Communication

Biological activities of extracts from aerial parts of *Salvia pachyphylla*

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Abstract: The antioxidant, antimicrobial, antiproliferative, and enzyme inhibitory properties of five extracts from aerial parts of *Salvia pachyphylla* were examined to assess the prospective of this plant as a source of natural products with therapeutic potential. Those properties were analyzed performing a set of standard assays. The extract obtained with dichloromethane showed the most variety of components, as yielded promising results in all completed assays. Furthermore, the extract obtained with ethyl acetate exhibited that greatest antioxidant activity as well as the best xanthine oxidase inhibitory activity. Remarkably, both extracts obtained with n-hexane or dichloromethane revealed significant antimicrobial activity against the Gram-positive bacteria; also, they showed greater antiproliferative activity against three representative cell lines of the most common types of cancers in women worldwide, and against a cell line that exemplifies cancers that typically develop drug resistance. Despite that other extracts were less active, such as the methanolic or aqueous, their results are promising for the isolation and identification of novel bioactive molecules.

Keywords: *Salvia pachyphylla*, plant extracts, antioxidant, antimicrobial, antiproliferative, enzyme inhibitory.

1. Introduction

The genus *Salvia* belongs to a large family of flowering plants, Lamiaceae, which comprises about 252 genera and 7,200 species [1-2]. Several species of *Salvia* are cultivated for their aromatic features and serve as flavorings, food condiments, cosmetics and perfume additives, and folk medicines [3]. Considering the latter, scrutiny of their chemical constituents have revealed the presence of a vast assortment of active compounds, some of them with antibacterial [4-7], antiviral [8-9], antitumor [10-13], antioxidant [14-17], antidiabetic [18-19], and antiparasitic [20] properties.
Also, some species have served for the treatment of mental and nervous illness [21] as well as for gastrointestinal conditions [22-23]. Furthermore, phytochemical studies have led to the isolation of many types of diterpenoids, such as abietane, ictexane, labdane, neoclerodane, and phenalenone [24-26], triterpenes and sterols [27], along with anthocyanins, coumarins, polysaccharides, flavonoids, and phenolic acids [22].

Salvia pachyphylla (blue sage) is a perennial herbaceous plant distributed from the state of California (USA) to the peninsula of Baja California (Mexico) [28]. The traditional medicine of Native-American communities has taken advantage of the curative goods of blue sage and, currently, serves to treat flu symptoms, menstrual depression and hysteria [29]. Several abietane diterpenoids with pharmacological properties have been isolated from the aerial parts of S. pachyphylla [30]. Considering the therapeutic potential of this plant, our study was directed towards identifying specific biological activities existing in different extracts from the aerial parts of S. pachyphylla. This approach represents the initial stage of a major survey aimed to isolate and identify phytochemicals with pharmacological potential.

Despite the recent dominance of synthetic chemistry as the foremost method to generate new or improved therapeutic agents, the potential of plants as a natural source of novel drugs is prevalent [31]. Interestingly, the chemical diversity of natural products is complementary to the diversity found in synthetic libraries. However, natural products are sterically more complex and have a greater diversity because of their long evolutionary selection process [32].

Examples of successful medicines derived from natural products include antibiotics, enzyme inhibitors, immunosuppressive drugs, and antiparasitic agents [33]. The antitumor area is likely the greatest impact of drugs derived from plants, where vinblastine, vincristine, taxol, and camptothecin have improved the effectiveness of chemotherapy of some of the deadliest cancers.

Here, we report the antioxidant, antimicrobial, antiproliferative, and enzyme inhibition properties of five extracts (obtained with n-hexane, dichloromethane, ethyl acetate, methanol, and water) from the aerial parts of S. pachyphylla. These properties were examined performing a set of standard in vitro assays.

2. Results

2.1. Antioxidant Screening

The antioxidant activity was evaluated using the β-carotene-linoleic acid assay and the DPPH radical-scavenging capacity assay (Figure 1-2). In the β-carotene-linoleic test, the best activity was detected in the ethyl acetate extract (84%) and immediately followed by the dichloromethane extract (83%); indeed, all five extracts showed higher activity, as compared with the reference compound α-tocopherol (8%). On the other hand, in the DPPH system, the ethyl acetate extract remained at the top in this activity, revealing an EC₅₀ of 0.28 mg/mL. In addition, the extracts obtained with n-hexane or water showed similar values (0.41 and 0.51 mg/mL, respectively).

