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RESEARCH ARTICLE

Biological Activities of *Salvia santolinifolia* Boiss. A Multifunctional Medicinal Plant

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Abstract: ***Background:*** *Salvia* species are used traditionally for treatment of many disorders all around the world. *Salvia santolinifolia* is a medicinal plant, traditionally used for treatment of inflammation, hypercholesterolemia, hemorrhoids and diarrhea.

Methods: In this study, antibacterial, antioxidant, toxicity, acetylcholinesterase and α-glucosidase inhibitory activities of several extracts of *S. santolinifolia* were evaluated. Total phenol and flavonoid contents of the extracts were also determined.

Results: The MeOH extract showed high antioxidant activity in DPPH scavenging and ferric reducing power assays. All samples exhibited high acetylcholinesterase (IC_{50} = 42-89 µg/ml) and α-glucosidase inhibitory activities (IC_{50} = 24-174 µg/ml). The MeOH extract showed great antibacterial and antifungal activities especially against *Bacillus cereus* and *Candida albicans* with MIC values from 10 to 200 µg/ml.

Conclusion: Our findings indicated that *S. santolinifolia* is a rich source of natural compounds with strong antimicrobial, antidiabetic and anti-Alzheimer activities. This plant has great potential for several applications as pharmaceuticals and functional foods.

Hassan Valizadeh

Keywords: *Salvia*, diabetes mellitus, alzheimer, antioxidant, antimicrobial, phenolic compounds.

1. INTRODUCTION

Plants have formed the basis of traditional medicine system for hundreds of years [1-4]. Folkloric medicine is a great source for discovery of new natural remedies [5-7] and the interest in Nature as a rich source of therapeutic compounds continues [8].

Salvia is an aromatic and medicinal genus and is characterized by occurrence of terpenoids [9, 10]. Moreover, it is a rich source of phenolic compounds some of which are unique to the genus [11, 12]. The genus *Salvia* produces rare terpenoids with unusual carbon skeletons [13, 14]. Several structurally interesting and bioactive sesterterpenoids and isoprenoids have been isolated from Iranian *Salvia* species in recent years [14-17].

S. santolinifolia Boiss grows wild in Pakistan, Afghanistan and south eastern of Iran, occurring in sandy and rocky areas [18, 19]. It is known as a medicinal plant. The seeds of the plant produce great amount of mucilage and are mixed with other medicinal herbs to making Ispaghol, a natural fiber, traditionally used for the treatment of hypercholesterolemia [20]. The aerial parts of *S. santolinifolia* are traditionally used as an anti-inflammatory herb [21]. It is used as a painkiller in hemorrhoids and diarrhea disorders [22]. Also, seeds of the plant are used for removing foreign bodies from eyes by the local people in Pakistan [20].

Traditional uses and biological effects of the genus prompted us for the investigation of *S. santolinifolia* for its potential in functional food, pharmaceutical and medical applications. To the best of our knowledge, biological properties and chemical composition of this plant have not been fully characterized yet. So, present study aims to assess the antioxidant, antimicrobial, α-glucosidase and acetylcholinesterase inhibitory activities and cytotoxicity of various extracts of the plant. Also, in order to have an insight on bioactive constituents, total phenolic and flavonoid contents of the extracts were determined.

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2. MATERIALS AND METHODS

2.1. Chemicals

6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, gentamicin, nystatin, Folin-Ciocalteu, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxy toluene (BHT), α-Glucosidase type I from Baker Yeast (EC 3.2.1.20), p-nitrophenyl-α-D-glucopyranose (*p*NPG), acarbose, podophyllotoxin, sea salt and ascorbic acid were purchased from Sigma-Aldrich (Germany). Dimethyl sulfoxide (DMSO), *n*-hexane, dichloromethane, methanol, ethanol, trichloroacetic acid, potassium phosphate, aluminum chloride, 4-nitrophenyl-α-D-glucopyranoside and $K_3Fe(CN)_6$ were purchased from Merck (Germany).

2.2. Plant Material

The aerial parts of *S. santolinifolia* were collected during the flowering stage in April 2014 from wild natural environment (Zahedan, southeastern of Iran). The plant was identified by Mr. Shahram Bahadori, taxonomist, and a voucher specimen was deposited at the herbarium of Urmia School of Pharmacy.

2.3. Extraction

The powdered aerial parts of *S. santolinifolia* (50 g) were extracted successively with *n*-hexane (3 × 300 ml), dichloromethane (3 × 300 ml) and methanol (3 × 300 ml) by maceration at room temperature. The solvent of the extracts was removed using rotary evaporator at 40 °C to afford crude *n*-hexane (1.5 g), dichloromethane (2.2 g) and methanol (4.5 g) extracts.

