Extracts of Sideritis species as inhibitors of monoamine transporters: A pharmacological mechanism for efficacy in CNS disorders like depressive disorders and attention-deficit hyperactivity disorder (ADHD)

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Botany


The important role of the leaves and flowering tops of *Sideritis* as traditional tea in the eastern mediterranean area and Spain (mountain tea, malotira, dag cay, té de puerto) has imposed the need of cultivation of *Sideritis* since the production from wild-collected plants was insufficient to cover the demand of market (Evstatieva and Koleva 2000; Goliaris 2000). In addition, about 20 wild grown *Sideritis* subspecies are considered as rare or threatened plants and are under governmental protection. In 1997, 74 t of wild-collected and 183 t of cultivated material was produced in Greece. In 1991, the share of *Sideritis* species in the total greek production of aromatic plants was 15% (13% cultivated, 2% wild-collected), which was slightly reduced in 2000 to 10% (7% cultivated, 3% wild). The producer prices for wild-collected *Sideritis* are between 3-7 €/kg (Stamou 2003).

*Sideritis* has become very fashionable in Germany recently and is found in a variety of shops marketed as “Bergtee” or “Griechischer Bergtee”. The herb is sold cut or sometimes even whole, the latter making identification much easier. The majority of species sold in Germany consists of *Sideritis syriaca*, *Sideritis scardica* and *Sideritis dichotoma*.

Chemical constituents

The chemical constituents of *Sideritis* have been investigated for a long time. The essential oil of all *Sideritis* species mainly consists of α-pinene, β-pinene, β-caryophyllene, caryophyllene oxide,
limonene, 1,8-cineole, carvacrol, myrcene, germacrene D, spathulenol, α-bisabolol, fenchone and sabinene. The main components vary between the species.

In addition, 8-hydroxyflavone allosylglucosides and p-coumaroylglucosides are found in several *Sideritis* species (Tomas-Barberan 1992). For some species the presence of phenylpropanoid glycosides is reported (Akcos 1999).

*Chemical constituents of selected Sideritis species*

*Sideritis scardica*

Monoterpene hydrocarbons form the largest group of compounds in the essential oil of *Sideritis scardica*: α-pinene (52.02%), β-pinene (12.87%), β-myrcene (12.88%) and β-phellandrene (4.46%). Alcohols form 4.23% of the essential oil. Other constituents include sesquiterpene hydrocarbons (4.58%), esters (0.36%), carbonyl compounds (1.36%), thymol (2.64%) and epoxypinane (0.11%) (Kokkalou 1987). Main components in the essential oil of *Sideritis scardica* grown in Macedonia and Bulgaria were α-cadinol (20%, macedonian species) and diterpenic compounds and octadecenol (over 20%, bulgarian species) (Kostadinova 2007).

*Sideritis* are rich in phenolic compounds such as hydroxycinnamic acids and flavonoids. Isoscufellarein, apigenin, 3’-methyl ether of hypolaetin and chryseriol were found in the ethyl acetate fraction of *Sideritis scardica* acetone extracts (Janeska et al. 2007). The occurrence of 8-hydroxyflavoneglucosides but no p-coumaroylglucosides was reported for a 70% ethanol extract of *Sideritis scardica* (Tomas-Barberan 1992).

The content of the individual phenolics and flavonoids in a water-ethanol extract from a cultivated hybrid *Sideritis scardica* × *Sideritis syrica* was analysed. The presence of chlorogenic acid, three phenylethanoid glycosides (lavandulifolioside, verbascoside and leucoseptoside A), and eight flavonoid glycosides (glycosides of isoscufellarein, isoscufellarein-4’-methyl ether, hypolaetin, hypolaetin-4’-methyl ether and apigenin) was established (Tisbranska 2010).

*Sideritis dichotoma*

In the hexane and acetone extracts of *Sideritis dichotoma* the diterpenes siderol, sideridiol, ent-7α-18-dihydroxybeyer-15-ene, ent-7α,15β,18-trihydroxykaur-16-en, ent-7α-acetoxy-15β,18-
Sideritis erythrantha var. cedretorum

In the essential oil of Sideritis erythrantha var. cedretorum 76 compounds were found and characterised that amount for 90% of the oil with myrcene (22-24%) and α-pinene (11-12%) as main constituents (Tabanca 2001). Among them are with an amount of more than 1%: β-pinene, germacrene D, bicyclogermacrene, δ-cadinene, epicubebol, cubebol, and α-bisabolol.

Biological activities

Plants of the genus Sideritis are widely used in folk medicine for the cure of cough due to cold and for the treatment of gastrointestinal disorders. This is due to their anti-inflammatory (Akcos 1999, Alcaraz 1989) and antibacterial and antifungal (Dulger 2006, Kilic 2006) activities. Studies on the pharmacological action of Sideritis revealed diuretic (Topcu 2002), antioxidant (Koleva 2003) and analgesic (Menghini 2005) effects.

Andalusol (ent-6α-8α-18-trihydroxy-13(16),14-labdadiene), a diterpene found in Sideritis foetens, has been shown to inhibit iNOS expression in macrophages. It is supposed that this effect is caused by a transcriptional mechanism. This inhibition of iNOS by andalusol and related substances might be responsible for the anti-inflammatory effect of Sideritis species, since iNOS is the enzyme responsible for the high-output NO synthesis (de las Heras 1999).

