Principal component and heat map analysis of the biological activities for some selected medicinal plants

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ABSTRACT

African and Asian countries have a gorgeous and prestigious heritage of herbal medicines. Ten well-known medicinal plants cultivated and grown under African environmental conditions (temperature, relative humidity, irradiance, photoperiod, wind and soil properties, etc) were extracted with methanol. Themethanolic extracts of these plants were screened for their antileishmanial, cytotoxic and free radical scavenging activities. The methanol extract of Colchicum autumnale and Alpinia officinarum showed high antileishmanial activity (IC₅₀ 61.85±2.09 and 66.42±1.60 µg/ml, respectively). A. officinarum exhibited the most potent cytotoxicity (IC₅₀ 8.66±2.30 and 3.66±1.52 µg/ml against Hep G2 and A549, respectively). Rosa damascena, A. officinarum, and Humulus lupulus showed potent DPPH radical scavenging activity with IC₅₀ values (2.0±0.23, 11.50±2.17, 14.0±2.0 µg/ml, respectively). Principal component analysis (PCA) was used as a statistical tool for experimental sets of obtained values for antileishmanial, antioxidant and anticancer activities performed statistics with person correlation matrix on plant samples. The results showed that there was a high correlation between anticancer activity against A549 and Hep G2 cells (0.743); moderate correlation between anticancer activity against A549 cells and antileishmanial activity (0.643); moderate correlation between anticancer activity against Hep G2 cells and antileishmanial activity (0.555), and low correlation between DPPH radical scavenging activity and anticancer against A549 cell (0.440). The heat map data set was based on values of the biological activities for the plants extract percentage. The results showed that C. autumnale and A. officinarum showed high antileishmanial activity, while A. officinarum exhibited the most potent cytotoxicity against Hep G2 and A549 cell lines. R. damascena, A. officinarum and H. lupulus showed potent DPPH radical scavenging activities. Hierarchical clustering analysis (HCA) data showed that some plant extracts have similar clustering results associated with their biological activities.

Keywords: Plant extract, antileishmanial, cytotoxicity, cancer cell line, DPPH radical scavenging activity, principal component analysis, heatmap, hierarchical clustering analysis, Rosa damascena, Alpinia officinarum, Humulus lupulus.

INTRODUCTION

Leishmania is a genus of trypanosomatid protozoa and is the parasite responsible for the disease leishmaniasis. It is spread through sandflies. Their primary hosts are vertebrates; Leishmania commonly infects hyraxes, Canidae, rodents and humans, and currently affects more than 12 million people. Leishmaniasis is a disease with a prolonged worldwide distribution in 98 countries where about third of the cases in the Americas, the Mediterranean

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basin and Western Asia with 70 to 75% of the cases recorded in Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, Costa Rica, Peru and Northern Sudan (Alvar et al., 2001).

Chemotherapy of leishmaniasis is still inspiring due to the limitation of the efficiency of the drug. N-methylglucamine, amphotericin B, and pentamidinethe drugs in use for the treatment of leishmaniasis. However, many parasites are resistant to these drugs. Medicinal plants are a good source of bioactive phytochemicals used for the treatment of parasitic diseases including leishmaniasis for people around the world (Bahmani et al., 2015).

Reactive Oxygen Species (ROS), including free radicals, are reported to cause several diseases like liver diseases, diabetes and cancer (Ames, 1983) which are considered the most dangerous in all countries around the world (Feher et al., 1987; Halliwell et al., 1989; Aruma, 1998; Suizane et al., 2009; Alzeer et al., 2014) and the cost of chemotherapy has become more expensive and the funds in many governments of some countries, particularly, the developing countries are unavailable for this treatment. Thus, it is important to investigate new drugs or compounds that could be associated with the traditional treatment. As such medicinal plants have provided some of the natural therapeutics for cancer (Alzeer et al., 2014). Furthermore, the use of plant extracts provides a standard gauge for screening especially in its initial phase (Ekta, 2013). The impact of environmental factors (temperature, relative humidity, irradiance, photoperiod, wind, soil properties, fertilization and harvest time) influence the composition of essential oils (terpenoids and aromatic compounds etc) (Kusumoto et al., 1995; Cordell, 1995; Baker et al., 1995; Nadia, 2013).

The aim of our work was to evaluate the in vitro activity of methanolic extracts of some medicinal plants such as antileishmanial activity and cytotoxic activity using two different cancer cell lines (Hep G2 and A549) and free radical scavenging activity with the DPPH assay.

