Chemical composition, antimicrobial and antileishmanial activity of essential oil of *Juniperus excelsa* M.Bieb. from Ziarat, Balochistan

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Abstract
Essential oils isolated from leaves, barriers and from their mixture of Juniper (*Juniperus excelsa* M.Bieb) growing in Ziarat district. Essential oils were obtained by Clevenger-type apparatus, and components identified by gas chromatography mass spectroscopy (GC-MS). A total 27 components were identified which were inconsistent in their composition in the essential oils of *J.excelsa*, where five major variable components were: limonene (15.5-28.59%), followed by α-pinene (5.01-14.04%), cedrol (4.68-14.5%), δ-elemen (6.9-13.01%) and δ-cadinene (5.2-12.06%). Essential oils were evaluated for their in vitro antimicrobial activity against five bacterial species by disk diffusion method. *J. excelsa* essential oils showed antibacterial activity against tested organisms whilst the highest inhibition zone was observed for *E. coli*. Antileishmanial activity of essential oils isolated from juniper leaves, barriers and their mixture were also determined by using different concentration from (0.01ng/ml to 100 μg/ml). The IC₅₀ value of all three essential oils were 0.0065 μg/ml, 0.0088 μg/ml and 0.0093 μg/ml respectively and demonstrated almost 100 times more antileishmanial activity in comparison to the standard Amphotericin B.

Keywords: *Juniperus excelsa*; Antimicrobial activity; Antileishmanial activity; Essential oil composition

Introduction
*Juniperus* forests along with certain plants and animals forms an ecosystem found in the Balochistan province southwest of Pakistan, which is unique in its qualities due to existence in dry and unfavorable weather...
conditions. *Juniperus* forests are not only scarce genetic asset but also a strong heritage of the province as considered in the most ancient species of trees due to which they are often named as “Living Forest Fossil” [1]. The elevation where *Juniperus* forests are abundant ranges from 2000-3350 meters. Their geographic location extends between latitude 30 degrees 18 N – 30 degrees 30 N and longitude ranging from 67 degrees 54 E – 67 degrees 67 E [2]. Koh I Khelifat 3475m is the highest peak falling under Ziarat district. Physical composition of the area includes asymmetrical hard ridges, upright terrain in addition to small valleys usually commencing from the east and ending towards west. Dry temperate climate is usually experienced in Ziarat district [3]. Few herbariums have classified *Juniperus* into 54 different species that are scattered all over the world which includes South Asia, West Asia, Central Asia, North Africa, Europe and North America [4]. Six species out of above mentioned 54 species including *J. excelsa* exists in Pakistan, a major portion of which lies in the northern areas of Pakistan and Balochistan province. Open canopy is one of the attributes of juniper forests. Their regeneration is usually poor and their growth rate is considered to be slowest as compared to others. They are spread out in an area of 141,000 hectares. The large area covering 100,000 hectares are spotted in Ziarat district and Zarghun area adjacent to the provincial capital Quetta [5]. Herboi hills located in Kalat district also offer a fair sum of area of junipers. Juniper forests in addition to the mountains cover almost 35,325 hectares of area of the whole Ziarat district which constitutes 54% of the total area of the district [5]. Personnel working with the field of forestry find these forests of great significance to their study which includes the age, sex, size and growth rates of different species.