Aqueous extracts of Sideritis euboea and Sideritis clandestina have been shown to stimulate osteoblastic cell differentiation and to exhibit antiestrogenic effect on breast cancer cells without proliferative effects on cervical adenocarcinoma cells (Kassi 2004).

Published data on the acute toxicology of selected Sideritis extracts


Determination of LD₅₀ in mice 24 h after subcutaneous administration in doses ranging from 2 to 14 g/kg body weight.
2. *Sideritis canariensis* var. *pannosa* (Hernandez-Perez 2002a):

Determination of LD<sub>50</sub> in mice 72 h after oral administration in doses ranging from 500 to 2000 mg/kg body weight.
Ethanol extract  no mortality up to a dose of 2 g/kg. The tested animals did not present any toxic manifestations.


Determination of LD<sub>50</sub> in mice 72 h after oral administration in doses ranging from 500 to 2000 mg/kg body weight.
Ethanol extract  no mortality up to a dose of 2 g/kg. The tested animals did not present any toxic manifestations.


Determination of LD<sub>50</sub> in mice 24 h after intraperitoneal administration.

<table>
<thead>
<tr>
<th>Extract Type</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; [CI&lt;sub&gt;95&lt;/sub&gt;]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform extract</td>
<td>1050 ± 198.60 mg/kg body weight</td>
</tr>
<tr>
<td>Ethylacetate extract</td>
<td>no mortality up to doses of 800 mg/kg body weight</td>
</tr>
<tr>
<td>Aqueous ethanol extract</td>
<td>3600 ± 129.19 mg/kg body weight</td>
</tr>
</tbody>
</table>

**Serotonin (5-HT) uptake experiments**

**Rat brain synaptosome experiments**

Male Wistar rats (250-300 g) were decapitated under CO<sub>2</sub> anaesthesia and the brain was quickly removed. Cortex was prepared on ice. The cortical tissue was homogenised in 10 volumes ice cold 0.32 M sucrose/10 mM HEPES pH 7.4. The homogenate was centrifuged for 10 min at 4° C and 900*g. The supernatant was centrifuged again for 10 min at 4° C and 11000*g. The supernatant was
discarded and the pellet was kept on ice. At the beginning of the experiment, the pellet was resuspended in buffer to yield a suspension with a total protein content of 20-30 µg/ml.

The uptake experiments were performed in 96 well filter plates (GF-C glass fiber filter, Millipore Multiscreen FB). Each well was washed with 250 µl of buffer containing 121 mM NaCl, 1.8 mM KCl, 1.3 mM CaCl₂, 1.2 mM MgSO₄, 25 mM NaHCO₃, 1.2 mM KH₂PO₄, 11 mM glucose, 0.57 mM ascorbic acid, saturated with 95% O₂/5% CO₂, final pH 7.4. 50 µM pargyline were added for the inhibition of MAO. 50 µl synaptosome preparation in buffer was added to each well and incubated with various concentrations of extracts dissolved in DMSO (final concentration of DMSO 10 µl/well) in a total volume of 240 µl for 10 min. After addition of 10 µl of a 100 nM 5-HT solution in buffer containing 0.1 µCi of [³H]5-HT (30 Ci/mmol [³H]5-HT) the plates were incubated for 10 min at room temperature, the uptake buffer was then rapidly filtered off, and the filter was washed three times with 250 µl buffer. The filters were punched out and transferred into scintillation vials for liquid scintillation counting. Nonspecific uptake was defined as uptake in the presence of 10 µM fluvoxamine.

**Cell culture experiments**

In addition, uptake experiments were performed with human placental choriocarcinoma cells (JAR) which constitutively express the human serotonin transporter hSERT.

JAR cells (DSMZ Braunschweig) were grown in RPMI-1640 medium containing L-glutamine, 10% fetal calf serum, 100 U/ml penicillin, and 100 mg/ml streptomycin at 37° C in an atmosphere of 5% CO₂, 95% air.

The uptake experiments were performed in poly(D-lysine)-coated 24-well plates (1 day after plating; 5*10⁴ - 2*10⁵ cells/well). Each well was washed twice with 1 ml of Krebs-Ringer-HEPES buffer containing 10 mM HEPES, 120 mM NaCl, 3 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, 5 mM glucose and 0.57 mM ascorbic acid final pH 7.3 and incubated with various concentrations of extracts dissolved in DMSO (final concentration of DMSO 10 µl/well) in a total volume of 1 ml. After addition of 10 µl of a 100 nM 5-HT solution in buffer containing 0.1 µCi of [³H]5-HT (30 Ci/mmol [³H]5-HT) the plates were incubated for 10 min at room temperature, the uptake buffer was then rapidly aspirated, and the cells were washed three times with 1 ml buffer. Cells were lysed with 0.5 ml of 0.5 M NaOH and transferred into scintillation vials for liquid scintillation counting. Nonspecific uptake was defined as uptake in the presence of 10 µM fluvoxamine.
Results

Serotonin uptake

Comparison of the effect of Sideritis scardica DMSO extract with Hypericum perforatum (St. John’s wort) extract on serotonin re-uptake into rat brain synaptosomes

*Sideritis scardica* was extracted in DMSO for one hour at room temperature. This extract was compared with a commercially available extract of *Hypericum perforatum* and the synthetic serotonin re-uptake inhibitor fluvoxamine in the rat brain synaptosome model of serotonin re-uptake.