Remarkably, neither of the extracts exhibited a comparative value with quercetin, the reference compound (0.003 mg/mL).
2.2. Antimicrobial activity

The antimicrobial activity was examined by determining the minimum inhibitory concentrations (MIC) using five bacterial strains and three antibiotics as the reference (Table 1). Interestingly, the extracts obtained with n-hexane or dichloromethane showed significant activity against the Gram-positive *S. aureus* and *E. faecalis*, as well as for the Gram-negative *E. coli*. Furthermore, *E. coli* also exhibited considerable sensitivity to the ethyl acetate extract. Remarkably, the methanolic and the aqueous extracts were inactive against the all bacteria tested. Moreover, *K. pneumoniae* and *A. baumannii* were insensitive to all *S. pachyphylla* extracts examined.
101 Table 1. In vitro antimicrobial activity of extracts from aerial parts of S. pachyphylla.

<table>
<thead>
<tr>
<th>Extracts or Controls</th>
<th>S. aureus</th>
<th>ORSA*</th>
<th>E. faecalis</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
<th>A. baumannii</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>62.5</td>
<td>125</td>
<td>250</td>
<td>250</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>62.5</td>
<td>125</td>
<td>250</td>
<td>250</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Methanol</td>
<td>1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Water</td>
<td>1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Oxacillin*</td>
<td>0.48</td>
<td>125</td>
<td>31.2</td>
<td>0.487</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Cephalothin*</td>
<td>0.48</td>
<td>62.5</td>
<td>31.2</td>
<td>1</td>
<td>&gt;1000</td>
<td>62.5</td>
</tr>
<tr>
<td>Vancomycin*</td>
<td>0.48</td>
<td>1.95</td>
<td>1.95</td>
<td>&gt;250</td>
<td>&gt;1000</td>
<td>250</td>
</tr>
</tbody>
</table>

*Used as a reference compound. *Oxacillin-resistant S. aureus.

2.3. Xantine and Acetylcholinesterase inhibitory assay

The enzymatic evaluation results are shown on Table 2. In the acetylcholinesterase inhibition assay, the extracts did not show a remarkable activity, only the dichloromethane extract presented a slight activity with an IC₅₀ of 191.7 µg/mL, however such result is far away from the positive control galantamine (0.278 µg/mL). In the xantine oxidase inhibition assay better results were obtained, standing out the IC₅₀ values for the ethyl acetate and methanol extracts with 11.7 and 19.5 µg/mL, although they not surpassed the drug allopurinol used as control (0.842 µg/mL); the rest of the extracts did not show significant activity.

Table 2. Acetylcholinesterase (AChE) and Xanthine Oxidase (XO) inhibitory activity of the extracts from aerial parts of S. pachyphylla.

<table>
<thead>
<tr>
<th>Extracts or Controls</th>
<th>IC₅₀ (µg/mL)</th>
<th>AChE</th>
<th>XO</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>&gt;400</td>
<td>254.5 ± 31.7</td>
<td></td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>191.7 ± 13.1</td>
<td>86 ± 6.0</td>
<td></td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>314.3 ± 43.2</td>
<td>11.7 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>&gt;400</td>
<td>19.5 ± 0.47</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>&gt;400</td>
<td>61.8 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Galantamine*</td>
<td>0.278 ± 0.01</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Allopurinol*</td>
<td>ND</td>
<td>0.842 ± 0.078</td>
<td></td>
</tr>
</tbody>
</table>

*Used as a reference compound. Values are mean ± SD, n=3. ND, not determined.

