Anticancer and Antibacterial Properties of *Verthemia Iphionoides* Essential Oil /Silver Nanoparticles

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Many medicinal herbs are widely used in traditional folk medicine in most countries of the world, including Jordan, to treat many human diseases including microbial infections, diabetes and cancer. This study was conducted to evaluate the anti-cancer and antibacterial effects of essential oil (EO) of *Varthemia Iphionoides*, as well as the synergistic effect of EO and biosynthesized silver nanoparticles (AgNPs) against many cancer lines including human breast cancer, leukemia, pancreatic cancer, and prostate cancer. The chemical composition of *V. iphionoides* essential oil was analyzed using GC/MS. Twenty-two compounds were identified representing 96.1% of the *V. iphionoides* EO. The anticancer effect of EO from *V. Iphionoides*, as well as its synergistic effect with AgNPs, were evaluated using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) for all mentioned cell lines, in which human periodontal ligament fibroblasts (PDL) was used for verification of selectivity. It is noted that the oil has anti-proliferative activity against many types of cancer cells. The study revealed that the combination of essential oil and AgNPs shows a synergistic, antiproliferative effect on all cancer cell lines at a low concentration whereas when they are used individually, the effect is only shown when using a high concentration. EO of *Varthemia Iphionoides* has clearly demonstrated its effectiveness on pancreatic cancer cells compared to doxorubicin and with a high selectivity rate on synergy with AgNPs. Regarding antibacterial assays using the well diffusion methods, EO of *Verthemia Iphionoides* was active against all five-tested bacteria, with inhibition zone ranged between 15 mm and 26.5 mm. The results of MIC for tested bacteria were between 50 and 370 µg/mL. The best synergistic capacity resulted from combination of AgNPs and EO of *Verthemia Iphionoides* was against *S. aureus*. Therefore, synergistic studies can be novel strategic plan by furnishing an interesting stage in the future for this kind of research.

**Keywords:** Varthemia Iphionoides, Anticancer, Antibacterial, AgNPs.

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*Verthemia species* belong to the family Compositae (*Asteraceae*), in which the most widely distributed species in Jordan and Middle east is *Verthemia iphionoides* (also known as *Chiliadenus iphionoides*). In fact, *V. iphionoides*, a 30-80 cm long bushy-perennial, is the most important
medical herb used in Jordan. In traditional herbal medicine, it plays a major role in the management of various diseases; relieving pain, healing wounds and in the treatment of eye complaints and urine retention. *V. iphionoides* has also been shown to exhibit some medicinal properties including antimicrobial, antiplatelet, and cytotoxic activities. Furthermore, it has been used for the treatment of many diseases, including cancer.

Recently, the application of nanotechnology has grown rapidly especially in the green synthesis of nanomaterials that have a wide range of medical applications. The green synthesis method of producing nanomaterials has rapidly grown in the nanotechnology field; including uses in drug delivery, ointments, nanomedicine, chemical sensing, data storage, cell biology, agriculture, cosmetics and textiles, the food industry and photocatalytic organic dye-degradation activity, antioxidants and antimicrobial agents. Since there is no effective treatment with a high level of selectivity for most cancer types, searching for bioactive chemicals in a traditional medicinal plant is a novel way to prevent patient suffering from different kinds of cancer. So, the aims of this study are to investigate the possible antiproliferative efficacy of the *V. iphionoides* essential oil and silver nanoparticles (AgNPs), which was synthesised using the culture of the supernatant of the fungal strain *Tritirachium oryzae* W5H, separately and in combination against different cancer cell lines in vitro.

Moreover, to study the broad-spectrum antibacterial activity of AgNPs used singly and in combination with EOs from the leaves of *V. iphionoides* plants. The effects of the *V. iphionoides* EO after synergizing with AgNPs were investigated against five bacterial strains using well diffusion and microdilution methods. This is the first report using EO of *V. iphionoides* combined with biosynthesized AgNPs for studying the antibacterial capacity and anticancer effect of different cell lines.