![Effect of DMSO extract of Sideritis scardica on serotonin reuptake into rat brain synaptosomes. Comparison with Hypericum perforatum and fluvoxamine](image)

Fig. 1: Effect of *Sideritis scardica* DMSO extract on the uptake of serotonin into rat brain synaptosomes (n=8; error bars represent the 95% confidence interval). The concentration of *Sideritis* extract was adjusted to the extract from 5 mg *Sideritis* per ml incubation buffer. *Hypericum perforatum* extract was used in a concentration of 500 µg/ml, fluvoxamine in a concentration of 10 µM.
**Effect of Sideritis scardica florescences, leaves and stems DMSO extracts on serotonin re-uptake into rat brain synaptosomes**

*Sideritis scardica* was divided into florescences, leaves and stems and the plant parts were extracted separately in DMSO for one hour at room temperature. The concentration of the extracts was adjusted to final concentrations in the uptake experiments of 5 mg, 0,5 mg and 0,05 mg *Sideritis* per ml incubation buffer.

Uptake experiments were performed with rat brain synaptosomes. Unspecific binding was determined with 10 µM fluvoxamine. The following specific uptake rates were obtained (mean ± CI₉₅, n=8):

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>1,0000 ± 0,2095</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Florescences</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mg/ml</td>
<td>-0,0091 ± 0,0298</td>
<td></td>
</tr>
<tr>
<td>0,5 mg/ml</td>
<td>0,6711 ± 0,1750</td>
<td></td>
</tr>
<tr>
<td>0,05 mg/ml</td>
<td>0,9023 ± 0,1148</td>
<td></td>
</tr>
<tr>
<td><strong>Leaves</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mg/ml</td>
<td>-0,0151 ± 0,0375</td>
<td></td>
</tr>
<tr>
<td>0,5 mg/ml</td>
<td>0,2467 ± 0,1125</td>
<td></td>
</tr>
<tr>
<td>0,05 mg/ml</td>
<td>0,6458 ± 0,0912</td>
<td></td>
</tr>
<tr>
<td><strong>Stems</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mg/ml</td>
<td>0,1441 ± 0,0503</td>
<td></td>
</tr>
<tr>
<td>0,5 mg/ml</td>
<td>0,6849 ± 0,0948</td>
<td></td>
</tr>
<tr>
<td>0,05 mg/ml</td>
<td>0,9666 ± 0,2042</td>
<td></td>
</tr>
<tr>
<td>10 µM fluvoxamine</td>
<td></td>
<td>0,0000 ± 0,0491</td>
</tr>
</tbody>
</table>

Inhibitory effects on the uptake of [³H]-serotonin were observed for all plant parts, the florescences, leaves and stems with a similar order of efficacy.
**Effect of DMSO extracts from several Sideritis species on serotonin re-uptake into rat brain synaptosomes**

Various *Sideritis* species were extracted in DMSO for one hour at room temperature, the concentration of the extracts was adjusted to a final concentration in the uptake experiments of 5 mg *Sideritis* per ml incubation buffer.

The species investigated were:

**Greek species:**
S. clandestina, S. euboea, S. raeseri, S. scardica, S.syriaca

**Turkish species:**
S. congesta, S. dichotoma, S. stricta, S. erythrantha var. cedretorum, S. argyrea, S. arguta, S. vuralii, S. condensata

**Spanish species:**
S. angustifolia

![Graph showing the effect of several Sideritis DMSO extracts on serotonin reuptake into rat synaptosomes](image)

Fig. 2: Effect of several *Sideritis* DMSO extracts on the uptake of serotonin into rat brain synaptosomes (n=8; error bars represent the 95% confidence interval). The concentration of *Sideritis* extracts was adjusted to the extract from 5 mg *Sideritis* per ml incubation buffer. Fluvoxamine was used in a concentration of 10 µM.
Based on these results and on the availability of the raw plant material, the following species were selected for the further studies:

*S. erythranta var. cedretorum*
*S. dichotoma*
*S. scardica.*

**Effect of various extracts from Sideritis erythranta var. cedretorum on serotonin re-uptake into rat brain synaptosomes**

*Sideritis erythranta var. cedretorum* was extracted with a variety of solvents. The fractions obtained were analysed for their biological activity. The following results were obtained.

<table>
<thead>
<tr>
<th>extraction condition</th>
<th>yield from 1 g</th>
<th>specific serotonin uptake (% controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50 µg/ml</td>
</tr>
<tr>
<td>Ethanol, 1 h reflux</td>
<td>112 mg oil</td>
<td>67,7 ± 11,5</td>
</tr>
<tr>
<td>Ethylacetate, 1 h reflux</td>
<td>60 mg oil</td>
<td>29,7 ± 5,5</td>
</tr>
<tr>
<td>Methylenechloride, 1 h reflux</td>
<td>64 mg oil</td>
<td>23,2 ± 3,4</td>
</tr>
<tr>
<td>Petrolether, 1 h reflux</td>
<td>69 mg oil</td>
<td>29,6 ± 6,3</td>
</tr>
<tr>
<td>Water, 1 h reflux</td>
<td>160 mg light brown powder</td>
<td>81,7 ± 11,6</td>
</tr>
<tr>
<td>Water, 1 h mazeration at 100 °C</td>
<td>170 mg light brown powder</td>
<td>69,9 ± 11,3</td>
</tr>
</tbody>
</table>