2.4. Antiproliferative activity

The antiproliferative activity was obtained by measuring the concentration needed to decrease cell propagation by 50% (GI₅₀) using six human cancer cell lines and three well-known anti-cancer drugs (Table 3). All extracts exhibited a degree of effectiveness against all cell lines tested. Specifically, extracts obtained with dichloromethane or n-hexane were the most active against all the evaluated cell lines, showing GI₅₀ values between 5.4 and 11 µg/mL. Both extracts showed higher cytotoxicity against cell lines SW1573, T-47D and WiDr, with concentrations of 6.6, 11 and 8.6 µg/mL and 7.7, 9.9 and 9.9 µg/mL for n-hexane and dichloromethane extracts, respectively; in both cases the extracts surpassed the positive control etoposide (GI₅₀ of 15, 22 and 23 µg/mL against SW1573, T-47D and WiDr) and cisplatin (GI₅₀ of 15 and 26 µg/mL against T-47D and WiDr).
Table 3. Antiproliferative activity of extracts from the aerial parts of *S. pachyphylla*.

<table>
<thead>
<tr>
<th>Extracts or Controls</th>
<th>A2780</th>
<th>HBL-100</th>
<th>HeLa</th>
<th>SW1573</th>
<th>T-47D</th>
<th>WiDr</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>n</em>-hexane</td>
<td>6.0</td>
<td>5.9</td>
<td>6.1</td>
<td>6.6</td>
<td>11</td>
<td>8.6</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>5.4</td>
<td>6.7</td>
<td>8.3</td>
<td>7.7</td>
<td>9.9</td>
<td>9.9</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>6.5</td>
<td>18</td>
<td>40</td>
<td>15</td>
<td>38</td>
<td>53</td>
</tr>
<tr>
<td>Methanol</td>
<td>34</td>
<td>64</td>
<td>71</td>
<td>70</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Water</td>
<td>52</td>
<td>55</td>
<td>77</td>
<td>74</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Cisplatin*</td>
<td>ND</td>
<td>1.9</td>
<td>2.0</td>
<td>3.0</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td>Etoposide*</td>
<td>ND</td>
<td>2.3</td>
<td>3.0</td>
<td>15</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>Camptothecin*</td>
<td>ND</td>
<td>ND</td>
<td>0.6</td>
<td>0.25</td>
<td>2.0</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Used as a reference compound. ND, not determined. Human cancer cell lines: A2780, ovarian carcinoma; HBL-100, breast carcinoma; HeLa, cervix adenocarcinoma; SW1573, lung carcinoma; T-47D, breast ductal carcinoma; and WiDr, colorectal adenocarcinoma.

3. Discussion

Oxidative stress plays a key role in the development of several pathophysiological conditions, as neurodegenerative and cardiovascular diseases, cancer and diabetes; natural antioxidants ingested in the daily diet protect the cells against the damage produced by an excess of ROS (reactive oxygen species). Several studies suggest a good antioxidant potential from Salvia species around the world, mainly because the presence of diterpenes such as carnosol and rosmanol [34]. In the present work, ethyl acetate extract showed a better activity for the 2 evaluated techniques, being these results in accordance to the obtained by Senol *et al.* (2010) and Loizzo *et al.* (2010) [35-36], where the intermediate polarity extracts (ethyl acetate) from different Salvia species presented better results for the same antioxidant techniques of β-carotene and DPPH.

In the antimicrobial evaluation, *n*-hexane and dichloromethane extracts presented a better activity against Gram-positive bacteria *S. aureus* and *E. faecalis*. Our results are consistent with those described by Vlietinck *et al.* [37], which suggest that Gram-positive bacteria are significantly more susceptible to plant-derived extracts, this may be attributed to the fact that cell wall in Gram-positive bacteria consist of a single layer, while Gram-negative cell wall is a multilayered and quite complex structure [38]. Previous studies suggest that the antibacterial activity from Salvia extracts over Gram-negative bacteria such as *E. coli* depends on the nature of the studied extract [39].