**MATERIALS AND METHODS**

**Plant material**

In 2018, the aerial parts of *V. iphionoides* were collected in June and July from Al Karak province, in the south of Jordan. The plant was identified as previously reported in Khlaifat et al. Preparation of the essential oil

A sample of 50g of the dried aerial part of the plant was hydro-distilled using a simple Clevenger apparatus for three hours. This procedure was repeated more than 20 times. The oil was extracted from the aqueous phase using diethyl ether. The diethyl ether was evaporated, and the oil was dried over anhydrous sodium sulfate. Finally, the extracted oil was stored at 4°C until further analysis.

**Preparation and characterization of AgNPs**

AgNPs were prepared in our laboratory as recently reported by Al-limoun et al.

**Gas chromatography/Mass spectrometry (GC/MS) analysis**

In this study, a Varian chrompack CP-3800 GC/MS-200 equipped with a split-splitless injector was used. The extracted components were separated on a DB-5 GC column and the mass detector was set to scan ions between 40-400 m/z using full scan mode and electron impact (EI, 70 eV). The temperature of the injector was set at 250°C with a split ratio of 1:10. The detector and transfer-line temperatures were 160°C and 230°C respectively. For separation of the different oil components, a linear temperature program was used. The heating rate was programmed at 3°C/min starting from 60°C (initial temperature) to 250°C (final temperature) and held at 250°C for 5 min with a total run time of 68 minutes 25 seconds. The identification of compounds was made by comparing the retention time with reference samples, based on their linear indices relative to a series of n-alkanes (C8-C20) in the same chromatographic conditions and mass spectra, by matching the essential oil constituents with NIST library and published reports. Whenever possible, the co-chromatography of certain standard compounds was performed under similar chromatographic condition.

**Bacterial strains**

For antibacterial activity five bacteria were employed involving three Gram-negative bacteria: E. aerogenes ATCC 13048, E. coli ATCC 11293 and the clinical isolate Extended Spectrum Beta-Lactamase producing *P. aeruginosa* (ESBL) and two Gram-positive bacteria: the clinical isolate Beta-Lactamase producing *S. aureus* (BL) and *S.
epidermidis ATCC 12228. The clinical isolates were obtained from the Department of Biology, Faculty of Science, Mutah University, Mutah, Jordan.

**Preparation of bacterial suspension**

Bacterial broth cultures were made following the M07-A9 procedure of the Clinical Laboratory Standards Institute (CLSI, 2012). One bacterial colony was cultivated in sterile 5 ml nutrient broth (NB) at 37 °C and incubated overnight. The resultant growth cultures were adjusted to 0.5 McFarland Standard using sterile NB broth. The adjustment of bacterial suspensions to the density of the 0.5 McFarland standard was done spectrophotometrically at (A 620 nm) to obtain a final absorption of 0.1.

**Antimicrobial activity**

The antibacterial activities of EO and AgNPs alone or in combination were evaluated using well diffusion method and microdilution method. In well diffusion method, EO, AgNPs and combination of AgNPs:EO were evaluated at concentrations equal to 10 mg, 172.6 mg and 86.28 µg : 5 mg per well, respectively. In microdilution method, three fold dilution were prepared from a stock solution of 100 mg, 1.726 mg and combination of 86.28 µg and 5 mg per mL for EO, AgNPs and AgNPs:EO, respectively.

**Well diffusion method**

Hundred µl of bacteria suspension adjusted to an equivalent of 0.5 McFarland standards (approximately 10¹⁸ CFU/mL) was spread on Muller Hinton agar then 100 µl from the prepared stock solutions were loaded into the well that previously was prepared. The prepared plates were incubated at 37°C for 24h and the inhibition zones were measured as mm diameter.

**Microdilution methods**

A three-fold dilution series was performed using Muller Hinton broth from the prepared stock solutions (100 mg per well, 1.726 mg per well and 86.28 µg: 5 mg per well for EO, AgNPs and AgNPs:EO (in combination), respectively) for each sample tested using 96-well plate. Then 10 µL of the bacterial suspension equivalent to 0.5 McFarland standards (approximately 10¹⁸ CFU/mL for bacteria) was added to each well. In addition to DMSO as negative control, three different antibiotics including chloramphenicol (Cm), kanamycin (Km) and ampicillin (Amp) were used as control. The inoculated plates were incubated at 37C for 24h. The growth of the microorganisms was monitored by the subculture of each well content on nutrient agar. The MIC values were defined as the lowest concentrations of the plant essential oil found to inhibit the growth of microorganisms 21.

