

## Effect of Ethanol Extract from Five Species of *Salvia* on the Spontaneous Contractile Activity of Isolated Rabbit Ileum

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### ABSTRACT

Nature has been recognized as a rich source of medicinal compounds for many years. The herb *Salvia* (Lamiaceae) is distributed all over West Bank different locations. In spite of the presence of several species of *Salvia*, only *S. fruticosa* has long been used locally by the native people to relief intestinal pains and diarrhea. Previous literature did not provide data considering the possibility of using other species of *Salvia* for the same purpose. The present study aimed to detect the effect of the ethanol extracts of five *Salvia* species on the spontaneous contractile activity of rabbit ileum using organ bath system. The investigated species were *S. dominica* L., *S. fruticosa* Mill., *S. judaica* Boiss., *S. lanigera* Poir. and *S. viridis* L. Ethanol extract from *S. fruticosa*, *S. dominica*, *S. judaica*, *S. viridis* and *S. lanigera* were prepared. They were obtained by soaking ten grams of each plant powder in 70 % ethanol for one week with intervals shaking for two days. Then the mixtures were centrifuged for 5 min at 5000 rpm. The effect of these extracts was studied on smooth muscle contractile activity from rabbit ileum. A 3cm piece of rabbit's ileum was suspended in 50 mL organ bath chamber filled with Tyrode's solution containing ((mM): 136 Sodium chloride (NaCl), 2.7 Potassium chloride (KCl), and 1.4 Calcium chloride dehydrate (CaCl<sub>2</sub> 2H<sub>2</sub>O), 0.5 Magnesium chloride Hexahydrate (MgCl<sub>2</sub> 6H<sub>2</sub>O), 11.9 Sodium Bicarbonate (NaHCO<sub>3</sub>), 0.42 Sodium dihydrogenorthophosphate (NaH<sub>2</sub>PO<sub>4</sub>) and 5.56 Glucose. The system was supplied continuously with 95% oxygen and 5% carbon dioxide and maintained at 37°C. One end of the isolated segment was tied to a holder at the bottom of the bath, while the other was connected to lever of kymograph on which the contractions were recorded with a pen. All the examined ethanol extracts) except *S. judaica*) produced concentration-dependent spasmolytic effect on the rabbit ileum, with the maximum was at 0.6 mg/ml concentration. All showed complete ileum contraction inhibition at 0.6 mg/ml concentration except *S. judaica*. In addition, the pretreatment of ileum with ethanol extract didn't affect the stimulatory responses of pilocarpine on the rabbit ileum. This observation could suggest that the spasmolytic effects of the ethanol extract were not mediated by cholinergic mechanism. This study could provide sound pharmacological basis for the medicinal use of *Salvia* species (Sage) in hyperactive gut disorders, such as abdominal colic and diarrhea. The recorded spasmolytic effect of the examined plant species might be the platform for their therapeutic effectiveness in the treatment of gastrointestinal disorders and future use in traditional medicine. Gastro-intestinal system

**KEYWORDS:** Traditional medicine Asia & Oceania, ileum, diarrhea, antispasmodic, alkaloids, *Salvia*, Lamiaceae, medicinal plants, plant extracts.

### CHEMICAL COMPOUNDS STUDIED IN THIS ARTICLE

Ethanol (PubChem CID: 702); Sodium chloride: NaCl (PubChem CID: 5234); Potassium chloride: KCl (PubChem CID: 4873); Calcium chloride dehydrate: CaCl<sub>2</sub> 2H<sub>2</sub>O (PubChem CID: 6093260); Magnesium dichloride hexahydrate: MgCl<sub>2</sub> 6H<sub>2</sub>O (PubChem CID: 25644); Sodium bicar-

bonate: NaHCO<sub>3</sub> (PubChem CID: 516892); Sodium dihydrogenorthophosphate: NaH<sub>2</sub>PO<sub>4</sub> (PubChem CID: 23672064); D-Glucose (PubChem CID: 53782692); Pilocarpine (PubChem CID: 5910).

The solvents and chemicals used were of analytical grade and purchased from Sigma-Aldrich Chem. (St. Louise, MI, USA). The stock solutions of these chemicals were pre-

pared by dissolving with distilled water. The solutions were prepared fresh on the day of experiments

## INTRODUCTION

*Salvia* species belong to the family Lamiaceae (Labiatae). *Salvia* (sage) is the largest and the most important genus of this family. High diversity in the secondary metabolites and their pharmacological effects were recorded in this genus [1]. *Salvia* genus has about 900 species, which spread throughout the world. It includes several ornamental, culinary and medicinal species [2]. The species of Sage were used traditionally to treat various conditions such as colic, diarrhea, common cold, cough, flu, liver sickness, bacterial infections, febrile attacks, sores in the body and abdominal trouble. In addition, they were used as a purgative. Also, they were recorded to be used for alimentary and cosmetic purposes [3- 5].

