IN VITRO BINDING STUDIES OF METHANOLIC EXTRACTS FROM DIFFERENT SALVIA SPECIES

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Lamiaceae are generally known for their multiple pharmacological effects including analgesic, anti-inflammatory, antioxidant, antimicrobial, antitumoral and central nervous system (CNS) depressant activities. The genus *Salvia* from the Lamiaceae family has numerous different species, *Salvia sclarea* L. is largely used in traditional medicine as antiseptic, for digestion disorders, in kidney disease. Furthermore, it has been reported that some compounds from *Salvia* ssp (specially *Salvia divinorum* Epling & Jativa) are able to induce allucinogenic activity.

To investigate the mechanism of action on CNS and in order to verify if pharmacological activity depends on species, we have studied five different species of *Salvia*. Therefore we have evaluated the affinity for the serotoninergic (5HT1A, 5HT2A and 5HT2C), noradrenergic (α1 and α2) and dopaminergic (D1 and D2) receptors of methanolic extracts of *Salvia sclarea* L. roots, *Salvia dominica* L. leaves, *Salvia dominica* L. flowers, *Salvia spinosa* L. aerial parts, *Salvia palaestina* Benth. aerial parts and *Salvia menthaefolia* Tenore roots.

Interesting results have been shown by *S. sclarea* extract with elevated affinity for the 5-HT2A receptors (IC50 value = 42.49 ± 0.591 µg/ml) and moderate affinity for the D2 receptors (46% as level of inhibition at the maximum concentration tested, 125 µg/ml). In addition *S. palaestina* extract showed high affinity for D1 and D2 receptors with IC50 values of 68.70 ± 2.421 µg/ml and 30.14 ± 3.643 µg/ml respectively, while *S. menthaefolia* extract displayed moderate affinity only for the 5-HT2A receptors with a level of inhibition of 48.3% at the maximum concentration tested (125 µg/ml). All remaining extracts showed low or no affinity for the examined receptors.

Our data disclosed the interactions with dopaminergic and serotoninergic receptors of methanolic extracts of *S. sclarea* and *S. palaestina* indicating some CNS effect. The divergences of results showed by this study of course underlined the differences among *Salvia* species tested.

**Key words:** *Salvia* species; central nervous system; receptor binding assay
Introduction

There is a recent increasing interest in biologically active compounds extracted from natural sources, due to their low or absent toxicity, complete biodegradability, availability from renewable sources, and, in most cases, to the low cost of production compared to pharmaceutical compounds obtained by total chemical synthesis. Many of the natural products in plants of medicinal value offer new sources of drugs, which have been used effectively for centuries in traditional medicine. Moreover, there are numerous chemically synthesized compounds used in medicine today, originally identified in plants (1-4).

The genus *Salvia* from the Lamiaceae family has numerous different species and from a phytochemical point of view, plants belonging to genus *Salvia* are of particular interest, due to the large diversity of secondary metabolites produced in these plants, such as flavonoids (5), monoterpenoids (6), triterpens (7) and several diterpens with abietane and clerodane skeleton (8-10).

Among *Salvia* species, *Salvia sclarea* L. is largely used in traditional medicine as antiseptic, for digestion disorders, in kidney disease and is the most well characterized species, mainly because of the great commercial value of the diterpen scareol, extracted from the leaves, which is precursor of the Ambrox, a compound largely used in the scent and tobacco industries (11).

Many diterpens, isolated from plants of several species of the genus *Salvia*, have been investigated for their pharmacological activities: analgesic, anti-inflammatory, hemostatic (12), antioxidant (13), antimicrobial (14) and as an antitumoral remedy (15). Some diterpens have been used efficiently against the treatment of coronary heart diseases, as angina pectoris and myocardial infarction (16,17) and recently it has been reported that some compounds from *Salvia* ssp (specially *Salvia divinorum* Epling & Jativa) are able to influence the central nervous system (CNS) inducing allucinogenic effects (18).

To investigate the mechanism of action on CNS and in order to verify if pharmacological activity depends on species, we have studied five different species of *Salvia* evaluating the affinity for the serotoninergic (5HT₁A, 5HT₂A and 5HT₂C), noradrenergic (α₁ and α₂) and dopaminergic (D₁ and D₂) receptors of methanolic extracts of *Salvia sclarea* L. roots, *Salvia dominica* L. leaves, *Salvia dominica* L. flowers, *Salvia spinosa* L. aerial parts, *Salvia palestina* Benth. aerial parts and *Salvia menthaefolia* Ten. roots.
Materials and methods

Plant materials
Leaves and flowers of *Salvia dominica* L. were collected in As-Salt (Jordan) in April 2003, aerial parts of *Salvia palaestina* Benth. were collected in Amman (Jordan) and aerial parts of *Salvia spinosa* L. were collected in Al-Hashemiyyeh (Jordan), both in April 2003. All these Jordanian *Salvia* species were identified by Dr. Ammar Bader, Al-Zaytoonah Private University of Jordan and their voucher specimens are deposited in the Herbarium of Laboratory of Pharmacognosy and Phytochemistry at Al-Zaytoonah Private University of Jordan.

