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# Chemical Analysis and Growth Inhibitory Effect Of Rosemary Plant on *Aspergillus niger*

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**Abstract:** ***The use of natural fungicides instead of synthetic ones covers is a present trend in mycological disease control. The quality and food safeties could be preserved by the addition of natural antifungal agents as essential oils. The aim of the present study was to assess the antifungal activity of certain essential oil against the genus of Aspergillus The oil of the aerial parts of Rosemary plant that collected from Sulaimani city was analyzed by using gas chromatography mass spectrometry. The antifungal activity of Rosemary plant extract against Aspergillus niger was assessed by using agar dilution method. The results showed identification of 17 compounds, with 80.62% of Benzene, 1-methoxy-4- (1-propenyl), 6.85% 1,3,6-Octatriene, 3,7-dimethyl-, (E), 5.16% 1,3,6-Octatriene, 3,7- dimethyl and 2.83% D-Limonene constitute the highest percentage of essential oil. The result of investigation the antifungal effect of Rosemary plant extraction dilutions showed that the essential oil has significant inhibitory effect on growth of Aspergillus niger in 10 %, 12%, 14%, 16%, 18%, 20%, 22%, 24%, 26%, and 28% dilutions with the average inhibitory growth percentage 25.91%, 25.60%, 28.96%, 28.96%, 30.18%, 32.31%, 28.04%, 28.65%, 31.40% and 31.70% respectively. Large percentage antifungal activities of Rosemary oil are related with Benzene, 1-methoxy-4-(1-propenyl) as the main compound. In conclusion, the essential oils exhibited a potential inhibition activity against toxic fungi and many invitro and invivo studies are requested to find more about the antifungal activity of these extracted oils.***

**Keywords:** Essential oils, GC-MS Analysis, *Rosmarinus officinalis, Aspergillus niger.*

## 1. INTRODUCTION

Pathogenic fungi are the most important agent that affects plants and cause severe quality disorders that related to nutritional value, shelf life termination and organoleptic characteristics [2] *Aspergillus niger* is a fungus which is geographically wide distributed and considers as a ubiquitous fungi in nature. It is one of the most common genuses of Aspergillus and is considered as asexual fungi. It causes black mold and rot diseases in many types of plants such as onion, peanut, grapes and it is considered as a food contaminant. In addition *A. niger* has been known as a causative agent of several diseases and allergies in human after inhaling its spore in air such as lung disorder [19]. Recently plant tissues such as seeds, flowers, leave, stems and roots have been used as antimicrobial agents against bacteria, fungi and infections in plants [5]. Moreover, in many places around world plant extracts that known as traditional medical plants have been used as medicine for different human infectious diseases[6]. *Rosmarinus officinalis* is a flowering plant and known as a member of *Lamiaceae* plant family. Different parts of Rosemary's plant have been used for obtaining its essential oil which has been considered as antibacterial and antifungal compounds [15]. Rosemary has been considered as a traditional medicine that used in different diseases such as arthritic pain, insomnia, gout and depression [21]. This plant recently has been used in sanitary, pharmaceutical and cosmetic industry and it's fresh and dried leave used as a flavor for meats and soups [1]. Rosemary is known as an antioxidant plant and this activity associated to two phenolic diterpenes: carnosic acid and carnosol [8].Furthermore, caffeic acid and its derivatives such as rosmarinic acid are component of Rosemary and are recognized as antioxidant compounds. The phenolic compound, rosmarinic acid, obtains one of its phenolic rings from phenylalanine via caffeic acid and the other from tyrosine via dihydroxyphenyl-lactic acid. Accordingly, it has been recorded that the essential oil from Rosemary plant showed strong antifungal activity against *Aspergillus flavus* fungi more than gentamycin antibiotic [12].

This study evaluated the chemical component of Rosemary plant extract and diagnosed the antifungal activity of Rosemary plant extract against growth of *A. niger* fungi in the laboratory.

