



## Original Article

# Antibacterial and Antioxidant [ Peroxynitrite and Singlet Oxygen Scavenging ] Capacities of *Rosmarinus officinalis*, L. and Screening Its Natural Chemical Constitutes

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### Abstract:

The antioxidant compounds found in herbs and plants include both vitamins (E and C) and glutathione together with enzymes and phenolic compounds. The aromatic plant from Lamiaceae family which originates from Mediterranean areas is known scientifically as *Rosmarinus officinalis* L. Nineteen peaks in the GC-MS chromatogram identified the present chemicals. The compounds mentioned are Octacosanol, D-alpha-Tocopherol, 9,13-dimethylnonacosane, n-Hentriacontane, 2-Pinene, L-Camphene, beta-Myrcene, n-Dotriacontane, beta-Amyrin, Lupeone, 1,8-Cineol, Alpha-Phellandrene, Limonene, Dolcymene, Alpha-Terpinene, Eugenol, Thymol, Carvacrol methyl ether and 3-Thujen-2-one. The current study examined the bioactivity of the Ethanolic extract of *Rosmarinus officinalis*, L. and the standard antibiotics CFO – Cefoxitin, EI - Erytromycin, and GN - Gentamicin against seven tested pathogens: *Enterococcus faecalis* (19.08±0.08, 26.11±0.07, 28.91±0.07, and 20.00±0.06), *Escherichia coli* (20.54±0.08, 24.00±0.05, 27.9±0.07 and 18.95±0.07), *Bacillus subtilis* (15.07±0.08, 25.00±0.07, 24.98±0.07 and 27.65±0.07). *Staphylococcus aureus* (13.19±0.06, 27.11±0.13, 21.00±0.09 and 16.00±0.08). *Staphylococcus epidermidis* (10.94±0.05, 18.00±0.09, 14.06±0.07, and 20.98±0.09). *Streptococcus pyogenes* (12.50±0.05, 18.45±0.09, 20.00±0.08 and 20.61±0.11). The metabolites of *Rosmarinus officinalis* exhibited significant activity against *Escherichia coli* (20.54±0.08).

**Keywords:** Natural Chemical Constitutes, Antibacterial, Peroxynitrite, Singlet Oxygen, *Rosmarinus officinalis*

### Introduction:

*R. officinalis* L. aerial parts from Lamiaceae which are utilized in aroma therapy have been routinely used through the ages of folk medicine to treat human pain symptoms. Scientific research

has documented that aroma therapy with this variety shows pain reducing properties which also reduces unpleasant sensations during pain in patients with arthritis while also testing results in

human-induced pain experiments [1]. A phytochemical evaluation of *R. officinalis* essential oil (RO) has already been conducted although its antinociceptive and anti-inflammatory properties remain unconnected. Previous experiments revealed that ethanol extract applied to aerial parts from *R. officinalis* showed antinociceptive as well as anti-inflammatory benefits across different pain models. We observe that this research represents the initial examination of RO as an anti-inflammatory agent and antinociceptive along with its underlying pharmacological action. The main objective of this present research included both antinociceptive effect testing of RO as well as identification of its active chemical components [2, 3]. Experts currently debate many challenges with the performance and security of medical and agri-food industry chemicals. Specialists identify the emergence of microorganism resistance against multiple antibiotics as a serious matter [4]. Additives containing antioxidants are believed to create health risks for those who consume them. Creating new therapeutic compounds represents a vital approach for managing bacterial resistance together with food oxidation [5]. At present essential oils attract much attention as a potential source of bioactive natural molecules because scientific studies investigate their potential to serve as an alternative treatment option for infectious diseases and food preservation against oxidation [6, 7]. The flavoring sector and cosmetics industry together with perfume production make use of essential oils as basic materials. Such compounds find applications in pharmaceuticals through their use as active pharmaceutical ingredients and as flavor components in various produced items [8]. The natural environment distributes essential oils throughout aromatic plant species worldwide. The medicinal plant *R. officinalis* L. (rosemary) exists within the Lamiaceae family that stands as one of the major groups of medicinal plants [9]. The research on *R. officinalis* continues to focus on pharmaceutical applications in combination with cosmetic development and food applications because this plant contains aromatic features

together with antioxidant effect and antimicrobial qualities and antispasmodic and anti-tumor features which have traditional medicinal uses.

### **Materials and methods:**

#### **Bioactive natural chemical components of *Rosmarinus officinalis* undergo spectral analysis by using (GC-MS) on sample collection data.**

The sample used in this research consisted of dried Rosemary (*Rosmarinus officinalis* L.) leaves extracted from a Hillah local market in Iraq. Researchers performed their analysis through GC-MS examination using Agilent 789 A equipment. J&W Scientific in Folsom California provided the DB-5MS column which served as the GC column. The column dimensions consisted of 30 m0.25 mm i.d. with 0.25  $\mu$ m film thickness. The oven temperature during the current investigation remained equivalent to what was used in the earlier examination. The selected carrier gas was helium at a flow rate measurement of one milliliter per minute. The GC column effluent was transferred through a heated line operating at 250 degrees Celsius before entering the MS instrument source. The ionization station operated at 70 electron volts eV while the source temperature remained at 230 degrees Celsius °C. Measurement occurred between atomic mass units ranging from 41 to 450 within the defined parameter.

