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Assessment of phytochemicals, antioxidant, antimicrobial and cytotoxic properties of *Salvia chudaei* Batt. et Trab. endemic medicinal plant from Algeria

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Abstract
The hydromethanolic extract of aerial parts of endemic *Salvia chudaei* Batt. et Trab. and its fractions were investigated for their phytochemical screening, total phenolic and flavonoid contents, antioxidant, antimicrobial and cytotoxic activities. For phytochemical screening, some common and available standard tests were done. Phytochemical screening revealed the presence of anthraquinones, triterpenes, saponins, flavonoids, tannins, *O*-heterosides, *C*-heterosides. Total phenolic and flavonoid contents of the extracts varied between 25.15-537.58 mg GAE/g extract and 1.43 - 4.68 mg QE/g extract, respectively. *In-vitro* antioxidant activities were performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reducing power, β-carotene–linoleic acid tests. The results of activity tests were compared with standards such as butylated hydroxytoluene (BHT), ascorbic acid and α- tocopherol. The results indicated that diethyl ether fraction exhibited stronger antioxidant activities than hydromethanolic crude extract and other fractions. Antimicrobial activity was examined against 9 bacteria and one yeast. Only two bacterial strains (*Salmonella enterica* and *Klebsiella pneumonia*) were not inhibited by plants extracts, and diethyl ether fraction was generally more active than others. Hydromethanolic extract was subjected to brine shrimp lethality bioassay for possible cytotoxicity. Concentration dependent increment of brine shrimp nauplii mortality caused by the extract was indicative of the presence of cytotoxic constituents in this extract.

Key words*:* *Salvia chudaei*, Antioxidant activity, Antimicrobial activity, Cytotoxic activity, phytochemical screening.

Introduction
Since ancient times, medicinal plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternatives remedies for the treatment of various diseases caused by oxidative stress, bacterial and/or viral infection; and for the preservation of food from lipid oxidation and microbial contamination. Plant products are rich sources of a variety of biologically active compounds, mainly phenolics, and these phytochemicals have been known to exhibit different biological properties [1, 2].
The genus *Salvia*, with about 700 species, is one of the largest members of the Lamiaceae family, and is widely distributed in various regions of the world. Some members of this genus are of economic importance since they have been used as food spices to flavor meats such as pork, sausage and poultry or flavouring agents in perfumery and cosmetics [3].
Since antiquity, *Salvia* species have been well known plants and widely used in folk medicines as antituberculosis, antibronchitis, antipyretic, antirheumatic, insecticidal, cold improver, sexual enhancer, carminative, wound healer, mental and nervous system elevator [3-5]. Modern scientific investigations have also confirmed several biological activities such as antimicrobial, anti-inflammatory, antioxidant, gastroprotective, antimutagenic, antituberculous, cardiovascular, liver protective, anticancer, neuroprotective, antiproliferative, anti-angiogenic, anti-nociceptive and hypoglycemic effects [6-15]. The phytochemical analysis of *Salvia* species revealed the presence of many compounds belonging mainly to the group of phenolic acids, phenolic glycosides, flavonoids, anthocyanins, coumarins, polysaccharides as well as essential oil [16-20].
Salvia chudaei (Tamahaq name: *aouit*, Arabic name: *tagrouft*) is an endemic plant of Algeria, where it grows only in central Sahara. The aerial parts of the plant are used locally in folk medicine for the treatment of dysmenorrhea, abdominal pains, spasms, sun stroke and gonorrhea [21].
According to the best of our knowledge, there are no experimental studies about phytochemical content or biological activity of this species. Thus, the aim of the present study was to chemically screen the hydromethanolic extract of *Salvia chudaei* as well as to determine the total phenolic and flavonoid contents, to investigate the antioxidant, antimicrobial and cytotoxic activities of this extract and its fractions.

Material and Methods

1. *Plant Material*

Arial parts of *Salvia chudaei* were collected from Hoggar, Southern Algeria (Coordinates; UTM: GF96; Latitude: 23°10'0"; Longitude: 5°49'60"), at the flowering stage, in July 2010. Plant identification was carried out by botanists, National Institute for Forest Research, Tamanrasset, Algeria and voucher specimen of the plant have been deposited (number LS/1/10). The plant aerial parts were cleaned and air-dried at room temperature in the shade, and then powdered.