## 2. MATERIALS AND METHODS

**Isolation and identification of the fungi:**

Fungus *A. niger* was collected from infected onion bulbs in the stores. Collected bulbs were brought to the plant pathology laboratory, department of plant protection, agricultural technical institute in Sulaimaniyah. Potato dextrose agar (PDA) was used for pathogen isolation [2]. *A. niger* spores from infected part of the onion bulbs were transferred to PDA plates and incubated at 25˚C ± 2˚C for 5-6 days for recovery of pathogen. Single spore method and

morphological characteristics were used for purification of fungi. Identification was done according to the standard keys [4, 13].

**Preparation of Plant Materials**

*Rosemary officinalis* leaves and young branches were collected from grown shrubs before blooming in Sulaimaniyah city from 15th Mar to 1st July 2016. The leaves and branches were dried in room temperature, and then the dry plants were milled and kept at appropriate position considering temperature and light until extracting the essential oil. Later, essential oil and Aqueous extract was taken from 100 g of the powdered sample in hydro distillation method with the help of Clevenger set in 500 mL of distal water, kept at 4 °C until use [12].

**Gas chromatography**

Gas chromatography mass spectrum analysis was done to the Rosemary plant extracted oil in the GC-analysis lab in the University of Basrah. The GC-MS instrument model was (QP 2010 Plus SHIMADZU), the computer control at 70 eV was used to analysis the plant extract. About 1 µl of the essential oils was injected into the GC-MS using a micro syringe and the scanning was done for 20 min. When plant oil components were separated they eluted from the column and entered a detector. In the detector an electronic signal was created whenever a compound was detected. All signals volume depended on the concentration of the compounds in the sample.

**Effect of plant extract on radial growth of *Aspergillus niger***

Agar dilution technic was performed for detecting the inhibitory effect of different concentration of Rosemary plant extract on radial growth of *A. niger*. Twenty different concentrations of Rosemary extract were added to PDA medium, that include (2%, 4%, 6% 8%, 10%, 12%, 14%, 16%, 18%, 20%) each treatment was replicated 3 times. Later, 1 cm diameter of fungal block from 4-day-old colony of *A. niger* was inoculated on the center of each Petri plate and incubated at 25˚C ± 2˚C. The diameter of fungal growth colony was measured after 4 days of inoculation. Three replicates in a completely randomized design were used within each treatment. The effect of Rosemary plant extract on fungal growth was calculated by using the following formula [17].

**[Growth inhibition% = [(growth in control – growth in sample)/growth in control] × 100].**

**Statistical Analysis**

The effect of Rosemary plant extract with different concentration on radial growth of *A. niger* evaluated by a one way analysis of variance (ANOVA). Mean differences between Rosemary plant extracts were separated by Fisher's [7] test significant difference (LSD) at 1% significant probability level.

## 3. RESULTS

Through the analyzation study of Rosemary plant essential oil by Gas chromatography, the results showed identification of 17 compounds (Table 1), with 80.62% of Benzene, 1-methoxy-4-(1-propenyl), 6.85% 1,3,6-Octatriene, 3,7-dimethyl-, (E), 5.16% 1,3,6-Octatriene, 3,7-dimethyl and 2.83% D-Limonene constitute the highest percentage of essential oil.

**Table (1): Chromatogram of essential oil plant *Rosmarinus officiall***

| Peak# | R.Time | Area | Area% | Name |
|---|---|---|---|---|
| 1 | 4.768 | 699515 | 0.81 | .alpha.-Pinene |
| 2 | 5.972 | 2441413 | 2.83 | D-Limonene |
| 3 | 6.048 | 5903202 | 6.85 | 1,3,6-Octatriene, 3,7-dimethyl-, (E)- |
| 4 | 6.172 | 4446370 | 5.16 | 1,3,6-Octatriene, 3,7-dimethyl- |
| 5 | 6.627 | 82116 | 0.10 | Guanosine, 2'-deoxy-N-(trifluoroacetyl)-, 3',5'-bis(trifluoroacetate) |
| 6 | 6.778 | 473836 | 0.55 | 1,6-Octadien-3-ol, 3,7-dimethyl- |
| 7 | 7.086 | 309810 | 0.36 | 2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)- |
| 8 | 7.324 | 350012 | 0.41 | Camphor |
| 9 | 7.594 | 305978 | 0.35 | Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)- |
| 10 | 7.897 | 69513832 | 80.62 | Benzene, 1-methoxy-4-(1-propenyl)- |
| 11 | 7.992 | 155404 | 0.18 | Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- |
| 12 | 8.693 | 376418 | 0.44 | Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester |
| 13 | 9.349 | 168388 | 0.20 | 3-Allyl-6-methoxyphenol |
| 14 | 9.750 | 687871 | 0.80 | Benzene, 1,2-dimethoxy-4-(2-propenyl)- |
| 15 | 9.972 | 86366 | 0.10 | Caryophyllene |
| 16 | 13.886 | 112893 | 0.13 | Naphthalene, decahydro-2,2-dimethyl- |
| 17 | 14.857 | 112839 | 0.13 | Methyl 10-trans,12-cis-octadecadienoate |
| | | 86226263 | 100.00 | |