Male and ovulate strobili are born at ends of short twigs or along the twigs, mostly on different trees (dioecious) or in a few species on the same tree (monoecious). Male flowers are visible in January while female flowers appear in the mid of March. Depending upon climatic conditions, the male flowers become mature up to April. In the meantime the female flowers also become ready to receive the pollen, which are very rapidly shed by the male flowers. Abundant pollen is produced and can be seen as yellowish powder in the air. Female flowers are greenish in colour and are found at the tips of new branches like the male flowers. The formation of small greenish globose berries takes place soon after pollination and can be observed in the month of May, very minute in size, growing progressively and become fully mature in November-December next year [1, 6, 7]. The *Juniperus* species are characterized by large amount of essential oil in berries and needles as well as in wood and seed. In the last decade the composition of the berries essential oils of *J. excelsa* was investigated and some data were published recently. The berries essential oil of subsp. *excelsa* is characterized by presence of very high amounts of α-pinene, followed by cedrol, L-verbenol and D-verbenol and/or as predominant components: 1,4-cineole and limonene, myrcene, limonene, caryophyllene, δ-elemene and cedrol, or cedrol, myrcene and limonene [8]. In addition to genetic makeup the oil composition of Junipers has also been reported to change due to geographic variation, age of plant, season of harvesting, and interspecies differences. Juniper berry oil is known to be a pain reliever and has been reported to cure tuberculosis, jaundice, and eczema among so many other ailments [9]. Juniper oil is of utmost importance to the pharmacists as the oil is used in different
medicines for treating different diseases relating to stomach, joints and muscles [10]. They are sometimes used as antiseptics as well. Juniper oil extracts its source from juniper berries botanically known as *Baccae Juniperi*. The same oil is also extracted from *J. communis* but the proportion is low as compared to the former [11, 13]. Majority portion up to 60% constitutes monoterpenes which are basically of utmost importance to the biologists.

Juniper is known for having insect repellent, disinfectant and antifungal properties, which made its use traditionally to use for the treatment of respiratory, gastrointestinal diseases, jaundice and tuberculosis [14, 15]. It is reported that due to volatile chemical components *J. excelsa* contain considerable antibacterial activity [16, 17]. In Iran, Lebanon and Oman *J. excelsa* also used traditionally to treat asthma, blood pressure, common cold, bronchitis, tuberculosis and jaundice [15, 18-26]. Different juniper species are also reportedly used in traditional medicine as well as for the treatment of bronchitis, hyperglycemia, intestinal worms, liver disease, pneumonia, tuberculosis, ulcers and wound healing [17].

Leishmaniasis is amongst the major abandoned diseases. According to the World Health Organization data, which affects 350 million people worldwide and Pakistan is also among the endemic regions. Some studies have already conducted on antileishmanial activity of leaf, fruit and their organic solvents fraction of leaf of *J. excelsa* on *L. major*. Crude methanolic extract in addition to its portions chloroform fraction and di-ethyl ether fraction of *J. excelsa* berries were evaluated biologically and significant antileishmanial activity was depicted by chloroform fraction [20]. The antileishmanial activity of leaf and fruit and their organic solvents extracts were studied and the extracts of leaf showed significant activity on *in vitro* and *in vivo* model [27].

Leaves extract and its solvent fraction showed maximum antileishmanial activity as compared to fruits and its fraction of Greek juniper [28]. Essential oil of *Juniperus* species was also studied for its antileishmanial activity and the result was satisfactory [29].

The aims of the study were to identify compounds present in essential oils from leaves, berries and their mixture of *Juniperus excelsa* M.Bieb., to assess their antimicrobial activity and to evaluate their antileishmanial activity on *Leishmania major*.

**Materials and methods**

**Collection of plant materials**

Leaves and barriers from several trees of *J. excelsa* were collected from Ziarat, Balochistan and air dried at room temperature in the shade for few weeks.

**Extraction of essential oil**

Plants dried parts were crushed and each specimen (100g) leaves, Barriers and their mixture was subjected to hydro distillation for four hours in a Clevenger-type apparatus. The obtained oils were dried and stored separately at 4 °C for further analysis. The extraction of essential oil was carried out in triplicate.

**Gas chromatography-mass spectrometry (GC-MS) analyses**

GC-MS analyses were carried out with a Varian 3400 GC-MS framework outfitted with a DB-5 combined silica section (30 m × 0.25 mm, film thickness 0.25 mm, J&W Scientific Corp.). The oven temperature was set at 50 to 260°C at a rate of 4°C/min. Exchange line temperature was 270°C, Helium was utilized as the transporter gas with a straight speed of 31.5 cm/sec., split proportion was 1:60, vitality of ionization was 70 eV, check time was 1 sec., and mass extent was from 40 to 300 amu (Resolution) recognizable proof of aggravates. Constituents were distinguished by examination of their mass spectra with those
in a PC library (LIBR-TR and Wiley-5 lib.) or by means of real mixes. Distinguishing
proof was affirmed by correlation of their maintenance files either with those of
credible mixes or with information in the writing [30].