Roots of *Salvia menthaefolia* Ten. were collected in Botanical garden of Palermo, in April 2002, identified by Prof. G. Venturella, University of Palermo and a voucher specimen is deposited at the Herbarium of the Botanical garden of Palermo.

Roots of *Salvia sclarea* L. were supplied by INDENA spa.

Extraction and Isolation
The powdered, dried parts of each *Salvia* species (50 g) were pretreated with n-exane and successively extracted with methanol (500 ml for 3 times). The extractive solutions were filtered and concentrated *in vacuum*, obtaining dried methanolic extracts.

The extracts were dissolved in dimethylsulphoxide (DMSO) 5% at initial concentrations of 1.25 mg/ml and subsequently diluted with appropriate buffer. These dilutions were tested in triplicate to final tube concentrations between 7.8 and 125 µg/ml.

Receptor binding experiments
The biological materials for binding assay (cerebral cortex, corpora striata) were taken from male Wistar rats. Experimental conditions for competition binding studies with seven different receptor preparations are reported in table 1 (19-24). Reactions were terminated by filtering the incubates through glass fiber filters (Whatman GF/B) which were rinsed twice with 5 ml aliquots of respective ice-cold buffer. The filters were added to 5 ml of liquid scintillation, and the radioactivity bound to the filters was measured by liquid scintillation counter. Specific binding is defined as the difference between binding in the absence or presence of $10^{-5}$ M cold ligand.

We had effected a control test to verify the effect of the solvent (DMSO 5%) on binding assay. There were not important variations.
Table 1- Methodological details for binding experiments

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Biological material</th>
<th>Tritiated ligand</th>
<th>Cold ligand</th>
<th>pH</th>
<th>Time (min)</th>
<th>Temp (°C)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT1A</td>
<td>Cerebral cortex</td>
<td>[3H]8-OH-DPTA</td>
<td>serotonin</td>
<td>7.7</td>
<td>30</td>
<td>37</td>
<td>19</td>
</tr>
<tr>
<td>5-HT2A</td>
<td>Cerebral cortex</td>
<td>[3H]ketanserin</td>
<td>cinanserin</td>
<td>7.4</td>
<td>30</td>
<td>37</td>
<td>20</td>
</tr>
<tr>
<td>5-HT2C</td>
<td>Cerebral cortex</td>
<td>[3H]mesulergine</td>
<td>mianserin</td>
<td>7.4</td>
<td>30</td>
<td>37</td>
<td>20</td>
</tr>
<tr>
<td>D1</td>
<td>Corpora striata</td>
<td>[3H]SCH-23390</td>
<td>(+)-butaclamol</td>
<td>7.1</td>
<td>15</td>
<td>37</td>
<td>21</td>
</tr>
<tr>
<td>D2</td>
<td>Corpora striata</td>
<td>[3H]spiperidol</td>
<td>(+)-butaclamol</td>
<td>7.1</td>
<td>15</td>
<td>37</td>
<td>22</td>
</tr>
<tr>
<td>α1</td>
<td>Cerebral cortex</td>
<td>[3H]prazosin</td>
<td>phentolamine</td>
<td>7.4</td>
<td>30</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>α2</td>
<td>Cerebral cortex</td>
<td>[3H]yohimbine</td>
<td>phentolamine</td>
<td>7.5</td>
<td>30</td>
<td>25</td>
<td>24</td>
</tr>
</tbody>
</table>
Results and Discussion

The affinity of *Salvia* spp methanolic extracts for the serotonergic (5HT\textsubscript{1A}, 5HT\textsubscript{2A} and 5HT\textsubscript{2C}), noradrenergic (\(\alpha_1\) and \(\alpha_2\)) and dopaminergic (D\textsubscript{1} and D\textsubscript{2}) receptors has been evaluated. The extracts affinity for receptor is definite as inhibition percentage (I\%) of radioligand/receptor binding and measured as the radioactivity of remaining complex radioligand/receptor.

The results obtained from *Salvia* spp. methanolic extracts for all receptors tested are reported in table 2 as the maximum effect observed (MEO) at the higher concentration tested (125 \(\mu\)g/ml).

The IC\textsubscript{50} values of extracts (concentration required to inhibit 50\% of radioligand specific binding) were obtained only in some cases. These values and IC\textsubscript{50} values of known compounds (positive control) are listed in table 3.