2. *Extraction Procedure*

Powdered plant material (10 g) was extracted for 48h with 100 ml of methanol–water (70%–30%) at room temperature. The solvent was then removed by filtration and fresh solvent was then added to the residue. The extraction process was third repeated. The combined filtrates were then concentrated under reduced pressure at 40°C using vaccum rotary evaporator to obtain dry extract (2.62 g). The hydromethanolic crude extract was subjected to fractionation using different solvents. The crude extract was first suspended with hot distillated water (100 ml) and kept at room temperature for 12 hours. Then the suspension was defatted using hexane (50 ml, three times) and then successively fractionated with equal volumes of chloroform, diethyl ether, ethyl acetate and n-butanol (50 ml, three times). These fractions were dried over anhydrous sodium sulfate, filtered and concentrated to dryness under vacuum using rotary evaporator. The yields of these fractions were 88 mg, 457 mg, 420 mg and 180 mg respectively. The fractions were then redissolved in methanol at a concentration of 10 mg/ml. All extracts obtained were kept in the dark at +4°C prior to use.

3. *Preliminary phytochemical screening*

The dried aerial parts of *S. chudaei* were subjected to preliminary phytochemical screening to identify the various active chemical constituents present in this species such as anthraquinones, triterpenes, saponins, flavonoids, tannins, *O*-heterosides, *C*-heterosides, alkaloids, coumarins, according to standard phytochemical methods as described by Lespagnol [22]; Harlay et al. [23] and Paris & Moyse [24].

4. *Total Phenolic contents*

The total phenolic contents of extracts were determined spectrophotometrically, using the Folin–Ciocalteu assay [25]. Briefly, an aqueous aliquot (0.25 ml) of the extract was added to 3.75 ml of distilled water in a test tube, followed by 0.25 ml of Folin-Ciocalteu's reagent. After 3 min, 0.75 ml of 20% sodium carbonate was added. Tube contents were vortexed and heated at 40°C for 40 min. the blue coloration was read at 760 nm. The concentrations of phenolic compounds were calculated according to the following equation that was obtained from the standard gallic acid graph:

Absorbance = 0.1035 gallic acid (µg/ml) + 0.1046 (R^2:0.98)

5. *Total Flavonoid contents*

The total flavonoid contents in the extracts were determined by a colorimetric method described by Lamairson and Carnet [26]. 1.5 ml of 2% $AlCl_3.6H_2O$ dissolved in methanol was added to equal volumes of the diluted extract. The mixture was shaken and the absorbance was read at 440 nm after 10 min incubation at room temperature. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:

Absorbance = 0.2829 quercitin (µg/ml) – 0.1155 (R^2:0.99)

6. *Antioxidant Activity*

6.1. DPPH Radical Scavenging Activity Assay

The method of Braca et al. [27] was used for determination of scavenging activity of DPPH free radical. Different methanolic dilutions of extracts (5 µg/ml to 1000 µg/ml) were mixed with equal volumes of freshly prepared DPPH methanol solution (0.004% w/v). The reaction mixture was vortexed thoroughly and then left to stand at room temperature in the dark for 30 min. and the absorbance was read at λ=517 nm using a blank containing the same concentration of extracts without DPPH. Ascorbic acid, α-tocopherol and BHT were taken as standards. Inhibition of the DPPH free radical in percent (I%) was calculated based on control reading, which contain equal volumes of DPPH solution and methanol without any test compound using the following equation:

$\%\ \text{inhibition} = [(A_C - A_S)/A_C] \times 100$

Where A_C is the absorbance of control reaction, and A_S is the absorbance of the sample. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of scavenging effect percentage against extract concentration.

6.2. *Reducing Power Assay*

The reducing antioxidant power of plant extracts was determined by the method of Oyaizu [28]. Different concentrations of plant extracts in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. then, 2.5 ml of trichloroacetic acid (10%) was added to mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer. Phosphate buffer (pH 6.6) was used as blank solution. Ascorbic acid, α-tocopherol and BHT were used as standards. Increased absorbance of the reaction mixture indicates increase in reducing power. IC_{50} value (µg ml^{-1}) is the effective concentration at which the absorbance was 0.5 for reducing power.

6.3. *β-Carotene/Linoleic Acid Bleaching Assay*

This test was carried out according to a described procedure [29], based on the aptitude of various extracts to decrease the oxidative discoloration of β-carotene in an emulsion. 2 mg of β-carotene was dissolved in 10 ml of chloroform (HPLC grade). 1 ml of this solution was pipetted into a round-bottom flask containing 20 mg of linoleic acid and 200 mg of Tween 40. Chloroform was completely evaporated using a vacuum evaporator. Then, 50 ml of distilled water was added slowly to the residue and the solution was vigorously agitated to form a stable emulsion. 4.8 ml of the obtained emulsion were transferred into different test tubes containing 0.2 ml of extract (2 mg/ml). The mixture was then gently mixed and placed in a water bath at 50°C for 120 min. Absorbance at 470 nm was measured every 30 min for 120

