Antibacterial effect of cinnamon essential oil in combination with traditional antibiotics

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ABSTRACT:
Drug-resistant microorganisms are on the rise, posing a danger to successful bacterial illness treatment and increasing the demand for new antibacterial drugs. Natural products are currently and will continue to be the principal source of antibacterial therapeutic agents. This study's aim was to assess the antibacterial effects of Cinnamon essential oil (EO) alone and in combination with several traditional antibiotics against multi-drug resistant Staphylococcus sp. The antibacterial efficacy was determined using the disc diffusion method. As a result, cinnamon oil possesses antibacterial properties with MICs were 1.25 mg/ml for S. epidermidis and 2.5 mg/ml for S. aureus, respectively. Commercial antimicrobials and essential oil work together most effectively when combined. Scanning electron microscopy revealed morphological alterations in Staphylococcus cells, indicating cell membrane damage. Cinnamon essential oil composition was assessed using GC/MS with polonicumtoxin B (14.71%), linalool (5.36%), cinnamaldehyde (3.37%), 5, 5-dimethyl-4-hydroxy-1-phenyl-1-hexen-3-one (6.47%), 2-methyl benzofuran (5.79%), and 1,2-propanediol (6.32%). This study presented a natural product as a substitute for chemical therapeutics, addressing the problem of antibiotic resistance.

KEYWORDS: MDR Staphylococcus, cinnamon oil, antimicrobials, synergy, GC/MS

1. INTRODUCTION:
Antibiotics and chemotherapy resistance have increased among many pathogenic bacterial strains (Papanicolas et al., 2018). Staphylococcus sp., particularly methicillin-resistant S. aureus (MRSA), is a type of bacteria that can cause a variety of illnesses, including endocarditis, wound infections, infections of the lower urinary tract, and osteomyelitis (Khaing, 2019). MRSA strains are resistant to a wide range of commonly used medicines in clinical research (Gatadi et al., 2019). Vancomycin, a glycopeptide antibiotic, is the most effective antibacterial drug for most strains; however, vancomycin resistant S. aureus (VRSA) strains have been reported (Adwan& Adwan, 2013).

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Due to the rise and spread of multidrug-resistant bacteria, bacterial infections have become a major healthcare concern, prompting greater interest in the development of novel antibacterial drugs (Hammer et al., 1999). Natural products collected from a variety of sources, including plants and microorganisms were used to generate potential antibacterial agents; but there has been an increase in interest in the plant's bioactive metabolites as a replacement for conventional antibiotics (El Atki et al., 2019).

Essential oils (EOs) are rich sources of natural components that could be used to develop novel antibacterial therapeutics. Several investigations have found that some essential oils have a high antimicrobial impact (Jalal et al., 2015: Marwa et al., 2017). Among these essential oils, Cinnamon's antibacterial effects have been well researched (Vasconcelos et al., 2018). Cinnamon oil has many defensive compounds that work against various pathogens as trans-cinnamaldehyde, eugenol, cinnamyl acetate, camphor, L-borneol, caryophyllene, α-cubebene, α-terpineol, terpinolene, and α-thujene. The presence and concentration of each compound vary depending on the distribution and the part of the plant (Tung et al., 2008: Tung et al., 2010). These compounds negatively affect the bacterial cells via alterations in cell membrane and its lipid profile, inhibition of cell division, inhibition of ATPase, inhibition of membrane porins, inhibition of motility and biofilm formation, and anti-quorum sensing effect (Hyldgaard et al., 2012: Wang et al., 2017).

Additionally, the combinations—whether made up of a single EO or a combination of pure major ingredients ensure that the target bacteria were exposed to a wide range of chemical compounds and typically result in higher activity (Shi et al., 2017). Compared to their pure EOs, the combination of cinnamon and several plants' EOs showed an additive action against bacterial species (Clemente et al., 2016: Fei et al., 2011). A few investigations on cinnamon and antibiotic combinations showed additive and synergistic benefits against a variety of bacteria (Van Vuuren et al., 2009). Many ailments such as respiratory illnesses, skin issues, nausea, vomiting, and a variety of vaginal infections have been described as being treated with medicinal herbs (Henry& Crowther, 1999: Maats& Crowther, 2002). Using natural medicines has been reported and is advocated by healthcare experts as a natural, safe alternative to artificial medicines that may harm patients (Henry& Crowther, 1999). As a result, the goal of this study was to see if cinnamon EO has any antibacterial properties that could be utilized for disease treatment. To the best of our knowledge, there is no data on the antibacterial effectiveness of cinnamon essential oil against multidrug-resistant *Staphylococcus* sp.