Regarding applied statistical analysis tool, we used principal component analysis (PCA) in order to obtain the initial solution reducing the original dataset. PCA expresses the data as a linear combination of its basic vectors. It is a non-parametric analysis, and the correlation is called loadings. The squared loadings are easier to interpret than the loadings (Horel, 1981). Pearson’s correlation is a normalization method where the standard deviation is in scaling the data between -1 to 1 (Janzen et al., 2006). Concerning the heat map analysis, it is a graphical representation of data where the individual values contained in a matrix are signified as colors. Fractal and tree maps both often use a comparable system of color-coding to represent the values occupied by a variable in a hierarchy.

The following plants Alpinia officinarum, Achillea millefolium, Colchicum autumnale, Chrysanthemum morifolium, Humulus lupulus, Matricaria chamomilla, Rosa damascena, Silybum marianum and Vitexagnus-castus were found of multiple biological activities and broad traditional uses against infectious and non-infectious diseases. A. officinarum is used in folk medicine as anticancer, antioxidant, antifungal and antimicrobial agent (Ray, 1976; Asolkar et al., 2005; An et al., 2005; Suja et al., 2008; Srividya et al., 2010). Traditionally, A. millefolium is used as antiseptic, antispasmodic, astringent, carminative, diaphoretic, digestive emmenagogue, stimulant, tonics, vasodilator, and vulnerary and also used against colds, cramps, fevers and kidney disorders (Stojanović et al., 2005; Yaeesh et al., 2006). C. autumnale is used as anti-inflammatory, antimitotic and antifibrotic and involved in the inhibition of microtubule formation (Boye and Brossi, 1992; Katzung, 2004). C. morifolium possesses antihepatotoxic and antigenotoxic effects (Lee et al., 2011). It exhibits an allelopathic activity (Beninger and Hall, 2005) and has anti-inflammatory, immunomodulatory humoral, cellular and mononuclear phagocytic activities (Cheng et al., 2005; Su et al., 2012). H. lupulus traditionally is used to relieve insomnia, anxiety, excitability, restlessness associated with tension, headache and gastrointestinal spasms (Newall et al., 1996; Schulz et al., 2001). M. chamomilla showed different pharmacological activities like anti-inflammatory, anticancer, anti-allergic activities and is used in the treatment of stress and depression (Shipchlev et al., 1981; Al-Hindawi et al., 1989). T. tomentosa have been used for diuretic, diaphoretic, antispasmodic, stomachic and sedative activities and have been taken for the treatment of flu, cough, migraine, nervous tension, ingestion problems, various types of spasms and liver disorders (Theallet, 1963; Baytop, 1984; Weiss, 1988; Toker, 1995). R. damascene has been used as cardiotonic (Hadjikakhoendi, 2009), mild laxative (Zargari, 1992), anti-inflammatory (Loghmani-Khozani et al., 2007), cough suppressant (Shafei et al., 2003), anti HIV (Mahmood et al., 1996), antibacterial (Basim and Basim, 2003) and antitussive (Shafei et al., 2003). S. marianum has hepatoprotective and antidepressant activities and used in the treatment of diabetes, varicose veins, senile congestions, amenorrhoea and uterine hemorrhage (Talcia, 2014; Evans, 2009; Barceloux, 2008). The essential oils of V. castus have antifungal and antimicrobial activities (Hoffman, 2003).

MATERIALS AND METHODS

Plant materials

The plants used in this study were collected from different cities of Egypt. Plants were identified by Engineer Esraa Mohamed, Department of Agricultural Chemistry, Faculty of Agriculture, Minia University, Egypt. The collected parts of plants were separated and cleaned from dust and placed in the shade inside a well-ventilated room until were completely dried and weight was obtained (Table 1). Dried parts of the plants were grounded to fine powder.

Solvents and chemicals

Dulbecco’s modified Eagle’s medium (DMEM), RPMI (R8758, SIGMA), penicillin-streptomycin (WAKO 119-00703), fetal bovine serum (FBS) and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) from (23547-21 1g, Nacalaitesque)Trolox, 2,2-Diphenyl-1-picyrylhydrazyl (DPPH) and Doxorubicin was obtained from
Preparation of plant extracts

The air-dried fine powders of the selected plants were extracted by methanol thrice. The methanolic extracts were concentrated under reduced pressure at 50°C using a rotary evaporator to yield viscous gummy materials, and then subjected to drying in vacuum desiccators (Oil pump). Finally, the weight of each plant extract was obtained.