2.4. Determination of Total Phenolic Content

Total phenolic content of the extracts was determined spectrophotometrically using the Folin-Ciocalteu method with some modifications [23]. Briefly, 20 µl of extracts solution in methanol (2 mg/ml) were mixed with 100 µl of 1:10 Folin-Ciocalteu reagent. After 6 minutes in the dark, 80 µl of sodium carbonate (7.5%) was added into the mixture. The absorbance was measured at 740 nm after 2 h of incubation in the dark at the room temperature. The total phenolic content of the extracts were expressed as milligrams of gallic acid equivalents per gram of dry weight of extracts (mg GAE/g DW) through the calibration curve with gallic acid. The calibration curve range was 1-1000 mg/L. All samples were analyzed in triplicates.

2.5. Determination of Total Flavonoid Content

The aluminum chloride colorimetric method was used to determine the total content of flavonoids [24]. 20 µl of extracts or a standard solution of quercetin (1 to 200 µg/ml) was diluted with 60 µl of methanol and 10 µl of 5% $AlCl_3$. Subsequently, 10 µl of 0.5 M potassium acetate was added to the mixture and the total volume was made up to 200 µl by distilled water. The solution was mixed well and the absorbance was read at 415 nm after 30 minutes. All tests were carried out in triplicate, and mean values of flavonoid content are expressed as milligrams of quercetin equivalents per gram of dry weight of extracts calculated according to the standard calibration curve.

2.6. DPPH Free Radical Scavenging Assay

The free radical scavenging activity was determined from the bleaching of purple-colored solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) [25]. 20 µl of samples or standard antioxidant, butylated hydroxy toluene (BHT), dissolved in methanol in several concentrations, were mixed with 180 µl of DPPH solution (0.1 mM). Discoloration of the mixtures was measured at 517 nm after 30 minutes using a microplate reader spectrophotometer (BioTek XS2 model). BHT was employed as the reference. Inhibition of DPPH in percent (I %) was calculated as given below:

$$I\ \% = [(A_0 - A_s) / A_0] \times 100$$

Where A_0 is the absorbance of the control reaction (containing all reagents except the test sample), and A_s is the absorbance of the extracts/reference. The IC_{50} values (concentration providing 50% inhibition) were also calculated using a calibration curve in the linear range by plotting the extract concentration *vs* the corresponding scavenging effect. All the assays were run in triplicates and the results were expressed as average values with the standard error of the mean (SEM).

2.7. Ferric Reducing Power Assay

The reducing capacity of various extracts of the plant was determined according to the method which was described in the literature [26]. Briefly, 1 ml of various sample solutions at different concentrations (0.25, 0.5, 1 and 1.5 mg/ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and $K_3Fe(CN)_6$ (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min and then a portion (2.5 ml) of trichloroacetic acid solution (10%) was added to the mixture, which was then centrifuged at 10000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with deionized water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The ascorbic acid was used as positive control and results were expressed as observed absorbance values.

2.8. α-Glucosidase Inhibition Assay

The α-Glucosidase inhibitory activity (AGI) was assessed according to a previously reported procedure [7]. In brief, 20 µl of 0.5 unit/ml α-glucosidase enzyme solution was mixed with 120 µl of 100 mM potassium phosphate buffer (pH = 6.9) and 10 µl of the extracts (25, 50, 100 and 200 µg/ml for *n*-hexane and dichloromethane extracts and 5, 10, 25 and 50 µg/ml for methanol extract) or acarbose as positive control (5, 10, 25 and 50 µg/ml). The mixtures were incubated at 37°C for 15 min and then enzymatic reaction was initiated by adding 20 µl of 5 mM 4-nitrophenyl-α-D-glucopyranoside (*p*NPG) in buffer. The plates were incubated at 37°C for another 15 min and the reaction was stopped by addition of 80 µl sodium carbonate solution (0.2 M). Finally, the absorbance of 4-nitrophenol released from *p*NPG was measured at 405 nm. The system without α-glucosidase was used as blank for correcting the background absorbance. The increasing of absorbance was compared with that of the control (buffer instead of sample solution) to calculate the inhibitory activity. IC_{50} values were also calcu-

lated and expressed as mean value ± SEM. The inhibition rate of the samples on α-glucosidase was calculated by the following formula:

% Inhibition = [(Absorbance $_{control}$ − Absorbance $_{sample}$)/Absorbance $_{control}$] × 100