2. MATERIALS AND METHODS:

Microorganisms

*Staphylococcus aureus* and *Staphylococcus epidermidis* with accession numbers MZ672019 and MZ672018 were obtained from the Microbiology Laboratory, Botany Department, Faculty of Science, Fayoum University. The cultures were maintained in nutrient agar slants, stored at 4°C and subcultured monthly

Multidrug-resistance (MDR) monitoring

10μg of Ciprofloxacin, Clindamycin, Fucidic acid, and Gentamycin discs and 30μg of Doxycycline and Tetracycline discs were used against *Staphylococcus aureus* and *Staphylococcus epidermidis*. MDR monitoring was carried out by positioning the discs on the surface of sterilized nutrient agar plates inoculated with *Staphylococcus aureus* and
Staphylococcus epidermidis. A 24-hour incubation at 37°C was carried out. For this test, Oxoid antimicrobial susceptibility antibiotic discs were used.

Essential oil (EO)
Cinnamon oil was extracted from the leaves of Cinnamomum cassia using steam distillation. The leaves were harvested from the trees are left to be dried for several days at room temperature, afterward; they go through a special steam distillation machine that extracts the oil. Distillation takes 3 to 4 hours. The extracted essential oil was kept in darkened bottles (Brown bottles) at 4°C until further investigation.

In vitro antimicrobial assay and MIC determination
The bactericidal activity of EO was tested in a sterile area using the disc diffusion technology (Goudjil et al., 2020). At 10 mgml⁻¹ concentration of EO, filter paper discs were impregnated. A negative control plate was made by soaking the filter paper disc with 70% ethyl alcohol, which was used to dilute the essential oil. The inhibitory growth zones' halo diameter (mm) was measured around the disc. The MIC was determined using the broth dilution method described by Wiegand et al. (2008) with cinnamon EO concentrations of 0, 0.5, 0.75, 1.25, 2.5, 3.75, 5, 6.25, 7.5, and 10 mgml⁻¹.

Antimicrobial interactions
The essential oil at starting stock concentration of 1.25 and 2.5 mgml⁻¹ For S. epidermidis and S. aureus, respectively combined with each antibiotic concentration prepared according to official Oxoid antimicrobial discs in seven different ratios i.e. 1 : 1; 2 : 1; 3 : 1; 4 : 1; 1 : 2; 1 : 3 and 1 : 4 of EO : antibiotic. The appropriate incubation conditions were used (37°C for 24 h). The study was carried out in three replicates.

Chemical composition of cinnamon oil using GC-MS
A Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column from Thermo Scientific was utilized for the GC/MS analysis (30m, 0.251mm, 0.1 mm film thickness). The quantification of all the observed components was explored using a percent relative peak area. By comparing the retention durations and mass spectra of the chemicals to the NIST, WILLY library data from the GC/MS instrument, the chemicals were tentatively identified.

Scanning electron microscope (SEM) analysis of the treated Staphylococcus sp cells
The bacterial cells were grown in nutrient broth for 24 hours, centrifuged (4000rpm for 5 minutes), and washed twice with phosphate buffer (0.1M and adjusted to pH 7.1). The cells were then treated with oil for 2 hours (incubated at 37°C), and the samples were centrifuged and fixed with paraformaldehyde (4%) and glutaraldehyde (2.5%) for 12 hours (Chao & Zhang, 2011). The cells were dehydrated with ethanol (15, 30, 70, and 100 percent), mounted, and dried (Jiang et al., 2015). A Carl Zeiss sigma 500 VP Jeol JSM – 6390 equipment was used to create the SEM images.

Statistical analysis
The one-way analysis of variance (ANOVA test) was used to statistically evaluate the data using SPSS Statistical Package Program version 23. Mean of the treatments were compared by Duncan multiple range test when the differences were significant. All tests had a P≤0.05 level of significance. The results are presented as means ± standard error (SE).
3. RESULTS AND DISCUSSION:

MDR monitoring and Antimicrobial effect of EO

Results in Table (1) show that two *Staphylococcus* sp resisted most of the used antibiotics. The inhibitory zones of the EO effectivity measured 19 mm against MDR *S. epidermidis* and 17 mm against MDR *S. aureus*. The MICs were 1.25 mgml\(^{-1}\) and 2.5 mgml\(^{-1}\) For *S. epidermidis* and *S. aureus*, respectively.