min. Blank solution was prepared in a similar way except that addition of β-carotene was omitted. Ascorbic acid, α-tocopherol and BHT were used as standards. The bleaching rate (R) of β-carotene was calculated according to first-order kinetics, as described in Al-Saikhan et al. [30]:

$$R=In\ (A_{t=0}/A_{t=t})/t$$

Where, ln = natural log, t is the time in minutes, $A_{t=0}$ is the initial absorbance of the emulsion immediately after sample preparation (t = 0 min) and $A_{t=t}$ is the absorbance at time t (30, 60, 90, and 120 min). The percent of antioxidant activity (AA) was calculated using the equation:

$$AA= (R_{control} - R_{sample})/ R_{control} \times 100$$

Where, $R_{control}$ and R_{sample} are average bleaching rates of the negative control and the antioxidant (plant extract, ascorbic acid or α-tocopherol or BHT), respectively.

7. *Antimicrobial Activity*

7.1. *Microbial Strains*

The hydro-methanolic crude extract and its fractions were individually tested against pathogenic microbes including four gram positive bacteria (*Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (CIP 7625), *Staphylococcus epidermidis* (CLM), *Listeria monocytogenes* (CIP 82110)), five gram negative bacteria (*Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (CIP A22), *Enterobacter cloacae* (E13), *Salmonella enterica* (CIP 81.3), *Klebseilla pneumonia* (CIP 82.91)); and one yeast (*Candida albicans* (IPA 200)). All microorganisms were obtained from The Microbiological laboratory, Department of Biology, ENS, Algiers, Algeria. Bacterial strains were cultured in Muller–Hinton agar (Institut Pasteur, Algeria) and yeasts were cultured in Sabouraud dextrose agar (Institut Pasteur, Algeria). All microbial strains were incubated for 24 h at 37°C.

7.2. Disc Diffusion Assay

Antimicrobial tests were carried out using the disk diffusion method. The microbial cultures were harvested and then suspended in sterile saline (0.9% NaCl) and the cell density was adjusted to 0.5 McFarland. Sterile 5.5 mm paper discs, impregnated with 10 µl of the extracts solutions (50 mg/ml) were placed on the inoculated surface. Before incubation, all Petri dishes were stored in the dark at +4°C for 1 hour, to allow the diffusion of the extracts from disc to medium without microbial growth. At the end of incubation time (18-24h at 37°C), the diameter of the zones of inhibition around each disc (in millimeters, diameter of the disc included) were used as a measure of antimicrobial activity. Levofloxacin (10 µg/disc) was used as positive control for bacteria and nystatin (10 µg/disc) for yeast.

7.3. *Agar Dilution Method*

MIC determination of extracts was carried out by the agar dilution method [5]. Appropriate amounts of the extract were added aseptically to sterile medium to produce the concentration range of 25–0.097 mg extract/ml medium. The resulting agar solutions were immediately mixed and poured into Petri plates. The plates were spot inoculated with 1 µl of microorganism. At the end of incubation period, the plates were evaluated for the presence or absence of growth. The MIC was defined as the lowest concentration of the extract needed to inhibit the growth of microorganisms.

8. Cytotoxic activity

Cytotoxicity of the plant extract was determined by brine shrimp lethality bioassay described by Turker & Camper [31]. Brine shrimp (*Artemia salina* Leach) eggs were obtained from CNRDPA, Algeria. Seawater was prepared by dissolving 36 g of sea salt in 1 l of distilled water and put in a shallow rectangular plastic container. Oxygen was supplied and 60-W lamp was positioned near the container to provide direct light and heat (~27- 28°C). About 1 g of shrimp eggs were placed in 1 l of sea water. After 10-12h, eggs began hatching. Two days was allowed for the shrimp to mature as nauplii (shrimp can be used 48- 72h after the initiation of hatching). After 72h they were discarded. Nauplii were harvested by turning off the aeration and letting the culture settle for about 10 min. Hatched, empty eggs floated on the surface and unhatched eggs sank to the bottom. Newly hatched nauplii concentrated just above the unhatched eggs on the bottom. Since the nauplii are positively phototropic (attracted to light), shining a light in the middle of the container and shading the container at the bottom helped direct them to an area where they can be easily harvested by siphoning or draining.