**Cancer cell lines culture**

Human breast cancer cell lines; namely MDA-MB-231 (mammary gland/breast; derived from metastatic site: pleural effusion. ATCC HTB-26, chronic myelogenous leukemia; namely K-562 (ATCC HTB-243), pancreatic cancer cell line Panc1 (ATCC CRL1469), human prostate cancer cell lines; namely PC3 (ATCC CRL-1435) and human periodontal ligament fibroblasts (PDL). All these cells were cultured in DMEM containing 10% FBS, HEPES Buffer (10 mM), L-glutamine (100 µg/mL), gentamicin (50 µg/mL), penicillin (100 µg/mL), and streptomycin (100 mg/mL).

**Cell harvesting and counting**

All cells were propagated in a humidified 5% CO2 incubator at 37æ°C. Firstly, all the cells were washed in 75 cm2 flasks with 3–5 ml of phosphate buffer saline (PBS), then 1–2 ml of trypsin was added to each flask until the cells detached. An equal amount of fresh media was added for each cell line, then gentle pipetting was performed to disturb any clumps and ensure a uniform single cell suspension. The frequency and ratio of the cell propagation was distinct for each cell line. The cells were propagated every 2-3 days, after reaching the desired number of cells. Cells were counted by mixing 100µl of 4% trypan blue dye and 25 µl of the harvested cells, then transferring the cell suspension to the edge of a hemacytometer counting chamber.

**Cytotoxicity assay**

The essential oil and AgNPs were assayed for cell toxicity. Cytotoxicity measurements were based on the viability of the cells present in the culture. Cells were seeded into 96-well plates at a density of 1×104 cells per well and incubated for 24 h at 37 C in DMEM, then incubated with DMEM containing different concentrations of oil and AgNPs for 48 hours. MTT assay was then performed as follows: the medium was removed and cells in each well were incubated with 20 µl of MTT solution (5 mg/ml) for 4 hours at 37ºC. MTT solution was then discarded and 200 µl of dimethyl sulfoxide (DMSO) were added to dissolve insoluble
formazan crystals. Optical density was measured at 570 nm and 630 nm. Data were obtained from triplicate wells. Human periodontal ligament fibroblasts (PDL) are a primary cell culture used for verification of selective cytotoxicity with the least antiproliferative IC50 value obtained. As a robust and classical antineoplastic reference agent, doxorubicin was used for comparison purposes. All the assays were performed in triplicate and the calculated IC50 antiproliferative activities were reported as the mean values ± SD (n=3).

**The combined Effects of V. iphipionides essential oil and AgNPs**

To assess the synergistic effects of the essential oil in combination with AgNPs, cells were seeded into 96-well plates at a density of 1×10^4 cells per well and incubated for 24 hours at 37 °C in DMEM. After incubation, all cell lines were treated with a combination of oil and AgNPs at IC25, I

C50, and IC75 values. After 48 hours the MTT solution (20 μL) was added to each well and incubated for 4 hours. The MTT-formazan crystals formed were dissolved in 100 μL of DMSO and the absorbance was measured at 570 nm and 630 nm. Data were obtained from triplicate wells. The combination index (CI) was calculated by CompuSyn software (Paramus, NJ, USA) to analyse the synergistic inhibitory effect.

**Statistical analysis**

The results were presented as means± standard deviation (SD) of three independent experiments. Statistical differences between a and different treatment groups were determined using GraphPad Prism ANOVA followed by Dunnett’s post hoc test. For all statistical analysis, a p-value of less than 0.05 was considered statistically significant. P values of less than 0.001 were considered of statistically highly significant.