2.9. Acetylcholinesterase Inhibition Assay

Acetylcholinesterase inhibitory activity of the extracts were spectrophotometrically determined with some modifications using a Shimadzu spectrophotometer (2550 UV/Vis) [27]. Briefly, 500 µl of sodium phosphate buffer (100 mM, pH = 8.0), 150 µl of DTNB (3.5 mM), 150 µl of substrate (acetylthiocholine Iodide) and 150 µl inhibitor solution were added into the reaction mixture in a 1 ml cell and incubated for 15 min at 37ºC. Thereafter, the reaction was started by addition of 50 µl of enzyme. After immediate mixing of the reaction mixture, the changing of the absorbance was measured at 412 nm for 10 min. The inhibition rate of the samples on acetylcholinesterase was calculated by the following formula:

Inhibition percentage = $[(A_c - A_s)/ A_c] \times 100$

The IC_{50} values were calculated from inhibition curves (inhibitor concentration vs. percent of inhibition). Galantamine was used as the standard drug and results were expressed as mean ± SEM of three independent experiments.

2.10. Antimicrobial Activity Assays

Antimicrobial activity of the extracts of the plant were tested against following microorganisms: *Bacillus cereus* (PTCC 1015), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (PTCC 1399), *Aspergillus niger* (PTCC 5012) and *Candida albicans* (PTCC 5027). Bacterial strains were cultured overnight at 37°C in Mueller Hinton agar and fungi were cultured overnight at 30°C in Sabouraud dextrose agar. All strains were obtained from the Pasteur Institute of Iran (IPI).

2.10.1. Disc Diffusion Method

The agar disc diffusion method was employed for the determination of antimicrobial activity of extracts of the plant [28]. Briefly, about 100 µl of the tested microorganisms suspensions, adjusted to 10^6-10^8 CFU/ml were spread on the solid media plates. The dried extracts were dissolved in dimethylsulfoxide (DMSO) at concentration of 400 µg/ml and filtered by 0.45 µm Millipore filters for sterilization. The paper discs (6 mm in diameter) impregnated with 10 µl of the extract solution, were placed on the inoculated agar. DMSO was used as negative control. These plates were incubated for 24 h at 37°C for bacterial strains and 48 h at 30°C for the yeasts. After the incubation period, the diameter of inhibition zone (IZ) was measured in mm. Gentamicin (10 µg/disc) and nystatin (50 IU) were used as positive controls for bacteria and fungi, respectively. All the assays were performed in triplicate and expressed as average values ± SEM.

2.10.2. Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of various extracts of the plant were determined through micro-well dilution assay method [29]. The inoculants of the microbial strains were prepared from freshly cultured bacteria that were adjusted to 0.5 McFarland standard turbidity. Serial dilutions of the extracts were made in a concentration range from 25 to 1000 µg/ml in 96-well plates, containing Mueller-Hinton broth for bacterial strains and Sabouraud dextrose broth for yeast. Gentamicin (for bacteria) and nystatin (for fungi) were used as standard drugs for positive control in conditions identical to test materials. The plates were covered with sterile plate sealers and then incubated at 37°C under normal atmospheric condition for 24 h for bacterial strains and at 30°C for 48 h for yeasts. The MIC value was considered as the lowest concentration of the sample required for inhibiting the growth of microorganisms.

2.11. *Artemia salina* Larva Toxicity

The experiments were carried out according to the method published previously with some modifications [30]. Brine shrimp (*Artemia salina*) eggs were allowed to hatch in a flask containing seawater (3.8% w/v salt in distilled water) for 48 h at 28°C under constant aeration. The plant samples were prepared in DMSO. Ten larvae were collected with a pipette and added to the two fold serially diluted solutions (15.6-1000 µg/ml extract) in the test tubes. After 48 h, a magnifying glass was used to count the number of killed larvae and the mortality percentage was calculated. The triplicate mean of percentage mortality was plotted against the concentrations logarithm using Microsoft Excel 2013 software. Equation and regression appeared on the graph, and LC_{50} values were determined from the linear equation by taking the antilogarithm. Podophyllotoxin was used as positive control, and pure DMSO as untreated control. Final DMSO concentration was 1%.

2.12. Statistical Analysis

The experiments were carried out in three or four replications. The results were expressed as mean ± SEM. Statistical comparisons were estimated by one-way ANOVA followed by Duncan's post-hoc test for multiple comparisons with control. Statistical analyses were performed using SPSS 16.0 software. A value of $p < 0.05$ was considered to indicate statistical significance.