The screening of natural products in the search of medically relevant enzyme inhibitors remains as a viable approach for isolation of novel compounds with specific pharmacological properties. Here, two activity assays were used to identify enzyme inhibitors, within each extract, with therapeutic potential (Table 2): xanthine oxidase (XO) and acetylcholinesterase (AChE) inhibition assays. Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and uric acid, but under certain conditions can generate superoxide. It has been proved that XO inhibitors can be helpful for the treatment of liver disease and gout [40]. Acetylcholinesterase catalyzes the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid [41]. Modulation of acetylcholine levels using acetylcholinesterase inhibitors is among the major strategies to address diverse neurodegenerative diseases [42]. Remarkably, extracts with a prospective inhibitory effect showed a concentration-dependent trend and IC₅₀ values were estimated, being the ethyl acetate extract the one which exhibited a significant effect over XO, while the dichloromethane extract showed a considerable effect on AChE. Unfortunately, other extracts showed a little or poor after-effect on either of the tested enzyme activities, hence considered as inactive.
Regarding the antiproliferative activity, it is noticeable the effects of the n-hexane and dichloromethane extracts over A2780 (ovarian carcinoma), HBL-100 (breast carcinoma), and HeLa (cervix adenocarcinoma), which represent three of the most common cancers in women worldwide. Remarkable results were also obtained against WiDr (colorectal adenocarcinoma), which exemplifies cell lines that typically show drug resistance [43]. Interestingly, these results are different from those previously reported by Cordova et al. [30]. They tested several pure compounds isolated from the aerial parts of two species of Salvia, being less effective than our extracts. We thought that the main difference resides in the nature of the sample, as our results were generated using total extracts, suggesting a possible synergistic effect. Despite that other extracts were less active, their results are promising for pursuing novel molecules with cytotoxic effect.

4. Materials and Methods

4.1. Plant material

The aerial parts (leaf, flower, and stem) were obtained from freshly harvested S. pachyphylla plants. All specimens were collected in lands of the Sierra Juarez- Constitution National Park, Ensenada, BC., México (Figure 3). A voucher specimen of S. pachyphylla (No. 9783) was deposited in the herbarium of the Autonomous University of Baja California, at Ensenada. Aerial parts (1.3 kg) were air-dried for a week, under shade (to reduce moisture content). The dried material was ground to fine powder and stored at 4 °C until use.

![Image](image.png)

Figure 3. Sierra Juarez- Constitution National Park (marked by red circle), Ensenada, B.C, México.

4.2. Preparation extracts

Crude extracts were obtained by performing the classical Soxhlet method. Five different thimbles were uniformly packed with 75 g of fine powder. The extraction was carried out using different solvents (250 mL): n-hexane (HX), dichloromethane (DC), ethyl acetate (EA), methanol (MT), and distilled water (AQ). The extraction process was stopped until the solvent in siphon tube
became colorless. Each extract was filtered and dried at 40 °C, using a rotary evaporator (Buchi Rotavapor® R-215), until a solid or semi-solid residue was yielded. All residues were further lyophilized to get a dry solid matter: HX = 60.3 g, DC = 7.9 g, EA = 24.2 g, MT = 9.9 g, and AQ = 14.5 g. All solids were kept in air tight bottles and stored at 4 °C until use.

4.3. β-Carotene-linoleic acid assay

The antioxidant activity was assayed by the coupled oxidation of β-carotene and linoleic acid as described by Burda and Oleszek [44] with minor modifications. 1 mL of a β-carotene solution (0.2 mg/mL in chloroform) was added to an emulsion containing 0.018 mL of linoleic acid and 0.2 mL of Tween-20. Chloroform was removed (under a nitrogen environment), 50 mL of aerated deionized water (DO of 9.7 mg/L) was slowly added, and the mixture was vigorously agitated to form a stable emulsion. 5 mL of this emulsion was transferred to test tubes containing the corresponding sample (2 mg) of each extract. Immediately, the absorbance was measured at 470 nm ($A_{470}$, zero time). All tubes were then incubated at 50 °C and $A_{470}$ values were registered every 15 min for 2 h. A control without the antioxidant was prepared aside and α-tocopherol was used as a reference compound. The antioxidant activity (AA) was expressed as percentage of inhibition of β-carotene bleaching, as compared to the control, and calculated using the following formula:

$$AA (\%) = [1 - (A_{50} - A_{120})/ (A_{c0} - A_{c120})] \times 100$$

Where $A_{5}$ and $A_{c}$ represent the $A_{470}$ value of the sample and control, respectively; while the superscript numbers denote the time of the initial and final measurement (0 and 120 min). All determinations were performed in duplicate and replicated at least three times.