Determination of the antileishmanial activity

The leishmanicidal activities of methanolic extracts were performed using the colorimetric MTT assay. Medium 199 supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 100 µg/ml of kanamycin was used as the cell culture medium. The methanolic extracts were dissolved in DMSO and added to each well of the 96-well micro-titration plates at 1% as the final concentration. Leishmania major cells (2×10⁵ cells/well) were cultured in a CO₂ incubator at 25°C for 72 h and then MTT solution was added to each well and the plates were incubated overnight at 25°C. The absorbance was measured at 540 nm using a Molecular Device Versamex tunable microplate reader. Amphotericin B was used as a positive control (Takahashi et al., 2004). The inhibition % was calculated using the equation:

\[
\text{% Inhibition} = \left\{1 - \frac{(A_{\text{sample}} - A_{\text{blank}})}{(A_{\text{control}} - A_{\text{blank}})}\right\} \times 100
\]

Where \(A_{\text{control}}\) is the absorbance of the control reaction mixture (containing DMSO and all reagents except for the methanolic extracts). \(IC_{50}\) was determined as the concentration of sample required to inhibit the formation of MTT formazan by 50%.

Cell lines

HepG2 cell (RIKEN Cell Bank: RCB1886) (Courtesy of Prof. Takano). The human cell line derived from hepatocyte carcinoma or hepatoblastoma of 15 years old male Caucasian cultured in RPM1640 (Sigma, 500 ml) was supplemented with 10% heat-inactivation Fetal Bovine Serum (FBS) and antibiotics cocktail and incubated in 5% CO₂ at 37°C.

Human lung cancer cell, A549 (RCB004B), was obtained from (RIKEN Cell Bank) cultured in Dulbecco’s modified Eagle’s medium (DMEM; Sigma, 500 ml) supplemented with 10% heat-inactivation Fetal Bovine Serum (FBS) and antibiotics cocktail and incubated in 5% CO₂ at 37°C

Antibiotics cocktail

Amphotericin B (Sigma A9528 100 mg) and kanamycin sulfate 5 g (WAKO 119 to 00703) are dissolved in 8.93 and 25 ml of sterilized MilliQ water, respectively. equal volume of this solution was combined with amphotericin B solution (8.93 ml) and kanamycin sulfate solution (8.93 ml), dispensed into microtube with 500 µl and stored at -20°C. Heat-inactivated FBS was kept at 56°C temperature for 30 min and stored in a 50 ml tube at -20°C.

Procedure for in vitro cytotoxicity assays of plants extract

This assay was performed using human lung cancer cell line (A549) and human hepatocyte carcinoma cell line (Hep G2) and the viability was estimated by the colorimetric MTT assay. Dulbecco’s modified Eagle medium (DMEM) supplemented with FBS 10% and antibiotics were used as the cell culture medium for A549 cell line and RPMI supplemented with 10% FBS and antibiotics for Hep G2 cell line. The test plant extracts were dissolved in DMSO and added to each well of the 96-well micro-titration plates at 1% as the final concentration. The plant extracts were tested at final concentrations of 100, 50, and 25 µg/ml. A549 and Hep G2 cells (5×10³ cells/well) were cultured in a 5% CO₂ incubator at 37°C for 72 h and thereafter, MTT solution was added to each well and the plates incubated for a further 1.5 h. Subsequently, the formazan precipitates were dissolved in DMSO and the optical density value for each well was measured at 540 nm with a microplate reader. Doxorubicin was used as a positive control. The cell growth inhibition was calculated.
results showed that the extracts had moderate antileishmanial activity (IC₅₀ >100 µg/ml), except for some extracts which had an IC₅₀ of 36.11±8.03 µg/ml. The antileishmanial activity of the plant extracts was evaluated using the equation:

\[
\text{Inhibition (\%) = } \frac{[1-(A \text{ sample- A blank})]}{(A \text{ control- A blank})}
\]

Where A_control is the absorbance of the control reaction mixture (containing DMSO and all reagents except for the test plant extracts). IC₅₀ was determined as the concentration of sample required to inhibit the formation of MTT formazan by 50% (Samy et al., 2014a and b).