**Investigation of Sideritis scardica and Sideritis dichotoma extracts**

For the further studies we used other *Sideritis* species, *Sideritis scardica* and *Sideritis dichotoma*. The extraction procedure also was altered, Sideritis was first extracted with hexane followed by an extraction of the residual plant material with methanol in order to assure the absence hydrophobic compounds in the extract. Investigations into cell viability with JAR and HELA cells (LDH assay, 50 µg/ml and 500 µg/ml extract) showed no alterations compared to controls.

This methanol extract was investigated for its effect on the uptake of serotonin into JAR cells. The results obtained are displayed in the following figure.
Fig. 7: Effect of a compound 1-free methanol extract of *Sideritis scardica* on the uptake of serotonin into human JAR cells (n=8; error bars and error of the EC$_{50}$ represent the 95% confidence interval).

With a EC$_{50}$ of 1.4 µg/ml and a maximum inhibition of about 80%, this extract of *Sideritis scardica* was even more active than the extracts of *Sideritis erythranta var. cedretorum* investigated earlier.

Another *Sideritis* species commonly sold as mountain tea is *Sideritis dichotoma*. The investigation of the methanol extracts of this species showed the following reuptake rates for serotonin into rat brain synaptosomes:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Specific serotonin uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.0000 ± 0.2503</td>
</tr>
<tr>
<td>Methanol extract 500 µg/ml</td>
<td>0.0148 ± 0.0208</td>
</tr>
<tr>
<td>Methanol extract 50 µg/ml</td>
<td>0.7701 ± 0.0958</td>
</tr>
<tr>
<td>Fluvoxamine 10 µM</td>
<td>0.0000 ± 0.0165</td>
</tr>
</tbody>
</table>

Methanol extracts from *Sideritis dichotoma* and *Sideritis scardica* revealed strong inhibitory effects on the uptake of serotonin into human cells and rat brain synaptosomes. This inhibition is caused by an interaction with the serotonin transporter and is not caused by an intrinsic cytotoxicity of substances.
present in the extracts. Both methanol extracts had no effect on extracellular LDH compared to controls even at the highest dose investigated (500 µg/ml).

Conclusions:

*Sideritis* extracts are inhibitors of rat and human serotonin transporters. The main indication for serotonin reuptake inhibitors is clinical depression but they are also frequently prescribed for anxiety disorders, such as social anxiety, panic disorders, obsessive–compulsive disorder, eating disorders and chronic pain. As serotonin uptake inhibitors, *Sideritis* extracts have the potential for a successful application in the treatment of these disorders.
Effect of the Sideritis scardica methanol extract on noradrenaline and dopamine uptake into rat brain synaptosomes

A methanol extract of *Sideritis scardica* was investigated for its possible effect on the uptake of the monoamines noradrenaline and dopamine. In the same series of experiments the effect on the uptake of serotonin was reinvestigated for an easier comparison of the results.

Noradrenaline and dopamine uptake experiments were performed as described earlier. The final concentrations of radiolabelled transmitter were 20 nM ([³H]-noradrenaline) and 10 nM ([³H]-dopamine). Unspecific binding was determined in the presence of 10 µM desipramine (noradrenaline uptake experiments) or 10 µM methylphenidate (dopamine uptake experiments). The plates were incubated for 15 min at 37° C.

The results obtained are presented together with the EC₅₀ values in the following figures.

![Serotonin uptake graph](image)

**EC₅₀:** 31.0 µg/ml [16.4; 58.6]

Fig. 8: Effect of a methanol extract of *Sideritis scardica* on the uptake of serotonin into rat brain synaptosomes (n=8; error bars and error of the EC₅₀ represent the 95% confidence interval).
Noradrenaline uptake

<table>
<thead>
<tr>
<th>[methanol extract µg/ml]</th>
<th>control</th>
<th>0.5</th>
<th>5</th>
<th>50</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>specific noradrenaline uptake</td>
<td>0.0</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

EC$_{50}$: 42.3 µg/ml [31.8; 56.4]

Fig. 9: Effect of a methanol extract of *Sideritis scardica* on the uptake of noradrenaline into rat brain synaptosomes (n=8; error bars and error of the EC$_{50}$ represent the 95% confidence interval).

Dopamine uptake

<table>
<thead>
<tr>
<th>[methanol extract µg/ml]</th>
<th>control</th>
<th>0.5</th>
<th>5</th>
<th>50</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>specific dopamine uptake</td>
<td>0.0</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

EC$_{50}$: 37.0 µg/ml [27.5; 49.8]

Fig. 10: Effect of a methanol extract of *Sideritis scardica* on the uptake of dopamine into rat brain synaptosomes (n=8; error bars and error of the EC$_{50}$ represent the 95% confidence interval).
The methanol extract from *Sideritis scardica* inhibited the uptake of all three neurotransmitters investigated.

