observed against the bacterial strains "Gram negative and Gram positive". The strongest inhibition zone was against *Escherichia coli* ATCC 6933 and *Escherichia coli* 0157:H7 ATCC 35150, those recorded 19.5 and 17.81mm, respectively, they were in significant differences with the other tested microorganism. This observation could be noticed for all the different dilutions of the essential oil .So, *Escherichia coli* is the most sensitive microorganism to the rosemary essential oil. Other sensitive microorganisms are *Bacillus subtilis* ATCC 33221, *Staphylococcus aureus* ATCC 25923 and *Salmonella typhimurium*, ATCC 20231, as a result of exposure to various dilutions of the essential oil. Our results indicated that *Staphylococcus aureus* was the least sensitive to the rosemary essential oil at different dilutions. Results of Prabuseenivasan *et al*. (2006) and Gachkar *et al*. (2007) are in accordance with our results. They attributed the antimicrobial property of the essential oil to the presence of α-pinene, 1,8 cineole, camphor ,verbenone and borneol. However, they indicated that,

borneol was being the most effective essential oil compound, followed by camphor and verbenone.

Results in Table ( 2 ) show also that the effect of rosemary essential oil was less sensitive on both yeast and mold than the bacterial strains. The action of essential oil was extended up to only the dilution 1:5. No action could be noticed for the other dilutions tested .Celiktas *et al*. (2007) reported that ,although α-pinene and 1,8 cineole contents of rosemary essential oil are very high, the inhibitory effect against yeast and mold may be related to the low content of camphor , borneol and verbenone .

**Table ( 2 ) Antimicrobial activity of *Rosemarinus officinalis* essential oil estimated by disc diffusion method**

| **Microbial strains** | **Source no** | **Diameter of inhibition zone(mm*) for different dilutions ** of essential oil** | | | |
|---|---|---|---|---|---|
| | | 1:1 | 1:5 | 1:10 | 1:20 |
| **bacteria** | | | | | |
| **Gram-negative** | | | | | |
| *Escherichia coli* | ATCC 6933 | $19.70^{a}$ | $17.56^{a}$ | $8.53^{ab}$ | - |
| *Escherichia coli* 0157: H7 | ATCC 35150 | $17.66^{ab}$ | $15.28^{ab}$ | $6.43^{b}$ | - |
| **Gram-positive** | | | | | |
| *Bacillus subtilis* | ATCC 33221 | $15.66^{bc}$ | $13.53^{bc}$ | $10.83^{a}$ | - |
| *Staphylococcus aureus* | ATCC 25923 | $13.76^{c}$ | $11.40^{c}$ | $7.60^{b}$ | - |
| *Salmonella Typhimurium* | ATCC 20231 | $15.63^{bc}$ | $12.71^{bc}$ | $8.73^{ab}$ | - |
| **Yeast** | | | | | |
| *Saccharomyces cerevisiae* | NRRLY 2034 | $7.53^{d}$ | $7.13^{d}$ | - | - |
| *Candida lypolitica* | NRRLY 1095 | $9.06^{d}$ | $7.33^{d}$ | - | - |
| **Molds** | | | | | |
| *Asperigllus niger* | NRRL 2322 | $9.53^{d}$ | $7.56^{d}$ | - | - |
| *Aspergillus flavus* | EMCC 100 | $8.23^{d}$ | $6.41^{d}$ | - | - |
| *Fusarium tricinctum* | **CBS 514478** | $8.53^{d}$ | $6.83^{d}$ | - | - |
| LSD (5%) | | 3.42 | 2.87 | 2.56 | - |

* Values represent the mean inhibition zones of three experiments
** Dilutions were made by volume: volume (50µl essential oil to the different of 10% aqueous dimethyl sulfoxide DMSO

Table ( 3 ): Free radical- scavenging activity percentage of *Rosmarinus officinalis* L. essential oil at different concentrations

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| Sample | DPPH Inhibition (%) |
|---|---|
| Rosemary essential oil | |
| 10 µl /ml (10000 ppm) | 65.91 |
| 25 µl /ml (25000 ppm) | 75.70 |
| 30 µl /ml (30000 ppm) | 82.33 |
| BHT | |
| 0.2 mg /ml (200ppm) | 80.40 |