The essential oil complex identified as restrictive index (RI), and quantitative percentage of thecompounds. The result of investigation the antifungal effect of Rosemary plant extraction dilutions showed that the essential oil has significant inhibitory effect on growth of fungi *A. niger* in10%, 12%, 14%, 16%, 18%, 20%, 22%, 24%, 26%, 28% dilution with average inhibitory growth percentage 25.91%, 25.60%, 28.96%, 28.96%, 30.18%, 32.31% , 28.04% , 28.65% , 31.40% and 31.70% respectively. (Figure 1)

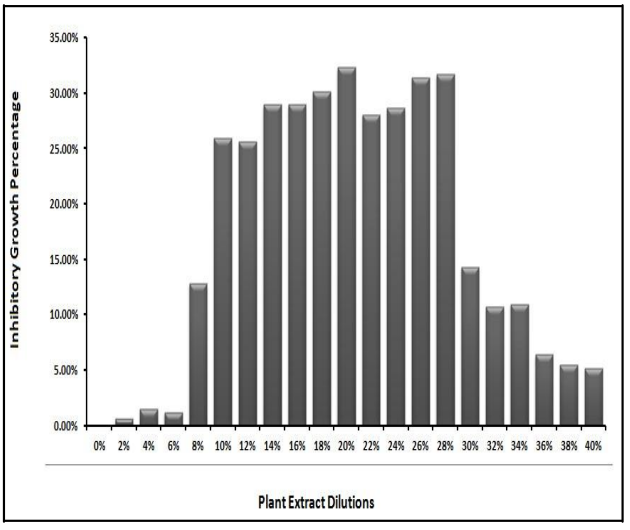


**Figure 1. Growth inhibitory effect of Rosemary extract with different dilutions on fungus *Aspergillus niger*.**

## 4. DISCUSSION

According to our results, it has been found that the largest antifungal activities of Rosemary essential oil are related to cis-Anethole (Benzene, 1-methoxy-4-(1-propenyl) as a major compound. Essential oil extracted from the aerial parts of (Rosmarinusofficinalis L.) by hydrodistillation. It was analyzed by gas chromatography/mass spectrometry and the components identified were (17) compound. The major constituents of the essential oil of (Rosmarinusofficinalis L.) from higher to lower were; cis-5.16%, D-Limonene 2.83%, α–Pinene 0.81%, methyl eugenol (Benzene, 1,2-dimethoxy-4-(2-propenyl)- 0.80%, Linalool (1,6-Octadien-3-ol, 3,7-dimethyl-) 0.55%, Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester 0.44%, Camphor 0.41%, Allocimene A (2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-)0.36%, (-)-Borneol ( Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)- 0.35%,Chavibetol (3-Allyl-6-methoxyphenol) 0.20%,Verbenone(Bicyclo [3.1.1] hept-3-en-2-one,4,6,6-trimethyl-) 0.18%, Naphthalene, decahydro-2,2-dimethyl- 0.13%, Methyl 10-trans,12-cis-octadecadienoate 0.13%, Guanosine2'-deoxy-N-(trifluoroacetyl)-,3',5'-bis(trifluoroacetate) 0.10% and Caryophyllene, 0.10%. The chemical composition of Rosemary plant oil has been studied broadly. It was obvious that the main compositions of Rosemary plant were different according to the countries and their environmental conditions. *Rosmarinus officinalis* oil essential was collected from various locations such as in Australia, USA, South Africa, Kenya, Nepal, and Yemen.The whole chemical compositions were not the same, however the main shared composition were alphapinene , cineole, (+)-verbenone, (-)-borneol, (-)-camphor, and racemic limonene [18].