#### **Evaluation of the antimicrobial activity of compounds derived from secondary metabolites against seven different types of harmful bacteria**

The agar received five millimetre diameter wells made by using a sterile cork-borer. Wells received a 25- $\mu$ l volume of metabolite solutions extracted from *Rosmarinus officinalis*. Scientists examined the Ethanolic extract of *Rosmarinus officinalis*, L. bioactivity when combined with standard antibiotics CFO – Cefoxitin and EI - Erytromycin and GN - Gentamicin to test seven different pathogens.

## Peroxynitrite scavenging

According to standard method I made a minor adjustment for the assay process. The test solution contained 50 mM buffer at pH 7.4 along with 0.1 mM DTPA and 90 mM NaCl and 5 mM KCl while Evans Blue reached a concentration of 12.5  $\mu$ M throughout a 1 ml reaction volume. Different amounts of plant extract (0–200  $\mu$ g/ml) combined with 1 mM peroxynitrite. The solution incubated at 25°C for 30 minutes allowed scientists to measure absorbance through the 611 nm wavelength. The ONOO- scavenging activity percentage resulted from evaluating the reaction results against the blank control. The experimental procedure was undertaken six times. The reference compound used for the experiments was gallic acid.

## Singlet oxygen scavenging

The researcher produced singlet oxygen through the interaction of NaOCl and H<sub>2</sub>O<sub>2</sub> while using 440 nm to track the RNO bleaching process. A reaction mixture that included 45 mM phosphate buffer (pH 7.1) and 50 mM NaOCl, 50 mM H<sub>2</sub>O<sub>2</sub>, 50 mM histidine, 10  $\mu$ M RNO, and different sample concentrations from 0 to 200  $\mu$ g/ml maintained in a 2 ml final volume served to determine single oxygen production. A solution containing 45 mM phosphate buffer (pH 7.1), 50 mM NaOCl, 50 mM H<sub>2</sub>O<sub>2</sub>, 50 mM histidine, 10  $\mu$ M RNO and various concentrations (0–200  $\mu$ g/ml) of sample in a final volume of 2 ml was incubated at 30°C for 40 min to measure RNO absorbance decrease at 440 nm. The antioxidant abilities of sample solution measured through tests were evaluated against the antioxidant properties of lipoic acid which served as reference standard. The experiment was conducted for each test six times.

## Statistical Analysis

The statistical analysis used ANOVA for comparison between mean values through SPSS 19.0 software (IBM, New York, NY, USA) at 95% or 99% confidence levels by applying

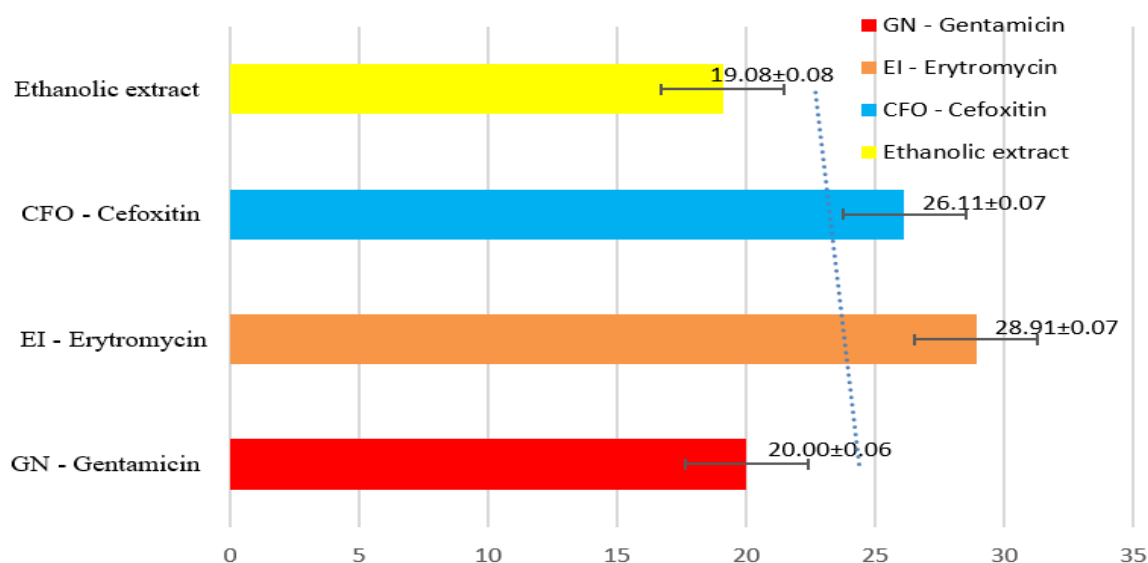
Tukey's honestly significant differences (HSD) test. The research considered any p-value below 0.05 as statistically significant.