3. RESULTS AND DISCUSSION

3.1. Total Phenolics and Flavonoids

Total phenol and flavonoid contents of *S. santolinifolia* extracts were determined according to the equations as gallic acid and quercetin equivalents (mg/g extract), respectively (y = 0.0095 × gallic acid (mg) + 0.1848, r^2 = 0.998 and y = 0.0044 × quercetin (mg) + 0.0533, r^2 = 0.999). The total phenolic amounts of *n*-hexane, dichloromethane and methanol extracts of *S. santolinifolia* were determined by Folin-Ciocalteu method and are listed in Table **1**. The TPC showed great differences in various extracts ranging from 19.3 to 284.1 mg GAE/g dry extract. The highest amount of phenolic contents was found in the MeOH extract followed by DCM and *n*-Hexane extracts (Fig. **1**).

The same trend was observed in the determination of the total flavonoid contents (Table **1**). The greatest amount of these compounds was found in the MeOH extract and the

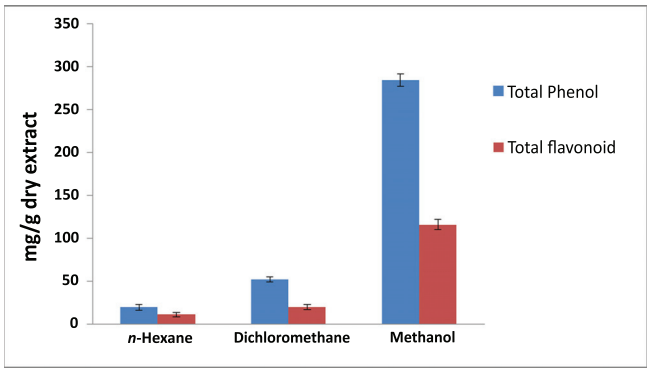

Fig. (1). Total phenolic and total flavonoid contents of *S. santolinifolia*.

values ranged from 10.7 to 115.8 mg QE/g dry extract. It was detected that TPC values were higher than TFC values in all of the extracts (Fig. **1**).

The amounts of phenolic and flavonoid contents, which were found in this study for the different extracts of *S. santolinifolia*, were higher than previous published studies. In a recent study the measurement of total phenol and flavonoid contents of nine *Salvia* species revealed the amount of 5.1-42.5 mg GAE/g dry extract and 2.2-32.2 mg catechin equivalents/g of dried extract, respectively [31]. In another study, TPCs were obtained as 12.4-30.3 mg equivalent gallic acid in 1 g dried plant for 80% methanol extracts of eleven *Salvia* plants [32]. Moreover, lower values for total phenolic amounts of twelve different extracts of *Salvia verbenaca* were reported the maximum level of which was 190.27 mg gallic acid equivalent (GAE)/g [33]. Also, total phenolic and flavonoid contents of the crude methanolic extract of *S. santolinifolia* (collected from Hormozgan province of Iran) were measured as 93.3 mg gallic acid/g dry extract and 148.9 mg catechin/g dry extract, respectively [34].

3.2. Antioxidant Potential

During the last decades it has been demonstrated that oxidative stress is considered to play a vital role in a wide range of human diseases [35]. Oxidative stress is defined as the imbalance between the generation of oxidants like reactive oxygen and nitrogen species (ROS/RNS) and endogenous antioxidants defenses [36]. However, the antioxidant compounds are characterized by the ability of reaction with free radicals and consequently, slowing and prevention of oxidation process. The search for new antioxidant agents, especially from natural resources has given rise, because most common synthetic antioxidants, such as BHT and BHA are suspected to be harmful to human health. Hydrogen atom transfer, single electron transfer and metal chelation are three main mechanisms through which the antioxidants counteract the oxidative process [37].

Antioxidant potential could not be described by a single method. For this reason the antioxidant activities were assayed using two different methods including DPPH radical scavenging activity as well as Fe reducing power assay which are fast, repeatable and reliable methods [1].

3.2.1. DPPH Radical Scavenging

The radical scavenging antioxidants react with active free radicals via two mechanisms: first donating hydrogen atom and second electron donating followed by proton transfer [38]. DPPH$^{\bullet}$ radicals scavenging assay is a simple and widely used method for evaluation of antioxidant potential of natural compounds [39].