Table 1. Inhibition zone diameter (mm) of cinnamon essential oil and antibiotics

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>S. aureus</em></th>
<th><em>S. epidermidis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamon</td>
<td>19.25±0.25 (S)</td>
<td>17±0.10 (S)</td>
</tr>
<tr>
<td>MIC= 1.25 mgml(^{-1})</td>
<td></td>
<td>MIC=2.5 mgml(^{-1})</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>6±0.5 (R)</td>
<td>6.85±0.15 (R)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6.75±0.25 (R)</td>
<td>6±0.5 (R)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>5.60±0.4 (R)</td>
<td>5.4±0.3 (R)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>7.4±0.25 (R)</td>
<td>7±0.4 (R)</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>14±0.5 (S)</td>
<td>14.6±1.5 (S)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>12±1 (S)</td>
<td>11±1.5 (S)</td>
</tr>
</tbody>
</table>

Data in table represent mean ± SE; (R) refers to resistant & (S) refers to sensitive Antimicrobials interactions

The combination profile for the Cinnamon essential oil with the commercial antimicrobials is presented in Table (2). A predominantly synergistic profile was noted against all studied pathogens. Synergy is best noted for five ratios (1:1, 2:1, 3:1, 4:1, and 1:2). for the ratio 1: 3, the synergy was observed only for the EO combination with tetracycline and Gentamicin. for 1:4 ratio, only the synergistic effect was detected for the combination with Gentamicin.
### Table 2. The inhibition zones (mm) of the different combinations' ratios of the cinnamon essential oil with the commercial antimicrobials

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1:1</th>
<th>2:1</th>
<th>3:1</th>
<th>4:1</th>
<th>1:2</th>
<th>1:3</th>
<th>1:4</th>
</tr>
</thead>
<tbody>
<tr>
<td>EO+ Doxycycline</td>
<td>10.70±</td>
<td>14.75±</td>
<td>15.75±</td>
<td>14.45±</td>
<td>15.00±</td>
<td>16.60±</td>
<td>15.75±</td>
</tr>
<tr>
<td>EO+ Ciprofloxacin</td>
<td>11.65±</td>
<td>10.25±</td>
<td>16.60±</td>
<td>15.65±</td>
<td>15.85±</td>
<td>17.25±</td>
<td>15.30±</td>
</tr>
<tr>
<td>EO+ Clindamycin</td>
<td>10.40±</td>
<td>10.10±</td>
<td>15.05±</td>
<td>15.35±</td>
<td>15.20±</td>
<td>17.85±</td>
<td>14.25±</td>
</tr>
<tr>
<td>EO+ Gentamicin</td>
<td>18.40±</td>
<td>23.00±</td>
<td>17.30±</td>
<td>16.25±</td>
<td>15.00±</td>
<td>18.75±</td>
<td>15.75±</td>
</tr>
<tr>
<td><strong>Ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- (a, b, c ..) Average in the same column having different superscripts are differ significantly (P0.05).

### Chemical composition of cinnamon oil

The GC/MS study of cinnamon oil revealed 52 organic components, including alkaloids, terpenes, alkanes/alkenes, aldehydes/ketones, esters, ethers, and organic acids (Table 3). Table 4 shows the major compounds, which include polonicumtoxin B (14.71%), linalool (5.36%), cinnamaldehyde (3.37%), 5, 5-dimethyl-4-hydroxy-1-phenyl-1-hexen-3-one (6.47%), 2-methyl benzofuran (5.79%), and 1,2-propanediol (6.32%).
### Table 3. Chemical composition of cinnamon oil