Several studies have concerned the influence of extracts taken from different *Salvia* species on smooth muscle contractions. Examination of the effect of fifteen different extracts from *S. officinalis*, *S. triloba* and *S. verbenaca* on guinea pig ileum was conducted. It revealed that *S. officinalis* and *S. triloba* extracts inhibit to various degree smooth muscle contractions induced by acetylcholine, histamine, serotonin and BaCl<sub>2</sub>. Whereas, *S. verbenaca* usually potentiate them [6]. Moreover, the effect of the organic and aqueous extracts of *S. fruticosa* on the spontaneous contractile activity of the rabbit ileum was detected [7]. In the former mentioned research, results showed that organic extracts inhibit the spontaneous activity of the rabbit ileum. While, the aqueous extracts showed no effect. The involvement of *Salvia* in the treatment of ileum troubles was supported by a study in which, *S. officinalis* crude extract possessed antidiarrheal and antispasmodic activities as it most probably was mediated through the dominant activation of voltage-dependent K<sup>+</sup> channels. The observed results provided sound pharmacological basis for *Salvia* species medicinal use in diarrhea and gut spasm [8]. Moreover, it was demonstrated that *S. miltiorrhiza* extracts cause ileum contraction through the Ca<sup>(2+)</sup>-calmodulin pathway [9]. A new secoisopimarane-

diterpenoid was extracted from *S. cinnabarina* which has non-specific spasmolytic activity. Its effect was on the contractions in the isolated guinea-pig ileum, which were induced by histamine, acetylcholine and barium chloride [10]. The evaluation of the effect of a standardized extract of *S. divinorum* on enteric cholinergic transmission in the guinea-pig ileum was conducted. It was concluded that *S. divinorum* depressed the enteric cholinergic transmission likely through activation of kappa-opioid receptors. This out finding may provide the pharmacological basis underlying its traditional anti-diarrheal use. Salvinorin A which is, the main active ingredient of *S. divinorum* might be the chemical ingredient responsible for this activity [11]. It was proved that Salvinorin A slows colonic motility *in vitro* and *in vivo* via influencing neurogenic ion transport. The specific regional action of Salvinorin A or its derivatives was the reason after the possibility of their use as drugs in the treatment of lower gastrointestinal disorders associated with increased gastrointestinal transit and diarrhea [12]. Accordingly, no previous inquiries considered the spasmolytic effect of *S. dominica* L., *S. judaica* Boiss., *S. lanigera* Poir. and *S. viridis* L. on rabbit ileum contraction. This lack of such information was the motivation behind this research. Therefore, an *in vitro* evaluation of the ethanol extracts influence of the former *Salvia* species on the contractile activity of an isolated rabbit ileum was carried out.

## MATERIALS AND METHOD

### Plant material

Aerial parts of *Salvia* species under study (*S. dominica*, *S. fruticosa*, *S. judaica*, *S. lanigera* and *S. viridis*) were collected from different locations in West Bank, Palestine. The plants species were classified and identified by Ghadeer Omar, Department of Biology and Biotechnology, An-Najah National University, Palestine. Representative plant specimens of the examined plant species were collected, pressed till drying, treated chemically, mounted on herbarium sheets and provided with voucher numbers (*S. dominica* 982, *S. fruticosa* 847, *S. judaica* 1367, *S. lanigera* 815 and *S. viridis* 642). Then they were deposited at An-Najah National Univer-

sity herbarium. The plant leaves were washed, air-dried, ground into powder using grinder and stored at room temperature until they were used.

### Extract Preparation

For ethanol extract preparation; ten grams of each plant powder were soaked in 70 % ethanol for one week with intervals shaking for two days. Then the mixtures were centrifuged for 5 min at 5000 rpm. The final concentrations of each extract was 100 mg/ml. The extracts were placed in a glass bottle and then stored at -20 °C up to used

### Animals

The research was conducted in accordance with the European Council Directive of November 24, 1986 (86/609/EEC). Male and female rabbits (1-2 kg, were used after a 24 h fasting with free access to water. The animals were scarified and the ilea were extracted.