Other extracts of several Sideritis plants with methanol (extracts 1, 2, 4) or water (extract 3) were investigated on their effect on noradrenaline and dopamine uptake into rat brain synaptosomes. The following results were obtained:

**Noradrenaline uptake:**

<table>
<thead>
<tr>
<th></th>
<th>Extract 1</th>
<th>Extract 2</th>
<th>Extract 3</th>
<th>Extract 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,00 ± 0,12</td>
<td>1,00 ± 0,17</td>
<td>1,00 ± 0,17</td>
<td>1,00 ± 0,17</td>
</tr>
<tr>
<td>Sideritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 µg/ml</td>
<td>0,96 ± 0,11</td>
<td>0,71 ± 0,32</td>
<td>0,78 ± 0,16</td>
<td>0,62 ± 0,20</td>
</tr>
<tr>
<td>50 µg/ml</td>
<td>0,48 ± 0,08</td>
<td>0,46 ± 0,26</td>
<td>0,22 ± 0,07</td>
<td>0,17 ± 0,26</td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>0,17 ± 0,02</td>
<td>-0,38 ± 0,05</td>
<td>-0,22 ± 0,13</td>
<td>-0,52 ± 0,11</td>
</tr>
<tr>
<td>EC\textsubscript{50}:</td>
<td>42,3 µg/ml</td>
<td>81,2 µg/ml</td>
<td>30,6 µg/ml</td>
<td>36,9 µg/ml</td>
</tr>
<tr>
<td></td>
<td>[31,8; 56,4]</td>
<td>[54,5; 120,8]</td>
<td>[25,1 37,5]</td>
<td>[27,3; 49,9]</td>
</tr>
</tbody>
</table>

**Dopamine uptake:**

<table>
<thead>
<tr>
<th></th>
<th>Extract 1</th>
<th>Extract 2</th>
<th>Extract 3</th>
<th>Extract 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,00 ± 0,11</td>
<td>1,00 ± 0,04</td>
<td>1,00 ± 0,04</td>
<td>1,00 ± 0,04</td>
</tr>
<tr>
<td>Sideritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 µg/ml</td>
<td>0,85 ± 0,04</td>
<td>1,02 ± 0,10</td>
<td>0,98 ± 0,05</td>
<td>1,02 ± 0,12</td>
</tr>
<tr>
<td>50 µg/ml</td>
<td>0,51 ± 0,07</td>
<td>0,73 ± 0,05</td>
<td>0,65 ± 0,23</td>
<td>0,79 ± 0,07</td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>0,16 ± 0,02</td>
<td>-0,13 ± 0,07</td>
<td>0,43 ± 0,06</td>
<td>-0,22 ± 0,01</td>
</tr>
<tr>
<td>EC\textsubscript{50}:</td>
<td>37,0 µg/ml</td>
<td>293,2 µg/ml</td>
<td>45,5 µg/ml</td>
<td>57,8 µg/ml</td>
</tr>
<tr>
<td></td>
<td>[27,5; 49,8]</td>
<td>[230,9; 372,3]</td>
<td>[31,4; 66,0]</td>
<td>[39,2; 85,2]</td>
</tr>
</tbody>
</table>

**Conclusions:**

In addition to their serotonin uptake inhibitory properties, *Sideritis* extracts are also inhibitors of noradrenaline and dopamine uptake.
Depressive disorders are characterised by an imbalance in monoaminergic neurotransmission. Based on findings from studies of antidepressants treatment, it may be possible to assign specific symptoms of depression to specific neurochemical mechanisms. Serotonin may be related to anxiety, obsessions and compulsions; noradrenaline to alertness and energy as well as anxiety, attention and interest in life; and dopamine to attention, motivation, pleasure and reward as well as interest in life (Nutt, 2008). Increasing any of these three neurotransmitters will elevate mood, but the other elements of depression may be particularly responsive to elevation of a certain neurotransmitter. It is therefore desirable to have a remedy which acts on as many monoaminergic systems as possible.

![Diagram of neurotransmitters and associated symptoms](image)

A recent study on the *Hypericum perforatum* extract Ze 117 showed that it interferes in three ways with the individual uptakes of the monoamine neurotransmitters. EC$_{50}$ values for this extract were 54 µg/ml for noradrenaline uptake, 350 µg/ml for dopamine uptake and 1600 µg/ml for serotonin uptake (Ruedeberg et al., 2010). Therefore, the potency of the noradrenaline uptake inhibition was around 30 times higher than that for serotonin, and seven times higher than that of the dopamine uptake inhibition.

In contrast to St. John’s wort, *Sideritis scardica* extract has EC$_{50}$ values for all three types of monoamine transporters in the range of 30-40 µg/ml. With this background, the pharmacological profile of *Sideritis scardica* extract might be beneficial for the therapy of depressive disorders. It elevates the extracellular concentration of all three monoamine neurotransmitters by inhibiting their transporters with similar potency and therefore might ameliorate the symptoms of depression better and more powerful than monospecific drugs. In allopathic pharmacology the debate about the advantage of dual action antidepressants like venlafaxine and duloxetine is still going on (Papakostas et al., 2007; Isaac, 2008).

In addition to the potential for the treatment of depressive disorders, this pharmacological profile suggests the application of Sideritis extracts for the treatment of other CNS disorders associated with an imbalance of monoaminergic neurotransmission. Among them is the attention-deficit hyperactivity
disorder (ADHD). This behavioural disorder is characterised by inattention or lack of focus, hyperactivity and impulsivity.