### Table 1. Chemical composition of V. iphipionides essential oil using GC/MS

<table>
<thead>
<tr>
<th>No</th>
<th>RT</th>
<th>Compound</th>
<th>KI exp</th>
<th>Ki let</th>
<th>Conc (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5.450</td>
<td>Santolina triene</td>
<td>913.57</td>
<td>908</td>
<td>0.76</td>
</tr>
<tr>
<td>2.</td>
<td>6.116</td>
<td>Alpha thujene</td>
<td>933.4</td>
<td>930</td>
<td>1.02</td>
</tr>
<tr>
<td>3.</td>
<td>7.505</td>
<td>Sabinene</td>
<td>977.0</td>
<td>969</td>
<td>0.69</td>
</tr>
<tr>
<td>4.</td>
<td>7.684</td>
<td>Beta pinene</td>
<td>982.9</td>
<td>974</td>
<td>0.79</td>
</tr>
<tr>
<td>5.</td>
<td>8.318</td>
<td>Yomogi alcohol</td>
<td>1001.6</td>
<td>999</td>
<td>0.71</td>
</tr>
<tr>
<td>6.</td>
<td>8.976</td>
<td>Alpha terpinene</td>
<td>1020.0</td>
<td>1014</td>
<td>0.72</td>
</tr>
<tr>
<td>7.</td>
<td>9.286</td>
<td>Para cymene</td>
<td>1027.9</td>
<td>1024</td>
<td>2.84</td>
</tr>
<tr>
<td>8.</td>
<td>9.434</td>
<td>Limonene</td>
<td>1032.2</td>
<td>1029</td>
<td>1.30</td>
</tr>
<tr>
<td>9.</td>
<td>9.608</td>
<td>Eucalyptol</td>
<td>1036.5</td>
<td>1031</td>
<td>53.65</td>
</tr>
<tr>
<td>10.</td>
<td>10.519</td>
<td>Gamma terpinene</td>
<td>1061.2</td>
<td>1054</td>
<td>1.88</td>
</tr>
<tr>
<td>11.</td>
<td>11.173</td>
<td>Cis-sabinenehydrate</td>
<td>1078.0</td>
<td>1065</td>
<td>1.71</td>
</tr>
<tr>
<td>12.</td>
<td>11.521</td>
<td>Artemisia alcohol</td>
<td>1087</td>
<td>1080</td>
<td>1.23</td>
</tr>
<tr>
<td>13.</td>
<td>12.480</td>
<td>1-terpineol</td>
<td>1112.7</td>
<td>1133</td>
<td>1.28</td>
</tr>
<tr>
<td>14.</td>
<td>14.435</td>
<td>Nerol oxide</td>
<td>1157.0</td>
<td>1173</td>
<td>0.58</td>
</tr>
<tr>
<td>15.</td>
<td>14.872</td>
<td>Cis-chrysanthanol</td>
<td>1167.6</td>
<td>1160</td>
<td>0.79</td>
</tr>
<tr>
<td>16.</td>
<td>15.145</td>
<td>Lavandulol</td>
<td>1174.9</td>
<td>1161</td>
<td>0.77</td>
</tr>
<tr>
<td>17.</td>
<td>15.529</td>
<td>Borenol</td>
<td>1183.2</td>
<td>1165</td>
<td>8.31</td>
</tr>
<tr>
<td>18.</td>
<td>15.767</td>
<td>Terpinen-4-ol</td>
<td>1188.9</td>
<td>1177</td>
<td>1.10</td>
</tr>
<tr>
<td>19.</td>
<td>16.491</td>
<td>Alpha terpineol</td>
<td>1206.1</td>
<td>1189</td>
<td>1.98</td>
</tr>
<tr>
<td>20.</td>
<td>16.660</td>
<td>Ethyl octanoate</td>
<td>1210.2</td>
<td>1196</td>
<td>1.27</td>
</tr>
<tr>
<td>21.</td>
<td>20.148</td>
<td>Bornyl acetate</td>
<td>1290.0</td>
<td>1284</td>
<td>11.26</td>
</tr>
<tr>
<td>22.</td>
<td>23.357</td>
<td>Neryl acetate</td>
<td>1365.2</td>
<td>1359</td>
<td>1.45</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>96.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Monoterpene hydrocarbons (9) 10.71
Oxygenated monoterpene (11) 82.67
Oxygenated sesquiterpene (1) 1.45
Aliphatic ester (1) 1.27
RESULTS

The chemical composition of V. iphionoides essential oil

The chemical composition of V. iphionoides essential oil was analyzed using GC/MS (Table 1). Twenty-two compounds were identified representing 96.1% of the V. iphionoides essential oil. The major classes of compounds were characterized as oxygenated monoterpenes (50%) and Monoterpenes hydrocarbons (41%). The result also showed that Eucalyptol (53.65) is the most dominant compound followed by Bornyl acetate (11.26) and Borenol (8.31%).