<table>
<thead>
<tr>
<th>Chemical compound</th>
<th>Retention time</th>
<th>Peak area (%)</th>
<th>Molecular weight</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propylamine, N,N,2,2-tetramethyl, N-oxide</td>
<td>5.04</td>
<td>0.20</td>
<td>131</td>
<td>C\textsubscript{7}H\textsubscript{17}NO</td>
</tr>
<tr>
<td>1,2Propanediol (CAS)</td>
<td>5.15</td>
<td>0.93</td>
<td>76</td>
<td>C\textsubscript{3}H\textsubscript{6}O</td>
</tr>
<tr>
<td>Acetaldehyde (CAS)</td>
<td>5.21</td>
<td>1.30</td>
<td>44</td>
<td>C\textsubscript{2}H\textsubscript{4}O</td>
</tr>
<tr>
<td>2-hydroxymethyl-3-methyl-1-oxirane</td>
<td>5.51</td>
<td>0.08</td>
<td>88</td>
<td>C\textsubscript{4}H\textsubscript{8}O</td>
</tr>
<tr>
<td>6,6”-Bis(chloromethyl)(4’,4&quot;:4&quot;,4”-terdibenzofuran</td>
<td>5.62</td>
<td>0.12</td>
<td>596</td>
<td>C\textsubscript{38}H\textsubscript{22}C\textsubscript{12}O\textsubscript{3}</td>
</tr>
<tr>
<td>Propanoic acid,2hydroxy,methyl ester (CAS)</td>
<td>5.94</td>
<td>0.14</td>
<td>104</td>
<td>C\textsubscript{4}H\textsubscript{8}O</td>
</tr>
<tr>
<td>Glycolic acid, trimethylsilyl ester</td>
<td>6.21</td>
<td>1.23</td>
<td>148</td>
<td>C\textsubscript{4}H\textsubscript{8}O\textsubscript{3}</td>
</tr>
<tr>
<td>1,2-Propanediol (CAS)</td>
<td>6.46</td>
<td>0.20</td>
<td>640</td>
<td>C\textsubscript{16}H\textsubscript{4}Br\textsubscript{4}S\textsubscript{4}</td>
</tr>
<tr>
<td>Formamide (CAS)</td>
<td>7.26</td>
<td>0.07</td>
<td>45</td>
<td>CH\textsubscript{3}NO</td>
</tr>
<tr>
<td>Urea</td>
<td>7.75</td>
<td>0.07</td>
<td>60</td>
<td>CH\textsubscript{4}N\textsubscript{2}O</td>
</tr>
<tr>
<td>Propanoic acid, 2-hydroxy, methyl ester (CAS)</td>
<td>7.86</td>
<td>0.68</td>
<td>104</td>
<td>C\textsubscript{4}H\textsubscript{8}O</td>
</tr>
<tr>
<td>Cyclopropanecis1,2,3d3-carboxylic Acid</td>
<td>8.01</td>
<td>0.37</td>
<td>86</td>
<td>C\textsubscript{4}H\textsubscript{8}D\textsubscript{3}O \textsubscript{2}</td>
</tr>
<tr>
<td>Isopropyl Alcohol</td>
<td>8.36</td>
<td>0.08</td>
<td>60</td>
<td>C\textsubscript{3}H\textsubscript{6}O</td>
</tr>
<tr>
<td>Thirane</td>
<td>8.53</td>
<td>0.12</td>
<td>60</td>
<td>C\textsubscript{2}H\textsubscript{4}S</td>
</tr>
<tr>
<td>1,2-Ethanediameine(CAS)</td>
<td>8.63</td>
<td>0.35</td>
<td>60</td>
<td>C\textsubscript{2}H\textsubscript{6}N \textsubscript{2}</td>
</tr>
<tr>
<td>Azetidine</td>
<td>8.88</td>
<td>0.11</td>
<td>57</td>
<td>C\textsubscript{4}H\textsubscript{8}N</td>
</tr>
<tr>
<td>Aziridine (CAS)</td>
<td>9.04</td>
<td>0.09</td>
<td>43</td>
<td>C\textsubscript{4}H\textsubscript{8}N</td>
</tr>
<tr>
<td>1,2-Propanediameine(CAS)</td>
<td>9.15</td>
<td>0.16</td>
<td>74</td>
<td>C\textsubscript{4}H\textsubscript{10}N \textsubscript{2}</td>
</tr>
<tr>
<td>1-Propanol,2,2-dimethyl acetate</td>
<td>9.53</td>
<td>0.18</td>
<td>130</td>
<td>C\textsubscript{4}H\textsubscript{14}O \textsubscript{2}</td>
</tr>
<tr>
<td>Acetic acid, hydroxy, methyl ester (CAS)</td>
<td>9.76</td>
<td>0.10</td>
<td>90</td>
<td>C\textsubscript{3}H\textsubscript{8}O</td>
</tr>
<tr>
<td>N,N-DiformylNbutane amine</td>
<td>9.94</td>
<td>0.13</td>
<td>129</td>
<td>C\textsubscript{6}H\textsubscript{11}NO \textsubscript{2}</td>
</tr>
<tr>
<td>Methanamine,N-hydroxy-N-methyl</td>
<td>10.41</td>
<td>0.11</td>
<td>61</td>
<td>C\textsubscript{2}H\textsubscript{7}NO</td>
</tr>
<tr>
<td>1,2-Propanediol (CAS)</td>
<td>10.74</td>
<td>6.32</td>
<td>76</td>
<td>C\textsubscript{4}H\textsubscript{10}O \textsubscript{2}</td>
</tr>
<tr>
<td>1,5-Diazatetracyclo(3.3.0 .0(2,8).0(4,6))octane</td>
<td>11.96</td>
<td>0.11</td>
<td>108</td>
<td>C\textsubscript{6}H\textsubscript{13}N \textsubscript{2}</td>
</tr>
<tr>
<td>Propionic acid,2,3-dihydroxy3phenyl</td>
<td>12.17</td>