DPPH radical scavenging activity of methanol, dichloromethane and *n*-hexane extracts of *S. santolinifolia* at different concentrations (0.05-4 mg/ml) were determined. In all of the tested samples a concentration dependent scavenging activity were demonstrated (Fig. **2**). As shown in Table **1** the MeOH extract of *S. santolinifolia* exhibited the strongest antiradical activity with IC_{50} value of 154.7 µg/ml. In accordance with this result the efficacy of 80% methanolic extract of *S. santolinifolia* to inhibit the DPPH free radicals, has been reported (IC_{50} value of 117 µg/ml) [32]. In addition, comparison of the activity obtained in this study with the data reported in the literature, revealed that methanolic extract of *S. santolinifolia* exhibits similar or stronger radical scavenging activity than studied eleven *Salvia* species [32]. This considerable DPPH radical scavenging abilities of methanolic extracts seem to be attributed to the high amount of phenolic and flavonoid compounds, which could be the electron and hydrogen donor and consequently can inactive free radicals and terminate radical chain reactions. Antioxidant potential of medicinal plants could be explained by their phenolic compounds [40]. Total phenolic and flavonoid contents of the extracts were correlated with their DPPH antiradical activity ($r^2 = 0.982$ and $r^2 = 0.972$, respectively). Compared with the synthetic antioxidant BHT (64 µg/ml), the DPPH radical scavenging activity of the non-polar extracts (*n*-hexane and dichloromethane) were nonsignificant. The IC_{50} values of non-polar extracts are higher than 500 µg/ml.

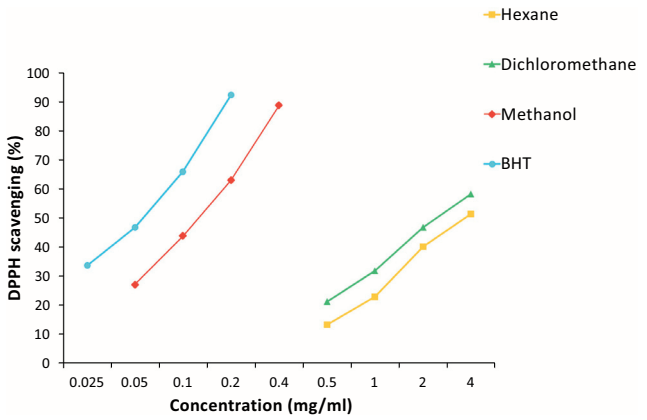

Fig. (2). DPPH radical scavenging activity of extracts of *S. santolinifolia*.

3.2.2. Reducing Power

The reducing potential of natural compounds, essential oils and various extracts derived from plants can be related to their abilities in electron transfer and can be used as a significant indicator of their antioxidant potency. In this study, the ferric reducing antioxidant power (FRAP) method was used to examine the reducing potential of *S. santolinifoli*a. In this assay, the amount of conversion of Fe^{3+} cyanide com-

Table 1. Antioxidant capacity in DPPH assay (IC_{50}), slop of trend line in reducing power assay (SRP), total phenolic content (TPC), total flavonoid content (TFC), α-glucosidase (AGI) and acetylcholinesterase (AChE) inhibitory activity and brine shrimp lethality assay (BSLA) of the extracts from Salvia santolinifolia[A].

Assay	*n*-Hexane	DCM	MeOH	Standard
DPPH (IC_{50} µg/ml)	na [B]	na	154.7 ± 8.4	64 ± 4.6
SRP	0.45 ± 0.06 ab	0.40 ± 0.10 a	0.59 ± 0.06 b	1.06 ± 0.13 c
TPC (mg GAE/g)	19.3 ± 3.3 a	52.0 ± 3.1 b	284.1 ± 6.9 c	- [C]
TFC (mg QE/g)	10.7 ± 2.8 a	19.5 ± 3.0 a	115.8 ± 5.9 b	-
AGI (IC_{50} µg/ml)	175.0 ± 3.0 a	116.5 ± 4.0 b	24.4 ± 1.3 c	17.1 ± 0.9 d
AChE (IC_{50} µg/ml)	67.6 ± 2.4 b	42.4 ± 2.1 c	89.7 ± 3.6 b	10.2 ± 0.4 a
BSLA (LC_{50} µg/ml)	75 ± 2.3 b	465 ± 12.6 c	350 ± 7.2 d	40 ± 1.5 b

[A] Values expressed are mean ± SEM of three replications; data marked with different letters indicate significant difference ($p < 0.05$). [B] not active. [C] not tested.

plex to the ferrous form was measured spectroscopically, via the change of the yellow color of the test solution to green and blue. Higher absorbance of the reaction mixture at 700 nm indicated increased reducing capability of the sample. Reducing power of the extracts of the plant was increased in a dose dependent manner (Fig. **3**). The order of decreasing potency in ferric reducing power of tested samples is MeOH > *n*-Hexane > DCM. The linear coefficient (r^2) of determination for extracts were between 0.985 and 0.995. Also, slopes of the trend lines (SRP) were calculated for all the samples that is a clearly indicator for ferric reducing power of the tested samples (Table **1**). The methanol extract of *S. santolinifoli*a exhibited the highest reducing power. Moreover, the activity of non-polar extracts was moderate in comparison with ascorbic acid, as a standard antioxidant (Fig. **3**).