Adequate catecholaminergic modulation of the prefrontal cortex, the brain region that plays an important role in the physiology of cognition and emotion, is essential for attention and vigilance. The dysfunction of the prefrontal cortex in ADHD has been attributed to a decreased catecholamine function affecting cognition and motor inhibition.

Since monoamine transporters are (at least in part) responsible for regulating extracellular concentrations of the catecholamines noradrenaline and dopamine, the common treatment of ADHD includes stimulant medications with drugs like methylphenidate. These stimulants block the reuptake of noradrenaline and dopamine thus rising their extracellular levels. It has been shown that simultaneous inhibition of the noradrenaline transporter attenuates some subjective and physiological effects of dopaminergic stimulants in humans (Sofuoglu, 2009). Currently, the only nonstimulant medication approved for the treatment ADHD is atomoxetine, which selectively enhances extracellular noradrenaline and dopamine levels within the prefrontal cortex (Bymaster et al., 2002). Although noradrenaline and dopamine are in the main focus of pharmacological therapy of ADHD, an additional contribution of the serotonergic system can not be excluded. Recent genetic and neuroimaging studies provide evidence for separate contributions of altered dopamine and serotonin function in this disorder (Oades, 2008). Most tricyclic antidepressants acting on serotonin and noradrenaline transporters are good remedies for managing behavioural and, to some extent, cognitive symptoms (Popper, 2000).

*Sideritis scardica* extracts inhibit all monoamine transporters with approximately the same potency. All other remedies used in ADHD treatment show selectivity towards only one of the three transporters. Methylphenidate has higher affinity to dopamine transporters than to noradrenaline transporters and very low affinity for serotonin transporters. Atomoxetine on the other hand selectively inhibits noradrenaline transport, its action at the serotonin transporter is weaker and it shows a low affinity towards dopamine transporters (Bymaster, 2002). *Sideritis scardica* extracts with their ability to inhibit dopamine, noradrenaline and serotonin uptake to the same extent may turn out to be beneficial for a phytochemical therapy of ADHD.
**Other pharmacological studies**

**GABA<sub>A</sub> receptor binding**

The methanol extract of *Sideritis scardica* showed no displacement of [³H]-flunitrazepam or [³H]-muscimol from their binding sites in rat brain homogenate. *Sideritis scardica* extracts do not interfere with the benzodiazepine or the GABA binding site at the GABA<sub>A</sub> receptor.

**α<sub>2A</sub> receptor binding**

Binding of a *Sideritis scardica* DMSO extract to α<sub>2A</sub> adrenoceptors was investigated using recombinant human receptors (Perkin Elmer Membrane Target Systems). The concentration of the extract was adjusted to the extract from 5 mg *Sideritis* per ml incubation buffer. Sideritis displaced [³H]-rauwolscine (final concentration 2.5 nM) from its binding site in a concentration-dependent manner with an EC<sub>50</sub> of 0.5 mg *Sideritis* plant per ml incubation buffer. The results are displayed in the following figure.

![Binding of a Sideritis scardica DMSO extract to human recombinant α<sub>2A</sub> adrenoceptors](image.png)

Fig. 11: Binding of a *Sideritis scardica* DMSO extract to human recombinant α<sub>2A</sub> adrenoceptors (n=8, error bars represent the 95% confidence interval). Unspecific binding was determined in the presence of 1 µM noradrenaline.
Several reports on the use of the $\alpha_2$-agonists guanfacine and clonidine show improvements in children with ADHD and improvements in hyperactivity, impulsiveness and inattention in children with tic disorders and pervasive developmental disorders (Scahill 2009). The binding of the *Sideritis scardica* extract to human $\alpha_{2A}$ adrenoceptors may contribute together with the inhibition of the monoamine uptake to the improvements observed in ADHD patients treated with mountain tea.

*MT$_1$ receptor binding*

Binding of a *Sideritis scardica* ethanol extract to human melatonin MT$_1$ receptors was investigated using recombinant receptors (Perkin Elmer Membrane Target Systems). *Sideritis* displaced [$^3$H]-melatonin (final concentration 0.4 nM) from its binding site in a concentration-dependent manner with an EC$_{50}$ of 147 $\mu$g/ml *Sideritis* extract per ml incubation buffer (CI$_{95}$: [125.1; 173.3]).

![Binding of a Sideritis scardica ethanol extract to recombinant human MT$_1$ receptors](image)

Fig. 12: Binding of a *Sideritis scardica* ethanol extract to human recombinant MT$_1$ receptors ($n=8$, error bars represent the 95% confidence interval). Unspecific binding was determined in the presence of 1 µM melatonin.
Currently available antidepressant agents such as tricyclic antidepressants act primarily through monoaminergic systems in the brain. Although the pathophysiology of depression is not completely understood, it is increasingly recognised that monoamine deficiency/disruption is not the only pathway involved. Recognition that circadian rhythm desynchronisation also plays a key role in mood disorders has led to the development of drugs like agomelatine, which is endowed with a novel mechanism of action distinct from that of currently available antidepressants. Agomelatine is an agonist of the melatonergic MT$_1$ and MT$_2$ receptors, as well as a 5-HT$_{2C}$ receptor antagonist (Popoli 2009). The binding of *Sideritis scardica* ethanol extract to melatonin MT$_1$ receptors may therefore also contribute to its antidepressant effects.