<td>0.75</td>
<td>182</td>
<td>C\textsubscript{4}H\textsubscript{10}O \textsubscript{4}</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>12.49</td>
<td>0.11</td>
<td>108</td>
<td>C\textsubscript{3}H\textsubscript{8}O</td>
</tr>
<tr>
<td>5’,5-Dihydroxy1,1-bicyclooctylidene</td>
<td>12.54</td>
<td>0.19</td>
<td>252</td>
<td>C\textsubscript{16}H\textsubscript{12}O \textsubscript{2}</td>
</tr>
<tr>
<td>Benzenemethanol,3-nitro</td>
<td>12.67</td>
<td>0.29</td>
<td>153</td>
<td>C\textsubscript{10}H\textsubscript{14}NO \textsubscript{3}</td>
</tr>
<tr>
<td>Benzenemethanol</td>
<td>13.20</td>
<td>2.26</td>
<td>108</td>
<td>C\textsubscript{3}H\textsubscript{8}O</td>
</tr>
<tr>
<td>Benzenemethanol,3-nitro</td>
<td>13.43</td>
<td>1.79</td>
<td>153</td>
<td>C\textsubscript{7}H\textsubscript{8}NO \textsubscript{3}</td>
</tr>
<tr>
<td>Linalool</td>
<td>13.62</td>
<td>5.36</td>
<td>154</td>
<td>C\textsubscript{10}H\textsubscript{15}O</td>
</tr>
<tr>
<td>Benzo[\textsubscript{a}]furan, 2-methyl</td>
<td>18.29</td>
<td>5.79</td>
<td>132</td>
<td>C\textsubscript{8}H\textsubscript{8}O</td>
</tr>
<tr>
<td>5,5-dimethyl-4-hydroxy-1-Phenyl-1-hexen-3-one</td>
<td>18.66</td>
<td>6.47</td>
<td>218</td>
<td>C\textsubscript{14}H\textsubscript{18}O \textsubscript{2}</td>
</tr>
<tr>
<td>Chemical compound</td>
<td>Retention time</td>
<td>Peak area (%)</td>
<td>Molecular weight</td>
<td>Molecular formula</td>
</tr>
<tr>
<td>--------------------------------------------------------</td>
<td>----------------</td>
<td>---------------</td>
<td>------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>19.26</td>
<td>3.37</td>
<td>132</td>
<td>C₉H₇O</td>
</tr>
<tr>
<td>Methyl 13-C-octadecanoate</td>
<td>20.35</td>
<td>0.14</td>
<td>298</td>
<td>C₁₈H₃₅O₂</td>
</tr>
<tr>
<td>HInden1-ol-2,3-dihydro(CAS)</td>
<td>20.56</td>
<td>0.46</td>
<td>134</td>
<td>C₉H₁₀O</td>
</tr>
<tr>
<td>Trans-Isoeugenol</td>
<td>21.12</td>
<td>0.13</td>
<td>164</td>
<td>C₁₀H₁₂O₂</td>
</tr>
<tr>
<td>4-Hydroxyaminocinnoline</td>
<td>21.43</td>
<td>0.23</td>
<td>161</td>
<td>C₈H₇N₂O</td>
</tr>
<tr>
<td>Polonicumtoxin B</td>
<td>21.59</td>
<td>14.71</td>
<td>223</td>
<td>C₁₀H₂₁NO₂</td>
</tr>
<tr>
<td>2-Propenoicacid, 3-phenyl, methyl ester</td>
<td>21.92</td>
<td>1.01</td>
<td>162</td>
<td>C₁₀H₁₀O₂</td>
</tr>
<tr>
<td>2-Propenoic acid-3-phenyl acetate(CAS)</td>
<td>23.11</td>
<td>0.15</td>
<td>176</td>
<td>C₁₁H₁₂O₂</td>
</tr>
<tr>
<td>2-Propenoic acid-3-phenyl methyl ester</td>
<td>23.70</td>
<td>1.17</td>
<td>176</td>
<td>C₁₁H₁₂O₂</td>
</tr>
<tr>
<td>Cinnamaldehyde propylene glycol acetal</td>
<td>24.08</td>
<td>0.26</td>
<td>190</td>
<td>C₁₂H₁₄O₂</td>
</tr>
<tr>
<td>2-D1-2-Tetralol</td>
<td>25.41</td>
<td>0.11</td>
<td>148</td>
<td>C₁₀H₁₁D₂O</td>
</tr>
<tr>
<td>Bicyclo(4.1.0)heptane,7-bicyclo(4.1.0)hept-7-ylidene</td>
<td>25.88</td>
<td>0.21</td>
<td>188</td>
<td>C₁₄H₂₀</td>
</tr>
<tr>
<td>à,à-Dideutero-à-(13C-cyano)2-cyanotoluene</td>
<td>25.94</td>
<td>0.08</td>
<td>142</td>
<td>C₉H₈N₂</td>
</tr>
<tr>
<td>1-Nitro-6-hydroxyazulene</td>
<td>26.18</td>
<td>23.43</td>
<td>189</td>
<td>C₁₀H₂NO₃</td>
</tr>
<tr>
<td>N-deutero-3-phenyl -2,6-dioxopiperidine</td>
<td>26.95</td>
<td>14.17</td>
<td>189</td>
<td>C₁₁H₁₀DNO₂</td>
</tr>
<tr>
<td>2-Butenoic acid,3-phenyl</td>
<td>29.85</td>
<td>0.08</td>
<td>230</td>
<td>C₁₅H₁₈O₂</td>
</tr>
<tr>
<td>1,2,3,4tetrahydro1(3'aminobenzyl)-7-methoxyN-methylisoquinolin-8-ol</td>
<td>36.42</td>
<td>4.04</td>
<td>298</td>
<td>C₁₈H₂₂N₂O₂</td>
</tr>
<tr>
<td>2-Propenoicacid, 3-phenyl, methyl ester</td>
<td>21.92</td>
<td>1.01</td>
<td>162</td>
<td>C₁₀H₁₀O₂</td>
</tr>
<tr>
<td>2-Propen-1-ol,3-phenyl acetate (CAS)</td>
<td>23.11</td>
<td>0.15</td>
<td>176</td>
<td>C₁₁H₁₂O₂</td>
</tr>
</tbody>
</table>

