Sedative, Muscle Relaxant-Like Effects, and Molecular Docking Study of Compounds Isolated from *Salvia leriifolia*

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Abstract

Salvia leriifolia Benth., Lamiaceae, an endemic medicinal plant of the northern region of Iran, has been documented as sedative. It has been used in traditional medicine for its muscle relaxation effect. The need for sedation and muscle relaxation are common problems which are usually treated with different synthetic drugs, which are associated with severe side effects. The aim of the current study was to investigate the muscle relaxation and sedative potency of compounds isolated from *S. leriifolia*. Open field and traction tests were employed to study the muscle relaxant and like effects of compounds **1** and **2** isolated from *S. leriifolia* in mice. Results revealed that each of these compounds (**1** and **2**) caused a considerable decrease in mice mobility at test doses of 10, 15, and 20 mg/kg *i.p.* in a dose-dependent manner. When these compounds were tested in the traction test, they exhibited significant dose-dependent skeleton muscle relaxation effect during various assessment times. Thus, it is concluded that compounds 1 and 2 exert significant sedative and muscle relaxant-like effects and therefore can be candidates for further detailed biological studies. This study strongly justifies the ethnopharmacological uses of *S. leriifolia* as sedative and muscle relaxant.

Keywords Abietane diterpenoids · Sedative effect · Open test · Skeleton muscle relaxant activity · Molecular docking

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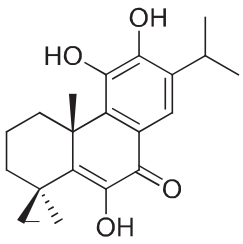
Introduction

Salvia leriifolia Benth., Lamiaceae, is a common plant which flourishes in the North West part of Iran. The plant exhibits a large number of biological activities, such as of morphine dependence (Hosseinzadeh and Lary 2000), antimicrobial and antioxidant (Abadi et al. 2016), and ischemia-reperfusion effect (Hosseinzadeh et al. 2007). Choudhary et al. (2012) isolated a number of compounds from *S. leriifolia* including salvialeriafone, a novel diterpene- norditerpene conjugate. Some of the isolated compounds, in cluding salvialeriafone, displayed *in vitro* anticancer activity against the human cervical cancer cell line (Hela), and cyto- toxicity against the human prostate cancer cell line (PC3). Based on what precedes, the aim of the present study was to conduct phytochemical investigation on the aforementioned Iranian medicinal plant (*S. leriifolia*), involving isolation and purification of compounds, followed by identification through various spectroscopic techniques, and to evaluate the sedative and muscle relaxant-like effects of isolated compounds using *in vivo* animal models. During the phytochemical investigation on the ethanol extract of the *S. leriifolia*, compounds **1** and **2** were purified and their chemical structures established by means of several spectroscopic techniques, including NMR and mass spectrometry (Kelecom and Dos Santos 1985). The isolated compounds (**1** and **2**) showed excellent muscle relaxant and sedative effects in an animal model.

Materials and Methods

The plant samples of *Salvia leriifolia* Benth., Lamiaceae, were obtained from the mountain region of North-West region of Iran. The plant specimen was identified by Prof. Jamzadeh, a senior researcher at the Botany Department, Shahid Beheshti University of Medical Sciences, Tehran, Iran. For identified plant specimen, the voucher No. (A.R. No.112) was placed in the herbarium of said department.

Approximately, 8.8 kg of air-dried powdered plant material was soaked in 20 l of ethanol/water (1:19) at 25 °C for 4 days, and the process was repeated thrice. The obtained extract was concentrated at low temperature and pressure which yielded 469 g of a brownish material. This material was suspended in methanol, and the soluble part (240 g) was concentrated at low temperature and reduced pressure and then assessed with vacuum liquid chromatography which yielded subfractions FR1− FR5. Based on the TLC profile, the subfraction FR3 was subjected to repeated column chromatography which yielded seven subfractions 1A−7A. The subfraction 2A (1.7 g) was subjected to pencil column chromatogram which by using chloroform/methanol (7:3) gave pale yellow crystals, which were further purified by washing with chloroform, yielding pure crystals of compound 1 (55.5 mg). The subfraction 3A was subjected to normal-phase column chromatography, eluted with hexane/chloroform (1:4), and hexane/acetone (9:1)] to give pure compound 2 (9.5 mg). The chemical structures of compounds 1 and 2 were elucidated by comparing the spectra data with reported information (Juan et al. 1983; Kelecom and Dos Santos 1985).