## Results and Discussion

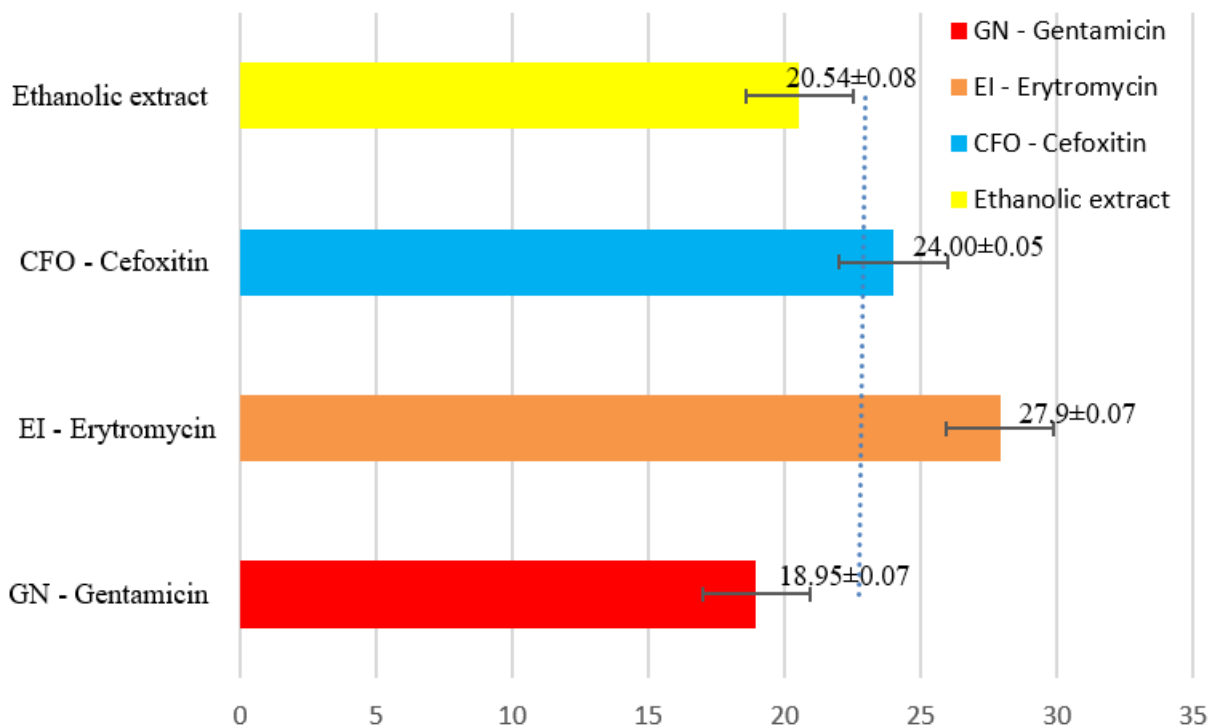
The GC-MS chromatogram displayed nineteen peaks corresponding to the identified chemicals. The compounds mentioned are Octacosanol, D-alpha-Tocopherol, 9,13-dimethylnonacosane, n-Hentriacontane, 2-Pinene, L-Camphene, beta-Myrcene, n-Dotriacontane, beta-Amyrin, Lupeone, 1,8-Cineol, Alpha-Phellandrene, Limonene, Dolcymene, Alpha-Terpinene, Eugenol, Thymol, Carvacrol methyl ether and 3-Thujen-2-one. The spice rosemary exists in commercial markets as an antioxidant ingredient. Medical professionals employ rosemary extracts for disease treatment due to its ability to protect the liver alongside its effects on Alzheimer's disease management and its suppression of new blood vessel development. The extracts find applications in food conservation by blocking both microbial growth and oxidative reactions. Foods could benefit from using rosemary extract as a possible replacement or reducer for synthetic food antioxidants. Technology and consumer benefits can be obtained from rosemary extracts when used as food preservatives. Scientists investigated the biological actions of *Rosmarinus officinalis*, L. Ethanolic extract against seven pathogens along with standard antibiotics CFO and GN and EI. *Enterococcus faecalis* (19.08±0.08, 26.11±0.07, 28.91±0.07, and 20.00±0.06), *Escherichia coli* (20.54±0.08, 24.00±0.05, 27.9±0.07 and 18.95±0.07), *Bacillus subtilis* (15.07±0.08, 25.00±0.07, 24.98±0.07 and 27.65±0.07). *Staphylococcus aureus* (13.19±0.06, 27.11±0.13, 21.00±0.09 and 16.00±0.08). *Staphylococcus epidermidis* (10.94±0.05, 18.00±0.09, 14.06±0.07, and 20.98±0.09). *Streptococcus pyogenes* (12.50±0.05, 18.45±0.09, 20.00±0.08 and 20.61±0.11). The metabolites of *Rosmarinus officinalis* exhibited significant activity against *Escherichia coli* (20.54±0.08) (Figure 1-6).