**5HT$_{2C}$ receptor binding**

Binding of a *Sideritis scardica* ethanol extract to human serotonin 5HT$_{2C}$ receptors was investigated using recombinant receptors (Perkin Elmer Membrane Target Systems). At a concentration of 500 µg/ml this *Sideritis* extract significantly enhanced the binding of [³H]-mesulergine to the receptor. Lower concentrations had no influence on ligand binding.

Specific [³H]-mesulergine binding (n=8; mean ± CI$_{95}$):

<table>
<thead>
<tr>
<th>Condition</th>
<th>Specific Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>control experiments</td>
<td>1,0000 ± 0,0802</td>
</tr>
<tr>
<td>5 µg/ml</td>
<td>0,9936 ± 0,0831</td>
</tr>
<tr>
<td>50 µg/ml</td>
<td>1,0013 ± 0,0892</td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>1,8662 ± 0,1150</td>
</tr>
<tr>
<td>100 µM mianserine</td>
<td>0,0000 ± 0,0109</td>
</tr>
</tbody>
</table>

Similar results were obtained for the binding of the endogeneous receptor ligand serotonin:

Specific [³H]-serotonin binding (n=4; mean ± CI$_{95}$):

<table>
<thead>
<tr>
<th>Condition</th>
<th>Specific Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>control experiments</td>
<td>1,0000 ± 0,1319</td>
</tr>
<tr>
<td>5 µg/ml</td>
<td>0,9500 ± 0,1511</td>
</tr>
<tr>
<td>50 µg/ml</td>
<td>0,9097 ± 0,1735</td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>1,4928 ± 0,1735</td>
</tr>
<tr>
<td>100 µM mianserine</td>
<td>0,0000 ± 0,0109</td>
</tr>
</tbody>
</table>

Numerous studies implicated the 5HT$_{2C}$ receptor in a number of diseases such as anxiety, obesity, depression, schizophrenia, and affective disorders (di Giovanni 2006). The receptor has become a therapeutic target for potential anxiolytics and antiobesity drugs. Much effort has been directed, so far,
to the discovery of selective agonists for its serotonin binding site. Another approach is the search of compounds that stimulate serotonin binding, so-called positive allosteric modulators. The enhancement of 5HT₂C ligand binding to their receptor by the *Sideritis scardica* extract at higher concentrations may be a beneficial part of the extract’s mode of action, but it also suggests the application of a special extract as an antiobesity drug.

*Inhibition of electric eel acetylcholinesterase*

Methanol extracts from several Sideritis species were investigated for their effect on electric eel acetylcholinesterase. The acetylcholinesterase assay is based on Ellman’s reaction (Ellman et al., 1961), in which thiocholine produced by the action of acetylcholinesterase forms a yellow colour with 5,5’-dithiobis-(2 nitrobenzoic acid). The intensity of the product colour, measured at 412 nm, is proportionate to the enzyme activity in the sample. Sideritis inhibited the activity of electric eel acetylcholinesterase in a concentration-dependent manner (Fig. 12).

![Inhibition of electric eel acetylcholine esterase by new Sideritis methanol extract](image)

**Fig. 12:** Inhibition of electric eel (*Electrophorus electricus*) acetylcholinesterase by a Sideritis methanol extract compared with the acetylcholinesterase inhibitor donepezil.
Inhibition of acetylcholinesterase and butyrylcholinesterase (a related enzyme) has also been reported for petrolether and acetone extracts of *Sideritis arguta*. The inhibitory effect was attributed to the kauranes present in the extracts (Ertas 2009).
Case reports on the use of *Sideritis* tea infusions in depressive disorders

In cooperation with the Universitätsklinik Freiburg we investigated the effect of *Sideritis scardica* on depressed patients. Two patients gave informed consent to an experimental treatment with “Greek mountain tea”. The results are described in the following case reports:

1. 47 years old female patient with recurrent depression, middle grade episode, and migraine for 20 years. The patient rejects therapy with classical antidepressants, wants to be treated with phytodrugs.  
   Prescription: 1 l mountain tea (*Sideritis scardica*) per day.  
   After 14 days clear elevation of mood, improvement of night sleep and motivation.

2. 43 years old male patient with recurrent depression and maintenance therapy with Venlafaxin 300 mg.  
   Add on mountain tea (*Sideritis scardica*), first 0.5 l, later 1 l per day because of the continuing lack of motivation. After 4 weeks he developed a intolerance for Venlafaxin with persisting diarrhoea, vomiting, transpiration and vertigo.  
   Reduction of the Venlafaxin dose over 6 weeks and stop of intake on behalf of the general practitioner. Since 6 months maintenance therapy for the depression with 1 l mountain tea per day.  
   One depressive episode over 4 weeks, compared to the patient history over the last 15 years significantly shorter episode with remission after 4 weeks. At the moment complete remission since 1 year.