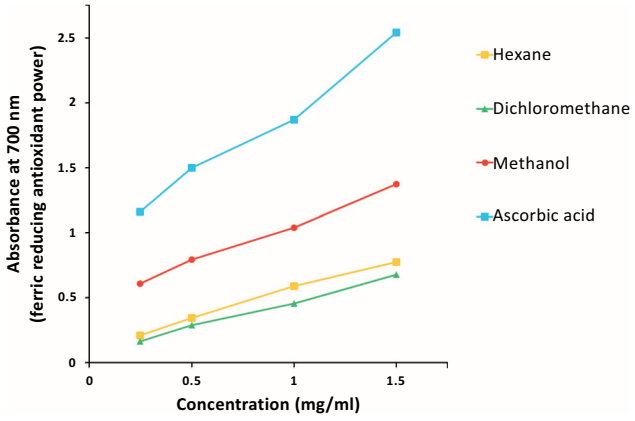

Fig. (3). Ferric reducing antioxidant power of extracts of *S. santolinifolia.*

3.3. Enzyme Inhibitory Activity

3.3.1. α-Glucosidase Inhibitory Activity

In the present work, the α-glucosidase inhibitory activities of the extracts of *S. santolinifolia* were determined (Table **1**). α-Glucosidase inhibitors are used for the treatment of metabolic disorders like diabetes. Our findings revealed that all of the tested extracts showed moderate to high α-glucosidase inhibition with IC_{50} values ranged from 24.4 to 175.0 µg/ml (Fig. **4**). Acarbose (IC_{50} = 17.1 µg/ml) was used as the positive control. The α-glucosidase inhibitory activity of tested extracts increased with the increment of sample concentrations (Fig. **4**). It is a worthy of note that a wide

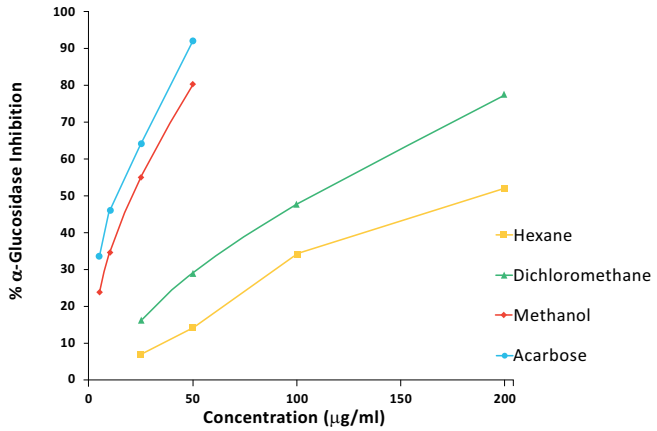

Fig. (4). α-Glucosidase inhibitory activity of extracts of *S. santolinifolia*.

range of natural and synthetic compounds like carbohydrates, flavonoids, steroids, terpenoids etc., have demonstrated to be α-glucosidase inhibitor [41-43]. In a previous study, an activity guided isolation of methanol extract of *Polygonum hyrcanicum* resulted in purification of thirteen phenolic compounds as strong α-glucosidase inhibitors [44]. In another work three flavonoids isolated from *Salvia chloroleuca* exhibited strong α-glucosidase inhibitory capacity [41]. Our findings showed that *S. santolinifolia* has high α-glucosidase inhibitory activity in comparison with previous studies. For example IC_{50} value of 200 µg/ml was reported for α-glucosidase inhibitory activity of methanolic extract of *Salvia acetabulosa* [45]. A very good correlation between α-glucosidase inhibitory effect and phenolic content (r^2 = 0.923) proved that phenolic compounds could be the major natural products in this plant responsible for enzyme inhibitory activity (Table **4**). There is no scientific evidence in the literature for the α-glucosidase inhibitory effect of this plant. Based on the obtained data, this plant could be a good

candidate for alternative and/or complementary medicine for diabetic patients. However, additional studies do need to determine the other sites of this pharmacological activity, the bioactive components involved and also any toxicological effects.