**Table 1. Compositional profile of *Rosmarinus officinalis* methanolic extract as analyzed by GC-MS.**

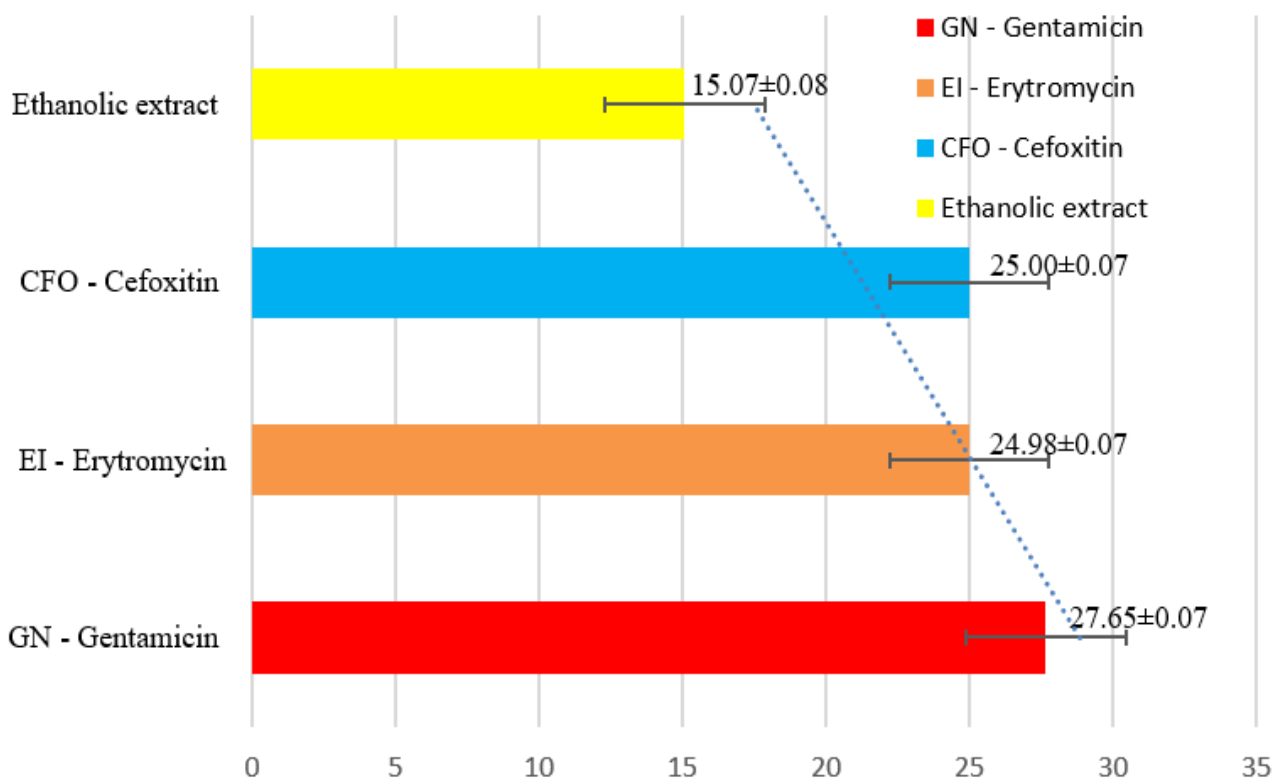
No.	Compounds	Molecular Formula	Molecular Weight
1.	Octacosanol	C <sub>28</sub> H <sub>58</sub> O	410.8 g/mol
2.	D-alpha-Tocopherol	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430.7 g/mol
3.	9,13-dimethylnonacosane	C <sub>31</sub> H <sub>64</sub>	436.8 g/mol
4.	n-Hentriacontane	C <sub>31</sub> H <sub>64</sub>	436.8 g/mol
5.	2-Pinene	C <sub>10</sub> H <sub>16</sub>	136.23 g/mol
6.	L-Camphene	C <sub>10</sub> H <sub>16</sub>	136.23 g/mol
7.	beta-Myrcene	C <sub>10</sub> H <sub>16</sub>	136.23 g/mol
8.	n-Dotriacontane	C <sub>32</sub> H <sub>66</sub>	450.9 g/mol
9.	beta-Amyrin	C <sub>30</sub> H <sub>50</sub> O	426.7 g/mol
10.	Lupeone	C <sub>30</sub> H <sub>48</sub> O	424.7 g/mol
11.	1,8-Cineol	C <sub>10</sub> H <sub>18</sub> O	154.25 g/mol
12.	Alpha-Phellandrene	C <sub>10</sub> H <sub>16</sub>	136.23 g/mol
13.	Limonene	C <sub>10</sub> H <sub>16</sub>	136.23 g/mol
14.	Dolcymene	C <sub>10</sub> H <sub>14</sub>	134.22 g/mol
15.	Alpha-Terpinene	C <sub>10</sub> H <sub>16</sub>	136.23 g/mol
16.	Eugenol	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	164.20 g/mol
17.	Thymol	C <sub>10</sub> H <sub>14</sub> O	150.22 g/mol
18.	Carvacrol methyl ether	C <sub>11</sub> H <sub>16</sub> O	164.24 g/mol
19.	3-Thujen-2-one	C <sub>10</sub> H <sub>14</sub> O	150.22 g/mol