*Aspergillus flavus* growth inhibition observed by Rosmary plant that could be due to the presence of its essential oil that contain α-pinene as a major phenolic compound. There have been concluded that the rosemary plant at 1, 1/2 and 1/4 oil dilutions exhibited strong antifungal activity, even more than gentamycin antibiotic on A. flavus and exhibited moderate of borneol was at 10% dilution. They reported that Forty-one compounds were identified in theessential oil concluded as 99.74% of the total oil. The main essential oil compounds that could be have that antifungal activity were; α-pinene (15.52%),camphor (11.66%), verbenone (11.10%) and 1, 8-cineole (10.63%), [12]. The antifungal activities of Rosemary essential oil was investigated against different plant pathogenic fungi Alternariaalternata, Botrytis cinerea and Fusariumoxysporum. The results of oil inhibition effects Anethole (Benzene, 1-methoxy-4-(1-propenyl), 80.62%, trans-β-Ocimene (1,3,6-Octatriene, 3,7-dimethyl-, (E)-)6.85%, cis-β-Ocimene (1,3,6-Octatriene, 3,7-dimethyl-)

On fungal growth varied depending on the levelsof essential oil used in experiment [16]. The inhibition effects of Rosemary extracts were investigated with regard to antioxidant (DPPH radical scavenging and total phenolic content – Folin-Denis reagent), antibacterial (Gramnegative bacteria – *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 and Gram-positive bacteria – *Staphylococcus aureus* ATCC 25923 Bacillus cereus ATCC 11778 – and) and antifungal (Candida albicans) activities. Antioxidant, antibacterial and antifungal activities of the SFE extracts were confirmed [9]. Moreover, Rosemary's essential oil as antimicrobial effects were tested against

different pathogenic bacteria, *S. aureus, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterococcusfeacalis, E. coli, Staphylococcus epidermidis, Bacillussubtilis and C. albicans*. Minimum inhibitory concentration (MIC) was used to find the essential oil inhibitory effects on these bacteria. The results showed that all bacteria were sensitive to the used essential oil; however the effect of the oil were different depending on the season and the location of the collecting plant [20]. According to another investigation on Rosemary essential oil antimicrobial effect, The 41 different components were found by using GC, the main constituents are α-pinene (15.52%), camphor (11.66%), and verbenone(11.10%) and 1, 8- cineole (10.63%).The antimicrobial effects of essential oil were investigated with Tetracycline antibiotic against nine strains of resistance pathogenic gram-positive and gram-negative bacteria. The results showed that the Rosemary's essential oil has a high antimicrobial effect against all used bacteria [11].Rosemary essential oil with Zatariamultiflora (thyme), Pelargonium graveolens (geranium), Artemisia sieberibesser (Artemisia) and lavandulastoechas (lavender) were tested to identify their antifungal effects against C.albicans. According to the GC, the main component of thyme, geranium, Rosemary, lavender and artemisia essential oil were Carvacrol (39.8%), Citronellol(45.2%), α- pinene (23.7%), 1, 8-cineol (30.2%) and, α- thujone (38.8%). The antifungal effect of Rosemary showed moderate effect [10]. The growth inhibition effect of Rosemary essential oil against *Aspergillus flavus* (PTCC=5004) showed strong controlling and antifungal effect [14].

## 5. CONCLUSION

According to this study, the antimicrobial effect of Rosemary essential oil against fungus *A. niger* showed positive result. It has been concluded thatthis plant's essential oil could be considered as antifungal compound in the nature. Moreover, this study showed the main chemical composition of Rosemary plant essential oil that was growing in Sulaimaniyah city, which was different from the plant composition in other countries with different environmental condition.

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