3.3.2. Acetylcholinesterase Inhibitory Activity

Alzheimer's disease (AD) is one of the major health problems of modern countries. AD leads to the loss of memory and finally, to death. The inhibition of cholinesterases (AChE and BChE) is a therapeutic strategy for treatment of AD. Common cholinesterase inhibitors are alkaloid natural products but they have some side effects and potentially are toxic [46]. Therefore, design and discovery of novel cholinesterase inhibitors with more activity and less toxicity is warranted.

Several *Salvia* species have been used to enhance memory in European traditional medicine [47]. Also, some *Salvia* plants have been reported as cholinesterase inhibitors [48, 49]. As mentioned previously, the genus *Salvia* is rich in terpenoids and there are several reports in the literature which have shown the neuropharmacological properties of *Salvia* diterpenes [50]. In this work, we evaluated the anticholinesterase potential of the extracts of *S. santolinifolia*. Results could be seen in Table **1**.

3.4. Antimicrobial Properties

There are hundreds studies on antimicrobial activities of medicinal plants [51, 52]. But this is the first report on antimicrobial activities of *S. santolinifolia* against human pathogenic microorganisms. In this study, the antimicrobial activity of the extracts of *S. santolinifolia* were evaluated against the following bacteria and fungi: Gram positive *Bacillus cereus* and *Staphylococcus aureus*, Gram negative *Escherichia coli* and *Pseudomonas aeruginosa*, fungi *Candida albicans* and *Aspergillus niger*. Antimicrobial activity at 400 µg/ml were evaluated by agar well diffusion method (Table **2**). The diameters of inhibition zones ranged from 8.8 to 33.7 mm including the diameter of paper disc (6 mm). Findings of the antimicrobial activity according to the disc diffusion method, demonstrated that various extracts of *S. santolinifolia* have moderate to high antimicrobial potential against Gram positive bacteria. *Pseudomonas aeruginosa* was resistant and just the MeOH extract could inhibit its growth moderately. The order of decreasing anti-fungal inhibitory activity of samples is MeOH > *n*-Hexane > DCM. From Gram positive bacteria, *B. cereus* was the most sensitive with inhibition zone ranged from 23.8 to 33.7 mm. From Gram negative bacteria, *E. coli* showed moderate sensitivity with inhibition zone ranged from 8.8 to 16.2 mm. Minimum inhibitory concentrations (MICs) were also determined and the above order was observed (Table **3**). The MIC values ranged from 10 to 1000 µg/ml. Gentamicin and nystatin were used as standard antibiotics in antibacterial and anti-fungal assays, respectively. MeOH extract showed an equal activity with Gentamicin against *B. cereus* with MIC value of 10 µg/ml.

The antibacterial activity of three *Salvia* species including *S. multicaulis S. sclarea* and *S. verticillata* have been evaluated [53]. The results showed diameters of inhibition zones ranging from 9 to 19 mm for eleven tested microorganisms. Another work reported MICs for some *Salvia* species ranging from 300 to 10000 µg/ml [32]. Diameters of inhibition zones have been reported for *Salvia tomentosa* ranging from 13 to 21 mm against seven bacterial strains [54]. In comparison, the extracts of *S. santolinifolia* have similar or stronger antimicrobial activities than those species reported in the mentioned literature. The existence of very good agreement between antibacterial activities (MICs and IZ) and phytochemical contents (TPC and TFC) of the plant (Table **4**) indicated that phenolic and flavonoid compounds have an important role in antimicrobial activity. Also terpenoids which are widely found in *Salvia* species could be responsible for antimicrobial activities. For example some diterpenoids from *Salvia adenophora* exhibited MIC values ranging from 4 to 128 µg/ml against several Gram-positive and Gram-negative bacteria [55].

Table 2. Antimicrobial activity of the extracts from *Salvia santolinifolia* at 400 µg/ml concentration by agar well diffusion method.

Microorganisms	Inhibition Zone (mm)				
	***n*-Hexane**	**DCM**	**MeOH**	**Gentamicin**	**Nystatin**
Gram-positive					
Bacillus cereus	25.2 ± 1.8	25.3 ± 1.3	33.7 ± 2.3	34.7 ± 2.1	nt[A]
Staphylococcus aureus	24.6 ± 1.2	30.5 ± 2.9	27.9 ± 2.5	31.8 ± 1.1	nt
Gram-negative					
Pseudomonas aeruginosa	na [B]	na	13.8 ± 1.3	28.6 ± 1.9	nt
Escherichia coli	8.8 ± 0.7	12.7 ± 0.6	16.2 ± 1.4	23.8 ± 1.3	nt
Fungi					
Aspergillus niger	na	na	18.4 ± 1.8	nt	24.2 ± 1.2
Candida albicans	9.9 ± 1.2	9.6 ± 0.8	21.7 ± 1.1	nt	27.0 ± 1.4

[A] not tested. [B] not active.