Antimicrobial screening of the essential oils
The antimicrobial activity of each extracted essential oil was assessed against five
different bacterial species by disk diffusion method. The obtained microorganisms were
from microbial culture collection unit of Microbiology Department, PCSIR, Lahore.
The microorganisms used in this study were Staphylococcus aureus, Thiobacillus
ferrooxidans (TBF), Leptospirillum ferrooxidans (LSF), Thiobacillus thiooxidans and Escherichia coli.

Disc diffusion method
Paper discs of 6 mm in diameter were
impregnated with 30 µl of essential oil
dissolved in dimethyl sulphoxide (DMSO)
at a final concentration of 5, 10, and 20% v/w and transferred onto the Mueller-Hinton
agar (Oxide UK) plates, the surface was
spread with 0.5 ml of bacterial suspension
adjusted to 3 × 10^8 CFU/ml (1 Mac-
Farland’s standard). For negative control
DMSO was used. Antibiotics Tetracycline
(30 µg/disk) and Gentamicin (30 µg/disk) of
commercial standard were used as positive
controls. The diameter of inhibition zone
was measured in millimeters (mm) after
incubation at 37 ± 1°C for 24 hrs. Tests
were carried out in triplicate. Sensitivity of
the bacterial species to the essential oils was
determined comparing the sizes of inhibitory
zones [31].

Antileishmanial activity
The antileishmanial screening of the essential oil was performed in flat-bottomed
plastic 96-well tissue plates. An amount 130 µl of 199 medium was added in wells of 96
well micro-titer plates (SPL Company). 70 µl of J. excelsa essential oil was added in
the first well and then serially diluted. 70µl
was discarded from the last well to keep the
final volume 130µl. 100µl parasite culture
was added in each well and 2 rows were left
for positive and negative control. DMSO
was taken as negative control and
successively diluted in the 199 medium.
Glucantime was taken as positive control
and was also serially diluted in 199 medium.
Micro-titer plates were kept in incubator
(DNP-9022) at 24°C for 72 hours. Assay
was performed in triplicate. After 72 hours,
20µl was picked from each dilution and
placed on Neubauer counting chamber and
live parasites were counted under
microscope. IC50 values of J. excelsa
essential oil having anti-promastigotes
activity were calculated by Graph Pad
Prism® software.

Results and discussion
The yield of the obtained essential oils by
hydro distillation of leaves, berries and
mixture of J. excelsa was 1.15, 1.17 and
1.14% respectively. The individual color of
the essential oil was light yellowish
transparent liquids with characteristic
turpentine like odor. The GC/MS analysis of
the juniper essential oil is detailed in Table 1
which showed that there were 27
compounds present in three essential oils.
The components might be assigned to three
diverse classes: monoterpene, diterpene, and
sesquiterpene hydrocarbons which were
present in all samples, while an alcoholic
compound was also present. In J. excelsa
essential oils of leaves, berries and mixture
ten common constituents were identified, all
analyzed samples were rich in limonene
(15.5 - 28.59%), followed by α –Pinene
(5.01-14.04%), cedrol (4.68-14.5%), δ-
elemen (6.9-13.01%), δ –cadinene (5.2-
12.06%), 6-epi-α-cubebene (2.85-8.31%),
terpine-4-ol (4.71-5.91%), bornyl acetate
(2.79-3.7%), cembrene A (1.29-1.58%) and
cembrene C (0.36-0.53%). Two common
constituents identified in leaves and berries
essential oils were: β-pinene (10.15%, 8.46%) and isoaromadendrene epoxide (1.07%, 1.09%) respectively. Three common constituents identified in essential oils of berries and mixture were: cis-β-terpinyl acetate (7.607%, 15.14%), γ–terpineol (4.52%, 5.64%) and β–eudesmol (1.83%, 1.51%) respectively. In essential oils of leave & mixture only one common component β-elemen (3.6%) was identified. The dissimilarities between leaves, berries and mixture essential oils exist. In essential oil of leaves six components: 2-carene (2.07%), perillene (3.26%), caren-4-ol (2.81%), cymene-8-ol (2.67%), spathulenol (1.4%) and 1-heptatriacotanol (0.43%) were identified, which were absent in essential oils of berries and mixture. In essential oil of berries 13-epi manoyl oxide (0.28 %) was identified which was not present in essential oils of leaves and mixture. Three components cis-verbenol (1.97%), ɤ-muurolene (2.33%), α-cadinol (1.09%) were identified in essential oil of mixture and they were absent in essential oils of leaves and berries.