**Antioxidant activity of rosemary essential oil:-**

The antioxidant activity of the essential oil of *Rosmarinus officinalis* L. at different concentration was determined by using the DPPH assay test .Results are shown in Table ( 3 ).It could be seen from the results that, as the concentration of rosemary essential oil increased, the percentage of DPPH inhibition increased. The highest percentage of DPPH inhibition was for 30 µl /ml (3000ppm) of rosemary essential oil that recorded 82.33 %. The DPPH inhibition of BHT is 80.40% that is comparable with the rosemary essential oil at 30 µl /ml. So, the antioxidant activities of all the tested samples were mostly related to their concentrations. Sacchetti *et al.* (2005) found that the DPPH radical scavenging activity of *Rosmarinus officinalis* at 10 µl /ml was 63.8%.Their results are in accordance with our results that was recorded 65.91%. DPPH inhibition (%) for the concentration of *Rosmarinus officinalis* at 10 µl /ml. Moreover, Wang *et al.*(2008), reported that ,it is very difficult to attribute the antioxidant effect of a total essential oil to one or a few active principles (1,8-cineole , α-pinene and camphor ) ,because an essential oil always contains a mixture of different chemical compounds .They also added that ,in addition to the major compounds , also minor compounds may make a significant contribution to the oil's activity. They also concluded that, results of antioxidant activity of

*Rosmarinus officinalis* L.essential oil is very much needed by the food industry in order to find possible alternatives to synthetic preservatives (BHT). *Rosmarinus officinalis* essential oil showed interesting results, being one of the best performing one in terms of ability to neutralize free radicals.

**Antidiabetic effect of rosemary essential oil:**

Acute and subacute effect of various doses of the *Rosmarinus officinalis* essential oil in diabetic animals was studied using alloxan – diabetic rats, results are shown in Table ( 4 and 5 ).

Table ( 4 ) shows the acute effect of *Rosmarinus officinalis* essential oil on the blood glucose in alloxan -induced diabetic rats through six hours /day. Significant difference could be shown for all the tested groups of rats. All the diabetic rats either non injected or injected with insulin or rosemary essential oil recorded the highest level of blood glucose after one hour that were in significant difference. The blood glucose levels decreased after 6 hours for all the diabetic rats either injected or not injected with insulin or rosemary essential oil. The blood glucose levels of rats after one hours of injection with 0.17 mg/kg rosemary essential oil were lower than the group of rats injected with 20 units /kg insulin and they were in significant difference .On the opposite side , the group of rats injected with 20 units /kg insulin "G3" after 3 h or 6 hr recorded the lowest level of blood glucose that was in significant difference .Moreover ,as the orally administrated doses of rosemary essential oil increased for the diabetic tested rats ,the blood glucose levels of the corresponded rats decreased. Bakiral *et al*. (2008) found that, at 6 h the magnitude of blood glucose reduction of tested rabbits injected with 200 mg /kg of rosemary extract was much closes to insulin where the blood glucose was

significantly reduced. .

The subacute effect of *Rosmarinus officinalis* essential oil on blood glucose in alloxan -induced diabetic rats for eight days is shown in Table ( 5 ). Significant difference is shown among all the tested samples. Blood glucose levels of diabetic rats were significantly higher than the rest of the other diabetic injected samples with insulin or rosemary essential oil. Each group individually of diabetic rats injected with insulin or administrated orally with rosemary essential oil recorded low level of blood glucose at the end of the 8$^{th}$ day of the experiment .On the other side, the highest reduction in blood glucose was observed on the 8$^{th}$ day for the diabetic rats, injected with 20 units / kg insulin that recorded 95 mg /dl, that was in significant difference. Obviously, the diabetic rats administrated orally with different doses of rosemary essential oil had also shown reduction in the blood glucose levels, on the 8$^{th}$ day of the experiment. The efficiency of rosemary ethanolic extract was examined by Hader *et al* (1994) and Bakiral *et al*. (2008) .They observed that the subacute effect of various doses of *Rosmarinus officinalis* extract on blood glucose levels in alloxan -induced diabetic rabbits was comparable with the other diabetic groups injected with insulin.

Table ( 4 ): Acute effect of *Rosmarinus officinalis* essential oil on blood glucose level in alloxan –induced diabetic rats

| **Groups** | **Mean blood glucose concentration (mg /dl)** | | | | |
|---|---|---|---|---|---|
| | 0h | 1 h | 3 h | 6h | LSD 5% |
| **G1: control** | $100^{cB}$ | $98^{eB}$ | $104^{fA}$ | $98^{fB}$ | 3.76 |
| **G2:Diabetic** | $314^{abC}$ | $330^{aA}$ | $320^{aB}$ | $304^{aD}$ | 3.76 |

| | | | | | |
|---|---|---|---|---|---|
| **G3:Diabetic injected with 20 units /kg insulin** | $316^{aB}$ | $316^{cA}$ | $213^{eB}$ | $163^{eC}$ | 3.76 |
| **G4:Diabetic administrated with 0.1mg /kg *Rosmarinus officinalis*** | $314^{aB}$ | $320^{bA}$ | $312^{bB}$ | $300^{bC}$ | 4.02 |
| **G5:Diabetic administrated with 0.15 mg /kg *Rosmarinus officinalis*** | $314^{abB}$ | $317^{bcA}$ | $280^{cC}$ | $265^{cD}$ | 3.76 |
| **G6:Diabetic administrated with 0.17 mg /kg *Rosmarinus officinalis*** | $310^{bA}$ | $309^{dA}$ | $260^{dB}$ | $240^{dC}$ | 4.02 |
| **LSD 5%** | 3.88 | 3.55 | 3.55 | 3.55 | |

*Capital letters (row) indicate about statistical analysis for the same group through the six hours
** Small letters (columns) indicate about statistical analysis for the different tested groups through each hour.