**DPPH radical scavenging activity**

The plant extracts were tested at a final concentration of 50, 25 and 12.5 µg/ml, respectively. The absorbance with various concentrations of the tested plant extracts dissolved in MeOH (100 µl) in a 96-well microtiter plate was measured at 515 nm as blank. Thereafter, 200 µM DPPH solution (100 µl) was added to each well, followed by incubation at room temperature for 30 min. The absorbance was measured again as a sample. The % inhibition was calculated using the equation:

\[
\text{Inhibition (\%) = } [1-(A \text{ sample- A blank})]/ (A \text{ control- A blank})
\]

Where A_control is the absorbance of the control reaction mixture (containing DMSO and all reagents except for the test plant extracts). IC₅₀ was determined as the concentration of sample required to inhibit the formation of the DPPH radical by 50% (Darwish et al., 2016).

**Data analysis**

**Principal component analysis (PCA)**

This was used as a statistical tool; it is used with the aim to evaluate the dataset, reducing its dimension and conserving most of the statistical information. The analysis was performed using data analysis and statistical application available for Microsoft Excel (XLSTAT 2018.3.16, Florida, USA).

**Heat maps**

The heat maps were created using the software (XLSTAT 2018.3.16, Florida, USA), based on their biological activities including antileishmanial, anticancer activity against (A549 and Hep G2 cell lines) and DPPH radical scavenging activity. The data treatment was based on several matrices containing different sets of information, depending on the interpretation. Hierarchical clustering analysis (HCA) was carried out for columns using Euclidean distance. Color scales were adopted for each individual case based on green (higher values), red (lower values), and black (intermediate values).

**RESULTS AND DISCUSSION**

The aim of this study was to evaluate the anti-leishmanial activity, DPPH radical scavenging activity and the anticancer activity of ten methanolic plant extracts against two different human cell lines (Hep G2 hepatoplastoma cell line and A549 human cancer cell line) using MTT assay.

**Antileishmanial activity of the plant extracts**

Table 1 and Figures 1 and 2 show that the methanolic extract of *C. autumnale*, *A. officinarum*, and *S. marianum* had moderate antileishmanial activity (IC₅₀ 61.85±2.09, 66.42±1.60 and 71.24±3.46 µg/ml, respectively) as compared to the standard amphotericin B (36.11±8.03 µM),

**Table 2: The anti-leishmanial activity of the plant extracts. Inhibition percentages are expressed as mean values± S.D. of 3 experiments.**

<table>
<thead>
<tr>
<th>Plant name</th>
<th>100 µg/ml</th>
<th>50 µg/ml</th>
<th>25 µg/ml</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alpinia officinarum</em></td>
<td>97.25±1.63</td>
<td>26.69±4.18</td>
<td>8.28±4.61</td>
<td>66.42±1.60</td>
</tr>
<tr>
<td><em>Rosa damascena</em></td>
<td>9.75±4.14</td>
<td>6.92±3.21</td>
<td>5.62±4.10</td>
<td>&gt;100</td>
</tr>
<tr>
<td><em>Silibum marianum</em></td>
<td>90.97±1.13</td>
<td>88.32±5.84</td>
<td>22.07±2.14</td>
<td>71.24±3.46</td>
</tr>
<tr>
<td><em>Colchicum autumnale</em></td>
<td>98.29±0.75</td>
<td>34.55±6.40</td>
<td>5.34±2.66</td>
<td>61.85±2.09</td>
</tr>
<tr>
<td><em>Humulus lupulus</em></td>
<td>28.82±6.04</td>
<td>2.48±2.19</td>
<td>4.28±2.41</td>
<td>&gt;100</td>
</tr>
<tr>
<td><em>Achillea millefolium</em></td>
<td>12.63±7.71</td>
<td>2.26±1.15</td>
<td>2.21±0.83</td>
<td>&gt;100</td>
</tr>
<tr>
<td><em>Chrysanthemum morifolium</em></td>
<td>7.54±1.97</td>
<td>3.86±1.93</td>
<td>3.25±0.94</td>
<td>&gt;100</td>
</tr>
<tr>
<td><em>Matricaria chamomilla</em></td>
<td>11.64±6.40</td>
<td>6.48±3.34</td>
<td>4.35±3.69</td>
<td>&gt;100</td>
</tr>
<tr>
<td><em>Tilia tomentosa</em></td>
<td>6.12±4.57</td>
<td>2.49±1.40</td>
<td>2.41±2.08</td>
<td>&gt;100</td>
</tr>
<tr>
<td><em>Vitexagnus</em></td>
<td>10.72±5.16</td>
<td>6.86±2.70</td>
<td>7.69±4.69</td>
<td>&gt;100</td>
</tr>
<tr>
<td><em>Amphtericin (B)</em></td>
<td>99.13±2.08</td>
<td>89.33±7.63</td>
<td>72.20±15.52</td>
<td>36.11±8.03</td>
</tr>
</tbody>
</table>
Figure 1: The anti-leishmanial activity of the plant extracts. Inhibition percentages are expressed as mean values ± S.D. of 3 experiments.