The composition of the essential oils of J. excelsa described in this study, numerous similarities and/or differences could be found. The overall composition of the essential oils is comparable to previously reported study by Khoury et al. [32]. Contents present in essential oil of J. excelsa leaves and by comparing with already published data the percentage of limonene in the present study is closed to a study that was conducted on J. excelsa growing wild in Greece which was (22.7%) [33]. While, in three different studies it has been reported that the predominant components identified in leaves essential oil from J. excelsa from Greece, Turkey and Iran contained α-pinene (22.5%, 29.7% and 32.34%) and cedrol (28.1%, 25.3% and 13.06%) respectively [34-36]. A study also reported that essential oil of J. excelsa leaves as a rich source of cedrol, a valuable component for perfumes [37]. Another study was conducted to find out if geographical variation can influence the presence of volatile components and a comparison was made among J. excelsa leaves essential oil samples from Bulgaria, Cyprus, Greece and Turkey, the component cedrol (11.3-35.8%) was found predominant in all oils [38]. The components α-pinene and cedrol and their percentage are higher as compare to the present study. A lot of other authors reported that in essential oil of J. excelsa berries α-pinene as a main component ranged from 34-47.64% [35, 36, 39-41] and up to 70.81% in the sample from south-east Macedonia [8]. The Serbian author reported that in berries essential oil of J. excelsa contained α-pinene (5.2%) [42], which is almost similar and terpinen-4-ol (1.15%) was a bit low to that of present study. In Iranian samples it was found that the amount of α-pinene, limonene and cedrol were (22.5%, 28% and 22.7%) respectively [33], by comparing the obtained components and their amount presented in Table 1, dissimilarities with respect to components percentage and their abundance were observed. Major and minor components present in Omani J. excelsa berries are almost same as reported in this study while their percentages are not same [43]. The components present in essential oil of mixture (leaves and berries) of J. excelsa the amount of the components limonene, cedrol and α-pinene followed a bit same pattern as that of essential oils of leaves and berries. Variation among the identified components in all three essential oils could be due to the interaction between different oil components that can be of four different kinds: antagonistic, synergistic, additive and/or indifferent. Antagonism means that the effects of one or both components are less when combined together than used separately. Synergism gives a better effect when two components are used together
than used individually. Additive interaction means that the effect is same when the two components are put together than apart. While indifferent means that there is no interaction at all [44]. As mentioned above when two components interact with each other, for example in the essential oil limonene was in leaves (15.5%), berries (25.35%) and in mixture (28.59%) respectively, according to the interaction between different essential oil components it is synergistic component because when leaves and barriers combined together for essential oil extraction the extracted mixture oil gives the better effect in term of percentage of a component as compare to essential oil of berries.

Table 1. Chemical composition (%) of the essential oils of *j. excelsa* from Ziarat, Balochistan