**RT:** retention time, peak area % represents the concentration, M. wt: molecular weight, M.formula: molecular formula.
### Table 4. The major detectable chemical compounds in cinnamon oil using GC-MS

<table>
<thead>
<tr>
<th>Chemical compound</th>
<th>RT</th>
<th>Peak area (%)</th>
<th>M. weight</th>
<th>M. formula</th>
<th>C. Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-Propanediol (CAS)</td>
<td>10.74</td>
<td>6.32</td>
<td>76</td>
<td>C₃H₆O₂</td>
<td></td>
</tr>
<tr>
<td>Linalool</td>
<td>13.62</td>
<td>5.36</td>
<td>154</td>
<td>C₁₀H₁₈O</td>
<td></td>
</tr>
<tr>
<td>2-methyl benzo-furan</td>
<td>18.29</td>
<td>5.79</td>
<td>132</td>
<td>C₉H₈O</td>
<td></td>
</tr>
<tr>
<td>5,5-dimethyl-4-hydroxy-1-Phenyl-1-hexen-3-one</td>
<td>18.66</td>
<td>6.47</td>
<td>218</td>
<td>C₁₄H₁₈O₂</td>
<td></td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>19.26</td>
<td>3.37</td>
<td>132</td>
<td>C₉H₈O</td>
<td></td>
</tr>
<tr>
<td>Polonicumtoxin B</td>
<td>21.59</td>
<td>14.71</td>
<td>223</td>
<td>C₁₃H₂₁NO₂</td>
<td></td>
</tr>
</tbody>
</table>

RT: retention time, peak area % represents the concentration, M. wt: molecular weight, M. formula: molecular formula, C. structure: compound structure.