Figure 2: Anti-leishmanial activity (IC_{50}) of the plant extracts. Inhibition percentages are expressed as mean values ± S.D. of 3 experiments.
Table 3: The cytotoxic activity (%) of plant extracts on human hepatocyte carcinoma cell line (HepG2) and human lung cancer cell line (A549). Inhibition percentages are expressed as mean values± S.D. of 3 experiments.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>HepG2 100 µg/ml</th>
<th>HepG2 50 µg/ml</th>
<th>HepG2 25 µg/ml</th>
<th>A549 100 µg/ml</th>
<th>A549 50 µg/ml</th>
<th>A549 25 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alpinia officinarum</em></td>
<td>98.00±0.57</td>
<td>94.00±0.56</td>
<td>85.00±5.50</td>
<td>93.66±6.35</td>
<td>70.66±3.78</td>
<td>58.00±13.11</td>
</tr>
<tr>
<td><em>Rosa damascena</em></td>
<td>93.33±3.21</td>
<td>57.33±9.45</td>
<td>17.33±4.04</td>
<td>82.00±6.55</td>
<td>51.66±11.59</td>
<td>29.66±15.50</td>
</tr>
<tr>
<td><em>Silybum marianum</em></td>
<td>86.33±7.09</td>
<td>21.00±7.80</td>
<td>21.66±4.72</td>
<td>85.00±19.0</td>
<td>18.33±3.78</td>
<td>3.66±1.52</td>
</tr>
<tr>
<td><em>Colchicum autumnale</em></td>
<td>88.00±9.50</td>
<td>43.67±5.50</td>
<td>27.66±4.93</td>
<td>52.00±7.0</td>
<td>42.66±2.68</td>
<td>42.00±21.07</td>
</tr>
<tr>
<td><em>Humulus lupulus</em></td>
<td>44.00±11.53</td>
<td>31.00±11.0</td>
<td>26.00±9.53</td>
<td>42.33±7.50</td>
<td>21.66±3.51</td>
<td>9.00±4.0</td>
</tr>
<tr>
<td><em>Achillea millefolium</em></td>
<td>25.00±11.35</td>
<td>15.00±5.0</td>
<td>5.66±1.52</td>
<td>44.66±2.60</td>
<td>6.66±2.51</td>
<td>2.33±0.57</td>
</tr>
<tr>
<td><em>Chrysanthemum morifolium</em></td>
<td>76.00±10.53</td>
<td>45.00±12.0</td>
<td>10.56±4.50</td>
<td>39.00±1.73</td>
<td>32.33±4.50</td>
<td>4.00±1.0</td>
</tr>
<tr>
<td><em>Matricaria chamomilla</em></td>
<td>45.33±3.51</td>
<td>26.00±7.02</td>
<td>3.33±1.52</td>
<td>20.66±1.52</td>
<td>5.66±2.08</td>
<td>3.00±1.73</td>
</tr>
<tr>
<td><em>Tilia tomentosa</em></td>
<td>76.66±11.23</td>
<td>33.00±4.0</td>
<td>6.00±1.0</td>
<td>38.00±4.0</td>
<td>14.66±6.42</td>
<td>6.66±1.52</td>
</tr>
<tr>
<td><em>Vitexagnus-castus</em></td>
<td>17.33±6.80</td>
<td>14.00±6.0</td>
<td>10.00±1.50</td>
<td>23.00±7.0</td>
<td>19.60±11.01</td>
<td>8.33±5.50</td>
</tr>
<tr>
<td><em>Doxorubicin</em></td>
<td>99.33±2.08</td>
<td>91.33±7.63</td>
<td>71.00±1.52</td>
<td>98.00±1.0</td>
<td>90.00±6.24</td>
<td>70.00±15.0</td>
</tr>
</tbody>
</table>

while the remaining tested extracts had no anti-leishmanial activity.