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Leaves</th>
<th>Berries</th>
<th>Leaves+ Berries (Mixture)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rt (%age)</td>
<td>Rt (%age)</td>
<td>Rt (%age)</td>
</tr>
<tr>
<td>Limonene</td>
<td>4.195 25.35</td>
<td>4.199 15.5</td>
<td>4.177 28.59</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>5.614 14.04</td>
<td>5.61 5.01</td>
<td>5.58-10.01</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>6.358 10.15</td>
<td>6.521 8.46</td>
<td>- -</td>
</tr>
<tr>
<td>2-Carene</td>
<td>7.193 2.07</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Perillen</td>
<td>7.388 3.26</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Caren-4-ol</td>
<td>8.337 2.81</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Terpine-4-ol</td>
<td>8.795 5.28</td>
<td>8.89 4.71</td>
<td>8.801 5.91</td>
</tr>
<tr>
<td>Cymene-8-ol</td>
<td>9.35 2.67</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Barnylacetate</td>
<td>10.094 3.7</td>
<td>10.15 2.84</td>
<td>10.117 2.79</td>
</tr>
<tr>
<td>β-Elenen</td>
<td>11.004 3.6</td>
<td>- -</td>
<td>11.05 3.13</td>
</tr>
<tr>
<td>δ-Cadinene</td>
<td>12.532 5.2</td>
<td>13.259 12.06</td>
<td>13.235 1.67</td>
</tr>
<tr>
<td>6-epi-Cubebene</td>
<td>12.961 2.85</td>
<td>12.675 8.31</td>
<td>12.566 3.77</td>
</tr>
<tr>
<td>10-epi-Elemol</td>
<td>13.602 2.52</td>
<td>- -</td>
<td>13.642 1.58</td>
</tr>
<tr>
<td>Cedrol</td>
<td>14.7 4.68</td>
<td>14.897 14.5</td>
<td>14.872 10.35</td>
</tr>
<tr>
<td>(-)-Spathulenol</td>
<td>15.942 1.4</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Iso aromadendrene epoxide</td>
<td>16.686 1.07</td>
<td>15.84 1.09</td>
<td>- -</td>
</tr>
<tr>
<td>Cembrene A</td>
<td>17.452 1.46</td>
<td>17.501 1.58</td>
<td>17.47 1.29</td>
</tr>
<tr>
<td>1-Heptatriacotanol</td>
<td>18.7 0.43</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Cembrene C</td>
<td>19.833 0.53</td>
<td>19.86 0.36</td>
<td>19.844 0.36</td>
</tr>
<tr>
<td>Cis-β-Terpinyl acetate</td>
<td>- -</td>
<td>6.498 7.607</td>
<td>6.569 15.14</td>
</tr>
<tr>
<td>Cis-Verbenol</td>
<td>- -</td>
<td>- -</td>
<td>8.423 1.97</td>
</tr>
<tr>
<td>β-Eudesmol</td>
<td>- -</td>
<td>16.736 1.83</td>
<td>16.646 1.51</td>
</tr>
<tr>
<td>γ-Terpineol</td>
<td>- -</td>
<td>7.203 4.52</td>
<td>7.25 5.64</td>
</tr>
<tr>
<td>x-Muurolene</td>
<td>- -</td>
<td>- -</td>
<td>12.297 2.33</td>
</tr>
<tr>
<td>Manoyl Oxide, epi-13</td>
<td>- -</td>
<td>18.733 0.28</td>
<td>- -</td>
</tr>
<tr>
<td>α-Cadinol</td>
<td>- -</td>
<td>- -</td>
<td>15.209 1.09</td>
</tr>
</tbody>
</table>

Rt: Retention time (min), – not detected

The result of the antibacterial activity of the *j. excelsa* essential oil by the disc diffusion method is given in the Table 2. The microorganisms tested were *Staphylococcus aureus*, *Thiobacillus ferrooxidans* (TBF), *Leptospirillum ferrooxidans* (LSF)
Thiobacillus Thiooxidans and Escherichia coli. All bacterial species tested are sensitive to the essential oils of J. excelsa leaves, berries and mixture with the inhibition zones ranging from 11.9 mm to 22.7 mm, 12.7 mm to 23.4 mm and 12.8 mm to 24.1 mm respectively. The lower values were found for the tested Gram-positive bacteria as compared to the tested Gram-negative bacteria. Omani authors reported that the essential oil of J. excelsa berries does not contain any antimicrobial activity against Staphylococcus aureus and Escherichia coli which is contradictory to the present study [43]. In another study the authors reported that J. excelsa essential showed less antimicrobial activity against the tested microorganisms [45]. It has been reported that the essential oils are usually believed to be active when they show minimum inhibitory concentration (MICs) of 128 mg/ml and/or below [46]. The essential oils isolated from leaves and twigs of J. excelsa growing wild in Lebanon showed antimicrobial activity against Staphylococcus aureus but Escherichia coli was resistant [32]. Therefore, the presented results of antimicrobial activity of J. excelsa essential oils similarities and dissimilarities are found in literature that could probably be due to the diverse percentage of chemical constituents present in essential oils.