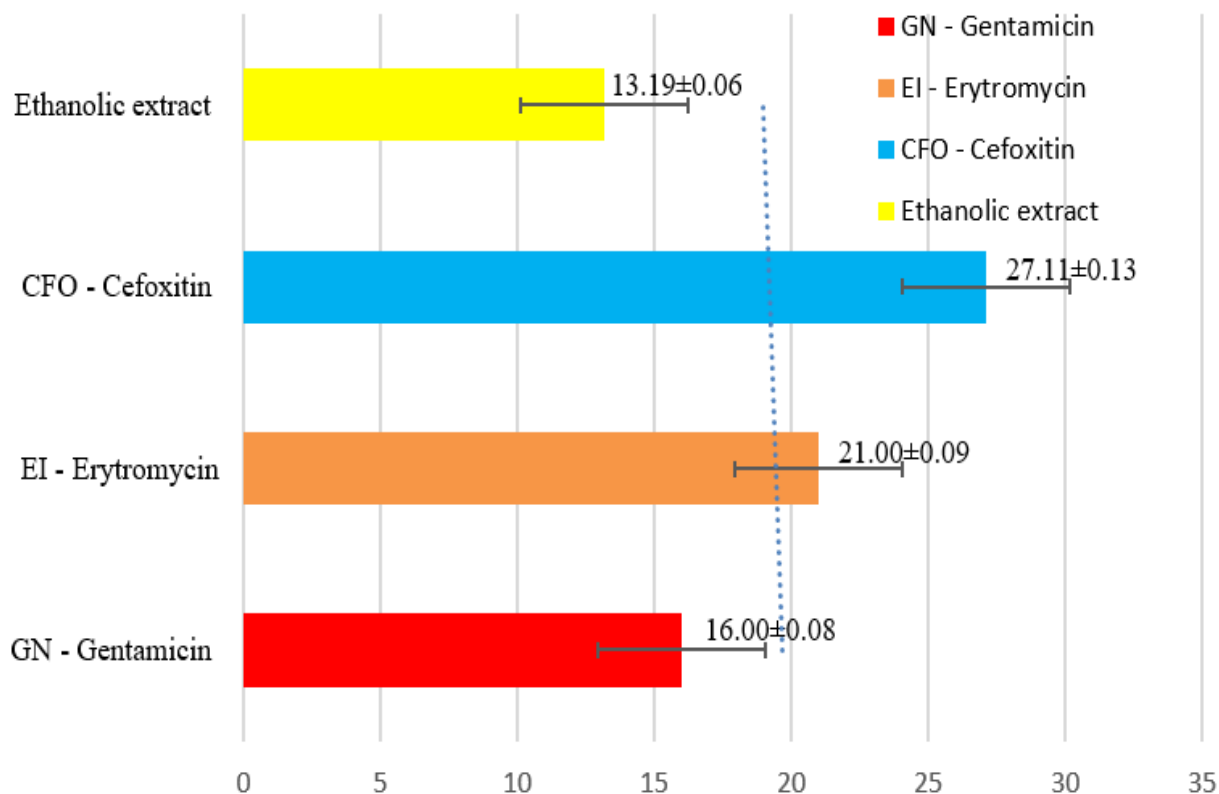
**Figure 1. Antibacterial activity of the ethanolic crude extract of *Rosmarinus officinalis* L. and standard antibiotics [CFO-Cefoxitin, EI-Erythromycin, and GN-Gentamicin] against *Enterococcus faecalis*.**



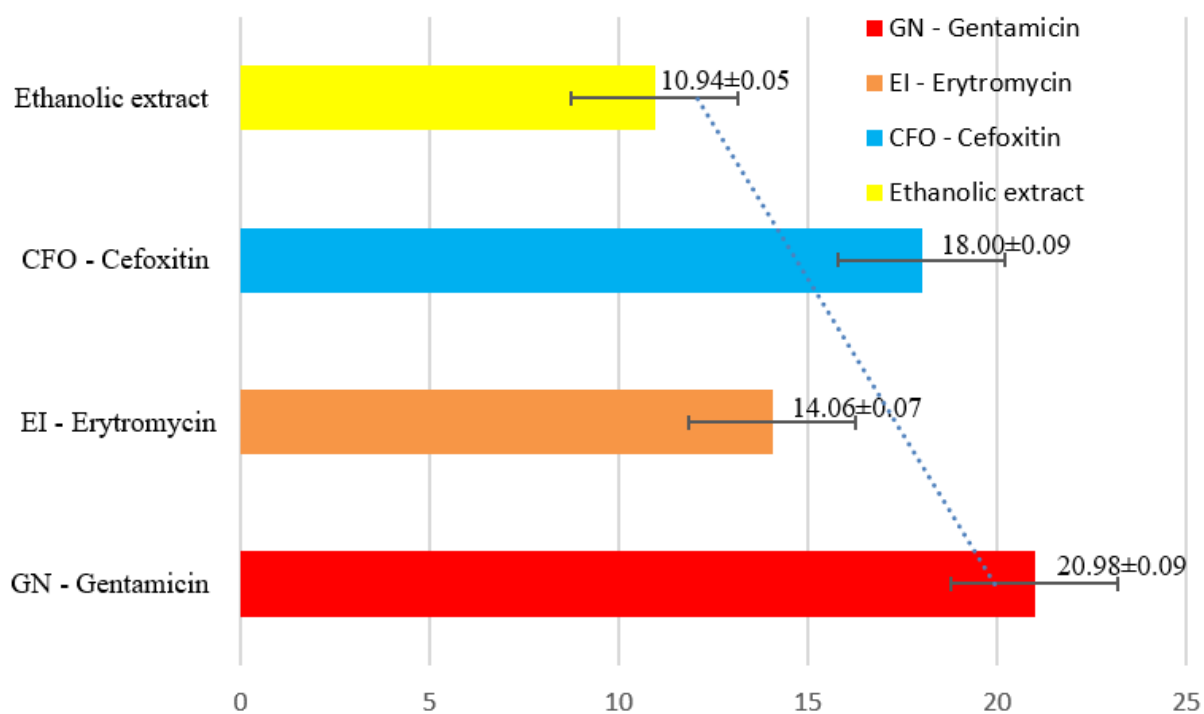
**Figure 2. Antibacterial activity of the ethanollic crude extract of *Rosmarinus officinalis* L. and standard antibiotics [CFO-Cefoxitin, EI-Erythromycin, and GN–Gentamicin] against *Escherichia coli*.**



**Figure 3. Antibacterial activity of the ethanollic crude extract of *Rosmarinus officinalis* L. and standard antibiotics [CFO-Cefoxitin, EI-Erythromycin, and GN–Gentamicin] against *Bacillus subtilis*.**