Interesting results (table 2-3) have been shown by *S. sclarea* extract with elevated affinity for the 5-HT\textsubscript{2A} receptors (IC\textsubscript{50} value of 42.49 ± 0.59 \(\mu\)g/ml) and fairly good affinity for the D\textsubscript{2} receptors (MEO 46.09 \%).

In addition *S. palaestina* extract showed high affinity for D\textsubscript{1} and D\textsubscript{2} receptors with IC\textsubscript{50} values of 68.70 ± 4.42 \(\mu\)g/ml and 30.14 ± 3.64 \(\mu\)g/ml respectively, while *S. menthaefolia* extract displayed moderate affinity only for the D\textsubscript{1} receptors with a level of inhibition of 48.32 \% at the maximum concentration tested (125 \(\mu\)g/ml).

All remaining extracts showed low or no affinity for the examined receptors (table 2).

Surely the differences of results showed by this study depend on *Salvia* species tested, but probably, they are also bound to the different plant parts used and, consequently, to their different composition, the other hand these parts of *Salvia* species were verified more actives in a previous study (25).

Our data disclosed the interactions with dopaminergic and serotonergic receptors of methanolic extracts of *S. sclarea* and *S. palaestina* indicating some CNS effects as other species belonging to *Salvia* genus. Establishing the mechanism of action and the receptors involved is important to understand in future the use of extracts and the role in same pathologies where are implicated the receptors of dopamine and serotonin.
Table 2 - Affinity of *Salvia* spp methanolic extracts expressed as the maximum effect observed (MEO) at the maximum concentration tested (125 µg/ml).

<table>
<thead>
<tr>
<th>Plant Extract</th>
<th>5HT&lt;sub&gt;1A&lt;/sub&gt;</th>
<th>5HT&lt;sub&gt;2A&lt;/sub&gt;</th>
<th>5HT&lt;sub&gt;2C&lt;/sub&gt;</th>
<th>D&lt;sub&gt;1&lt;/sub&gt;</th>
<th>D&lt;sub&gt;2&lt;/sub&gt;</th>
<th>α&lt;sub&gt;1&lt;/sub&gt;</th>
<th>α&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salvia sclarea</em> roots</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
<td></td>
<td>46.76%</td>
<td>inactive</td>
<td>inactive</td>
</tr>
<tr>
<td><em>Salvia menthaefolia</em> roots</td>
<td>inactive</td>
<td>18.04%</td>
<td>inactive</td>
<td>48.32%</td>
<td>18.82%</td>
<td>inactive</td>
<td>25.28%</td>
</tr>
<tr>
<td><em>Salvia dominica</em> leaves</td>
<td>inactive</td>
<td>31.02%</td>
<td>inactive</td>
<td></td>
<td>29.86%</td>
<td>inactive</td>
<td>28.26%</td>
</tr>
<tr>
<td><em>Salvia dominica</em> flowers</td>
<td>inactive</td>
<td>25.06%</td>
<td>29.84%</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
</tr>
<tr>
<td><em>Salvia spinosa</em> aerial parts</td>
<td>inactive</td>
<td>inactive</td>
<td>34.11%</td>
<td>inactive</td>
<td>39.38%</td>
<td>inactive</td>
<td>inactive</td>
</tr>
<tr>
<td><em>Salvia palaestina</em> aerial parts</td>
<td>inactive</td>
<td>17.54%</td>
<td>29.67%</td>
<td>84.22%</td>
<td>93.45%</td>
<td>inactive</td>
<td>inactive</td>
</tr>
</tbody>
</table>
### Table 3 - Concentration (µg/ml as mean ± standard deviation) required to inhibit 50% of radioligand binding (IC₅₀).

<table>
<thead>
<tr>
<th>Extract/Compound</th>
<th>5HT₁A</th>
<th>5HT₂A</th>
<th>5HT₂C</th>
<th>D₁</th>
<th>D₂</th>
<th>α₁</th>
<th>α₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salvia sclarea roots</td>
<td>-</td>
<td>42.49± 0.59</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salvia palaestina aerial parts</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>68.70±4.42</td>
<td>30.14±3.64</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>0.70±0.089 (x 10⁻³)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>-</td>
<td>0.93±0.036 (x 10⁻³)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cinanserin</td>
<td>-</td>
<td>0.54±0.096 (x 10⁻³)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mesulergine</td>
<td>-</td>
<td>-</td>
<td>0.48±0.029 (x 10⁻³)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spiperidol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.55±0.106 (x 10⁻³)</td>
<td>1.98±0.136 (x 10⁻³)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Prazosin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.54±0.099 (x 10⁻³)</td>
<td>-</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.59±0.182 (x 10⁻³)</td>
</tr>
</tbody>
</table>
References


2. Tringali C. (Ed), 2000 Bioactive Compounds from Natural Sources; London, UK