4.4. DPPH radical-scavenging capacity assay

The radical-scavenging activity was performed as described by Burda and Oleszek [44] with slight modifications. For the evaluation of each extract, a stock solution (4 mg/mL) was prepared and serially two-fold diluted (down to 0.003 mg/mL) with methanol. An aliquot of each dilution (1 mL) was mixed with 1 mL of a methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH at 0.03 mg/mL). At the same time, a control containing 1 mL of methanol and 1 mL of the DPPH solution was prepared. The mixtures were incubated at room temperature in the dark for 5 min. Using methanol as a blank, the absorbance was quantified at 517 nm ($A_{517}$). The radical-scavenging activity was calculated as percentage of DPPH decoloration using the following formula:

$$DPPH (\%) = [1 - (A/B)] \times 100$$

Where $A$ and $B$ represent the $A_{517}$ value of the control and sample, respectively. All determinations were performed in duplicate and replicated at least three times. For each extract, the percentage of DPPH decoloration was plotted against the concentration of each dilution. The concentration required to decrease the absorbance of DPPH by 50% was obtained by interpolation, from a linear regression analysis, and expresses the EC$_{50}$ value. Quercetin was used as a reference compound.

4.5. Antimicrobial assay

Antibacterial activity was tested using a microdilution assay following the National Committee for Clinical Laboratory Standards (NCCLS) [45-46] and minimal inhibitory concentration (MIC) was defined as the lowest concentration that prevents visible growth of bacteria. Acinetobacter baumannii, Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, and Staphylococcus aureus strains were provided by the Regional Center for Infection Diseases, School of Medicine, Autonomous University of Nuevo Leon (Monterrey, Mexico). All strains were plated on Müeller–Hinton agar (Becton Dickinson) and incubated at 37°C for 24 hours. Four or five colony
forming units were suspended in saline solution and the optical density was adjusted to the
turbidity of the 0.5 McFarland Standard. Working suspensions were prepared by a 1:50 dilution in
Müeller–Hinton broth. For the evaluation of each extract, a stock solution (6 mg/mL in 5% DMSO)
was prepared and serially two-fold diluted, with Müeller–Hinton broth, in a 96-well microtiter
plate (down to 0.5 μg/mL). One volume (0.1 mL) of working suspension was added to each well.
The antibiotics cephalothin, oxacillin, and vancomycin were used as reference compounds. Controls
without bacterial cells (medium control) and without extract or antibiotic (growth control) were
prepared aside. Plates were incubated at 37 °C for 48 h and growth was visually examined.

4.6. Xanthine oxidase (XO) inhibition assay

Inhibition of the XO activity was evaluated using the protocol described by Havlik et al. [47]. A
reaction solution containing 0.4 mL of 120 mM phosphate buffer (pH 7.8) and 0.33 mL of 150 mM of
xanthine was supplemented with 0.25 mL of inhibitor solution (extract or reference) and mixed
thoroughly. The reaction was started by adding 0.02 mL of XO enzyme solution (0.5 U/mL). After
3 min of incubation at 24 °C, the uric acid formation was determined by measuring the absorbance
at 295 nm (A_{295}). A reaction without inhibitor was used as control and allopurinol served as a
reference compound. The inhibition percentage of XO activity was calculated using the following
formula:

\[
\text{XO inhibition (\%)} = \left[ 1 - \left( \frac{A_{SCS}}{A_{CCc}} \right) \right] \times 100
\]

Where \( A_S \) and \( A_C \) represent the initial velocity of reactions with sample and control,
respectively. All determinations were performed in duplicate and replicated at least three times.
The concentration required to decrease the activity of XO by 50% was obtained by interpolation,
from a sigmoidal regression analysis, and expresses the IC_{50} value.