**Figure 4. Antibacterial activity of the ethanolic crude extract of *Rosmarinus officinalis* L. and standard antibiotics [CFO-Cefoxitin, EI-Erythromycin, and GN–Gentamicin] against *Staphylococcus aureus*.**



**Figure 5. Antibacterial activity of the ethanolic crude extract of *Rosmarinus officinalis* L. and standard antibiotics [CFO-Cefoxitin, EI-Erythromycin, and GN–Gentamicin] against *Staphylococcus epidermidis*.**

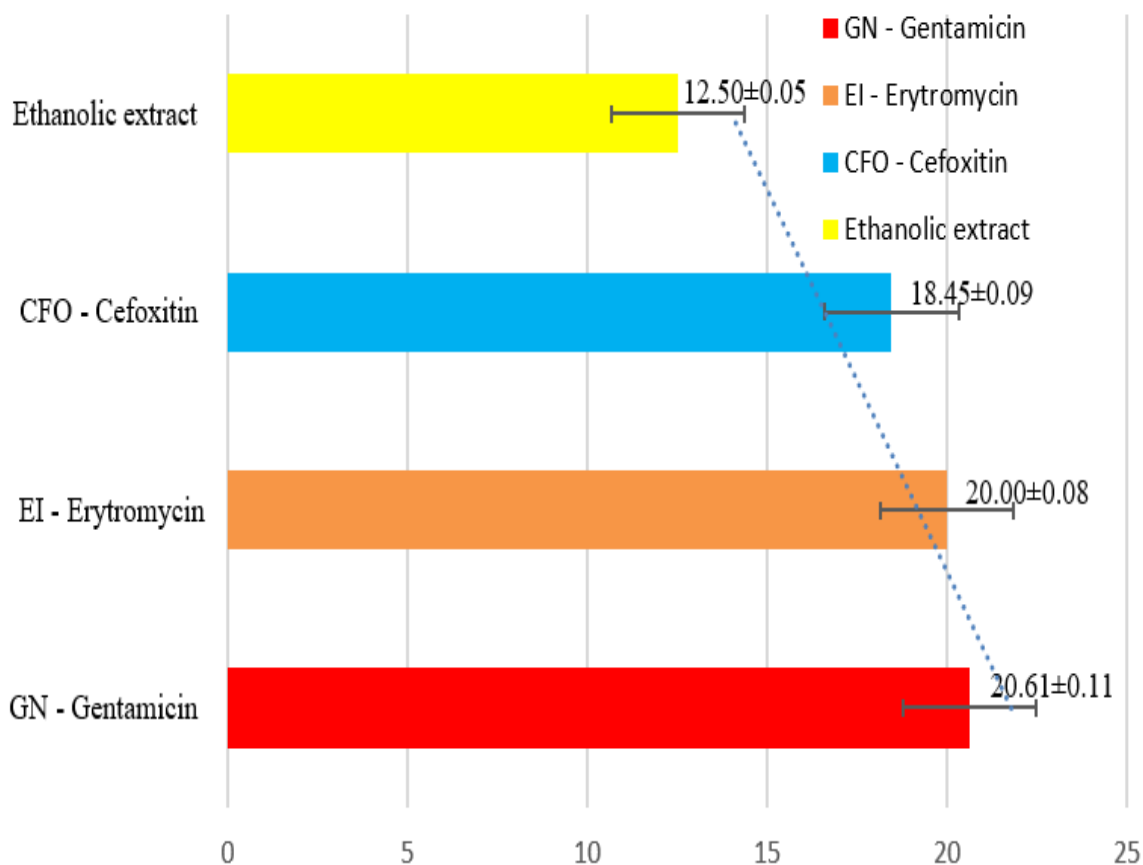
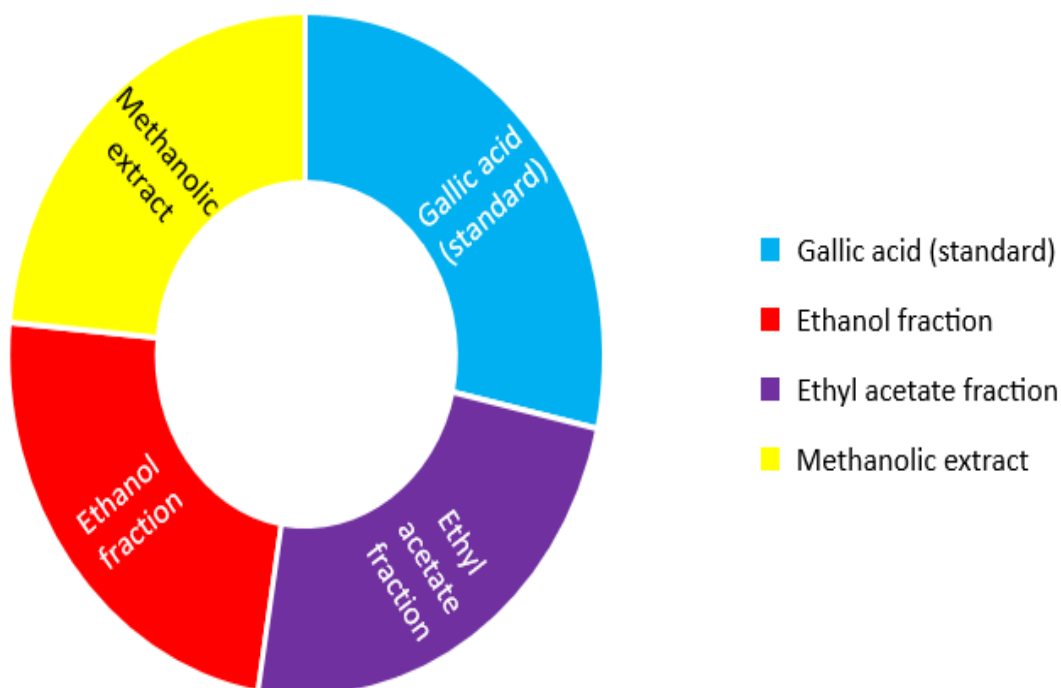
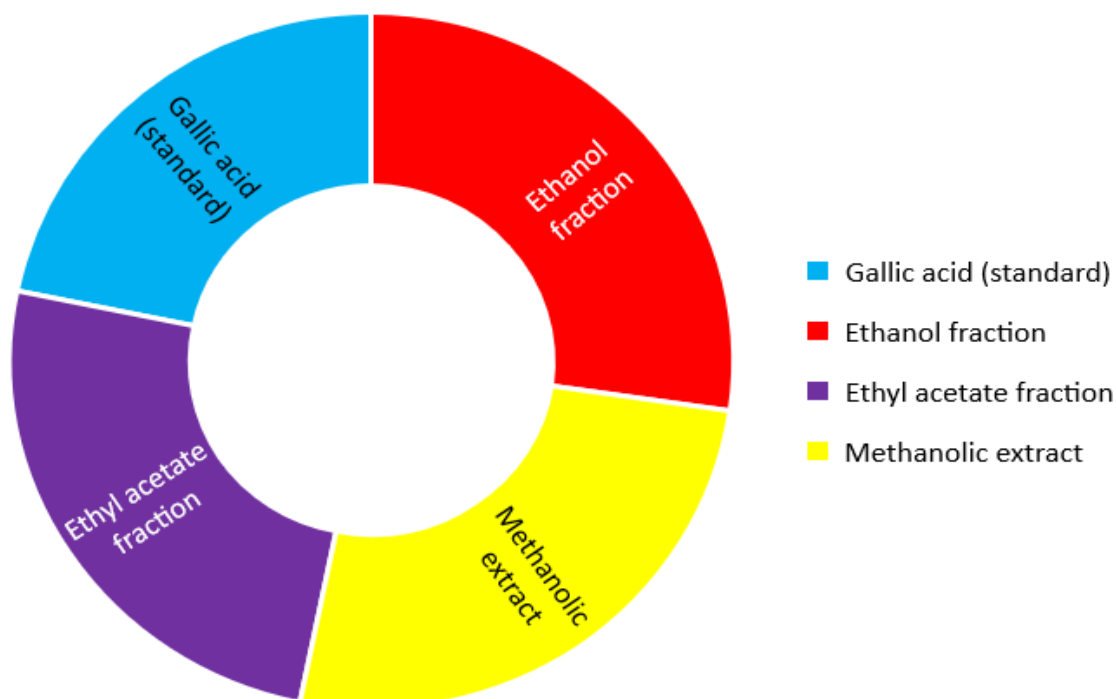