Growth Inhibition values in cell cultures for plant extracts

The plant extracts were screened for their cytotoxic activities in two different cancer cell lines using MTT assay. This assay showed highly potent activities exhibited by *A. officinarum*, *R. damascena*, *S. marianum* and *C. autumnale* at concentration 100 µg/ml against HepG2 cell line (Figure 3), and *A. officinarum*, *R. damascena* and *S. marianum* against A549 cell line, as shown in Table 3 and Figure 4.

IC<sub>50</sub> values in cell cultures

The IC<sub>50</sub> values confirmed the highly potent cytotoxic activities against HepG2 and A549 cell lines showed by *A. officinarum* (8.66±2.30 and 3.66±1.52 µg/ml, respectively) and *R. damascena* and (37.32±17.0 and 44.60±23.18 µg/ml,
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![Figure 4](image_url)

**Figure 4:** The cytotoxic activity of plant extracts on human lung cancer cell line (A549). Inhibition percentages are expressed as mean values± S.D. of 3 experiments.

**Table 4:** *In vitro* cytotoxicity IC₅₀ values for the ten different plant extracts.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>HepG2 IC₅₀ (µg/ml)</th>
<th>A549 IC₅₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alpinia officinarum</em></td>
<td>8.66±2.30</td>
<td>3.66±1.52</td>
</tr>
<tr>
<td><em>Rosa damascena</em></td>
<td>37.32±1.00</td>
<td>44.60±2.18</td>
</tr>
<tr>
<td><em>S. marianum</em></td>
<td>80.3±1.89</td>
<td>75.0±9.64</td>
</tr>
<tr>
<td><em>Colchicum autumnale</em></td>
<td>57.00±6.08</td>
<td>74.0±2.28</td>
</tr>
<tr>
<td><em>Humulus lupulus</em></td>
<td>113±3.0</td>
<td>99.0±1.50</td>
</tr>
<tr>
<td><em>Achillea millefolium</em></td>
<td>74.08±30.0</td>
<td>116.3±4.64</td>
</tr>
<tr>
<td><em>Chrysanthemum morifolium</em></td>
<td>80.66±4.10</td>
<td>114.6±4.40</td>
</tr>
<tr>
<td><em>Matricaria chamomilla</em></td>
<td>105.3±1.89</td>
<td>166.0±8.61</td>
</tr>
<tr>
<td><em>Tilia tomentosa</em></td>
<td>86.33±3.16</td>
<td>139.3±7.15</td>
</tr>
<tr>
<td><em>Vitexagnus-castus</em></td>
<td>391.6±1.45</td>
<td>234.6±7.84</td>
</tr>
<tr>
<td>Doxorubicin (µM)</td>
<td>7.33±3.51</td>
<td>8.66±3.21</td>
</tr>
</tbody>
</table>

respectively) (Table 4, Figures 5 and 6).

**DPPH radical scavenging activity**

The plant extracts were examined for their 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities showed high activity in the DPPH radical scavenging activities for *R. damascena, A. officinarum* and *H. lupulus* with (IC₅₀ 2.0±0.23, 11.50±2.17 and 14.0±2.0 µg/ml, respectively) as compared with the standard trolox (IC₅₀ 16.0±1.0 µM) (Table 5 and Figure 7).