### Antispasmodic activity Assay

The spasmolytic activity of the test plant extracts was investigated on an isolated rabbit ileum. The ileum was dissected out, immersed in Tyroide's solution and cleaned off the mesentery. Respective segments of 2-3cm long were suspended in 50 ml organ bath chamber filled with Tyrode's solution containing (mM): 136 Sodium chloride (NaCl), 2.7 Potassium chloride (KCl), and 1.4 Calcium chloride dehydrate ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ), 0.5 Magnesium chloride Hexahydrate ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ), 11.9 Sodium Bicarbonate ( $\text{NaHCO}_3$ ), 0.42 Sodium dihydrogenorthophosphate ( $\text{NaH}_2\text{PO}_4$ ) and 5.56 Glucose. The system was supplied continuously with 95% oxygen carbon dioxide and maintained at 37°C [13]. One end of the isolated segment was tied to a holder at the bottom of the bath, while the other end was connected to lever of kymograph on which the contractions were recorded with a pen. At the beginning of the experiment, the ileum segments were allowed to equilibrate for 30 min until it exhibited spontaneous rhythmic contractions allowing test-

ing of the relaxant (spasmolytic) activity directly without the use of any agonist. To study the effect of the ethanol extracts on ileum contraction, 0.2, 0.4 and 0.6 mg/ml concentrations were added to the rabbit isolated ileum. Ethanol solution was used as a control. Moreover, the ileum was subjected to high dose (1 mg/ml) of each extract for 10 min to ensure complete saturation of the receptors, followed by the addition of  $1.5 \times 10^{-4}$  M pilocarpine drug.

The experiment was conducted in triplicates using three different segments of ileum at different periods of time, using the same prepared extracts.

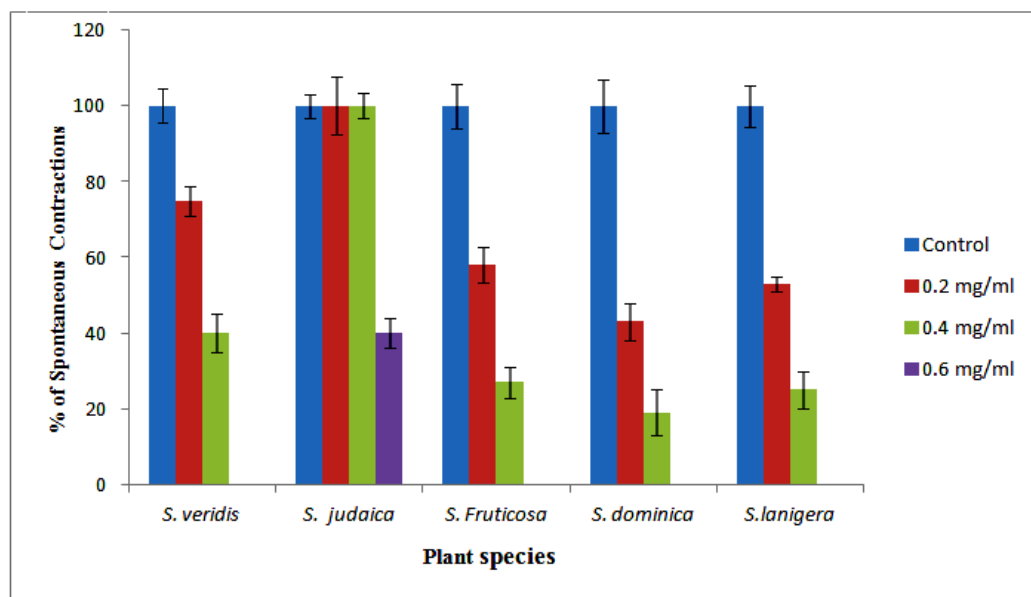
### STATISTICAL ANALYSIS

Results are presented as means  $\pm$  S.E.M. The data were analyzed by using the SPSS version 20.0 statistical software. The data were evaluated by ANOVA, Tukey test.  $P < 0.05$  was considered as significant.

### RESULTS

Ethanol extracts prepared from *S. dominica*, *S. fruticosa*, *S. judaica*, *S. lanigera* and *S. viridis* were examined for spasmolytic activity on the rabbit ileum. Effect of the ethanol extracts of *S. dominica*, *S. fruticosa*, *S. lanigera* and *S. viridis* (0.2, 0.4 and 0.6 mg/ml) on the contractile activity of the rabbit ileum exhibited a significant concentration-dependent spasmolytic effect on the rabbit ileum. *S. judaica* is an exception in which it shows significant spasmolytic effect at concentration of 0.6mg/ml (Fig. 1). Complete inhibition of contraction was induced by the extracts of *S. dominica*, *S. fruticosa*, *S. lanigera* and *S. viridis* at concentration 0.6mg/ml.

In addition, the obtained results indicated that there is no toxicity of the different concentrations of the ethanol extracts of all *Salvia* species under study. This was referred to the ileum return to the normal control contraction after being washed with the physiological solution.