### SEM screening

Micrographs of treated *S. epidermidis* and *S. aureus* cells revealed membrane rupture, irregularly shaped cells, damaged cell sections, and cellular damage (Figure 1). From the images, it is observed that the effect of cinnamon oil against *S. epidermidis* exceeded the activity towards *S. aureus*. The micrographs of untreated cells showed no significant differences in the cell morphology of both bacterial strains (Figure 1).
Fig 1. SEM micro-images of the inhibitory effect of EO on bacterial cells versus control cells. Control (a) normal bacterial cells of *S. aureus* strain, (b) the treated cells of *S. aureus* appeared destructed and torn, control (c) normal bacterial cells of *S. epidermidis* strain, and (d) the treated abnormal cells of *S. epidermidis* appeared destructed.
Using natural medicines has been advocated by healthcare experts as a natural and safe alternative to artificial medicines that may harm patients (Henry & Crowther, 2000). Based on the findings, it was determined that cinnamon oil has the ability to inhibit both tested strains; these findings are in line with previous reports (Utchariyakiat et al., 2016). An explanation of high antibacterial activity of cinnamon essential oil may be owing to the action of its constituents, particularly trans-cinnamaldehyde, which is one of the major compounds. It has been reported that trans-cinnamaldehyde possesses the highest antimicrobial in comparison with other constituents of cinnamon oil (AL-Jabri & Hossain, 2018; Cheng et al., 2006). The alkaloid Polonicumtoxin B is the major compound in the oil sample analysis with 14.71% and that result is in conflict with other studies that showed that the major compounds of cinnamon oil are trans-cinnamaldehyde, cinnamyl acetate, terpinolene, eugenol, L-borneol, and camphor (Tung et al., 2010); the chemical composition of the oil depend on plant type and age, geographical locations, and extraction methodology (Olise et al., 2020). Another explanation of bacterial growth retardation is due to the toxicity effect of Polonicumtoxin B. Some authors had determined the antibacterial effect of cinnamon EO combinations; Utchariyakiat et al. (29) revealed that cinnamon EO mixed with various antimicrobials had a synergistic impact against multidrug resistant pathogenic bacteria. According to Mahadlek et al. (2012), cinnamon oil combined with ciprofloxacin, doxycycline, and metronidazole demonstrated synergy against S. aureus ATCC 6538P. The current work provided confirmation for these data. It was noted that the combination of cinnamon oil and various conventional antibiotics showed a synergistic effect against E. coli and Staphlococcus sp. (Ali et al., 2005). SEM scans revealed that the treated bacterial cell surface differed significantly from the untreated cells in terms of structure, which was attributable to cell membrane disruption, which resulted in bacterial cell wall lysis and the loss of intracellular dense material (Vasconcelos et al., 2018). The treated cells had a reduced negative charge, and it was hypothesized that the damage was produced by acidification and protein denaturation of the cell membrane as a result of an aggregation of essential oil components. Cinnamon oil has been shown to have antimicrobial activity against a variety of pathogenic bacteria in previous studies; however, we focused on MDR Staphylococcus sp. The antimicrobial effect of cinnamon oil may be due to degradation of the cell wall, flowing of cellular content, and thickening of cytoplasm (Radaelli et al., 2016).

**Conclusion:**
The current study provided a natural product as a substitute for chemical therapeutics, addressing the problem of antibiotic resistance. Cinnamon oil was found to have antibacterial action against MDR S. epidermidis and S. aureus, indicating that it might be used to generate alternative bioactive agents or added to existing antibiotics to improve antimicrobial activity and create new products.
4. REFERENCES:


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Wiegand, I., Hilpert, K., & Hancock, R. E. 2008. Agar and broth dilution methods to
The effect of the traditional and essential oils and the mixture of the antimicrobial activity on Staphylococcus sp against the traditional and essential oils.

Amna Mohamed Rashed

Objective

The objective of this study was to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols, 3(2), 163-175.

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