The following subjective impression of a patient was found in an internet blog on antidepressants in pregnancy (http://www.urbin.de/forum/index.html?area=thread&bid=2&tid=2910752):

```
Hallo!


Hört sich bloß an, aber mir tat er echt gut. Den kann man bei Tee Gschwender kaufen/online bestellen.


Frage mal deinen Psy.

Ich habe 100cm (sind so Stängel) auf 1 Liter über den Tag verteilt getrunken

Lö und Glückwünsche zu S9

Leelala
```
**Shift of the fluvoxamine dose-response curve by a Sideritis scardica ethanol extract**

The observation with the second patient (development of the Venlafaxin intolerance) led us to the following experiment. We assumed a synergistic effect of *Sideritis* on the action of synthetic serotonin uptake inhibitors. Therefore we investigated the effect of various concentrations of a *Sideritis scardica* ethanol extract on the inhibition of serotonin uptake by fluvoxamine. The dose-response curve of fluvoxamine was shifted to the left by a concentration of *Sideritis* which was not pharmacologically active alone (10 µg/ml). Higher concentrations of *Sideritis* (50 µg/ml, 100 µg/ml) lead to a almost total inhibition of specific serotonin uptake in the whole fluvoxamine concentration range investigated.

Fig. 11: Shift of the fluvoxamine dose-response curve to the left in the presence of 10 µg/ml and 50 µg/ml *Sideritis scardica* extract. 10 µg/ml *Sideritis* alone showed an inhibition of serotonin uptake of 0.0732 ± 0.1761 (remaining uptake rate: 0.9268 ± 0.1761). 50 µg/ml *Sideritis* alone showed an inhibition of 0.5193 ± 0.2034 (remaining uptake rate: 0.4807 ± 0.2034).
Case reports on the use of *Sideritis* tea infusions in attention-deficit-hyperactivity disorder

1. Thirteen years old girl, ADHD with conduct disorder (ICD-10: F 90.1)

   At baseline, the girl’s psychopathological assessment showed mild to moderate impairment of concentration, attention and perception at school and at home, pronounced fluctuations of motivation, procrastination of tasks that require sustained mental effort. She does not listen when her mother is talking to her, often fails to finish her duties, forgetful in daily activities and often loses things from her personal belongings or for tasks. The girl showed intense physical tension, restlessness and irritability as well. The patient shows frequently verbal and physical aggressive behaviour due to low stress tolerance. The mother complained that the girl does not listen, often defies and refuses the parents’ requests and rules. Under high distress conditions the girl shows self-injurious behaviour by superficial cutting of her skin with pieces of broken glass or metal sheets. She complains about emotional instability with frequent mood swings. The girl shows a high motoric drive and talked excessively.

   Individual study started with one litre of mountain tea (3 g *Sideritis scardica*) per day. Within the first week of active treatment the parents reported several changes on their daughter: reduction of physical tension, improvement of active listening, less interruptive in talks, and less conflicts in daily family life. Patient’s performance at school improved through increased ability to sustain attention. Although the patient and the parents reported improvements, respectively, the girl became non-compliant at week four of treatment. She complained about the taste of the tea and refused to keep on drinking it every day. The patient asked for a ready-made capsule or tablet to go on with the treatment because of the improvement of her state during the active treatment phase.

   CGI Baseline: 6  
   CGI last observation week three: 4 – 5

2. 35 years old male proband with persistent adult attention-deficit-hyperactivity disorder, hyperkinetic type, treatment naive (ICD-10: F 90.0)

   At baseline, the man’s psychopathological assessment showed a moderate impairment of attention, concentration and perception with high interference for tonic alertness, high distractability for divided attention, often loses his personal belongings, often talked excessively and interrupts on others, longwinded when talking. His formal thinking showed excessive associative loosening, mental leaps and some incoherence. He often fidgets with hands and feet, can remain seated for longer time with high physical tension. His mood was instable with high frequent reactive mood swings. His interpersonal skills were impaired
through limited stress tolerance, intermittent explosive behaviour and never keeping one’s distance resulting in lack of confidence and remarked sense of inferiority as well.

Individual study started with two litres of mountain tea (6 g *Sideritis scardica*) per day. Within the first week of active treatment there was a significant improvement of concentration, attention and perception and behaviour as well. The proband is able now to focus in talks, reporting facts concisely and structured without interrupting the dialogue partners. The man is relaxed with gain of impulse control, stress tolerance and mood stability. Sustained effect of continuous treatment for several months as long as the patient is compliant. During wash out for two weeks worsening of the psychopathology without reaching the magnitude of baseline assessment.

CGI Baseline: 5  CGI week three: 3  CGI month seven: 3
Summary

Plants and extracts of *Sideritis* species are able to inhibit the uptake of serotonin, noradrenaline and dopamine into neurons. Together with their affinity to several receptors, this is a biochemical and pharmacological mode of action which allows the use of *Sideritis* for the treatment of CNS disorders like

- depression
- chronic neuropathic pain
- panic disorders
- anxiety disorders
- obsessive-compulsive disorders
- eating disorders
- attention-deficit/hyperactivity disorder (ADHD)
- Morbus Parkinson

among others.

Due to the mild inhibition of acetylcholinesterase, *Sideritis* may be beneficial as a cognitive enhancing drug in healthy people or for the treatment of diseases like

- mild cognitive impairment (MCI)
- senile dementia
- cerebrovascular dementia
- Alzheimer’s disease
- Levy body dementia
- dementia as a result of vascular stroke.

*Sideritis* has already been shown to be effective in patients for the treatment of depression and ADHD.
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