IN VITRO BINDING STUDIES OF METHANOLIC EXTRACTS FROM DIFFERENT *SALVIA* SPECIES

Cavallo Federica, [1]Nencini Cristina, Capasso Anna,
De Tommasi Nunziatina, Leone Antonella, [1]Micheli Lucia

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno.
[1]Dipartimento di Farmacologia "Giorgio Segre", Università degli Studi di Siena.

Corresponding author:
Dr. Federica Cavallo
Dipartimento di Scienze Farmaceutiche
Università di Salerno
Via Ponte Don Melillo (84084) Fisciano, Italy
E-mail: federicacavallo1@yahoo.it

Summary

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 ($5HT_{1A}$, $5HT_{2A}$ and $5HT_{2C}$), noradrenergic (α_1 and α_2) and dopaminergic (D_1 and D_2) 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-HT_{2A} receptors (IC_{50} value = 42.49 ± 0.591 μg/ml) and moderate affinity for the D_2 receptors (46% as level of inhibition at the maximum concentration tested, 125 μg/ml). In addition *S. palaestina* extract showed high affinity for D_1 and D_2 receptors with IC_{50} 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-HT_{2A} 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 ($5HT_{1A}$, $5HT_{2A}$ and $5HT_{2C}$), noradrenergic (α_1 and α_2) and dopaminergic (D_1 and D_2) 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

Receptor	Biological material	Tritiated ligand	Cold ligand	Incubation			*References*
				pH	Time (min)	Temp (°C)	
5-HT$_{1A}$	Cerebral cortex	[^{3}H]8-OH-DPTA	serotonin	7.7	30	37	[19]
5-HT$_{2A}$	Cerebral cortex	[^{3}H]ketanserin	cinanserin	7.4	30	37	[20]
5-HT$_{2C}$	Cerebral cortex	[^{3}H]mesulergine	mianserin	7.4	30	37	[20]
D$_1$	*Corpora striata*	[^{3}H]SCH-23390	(+)-butaclamol	7.1	15	37	[21]
D$_2$	*Corpora striata*	[^{3}H]spiperidol	(+)-butaclamol	7.1	15	37	[22]
α_1	Cerebral cortex	[^{3}H]prazosin	phentolamine	7.4	30	25	[23]
α_2	Cerebral cortex	[^{3}H]yohimbine	phentolamine	7.5	30	25	[24]

Results and Discussion

The affinity of *Salvia* spp methanolic extracts for the serotoninergic ($5HT_{1A}$, $5HT_{2A}$ and $5HT_{2C}$), noradrenergic (α_1 and α_2) and dopaminergic (D_1 and D_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 μg/ml).

The IC_{50} values of extracts (concentration required to inhibit 50% of radioligand specific binding) were obtained only in some cases. These values and IC_{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 $5\text{-}HT_{2A}$ receptors (IC_{50} value of 42.49 ± 0.59 μg/ml) and fairly good affinity for the D_2 receptors (MEO 46.09 %).

In addition *S. palaestina* extract showed high affinity for D_1 and D_2 receptors with IC_{50} values of 68.70 ± 4.42 μg/ml and 30.14 ± 3.64 μg/ml respectively, while *S. menthaefolia* extract displayed moderate affinity only for the D_1 receptors with a level of inhibition of 48.32 % at the maximum concentration tested (125 μg/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 serotoninergic 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).

Plant Extract	**$5HT_{1A}$**	**$5HT_{2A}$**	**$5HT_{2C}$**	**D_1**	**D_2**	**α_1**	**α_2**
Salvia sclarea roots	inactive	88.39%	inactive	inactive	46.76%	inactive	inactive
Salvia menthaefolia roots	inactive	18.04%	inactive	48.32%	18.82%	inactive	25.28%
Salvia dominica leaves	inactive	31.02%	inactive	inactive	29.86%	inactive	28.26%
Salvia dominica flowers	inactive	25.06%	29.84%	inactive	inactive	inactive	inactive
Salvia spinosa aerial parts	inactive	inactive	34.11%	inactive	39.38%	inactive	inactive
Salvia palaestina aerial parts	inactive	17.54%	29.67%	84.22%	93.45%	inactive	inactive

Table 3 - Concentration (μg/ml as mean ± standard deviation) required to inhibit 50% of radioligand binding (IC_{50}).

Extract/ Compound	**$5HT_{1A}$**	**$5HT_{2A}$**	**$5HT_{2C}$**	**D_1**	**D_2**	**α_1**	**α_2**
Salvia sclarea roots	-	42.49± 0.59	-		-	-	-
Salvia palaestina aerial parts	-	-	-	68.70±4.42	30.14±3.64	-	-
8-OH-DPAT	0.70±0.089 (x 10^{-3})	-	-	-	-	-	-
ketanserin	-	0.93±0.036 (x 10^{-3})	-	-	-	-	-
cinanserin	-	0.54±0.096 (x 10^{-3})	-	-	-	-	-
mesulergine	-	-	0.48±0.029 (x 10^{-3})	-	-	-	-
spiperidol	-	-	-	1.55±0.106 (x 10^{-3})	1.98±0.136 (x 10^{-3})	-	-
prazosin	-	-	-	-	-	0.54±0.099 (x 10^{-3})	-
yohimbine	-	-	-	-	-	-	8.59±0.182 (x 10^{-3})