The modulation of the proliferation of different cancer cell lines and fibroblasts by V. iphionoides EO and AgNPs

The antiproliferative efficacies of doxorubicin on breast, pancreatic and prostate cancer and leukaemia cell lines are further illustrated in table 2. Nevertheless, doxorubicin lacked selective cytotoxicity in fibroblasts. Table 2 further displays the antiproliferative efficacies of V. iphionoides essential oil and AgNPs against the same cancer cell lines. Moreover, the V. iphionoides essential oil has an equipotent effect to doxorubicin against all cancer cell lines, except pancreatic cancer, where the essential oil showed promising
antiproliferative effects compared to doxorubicin (table 2). The AgNPs also showed cytotoxicity effects against all the cancer cell lines. Surprisingly, the AgNPs were not as potent as doxorubicin or V. iphionides essential oil. In cancer cell lines such as PANC1, K562 and PC3, the V. iphionides essential oil and the doxorubicin showed significantly antiproliferative effects compared to the AgNPs. Nevertheless, both V. iphionides and AgNPs had selective efficacies in the PC3 cell line (see figure 1).

The antiproliferative activity of the combination of V. iphionides essential oil and AgNPs is shown in figure 2. It was clear that

Table 2. IC$_{50}$ values (µg/ml) of in vitro antiproliferative activity of oil and Nano on different cancer cell lines

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fibroblast</td>
</tr>
<tr>
<td>oil</td>
<td>0.3±0</td>
</tr>
<tr>
<td>Nano</td>
<td>15.2±1.4</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.03±0.01</td>
</tr>
</tbody>
</table>

Results are mean ± SD (n = 3-4 independent replicates). IC$_{50}$ values (concentration at which 50% inhibition of cell proliferation took place in comparison to non-induced basal 72 h incubations)

Fig. 2. Effect of the combination between V. iphionides oil and AgNPs on PANC1, K562, PC3, MDA-MB-231 and fibroblast cell lines, values are the mean of three dependent replicates ± SD
Table 3. Antibacterial activity of *V. iphionoides* EO, AgNPs and EO:AgNPs (1:1) on the tested bacterial strains using well diffusion method

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>AgNPs:EO (1:1)</th>
<th>EO</th>
<th>AgNPs</th>
<th>Bacterial strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21±0.57</td>
<td>19.2±1.0</td>
<td>20±1</td>
<td><em>E. aerogenes</em></td>
</tr>
<tr>
<td></td>
<td>23±1</td>
<td>15.6±0.8</td>
<td>22±0.57</td>
<td><em>P. aeruginosa</em></td>
</tr>
<tr>
<td></td>
<td>18±0.50</td>
<td>26.5±1.40</td>
<td>18±0</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td></td>
<td>22±0.50</td>
<td>24±1.08</td>
<td>20±0.57</td>
<td><em>S. epidermidis</em></td>
</tr>
<tr>
<td></td>
<td>22±0.60</td>
<td>19±0.88</td>
<td>16±0.57</td>
<td><em>S. aureus</em></td>
</tr>
</tbody>
</table>

Each well contains 10 µg of EO, 172.56 µg AgNPs or 5 µg EO: 86.28 µg AgNPs.
Data are expressed as mean ± SD.