Table 3. Minimum Inhibitory Concentrations (MICs) of the extracts from *Salvia santolinifolia*.

Microorganisms	MIC (µg/ml)				
	***n*-Hexane**	**DCM**	**MeOH**	**Gentamicin**	**Nystatin**
Gram-positive					
Bacillus cereus	100	100	10	10	nt[A]
Staphylococcus aureus	200	25	50	10	nt
Gram-negative					
Pseudomonas aeruginosa	>1000	>1000	>1000	50	nt
Escherichia coli	>1000	>1000	800	25	nt
Fungi					
Aspergillus niger	>1000	>1000	800	nt	50
Candida albicans	>1000	>1000	200	nt	50

[A] not tested.

Table 4. Correlation coefficients of biological activities, total phenolic and total flavonoid content of the extracts from *Salvia santolinifolia.*

	DPPH	TFC	TPC	FRAP[A]	AGI	AChE	IZ[B]	MIC[B]	BSLA
DPPH	1	-0.972	-0.982	-0.797	0.977	-	-0.946	0.942	0.2104
TFC		1	0.999	0.914	-0.901	0.646	0.996	-0.994	0.092
TPC			1	0.891	-0.923	0.609	0.989	-0.987	0.115
FRAP				1	-0.660	-0.91	0.947	-0.951	9×10^{-5}
AGI					1	0.332	-0.859	0.852	0.3453
AChE						1	-0.707	-0.716	0.101
IZ							1	0.999	0.0578
MIC								1	0.0531
BSLA									1

[A] Absorbance values related to concentration of 200 µg/ml were used for calculations. [B] Values related to *Bacillus cereus* were used for calculations.

3.5. General Toxicity

Medicinal plants are widely investigating for the discovery of anticancer natural drugs [56, 57]. The genus *Salvia* have been studied for its cytotoxic activities and several bioactive terpenoids have been isolated from Iranian *Salvia* species [10, 58]. Also, several Lamiaceae plants have been studied for their cytotoxicity [59]. Brine shrimp lethality assay could be used for the screening of natural and synthetic compounds for cytotoxic agents. In the present study, the LC_{50} values of extracts were determined against brine shrimp larvae (*Artemia salina*). Values were expressed as an average of triplicates ± SEM and the results are summarized in Table **1**. The *n*-hexane extract showed LC_{50} value of 75 µg/ml and maximum mortality (100%) was observed at 200 µg/ml. So, it could be considered highly toxic. DCM and MeOH extracts demonstrated low toxicity against *Artemia salina* larvae compared to the positive control, podophyllotoxin (LC_{50} = 40 µg/ml). The mortality rate of brine shrimp larva was found to be concentration dependent. In general, brine shrimp cytotoxicity activity of crude extracts with LC_{50} values less than 1000 µg/ml are considered to be active [30]. In comparison with previous studies *S. santolinifolia* exhibited moderate to high brine shrimp toxicity. LC_{50} values have been reported for eight crude extract of five *Salvia* species ranging from 128 to 291 µg/ml [60]. The crude acetone extract of *S. moorcraftiana* had no activity in brine shrimp cytotoxicity bioassay [61]. Diterpenes isolated from *Salvia* species have exhibited various biological activities, particularly cytotoxic and anti-proliferative properties [62]. Accordingly, diterpenoid compounds may be the responsible bioactive metabolites in this assay. To the best of our knowledge, this is the first report on brine shrimp toxicity of the extracts of *S. santolinifolia.* Although, more bioassays against human cancer cell lines are necessary for evaluation of cytotoxic activity of *S. santolinifolia*.

CONCLUSION

The extracts of *S. santolinifolia* showed high acetylcholinesterase and α-glucosidase inhibitory activities. Also,

some of the tested samples exhibited moderate to high antimicrobial activity against the selected microorganisms. The *n*-hexane extract exhibited high cytotoxicity and MeOH extract showed the strongest activity in antioxidant assays and had high amounts of phenolic and flavonoid compounds. Our findings indicated that *S. santolinifolia* is a multifunctional medicinal plant and is a valuable source of natural compounds. Accordingly, *S. santolinifolia* could be considered for some applications in food and pharmaceutical industries.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest concerning this article.

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