References

1. Atta-ur-Rahman, Choudhary MI, eds. New Trends in Natural Products Chemistry. Amsterdam, The Netherlands: Harwood Academic Publishers, MI OPA, 1998.
2. Tringali C. (Ed), 2000 Bioactive Compounds from Natural Sources; London, UK
3. Kuo YH, King ML. Antitumor drugs from the secondary metabolites of higher plants. In: Tringali C, ed. Bioactive Compounds from Natural Sources, London, UK: Taylor & Francis, 2000:189-282.
4. Clark AM. Natural products as a resources for new drugs. Pharm Res 1996;13:1133-1138.
5. Barberan FAT. The flavonoid compounds from Labiatae. Fitoterapia 1986;57:67-95.
6. Emboden WA, Lewis H. Terpenes as taxonomic characters in Salvia section Audibertia.Brittonia 1967;19:152-160.
7. Bruno M, Savona G, Hueso-Rodriguez JA, Pascual C, Rodriguez B. Ursane and oleane triterpenoids from *Salvia argentea*. Phytochemistry 1987;26:497-501.
8. Luis JG. Ecological Chemistry and Biochemistry of Plant Terpenoids. In: Harborne JB, Tomas-Barberan FA eds. Proceedings of Phytochemical Society of Europe, 1st ed. Oxford, UK: Clarendon Press, 1991;31:63.
9. Rodriguez-Hahn L, Esquivel B, Cardenas J, Ramamoorthy TP. Advances in Labiatae Science. In: Harley RM, Reynolds T eds. The Royal Botanic Gardens, Kew, UK, 1992:335-347.
10. Rodriguez-Hahn L, Esquivel B, Cardenas J, Progress in Chemistry of Organic Natural Products, vol. 63, ed. Herz W, Kirby GW, Moore RE, Steglich W, Tamm C. Wien: Springer, 1994;107.
11. Ohloff G. The Fascination of Odors and Their Chemical Perspectives. Scent and Fragances. Berlin, Germany: Springer-Verlag, 1974;238.
12. Hernandez-Perez M, Rabanal RM, de La Torre MC, Bodriguez B. Analgesic, anti-inflammatory, antipyretic and haematological effects of aethiopinone, an o-naphthoquinone diterpenoid from *Salvia aethiopis* roots and two hemisynthetic derivatives. Planta Med 1995;61(6, suppl):505-509.
13. Cuppett SL, Hall CA. Antioxidant activity of the Labiatae. Adv Food Nutr Res 1998;42:245-271.
14. Ulubelen A, Oksuz S, Topcu G, Goren AC, Voelter W. Antibacterial diterpenes from the roots of *Salvia blepharochlaena*. J Nat Prod 2001;64:549–551.
15. Qing C, Jiang C, Zhang JS, Ding J. Induction of apoptosis in human leukemia K-562 and gastric carcinoma SGC-7901 cells by salvicine, a novel anticancer compound. Anti-cancer Drugs 2001;12:51-56.

16. Ji XY, Tan BK, Zhu YZ. Salvia miltiorrhiza and ischemic diseases. Acta Pharmacol Sin 2000;21(12, suppl):1089-1094.
17. Ulubelen A. Cardioactive and antibacterial terpenoids from some Salvia species. Phytochemistry 2003;64(2, suppl):395-399.
18. Bucheler R, Gleiter CH, Schwoerer P, Gaetner I. Use of non prohibited hallucinogenic plants: increasing relevance for public health? A case report and literature review on the consumption of *Salvia divinorum* (Diviner's Sage). Pharmacopsychiatry 2005;38:1-5.
19. Schlegel JR, Peroutka SJ. Nucleotide interactions with 5-HT_{1A} binding sites directly labeled by [^{3}H]-8-hydroxy-2-(di-n-propylamino)tetralin ([^{3}H]-8-OH-DPAT). Biochem Pharmacol 1986;35:1943-49.
20. Herndon JL, Ismaiel A, Ingher SP, Teitler M, Glennon RA. Ketanserin analogues: structure-affinity relationships for 5-HT_2 and 5-HT_{1C} serotonin receptor binding. J Med Chem 1992;35:4903-10.
21. Billard W, Ruperto V, Crosby G, Iorio LC, Barnett A. Characterization of the binding of [^{3}H]-SCH 23390, a selective D_1 receptor antagonist ligand, in rat striatum. Life Sci 1984;35:1885-1893.
22. Cresee I, Schneider R, Snyder SH. ^{3}H-Spiroperidol labels dopamine receptors in pituitary and brain. Eur J Pharmacol 1977;46:377-381.
23. Greengrass P, Bremner R. Binding characteristics of [^{3}H]-prazosine to rat brain alpha-adrenergic receptors. Eur J Pharmacol 1979;55:323-326.
24. Perry BD, U'Prichard DC. [^{3}H]-rauwolscine (alpha-yohimbine): a specific antagonist radioligand for brain alpha2-adrenergic receptors. Eur J Pharmacol 1981;76:461-464.
25. Fiore G, Nencini C, Cavallo F, Capasso A, Bader A, Giorgi G, Micheli L. *In vitro* antiproliferative effect of six *Salvia* species on human tumor cell lines. Phytother res 2006; in press.