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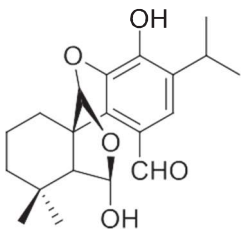

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The details on animals and procedures used for muscle relaxant-like effects and molecular docking are given in supplementary file (Please see supplementary file).

Results and Discussion

The methanolic extract was subjected to column chromatography, which yielded compounds 1 and 2. Shown in Table 1 are results pertaining to the effect of compounds **1** and **2** in the open field test. Results show that pretreatment of animals with compound **1** caused a significant ($p < 0.05$) dose-dependent reduction in mobility at

the three used doses. Similarly, injection of compound 2 equally reduced the mobility ($p < 0.05$) in test animals. On the other hand, treatment with bromazepam caused considerable sedative effect, and animals were almost immobile.

Listed in Table 2 are results related to the effect of isolated compounds **1** and **2** in traction test during various assessment times. Administration of compound **1** at various doses caused a significant muscle relaxant-like effect during various assessment times, and the effect was maximum after 90 min. In a similar way, compound **2** exhibited considerable effects during various times, and the effect was maximum after 90 min.

Table 1 Effect of isolated compounds 1 and 2 from *Salvia leriifolia* in open field test (sedative activity)

Sample	Dose (mg/kg)	No. of lines crossed	% effect
Normal saline water	10 ml/kg	129.1 ± 1.58	13.6
S7	10	67.2 ± 1.3*	26.19
	15	52.8 ± 1.3*	33.34
	20	35.8 ± 1.79**	49.16
S8	10	72.2 ± 3.67	24.37
	15	54.8 ± 2.24*	32.11
	20	36.4 ± 2.51**	48.35
Bromazepam	5	17.6 ± 1.67***	100

Values represent the percentage effect in the open field test after treatment in six mice with distilled water (10 ml/kg), compounds **1** and **2** (10, 15, and 20 mg/kg) or diazepam (0.25 mg/kg). Data presented as mean ± SEM ($n = 6$) *$p < 0.050$ and ** $p < 0.01$, ***$p < 0.001$ compared with controls.

The isolated compounds (**1** and **2**) were primarily docked into the $GABA_A$ receptor, which possesses properties like host unit. Then for qualitative investigation, the positions with maximum value for the protein contact energy were also designated. The results achieved from blind docking analysis of compounds (1 and 2) are listed in Fig. 1. The obtained results showed that the key stabilizing links for bromazepam are due to pi-pi interaction among Tyr62B, Phe200C as well as aromatic scaffold of the ligand, while the hydrophobic connections are with the similar residue Tyr62B, as well as hydrogen bond with Gln64B. The huge number of protein-ligand connections elucidates the reason that bromazepam interacts so strongly with $GABA_A$. Furthermore, docking results for compounds 1 and 2 revealed that both of them bind to the protein in the same area as bromazepam, and with hydrophobic inter- actions with Phe200C, Asp43B and Tyr62B, as well as with hydrogen bonds with Thr176B and Asn41B (compound **1**) and with Tyr205C (compound **2**).

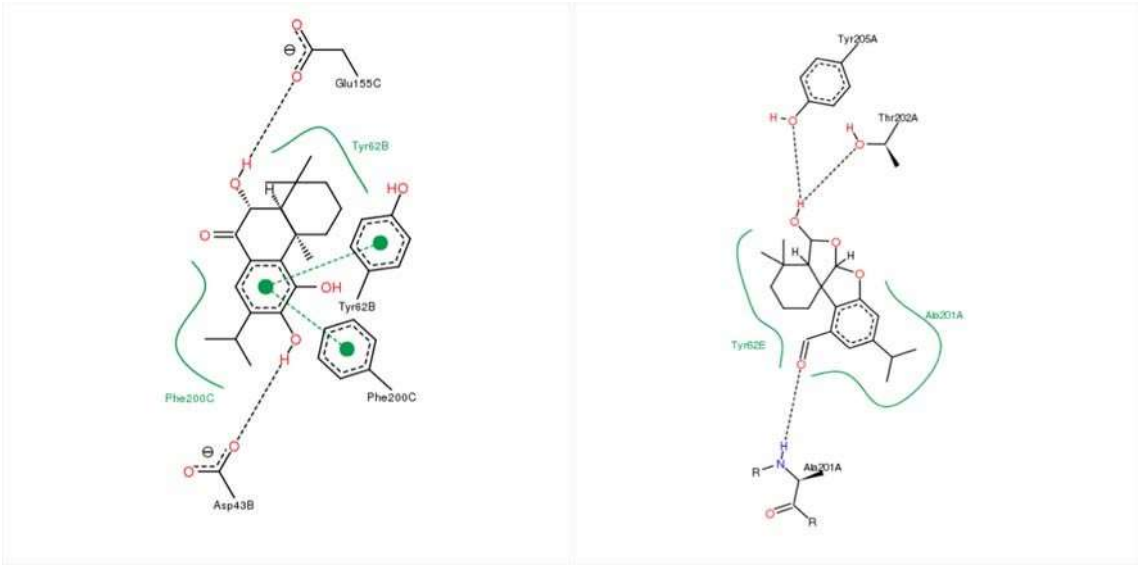
Table 2 Percent effect of isolated compounds 1 and 2 from *Salvia leriifolia* in traction test