Table 2. Antimicrobial activity of J. excelsa essential oils determined by diffusion method

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Leaves</th>
<th>Berries</th>
<th>Leaves + Berries (Mixture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>11.9 ± 04</td>
<td>12.7 ± 0.7</td>
<td>12.8 ± 0.5</td>
</tr>
<tr>
<td>Thiobacillus ferrooxidans (TBF)</td>
<td>19.8 ± 0.7</td>
<td>23.3 ± 12</td>
<td>24.1 ± 0.5</td>
</tr>
<tr>
<td>Leptospirillum ferrooxidans (LSF)</td>
<td>17.0 ± 0.9</td>
<td>14.9 ± 0.7</td>
<td>15.9 ± 0.7</td>
</tr>
<tr>
<td>Thiobacillus Thiooxidans</td>
<td>16.8 ± 0.5</td>
<td>17.2 ± 0.6</td>
<td>17.3 ± 0.8</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>22.7 ± 0.5</td>
<td>23.4 ± 0.7</td>
<td>23.2 ± 0.7</td>
</tr>
</tbody>
</table>

Table 3 displays IC50 values of J. excelsa essential oil studied for antileishmanial activity. The essential oil of leaves, berries and mixture were checked for their activity and possible mode of action against Leishmania major parasites at different concentrations, i.e., from (0.01ng/ml to 100 μg/ml). The IC50 of J. excelsa leaves essential oil was in the range of 0.0065μg/ml, while the IC50 value of berries was 0.0088μg/ml and the IC50 value of mixture was in the range of 0.0093μg/ml. The J. excelsa mixture essential oil was 10 times more active than the oil extracted from barriers and leaves (Table 3). Further, all the three essential oils (leaves, berries and mixture) were found more active than the standard drug Amphotericin B (0.34 μg/ml). A Juniperus species (J. oxycedrus) berries and leaves essential oil showed IC50 values of 51 and 127 μg/mL respectively [29], the concentrations were much higher to inhibit Leishmania growth than J. excelsa leaves, berries and mixture essential oils reported in present study. Antileishmanial activities of Greek J. excelsa leaf and fruit solvent extracts and their related fractions showed strong inhibitory effects against Leishmania major promastigotes [28]. The obtained result of antileishmanial activity presented in this study is comparable to the previously reported (PEGylated silver doped zinc oxide nanoparticles as novel Photosensitizers for photodynamic therapy against Leishmania [47].
Table 3. In vitro activity of essential oils of *J. excelsa* against *Leishmania major* (promastigote)

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC₅₀(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>0.0065</td>
</tr>
<tr>
<td>Berries</td>
<td>0.0088</td>
</tr>
<tr>
<td>Leaves + Berries (Mixture)</td>
<td>0.0093</td>
</tr>
<tr>
<td>Amphotericin B*</td>
<td>0.34</td>
</tr>
</tbody>
</table>

*Reference drug. IC₅₀ values express the concentration of the sample to inhibit cell growth or degradation by 50%.

Conclusions

Twenty-seven compounds were identified in *J. excelsa* essential oils from the leaves, berries and mixture. Most abundant components in three oils were: limonene, α-pinene, cedro, δ-elemen, δ-cadinene, 6-pi-α-cubebene and terpine-4-ol. All three obtained oils showed antimicrobial activity against five bacterial strains with the inhibition zones from 11.9 mm to 24.1 mm. The results revealed that antimicrobial activity of mixture was higher than those of the leaves and berries. Antileishmanial activity of *J. excelsa* essential oils at low concentrations as shown in the present study seems more advantageous if compared to other essential oils and/or photosensitizers tested against *Leishmania major* parasites. The IC₅₀ values described here showed that *J. excelsa* essential oils possess an interesting antileishmanial activity.

Further studies will be performed as all three essential oils from *J. excelsa* demonstrate antimicrobial and antileishmanial activities. Obtained essential oils contain a strong and lasting aroma which could possibly be used for a number of applications such as in cosmetics, agrochemicals, foods and medicines.

Authors’ contributions

Conceived and designed the experiments: H Kakar, A Sajjad, S Rizwan & K Mahmood, Performed the Experiments: H Kakar, I Hafeez & A Nada, Analyzed the Data: A Sajjad, K Mahmood & A Nada, Contributed reagents/ materials/ analysis tools: Z Mahmood, M Azam, A M Sarangzai & M Yasinzai, Wrote the paper: H Kakar & A Sajjad.

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