Figure 6. Antibacterial activity of the ethanolic crude extract of *Rosmarinus officinalis* L. and standard antibiotics [CFO-Cefoxitin, EI-Erythromycin, and GN-Gentamicin] against *Streptococcus pyogenes*.

Figure 7. Antioxidant [Peroxynitrite Scavenging] Capacities of Crude, Ethyl acetate and Ethanol fraction of *Rosmarinus officinalis*.



**Figure 8. Antioxidant [Singlet Oxygen Scavenging] Capacities of Crude, Ethyl acetate and Ethanol fraction of *Rosmarinus officinalis*.**



Antioxidant [Peroxynitrite Scavenging] Capacities of Crude, Ethyl acetate and Ethanol fraction of *Rosmarinus officinalis* were  $731.00 \pm 30.45$ ,  $748.91 \pm 33.01$ ,  $743.05 \pm 32.00$  and  $885.00 \pm 36.50$  respectively (Figure 7). While recorded  $51.07 \pm 4.20$ ,  $49.65 \pm 4.09$ ,  $54.00 \pm 4.31$  and  $43.03 \pm 3.29$  for The antioxidant [singlet oxygen scavenging] capacities appear in Figure 8. The cyclical diterpene diphenols carnosolic acid together with carnosol function as the most potent antioxidant components found in rosemary. The extract of rosemary incorporates carnosic acid, epirosmanol, rosmanol together with methylcarnosate and isorosmanol. The distillation process through steam extraction produces oils from rosemary plant twigs and fresh leaves. The three main chemical components in rosemary essential oil are 1,8-cineole with 46.4%, camphor at 11.4% followed by  $\alpha$ -pinene at 11.0%. The major compounds of rosemary essential oil consisted primarily of 1,8-cineole at 26.54% together with  $\alpha$ -pinene at 20.14%. The primary components found in this essential oil included 37.6% camphor alongside 10.0% 1,8-cineole and 7.8% p-cymene-7-ol and 5.4% borneol. The medicinal plant *Rosmarinus officinalis*, L. possesses abundant phenolic compounds that