Sample	Dose (mg/kg)	Traction test %		
		30 min	60 min	90 min
Normal saline	10 ml/kg	0 ± 0.00	0 ± 0.00	0 ± 0.00
S7	10	38.89 ± 0.58	44.44 ± 0.58	55.56 ± 3.58
	15	55.56 ± 0.58	44.44 ± 0.58	61.11 ± 4.00*
	20	61.11 ± 0.58	66.67 ± 0.58	77.78 ± 5.58**
S8	10	44.44 ± 0.58	50.00 ± 0.58	61.11 ± 4.58*
	15	61.11 ± 1.00	55.56 ± 0.58	72.22 ± 4.00**
	20	66.67 ± 0.58	77.78 ± 1.00	88.89 ± 5.58**
Diazepam	5	100 ± 0.00	100 ± 0.00	100 ± 0.00***

Values represent the percentage effect of mice ($n = 6$) in the traction test 30, 60, and 90 min after treatment with distilled water (10 ml/kg), compounds 1–2 (10, 15, and 20 mg/kg) or diazepam (0.25 mg/kg). Data presented as mean ± SEM ($n = 6$) *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with controls

Although there are not as many connections for compounds **1** and **2** as with bromazepam, there is, however, a higher shape complementarity of the two compounds with the binding site. Therefore, hydrogen bonds, hydrophobicity, and

shape complementarity allowed us to define the experimentally observed affinity of $GABA_A$ to these phytochemicals. Results from this study show excellent muscle relaxant and seductive effects of both tested compounds (**1** and **2**) from *S. leriifolia* in open field and traction tests, respectively.



1 **2**

Fig. 1 2D representation of docking results of compounds 1 and 2 into GABAA receptor. Dashed black lines represent hydrogen bonds, green dashed lines are pi-pi interactions, and green continuous lines are related with hydrophobic interactions

The open field screening test is one of the simplest *in vivo* screening protocols for sedative-like activity of test substances (Rauf et al. 2015, 2016). Our findings indicated that both isolated compounds 1 and 2 cause significant reduction in locomotion of test animals, suggesting the sedative-like effect of the compounds. Reduction in the frequency of crossing the field lines implied that these compounds interfere with the central nervous system of test animals.

The traction test is one of the commonly used in animal models to evaluate muscle relaxant-like effects of test trainings (Hosseinzadeh et al. 2003). Administration of pure com- pounds (1 and 2) exhibited promising relaxation of the skeleton muscle. The sedative and muscular relaxation properties of benzodiazepines (BDZ) such as bromazepam are generally recognized to interfere with the act of gamma aminobutyric acid ($GABA_A$), suggesting that BDZ is connected to the gam- ma subunit of the $GABA_A$ receptor. This process is accompanied with a structural alteration of the receptor along with a rise in $GABA_A$ receptor activity (Mele et al. 2019). Benzodiazepines (BDZ) connect to the gamma subunit of the GABA-A receptor. Their binding produces an allosteric modification of the receptor which results in an increase in GABA-A receptor activity. Benzodiazepines do not substitute for GABA, which connects the alpha subunit but increases the frequency of channel initial events principals to a rise in chloride ion conductance and inhibition of the act potential.

Thus, we can assume that our tested compounds exert their sedative and muscle relaxant effects through a mechanism similar to that of BDZ. However, further detailed studies are required to determine the exact mechanism of action of these compounds and their clinical applications.

Conclusion

It is concluded that compounds 1 and 2 isolated from *S. leriifolia* showed noticeable muscle relaxant and sedative effects and thus could be strong candidates in the search for novel and effective compounds for clinical use. However, additional work is necessary to establish the safety and efficacy of these compounds before their introduction into clinical trials on human subjects.

Authors' Contribution AH, AR, and TA-I performed the experimental part. MI, SA, HK, and B did English editing. JPC-C, HP-S performed the docking study. MIC and MSM supervised this project. MAS, YNM, and M-LB-K did statically analysis and give the final shape to this paper.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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