Table ( 5 ): Subacute effect of *Rosmarinus officinalis* essential oil on blood glucose level in alloxan –induced diabetic rats

| **Groups** | **Mean blood glucose concentration (mg /dl)** | | | | |
|---|---|---|---|---|---|
| | $1^{st}$ day | $3^{rd}$ day | $5^{th}$ day | $8^{th}$ day | LSD 5% |
| **G1: control** | $100^{dA}$ | $98^{fAB}$ | $101^{eA}$ | $95^{eB}$ | 3.76 |
| **G2:Diabetic** | $317^{abB}$ | $340^{aA}$ | $318^{aB}$ | $340^{aA}$ | 4.02 |
| **G3:Diabetic injected with 20 units /kg insulin** | $320^{aA}$ | $150^{eB}$ | $110^{dC}$ | $95^{eD}$ | 3.76 |
| **G4:Diabetic administrated with 0.1mg /kg *Rosmarinus officinalis*** | $318^{aA}$ | $205^{bB}$ | $180^{bC}$ | $150^{bD}$ | 3.76 |
| **G5:Diabetic administrated with 0.15 mg /kg *Rosmarinus officinalis*** | $312^{cA}$ | $195^{cB}$ | $180^{bC}$ | $134^{cD}$ | 4.03 |

| | | | | | |
|---|---|---|---|---|---|
| **G6:Diabetic administrated with 0.17 mg /kg *Rosmarinus officinalis*** | $314^{bcA}$ | $180^{dB}$ | $165^{cC}$ | $125^{d\ D}$ | 3.76 |
| **LSD 5%** | 3.55 | 3.55 | 3.72 | 3.72 | |

*Capital letters (row) indicate about statistical analysis for the same group through the eight days.
** Small letters (columns) indicate about statistical analysis for the different tested groups through each day.

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# التركيب الكيميائي لزيت الروزمارى و النشاط المضاد للميكروبات و المضاد للاكسدة والمخفض لمرض السكر

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تم استخلاص زيت الروزمارى عن طريق التقطير بالبخار. وكانت كمية الزيت المتحصل 0.35 % وزن /حجم . وقد تم التعرف على المركبات الموجودة بزيت الروزمارى باستخدام GC/MS واظهرت النتائج وجود المركبات الاساسية مثل الفا بينين ( 25.21% ) ، 1.8 سينيول ( 17.87% ) ، كامفور ( 10.69% ) ، فيربينون ( 4.40% ) ، كمفين ( 4.32%) و بورنيول ( 4.01%) على التوالى . كما تم اختبار زيت الروزمارى كمضاد للميكروبات وذلك باستخدام خمس سلالات بكترية وخمس سلالات فطرية وذلك عن طريق استخدام – Disc Diffusion .اشارت النتائج الى ان سلالات البكتريا الموجبة لجرام كانت اكثر حساسية لزيت الروزمارى من تلك السالبة لجرام . على الجانب الاخر اظهرت خميرة السكاروميسز سيرفيسيا تأثيرا مقاوما لزيت الروزمارى .الفطريات ممثلة فى الاسبراجلس نيجر واسبراجلس فلافيس اظهرت حساسية عالية. وقد لوحظ ان التاثير المحتمل يعتمد على تركيز الزيت العطرى .تم تقدير نشاطة على الشقوق الحرة بواسطة DPPH . اظهر زيت الروزمارى نشاطا مضاد للاكسدة بنسبة ( 82.33%) عند 30 ميكرولتر /مل مقارنة بمادة BHT التى اعطت( 80.40% ) عند استخدامها بتركيز 0.2 ملجم / مل . وقد تم تصميم هذة الدراسة ايضا لاختبار تاثير جرعات زيت الروزمارى 0.1- 0.15- 0.17 ملجم / كيلو جرام من وزن الجسم على خفض سكر الدم . واشار التاثير الحاد لزيت الروزمارى بانه كلما زاد تركيز الزيت المستخدم ادى الى خفض اكبر فى نسبة سكر الدم لفئران التجارب. كما تم اختبار التاثير دون الحاد من زيت الروزمارى لمده 8 ايام . حيث اظهرت النتائج انخفاض كبير فى مستويات سكر الدم فى اليوم التامن من التجربة.