Table 4. Minimum Inhibitory concentration (MIC) of AgNPs, *V. iphionoides*, EO and combination of AgNPs:EO

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>AgNPs µg/mL</th>
<th>EO µg/mL</th>
<th>AgNPs:EO µg/mL</th>
<th>Cm</th>
<th>Km</th>
<th>Amp</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. aerogenes</em></td>
<td>19.15</td>
<td>50</td>
<td>10.63:61</td>
<td>0.75</td>
<td>0.20</td>
<td>2.30</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>6.38</td>
<td>370</td>
<td>3.20:61</td>
<td>1.10</td>
<td>0.30</td>
<td>2.5</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>19.15</td>
<td>50</td>
<td>6.4:184</td>
<td>0.80</td>
<td>0.15</td>
<td>0.20</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>6.38</td>
<td>123</td>
<td>3.20:61</td>
<td>0.16</td>
<td>0.28</td>
<td>0.32</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>6.38</td>
<td>50</td>
<td>3.20:61</td>
<td>0.15</td>
<td>0.32</td>
<td>0.25</td>
</tr>
</tbody>
</table>

the synergistic effect only appeared when the *V. iphionoides* essential oil and AgNPs were incubated with different cancer cell lines at low concentration. However, at high concentrations the combination between *V. iphionoides* essential oil and AgNPs showed an antagonistic effect.

**Antibacterial activity using well diffusion method**

The increase in the resistance to different antibiotics can be controlled using new methodologies, such as synergy with either AgNPs or the EOs of plants. The synergistic effect of AgNPs and EO was evaluated using well diffusion method (Table 3). The results showed that there were notable increasing in antibacterial activity of AgNPs:EO except against *E. coli*. When AgNPs and EO were examined separately, the inhibition zones exhibited by AgNPs against *E. aerogenes*, *P. aeruginosa* and *S. epidermidis* and *S. aureus* were 20, 22, 20 and 16 mm, respectively. EO of *V. iphionoides* against same bacteria exhibited 19.2, 15.6, 26.5 and 19 mm, respectively. After combining of AgNPs and EO in a proportion of 1:1, the inhibition zone resulted is 21, 23, 22 and 22 mm, respectively showing presence of synergistic effect among them.

**Minimal Inhibitory Concentration and Growth Curves**

Table 4 shows MIC values of AgNPs and EO of *V. iphionoides* against *E. aerogenes*, *P. aeruginosa*, *E. coli*, *S. epidermidis* and *S. aureus*. MICs of AgNPs against these bacterial strains in order were 19.15, 6.38, 19.15, 6.38, 6.38 µg/mL, respectively as demonstrated by broth dilution method. MICs of EO against the same bacteria were 50, 370, 50, 123 and 50 µg/mL, respectively.

**DISCUSSION**

For 20 years, the extracts and phytoconstituents of *V. Iphionoides* have been known to possess many biological effects including; antibacterial 4, anti-diabetic 22, antiplatelet 5 and anticancer activities against many cancer cell lines 23,24. In this study, we analyzed the chemical composition of *V. iphionoides* essential oil using GC/MS, and it was clear that oxygenated monoterpene was the major compound class...
identified in V. iphionoides essential oil and Eucalyptol (53.65), with Bornyl acetate (11.26) and Borenol (8.31%) the most dominant compounds identified. It has been reported that V. iphionoides essential oil collected from Jordan Valley, was rich in oxygenated monoterpenic compounds and Borenol (49.3%), with bornyl formate (3.6%) and bornyl acetate (2.9%) the most dominant compounds (Avato et al., 2004). Al-Douri and co-authors 25 qualitatively identified the presence of 1,8-cineole (8.4%), and camphor (3.7%) in V. iphionoides oil using Thin Layer Chromatography (TLC) and Gas Chromatography (GC) analysis.