4.7. Acetylcholinesterase (AChE) inhibition assay

Inhibition of the AChE activity was estimated using the method described by Adewusi et al. [48]
[with a slight modification. For each determination, wells of a microtiter plate were filled with
25 μL of 15 mM acetylthiocholine iodide (in water), 125 μL of 3 mM DTNB in buffer C (50 mM
Tris-HCl, pH 8.0, containing 0.1 M NaCl and 0.02 M MgCl_2·6H_2O), 72.5 μL of buffer B (50 mM
Tris-HCl, pH 8.0, containing 0.1 % BSA) and 2.5 μL of inhibitor solution (extract or reference, in
DMSO) and mixed thoroughly. Absorbance was measured at 412 nm (A_{412}) every 45 s, three times
consecutively. Thereafter, 25 μL of AChE enzyme solution (0.2 U/ml) was added to each well and
A_{412} was measured five times consecutively every 45 s. A reaction without inhibitor was used as
control and galantamine served as the reference compound. Any increase in absorbance due to the
spontaneous hydrolysis of the substrate was corrected by subtracting the A_{412} before adding the
enzyme. The inhibition percentage of AChE activity was calculated using the following formula:

\[
\text{AChE inhibition (\%)} = \left[ 1 - \left( \frac{A_{SCS}}{A_{CCc}} \right) \right] \times 100
\]

Where \( A_S \) and \( A_C \) represent the initial velocity of reactions with sample and control,
respectively. All determinations were performed in duplicate and replicated at least three times.
The concentration required to decrease the activity of AChE by 50% was obtained by interpolation,
from a sigmoidal regression analysis, and expresses the IC_{50} value.

4.8. Cell lines and culture conditions

Five human cancer cell lines were used in this study: A2780 (ovarian carcinoma), HBL-100
(breast carcinoma), HeLa (cervix adenocarcinoma), SW1573 (lung carcinoma), T-47D (breast ductal
carcinoma), and WiDr (colorectal adenocarcinoma). All line cells were maintained in RPMI 1640
media supplemented with 5% heat-inactivated FCS and 2 mM L-glutamine at 37 °C, 5% CO2, and
95% humidity. Exponentially growing cells were trypsinized and resuspended in medium
containing 2% FCS and antibiotics (100 U/mL of penicillin G and 0.1 mg/mL of streptomycin). Single cell suspensions showing >97% viability, by trypan blue dye exclusion assay, were subsequently counted. After counting, dilutions were made to give the appropriate cell densities required for antiproliferative testing.

4.9. Antiproliferative assay

Antiproliferative testing was performed using the Sulforhodamine B (SRB) assay of the National Cancer Institute (NCI, NIH, USA) as reported by Miranda et al. [43] with slight modifications. Each extract was initially dissolved in DMSO at 400 times the desired maximum concentration to test. Six thousand cells were inoculated to each well of a microtiter plate (100 μL of a suspension of 6 x 10⁴ cells per mL). One day after plating, all testing samples and reference compounds were added to corresponding wells (triplicated). Control cultures were tested against equivalent concentrations of DMSO (0.25% as a negative control). After 48 h of incubation at permissive conditions, cell cultures were treated with 25 μL ice-cold 50% TCA and fixed at 4 ºC for 60 min. Thereafter, the SRB assay was performed. The absorbance of each well was measured at 492 nm (A₄₉₂). All absorbance values were corrected for background A₄₉₂ (control wells containing just culture media). As recommended by the NCI, the concentration that causes 50% growth inhibition, GI₅₀ value, was corrected by count at time zero; thus, GI₅₀ is the concentration where [(T-T₀)/(C-T₀)] = 0.5. The absorbance of the test well after 48 h is T, the absorbance at time zero is T₀, and the absorbance of the control is C.

5. Conclusions

In conclusion, aerial parts of S. pachyphylla have prospective potential as a source of natural products, which could act as antioxidant, antimicrobial, antiproliferative or enzyme inhibitors. Although our findings could be the outcome of a synergistic effect, they support the notion of aiming our next approach towards the isolation and identification of novel molecules with therapeutic potential.


Funding: This research received no external funding.

Acknowledgments: The authors would like to thank the Facultad de Ciencias Químicas e Ingeniería, Universidad Autónoma de Baja California, for the support given and for allowing the use of their facilities during this investigation. Additionally, to CONACYT, who supported G.A.-T. with a scholarship during her PhD studies during the realization of this investigation.

Conflicts of Interest: The authors declare no conflict of interest.

References