**Figure (1):** Effect of the ethanol extracts (0.2, 0.4 and 0.6 mg/ ml) of the five *Salvia* species on the spontaneous contraction of isolated rabbit ileum.

One of the possible spasmolytic effect mechanisms of the studied plant species extracts was investigated. This aim was achieved by treating the rabbit ileum with high dose (1 mg/ml) ethanol extract for 10 min separately followed by pilocarpine drug  $1.5 \times 10^{-4}$  M (cholinergic muscarinic drug) was added. This drug agonizes the action of acetylcholine on Muscarinic cholinergic receptors. The recorded data indicated that the ethanol extracts from the *Salvia* species did not affect the contraction induced by pilocarpine, suggesting that cholinergic receptors most probably are not involved.

## DISCUSSION

Nature has been recognized as a rich source of medical compounds for hundreds to thousands of years. Today a wide range of drugs, which represent the cornerstones of modern pharmaceutical care, are either natural products or have been derived from them. Natural extracts have been proved to be useful in different human pathological conditions. It is estimated that about one third of currently marketed drugs are related to natural products [14, 15]. In this study, the effect of the ethanol extract from five *Salvia* species (*S. dominica*, *S. fruticosa*, *S. judaica*, *S. lanigera* and *S. viridis*) was examined on the

spontaneous contractile activity of rabbit ileum. The ethanol extracts of the five *Salvia* species showed spasmolytic effect on rabbit ileum. Flavonoids which are obtained from different medicinal plants, including *Salvia* species have been reported to have spasmolytic activity on isolated tissues like guinea-pig trachea and ileum [16]. Ethanol extract showed intense relaxation effect as all extracts from *S. dominica*, *S. fruticosa*, *S. lanigera* and *S. viridis* at concentration 0.6 mg/ml that produced complete inhibition of ileum contraction. This could be referred to that all of the identified components from plants with relaxation effects on smooth muscles, aromatic saturated organic compounds are most often obtained through initial ethanol extraction [17]. The extract from *S. judaica* was an exception compared with other species used in this study; it has spasmolytic effect only at concentration 0.6 mg/ml with about 60 percent inhibition of contraction compared to control. This could be due to different species of *Salvia* contain different content of flavonoids [30].

Acetylcholine, a well known neurotransmitter; regulates the peristaltic movements of the gut via contraction through muscarinic receptors [18]. Pilocarpine is a

para-symphathomimetic alkaloid obtained from the leaves of tropical American shrubs from the genus *Pilocarpus*. It is a non-selective muscarinic receptor agonist that agonized acetylcholine action on Muscarinic receptor [19]. Therefore, the involvements of the cholinergic mechanism in the spasmolytic effect of the plant extract were detected.

In this research, pretreatment of the ileum with ethanol extract didn't affect the stimulatory responses of pilocarpine on the rabbit ileum suggesting that the spasmolytic effects of extract were not mediated by cholinergic mechanisms. Several studies showed that the spasmolytic effect of medicinal plants is usually mediated through K<sup>+</sup> channel opening [20, 21] or Ca<sup>++</sup> channel blockade [22, 23, 24]. Thus, it is possible to speculate that extracts of *Salvia* species used in this study, might act through K<sup>+</sup> channel opening or Ca<sup>++</sup> channel blockade.

There is growing evidence that the spasmolytic effect of the plant extract is associated with the presence of phenolic compounds. Flavonoids are one of the most numerous and widespread group of phenolics in higher plants. Some of them inhibit the intestinal motility in vitro. Quercetin produces relaxation in ileum contracted by KCl [25]. Apigenin and luteolin inhibited the contractions of isolated intestine [26, 27]. These substances have been reported to exhibit calcium antagonist and anti-cholinergic activities [28, 21].

*Salvia* species contains a large amount of flavonoids and tanning materials such as; caffeic acid, chlorogenic acid, ellagic acid and gallic acid [29]. As a result, the medicinal properties of *Salvia* species could be mainly attributed to the presence of flavonoids [30].

## CONCLUSION AND RECOMMENDATION

In conclusion, the obtained data suggest that the crude extract of the examined five *Salvia* species possesses spasmolytic effect. This out finding provides sound pharmacological basis for the medicinal use of *Salvia* species (Sage) in hyperactive gut disorders, such as abdominal colic and diarrhea. The therapeutic effectiveness of this genus spe-

cies and their spasmolytic effect in the treatment of gastrointestinal disorders may be a good recommendation for their use in the traditional medicine. Further experimentation is needed in order to understand the precise mechanism of action in smooth muscles by the extracts. Also analysis of photochemical constituents of the five species is recommended, so the relation between the flavonoids percentage and the intestinal motility effect can be determined.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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