**PCA Analysis**

Performed statistics with Pearson Correlation matrix on plant samples based on the antileishmanial, anticancer and DPPH radical scavenging activities showed that there was a strong correlation between anticancer activity against A549 cells and anticancer activity against Hep G2 cells (0.743), moderate correlation between anticancer activity against A549 cells and antileishmanial activity (0.643), moderate correlation between anticancer activity against Hep G2 cells and antileishmanial activity (0.555), and low correlation between DPPH radical scavenging activity and anticancer...
against A549 cell (0.440) as shown in Figure 8.
Eigen values for the first and the second factor are higher (2.404 and 0.985, respectively) (Figure 9) as compared to values for the third and fourth factor (0.459 and 0.151, respectively). So, the first two factors must be used to explain the obtained variabilities (60.11, 24.62, 11.48 and 3.78%, respectively). Some values for factors (F1, F2, F3 and F4) are positive, while some are negative. Observation plot based on the activity it is shown that the highest DPPH activity is in H. Lupulus, R. damascene and A. officinarum extracts. The highest antileishmanial activity is in C. autumnale, A. officinarum, and S. marianum extracts. The
Table 5: DPPH Inhibition % are expressed as mean values± S.D. of 3 experiments and IC₅₀ for the ten different plants extract.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>% of inhibition</th>
<th>IC₅₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 µg/ml</td>
<td>25 µg/ml</td>
</tr>
<tr>
<td>Silybum marianum</td>
<td>55.03±10.14</td>
<td>34.66±5.54</td>
</tr>
<tr>
<td>Matricaria chamomilla</td>
<td>68.13±5.10</td>
<td>55.03±14.02</td>
</tr>
<tr>
<td>Vitexagnus-castus</td>
<td>42.86±1.09</td>
<td>23.53±1.70</td>
</tr>
<tr>
<td>Alpinia officinarum</td>
<td>84.56±0.83</td>
<td>70.60±1.57</td>
</tr>
<tr>
<td>Tilia tomentosa</td>
<td>12.20±2.95</td>
<td>6.96±4.63</td>
</tr>
<tr>
<td>Achillea millefolium</td>
<td>40.56±3.19</td>
<td>22.96±2.45</td>
</tr>
<tr>
<td>Chrysanthemum morifolium</td>
<td>12.30±5.28</td>
<td>9.10±2.53</td>
</tr>
<tr>
<td>Rosa damascena</td>
<td>94.33±2.02</td>
<td>90.0±0.05</td>
</tr>
<tr>
<td>Colchicum autumnale</td>
<td>27.96±3.52</td>
<td>13.26±1.60</td>
</tr>
<tr>
<td>Humulus lupulus</td>
<td>94.80±1.13</td>
<td>81.13±6.31</td>
</tr>
<tr>
<td>Trolox (µM)</td>
<td>96.0±2.51</td>
<td>70.0±5.0</td>
</tr>
</tbody>
</table>

Figure 7: DPPH radical scavenging activity of the tested plant extracts. Inhibition percentages are expressed as mean values± S.D. of 3 experiments.

The highest anticancer activity against Hep G2 cells is in A. officinarum, R. damascena, and S. marianum extract. The highest anticancer activity against A549 cells is in A. officinarum, S. marianum and R. damascena extracts. The correlations between plant samples and antileishmanial, anticancer and DPPH radical scavenging activity (Figure 10) showed that there is a strong correlation between the first factor (F1) and anticancer activity against A549 cells (r = 0.941), (Figures 6 and 7) and strong correlation between the second factor (F2) and DPPH radical scavenging activity (r =0.900) and a moderate correlation between F1 and anticancer activity against Hep G2 cells (r =0.836) and moderate correlation between the F1 and antileishmanial activity (r =0.804). The results showed low correlation between the third factor (F3) and antileishmanial activity (r =0.544) and low correlation between F1 and DPPH activity (r = 0.417). It can be concluded that the highest activity among the investigated
Figure 8: Principal component score plot (PC1 and PC2) of Contribution of values %.

Figure 9: The importance of factors and values of cumulative variabilities.
Figure 10: Principal component score plot (F1 and F2) of the studied plant samples based on their bioactivities.

Figure 11: Heat map of the biological activities of the methanolic plants extract. Annotations on the left side of the heat map show clustering of samples.
samples is present in the methanolic extract of A. officinarum.

Heat map analysis

The default color gradient sets the lowest value in the heat map to bright red, the highest value to a bright green, and mid-range values to black, with a corresponding transition (or gradient) between these extremes; the data showed that C. auramale, A. officinarum, and S. marianum exhibited high antileishmanial activity (Figure 11). A. officinarum, R. damascena and S. marianum exhibited the most potent cytotoxicity against Hep G2 and A549 cell lines. R. damascena, A. officinarum, and H. lupulus showed potent DPPH radical scavenging activity. Hierarchical clustering analysis (HCA) was carried out for columns using Euclidean distance and can be used to identify clusters of rows with similar values, as these are displayed as areas of similar color. The data showed that there is a high similarity in values between T. tomentosa and C. morifolium and also between V. castus and A. folium and a moderate similarity between A. officinarum and S. marianum. A low similarity was also observed between M. chamomilla and H. lupulus. Taken together, these results demonstrate that the medicinal plants are a good source of new antileishmanial, antioxidant and anticancer drugs. PCA and heat map analysis obtained the initial solution reducing the original dataset.

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