produce their properties through extractions and essential oils according to research reports [10, 11]. Rosemary extracts serve double purposes as medical treatments and food preservation agents. The extracts from rosemary plants possess antioxidant components that include the phenolic acids alongside flavonoids and diterpenoids [12-15] in addition to their volatile compounds. The antioxidant substances identified in rosemary include seven different types of flavonoids which can be found in all leaf, flower, root and stem parts of the plant. This research explored seven antioxidant components including 7-O-glucoside as well as hispidulin and diosmin and hesperidin with 30 -O- $\beta$ -D-glucuronide and genkwanin and isoscutellarein 7-O-glucoside. The research confirmed that hesperidin and diosmin exist abundantly in vascular tissues. Research shows rosmarinic acid along with hydroxyhydrocaffeic acid among the rosemary-derived compounds exhibit antioxidant effects in combination with other identified compounds. The extract of rosemary includes other chemical compounds which derive from caffeic acid. The compounds form chelates with current metal ions then stabilize peroxide radicals by reacting with them. People use rosemary oil as a food spice because

its chemical compounds produce antibacterial effects as well as antifungal properties and act as antioxidants. The traditional medical field demonstrates that rosemary oil serves as a treatment solution for various diseases including inflammatory conditions and diabetes mellitus [16, 17]. The bioactivities derived from rosemary extracts span across multiple properties including hepatoprotective activity as well as antidiabetic effects and antimicrobial properties and anti-inflammatory response. A combination of phenolic compounds including caffeic acid and rosmarinic acid and carnosic acid within rosemary extract gives rise to its bioactivities.

The antioxidant effects in rosemary stem from its content of isoprenoid quinones that both stop free radical chain reactions and bind reactive oxygen species (ROS). The primary antioxidants found in rosemary extract commercial formulations convert lipid and hydroxyl radicals into stable products through interactions with phenolic compounds [18-21]. The compounds serve as Fe<sup>+2</sup> chelators to decrease the formation of oxygen-derived reactive species. Carnosic acid and carnosol act as potent scavengers of peroxy radicals. Membrane lipid peroxidation shows greater inhibition from both compounds than artificial antioxidants including BHA, BHT and propyl gallate [22, 23]. The antioxidant properties of the compound are primarily due to carnosic acid and carnosol which constitute 90% of its total properties. These substances demonstrate lipid peroxidation blocking activity in both liposomes and microsomal systems while showing high absorption capacity against peroxy radicals CCl<sub>3</sub>O<sub>2</sub> and reducing cytochrome c and destroying hydroxyl radicals. Carnosic acid functions as both a molecule that scavenges H<sub>2</sub>O<sub>2</sub> and serves as an acceptable substrate for the peroxidase system. The antioxidant capacity of the extract shows a direct correlation with polyphenol content because these compounds increase through carnosol and rosmarinic acid and hesperidin while the fruiting stage progresses. The essential connection between compounds known as diterpenes and radical-scavenging properties stands among the major characteristics of

antioxidant rosemary function. The essential structural components of rosemary include the aromatic ring from C11 to C12 in the catechol group together with the connected arrangement of three basic rings.

The essential oil rich in carvacrol at 67.0% along with  $\gamma$ -terpinene at 15.3% displayed effective Gram-negative inhibition against *Escherichia coli* using the broth microdilution method with MIC values ranging from 0.025  $\mu$ L/mL to 0.78  $\mu$ L/mL. Rosemary essential oil demonstrates a powerful effect as an inhibitor of *E. coli* ATCC 25922. Lab tests confirmed that the necessary dose of rosemary oil to inhibit *E. coli* growth remained above 6.4 mg/L. Scientists revealed through different studies that rosemary oil possesses antibacterial properties which successfully combat *E. coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus aureus*, *Clostridium perfringens*, *Aeromonas hydrophila*, *Bacillus cereus* and *Salmonella choleraesuis* [24]. Researchers discovered that the addition of essential oil to meat resulted in antibacterial effects against *Brochothrix thermosphacta* and *Enterobacteriaceae*. The inhibitory nature of rosemary results from rosmarinic acid and other substances including rosmaridiphenol along with carnosol, epirosmanol, carnosic acid and rosmanol and isorosmanol. The cell membrane experiences changes at genetic and nutrient levels and electron transport disruptions and cellular component leaks together with fatty acid production changes from their interactions. Additionally the inhibitory substance caused protein-membrane interaction leading to functional and morphological deterioration of the membrane structure. The antimicrobial abilities of carnosic acid surpass those of all other predominant extract compounds including rosmarinic acid. Various scientific works diverge in their explanations about any possible connections between the arrangement of polyphenolic extract compounds and their antimicrobial effects. The degree of antimicrobial activity among these extracts directly correlates with the carnosic acid concentration levels alongside carnosol diterpene amounts. Cell membranes become disordered due to terpene

action which leads to membrane lysis according to research findings [25]. The essential oil of rosemary's effectiveness against *E. coli* bacteria emerges from numerous minority components of its volatile fraction working in concert according to report [26]. Various authors state that *E. coli*, *L. monocytogenes* and *S. aureus* demonstrate high resistance to antimicrobial agents and the correct chemical mixture of oil ingredients is crucial to achieve maximum inhibitive effect.

### Conclusion;

The GC-MS analysis detected nineteen peaks from the identified chemical substances. Rosemary extracts contain defensive antioxidant compounds that inhibit lipid oxidation both in biological organisms and food systems. Functional foods and pharmaceutical products alongside plant products and food preservation utilize rosemary extracts. As a cheap herb which remains easily available and poses no toxicity risk to humans. The metabolites derived from *Rosmarinus officinalis* effectively subdued *Escherichia coli* with a potency value of  $20.54 \pm 0.08$ .

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