In addition, in this study, we also reported the cytotoxicity effect of the essential oil extracted from V. iphionoides and the cytotoxicity effect of AgNPs, (which were synthesized from fungal strain Tritirachium oryzae W5H) against breast, pancreatic and prostate cancer and leukaemia cell lines. 75 cm2 his suggests that Eucalyptol, Bornyl acetate and Borenol play a major role in this effect and we also documented that Eucalyptus has antiproliferative activity against leukemia by inducing apoptosis 26, as well as against colorectal cancer by inhibition of P13K/Akt pathway and inducing caspase-3 cleavage leading finally to apoptosis 27. On the other hand, it has been reported that AgNPs exert antiproliferative activity against breast cancer 28, lung cancer 29, colon cancer 30 and cervical cancer 28, through the inactivation of P13K/Akt signalling pathways 27. In our study we revealed the co-incubation effect between V. iphionoides essential oil and AgNPs against the same cell lines, and our result showed the synergistic effect occurred when V. iphionoides essential oil and AgNPs were co-incubated at low concentrations. However, the cytotoxicity shows antagonistic effect at high concentrations. Consequently, we suggested that high concentrations of V. iphionoides essential oil and AgNPs causes hyperactivation in the mTOR pathway leading to activation of P13K/Akt signalling pathways, which enhance proliferation. In contrast, at low concentration of V. iphionoides essential oil and AgNPs, the activation of the mTOR pathway is reduced significantly, leading to the inactivation of the P13K/Akt signalling pathways, which increases the apoptosis and reduces the cells proliferation.

Antibacterial activity was estimated using a well diffusion assay (table 1). In general, EO and AgNPs revealed different effects against bacteria. EO and AgNPs exhibited reasonable antibacterial activities against the investigated bacteria, both G positive and negative bacteria. However, AgNPs exhibited stronger antibacterial activity than EO for V. iphionoides (excluding E. coli). AgNO3 showed a lower inhibition region (data not shown) compared to a similar concentration of AgNPs, which indicates that antibacterial activity is due to AgNPs no other thing. AgNPs are known to increase the surface area leading to greater surface contact with bacteria and thus a better bactericidal effect against bacteria under test 31. Previous studies, appeared that AgNPs had antibacterial activity and inhibitory zone extended between 16-22 mm against E. aerogenes, P. aeruginosa, E. coli, S. epidermidis and S. aureus at 100 µl (172.56 µg AgNPs per well) of AgNPs 18. The most susceptible strain to EO of V. iphionoides was E. coli (26.5 mm). P. aeruginosa (ESBL) showed lowest sensitivity (16.5 mm) to the EO. It might be reflect the effect of AgNPs through perforating and lysing the bacterial cell wall followed by generation of free radicals 31 and disintegration of DNA 32.

The MICs of the NPs varied from 6.4 – 19.2 µg/mL . The MIC values for the various bacteria were as follow: 6.4 µg/mL for S. aureus, S. epidermidis and P. aeruginosa; and 19.2 µg/mL for E. coli and E. aerogenes. The results of present study showed that V. iphionoides oil exhibited marked inhibition activity against Gram positive and Gram-negative bacteria with inhibition zone ranged between 16.5 and 26.5 mm and MIC value as low as 50 µg/mL. The EO appears to be more substantial determinant for the synergetic results of antibacterial activities by AgNPs and EO of V. iphionoides, especially against S. aureus, S. epidermidis, P. aeruginosa and E. aerogenes. Although many works have been published studying AgNPs, these synergistic applications have infrequently been investigated for the dealing with the resistant bactericidal activity. However, no data have been previously reported on the potential antibacterial effects of such cooperative interaction between biosynthesized AgNPs from Tritirachium oryzae, and the EO of V. iphionoides against the tested bacteria. It has been mentioned that the absorption of a drug is increased several times in the presence of nanoparticles, suggesting that AgNPs could be used as potential drug delivery system.
19. For example, the antimicrobial effect of some antibiotics including trimethoprim, amoxicillin, vancomycin and cefotaxime were much greater than the antibacterial effect of AgNPs alone. However, combinations between these antibiotics and AgNPs led to the boosting of antibacterial activity which indicates the synergistic effect of these components with the AgNPs. Moreover, nanoparticle-EO associations could reduce the amounts of combined agents necessary, herewith lowering noxiousness and elevating antimicrobial prospect. Thus, the raises in the scale of inhibition against these antibiotic-resistant bacteria or still antibiotic-sensitive bacteria is seen a remarkable result and an effective therapeutic master plan. However, further studies are necessary in order to determine the precise pathway when different concentrations of V. iphionoides essential oil and AgNPs are co-incubated with different cancer cell lines.

REFERENCES