Stock solutions of extract were prepared by suspending dried extract in saltwater to prepare a 10 000 µg/ml solution. The suspension was mixed for 5 min; then, 1000, 100, 10, 1µg/ml solutions were prepared by dilution. A suspension of nauplii was removed and 10 nauplii were placed into each of the test tubes and 2.5 ml of appropriate concentration of extract/salt mixture was added. Uncovered tubes were incubated for 24h at room temperature under illumination. Three replicates were prepared for each concentration. The same saline solution used to prepare the stock test sample solution was used as a negative control. After 24h, the surviving nauplii were counted with the aid of a 3x magnifying glass, and the percentage of deaths was determined. The median lethal concentration, LC_{50} value of the plant extract was determined.

9. *Statistical Analysis*

All experiments were carried out in triplicate. Data were expressed as means ± S.D. Differences were evaluated by one-way analysis of variance (ANOVA) test completed by a Student's test. Differences were considered significant at $p<0.05$. The correlations between methods were determined using analysis of variance (ANOVA) and quantified in terms of the correlation factor. LC_{50} value was obtained by a plot of percentage of dead shrimps against the logarithm of the sample concentration using Microsoft Excel.

Results and discussion

1. *Preliminary phytochemical screening*

Phytochemical constituents in the plants are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal, and anticancer [32]. In this study, the preliminary phytochemical screening of plant extracts had shown the presence of anthraquinones, triterpenes,

saponins, flavonoids, tannins, *O*-heterosides, *C*-heterosides. Alkaloids and coumarins were not detected; the summary of the results are presented in table 1.

Table 1. Phytochemicals detected in aerial parts of *S. chudaei*

Phytochemicals	**Results**
Anthraquinones	+
Alkaloids	-
Triterpenes	+
Saponins	+
Coumarins	-
Flavonoids	+
Tannins	+
O-heterosides	+
C- heterosides	+

Key: += present, - = absent

2. *Amount of Total Phenolic contents*

The systematic literature collection pertaining to this investigation indicates that the plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical scavengers. Therefore, it is worthwhile to determine their total amount in tested extracts.

Based on the absorbance values of the various extract solutions reacted with Folin-Ciocalteu's reagent and compared with the standard solutions of gallic acid equivalents as described above, the total phenolic in the crude extract and their derived fractions of *S. chudaei* were determined (Table 2). Results showed that phenolic contents varied significantly as function of solvent nature ($P < 0.05$). The diethyl ether fraction showed the highest polyphenol content with 537.58 mg of GAE/g, followed by ethyl acetate fraction (54.49 mg GAE/g) and hydro-methanol crude (39.03 mg GAE/g). However, the chloroform and n-butanol fractions showed weaker polyphenol content (32.38 and 25.15 mg GAE/g respectively) among the solvents used.

3. *Amount of Total Flavonoid contents*

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities [33]. The hydromethanolic crude extract and ethyl acetate fraction showed the highest flavonoid contents with 4.68 mg QE/g and 4.41 mg QE/g, respectively; while diethyl ether fraction showed the lowest one with 1.43 mg QE/g (Table2).

Table 2. Total phenolic, flavonoid contents (mean ± SD) of extracts from *S. chudaei*

Extracts	Total phenolic contents[a,b]	Total flavonoid contents[a,c]
hydromethanolic crude	39.03±4.83	4.68±0.05
chloroform	32.38±1.37	2.79±0.01
diethylether	537.58±16.92	1.43±0.01
ethyl acetate	54.49±6.76	4.41±0.01
n-butanol	25.15±2.74	1.95±0.01

[a]Each value is presented as mean ± SD (n = 3)
[b]Total phenolic content was expressed as mg gallic acid equivalents/g dried extract
[c]Total flavonoid content was expressed as mg quercitin equivalents/g dried extract

4. *Antioxidant Activity*

4.1. *DPPH Assay (Radical Scavenging Activity)*

DPPH radical scavenging activity assay is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of many plant extracts or compounds [34]. DPPH is a stable free radical which exhibits a deep purple color with maximum absorption at 517 nm. Antioxidant molecules react with the free radical by hydrogen or electron donation, resulting in discoloration of DPPH because of their conversion into yellow colored diphenylpicryl hydrazine [27]. As shown in Fig.1, the DPPH radical scavenging activities of five extracts of *S. chudaei* were concentration-dependent. hydromethanolic

crude and chloroform, diethyl ether, ethyl acetate, n-butanol fractions exhibited high scavenging activity toward DPPH (93.02%, 93.38%, 96.87%, 95.93% and 74.02%, respectively) at 0.2 mg/ml. IC_{50} values were found to be in the following order: ascorbic acid <diethyl ether <ethyl acetate <α-tocopherol <hydromethanolic crude < BHT < chloroform < n-butanol (Table 3).

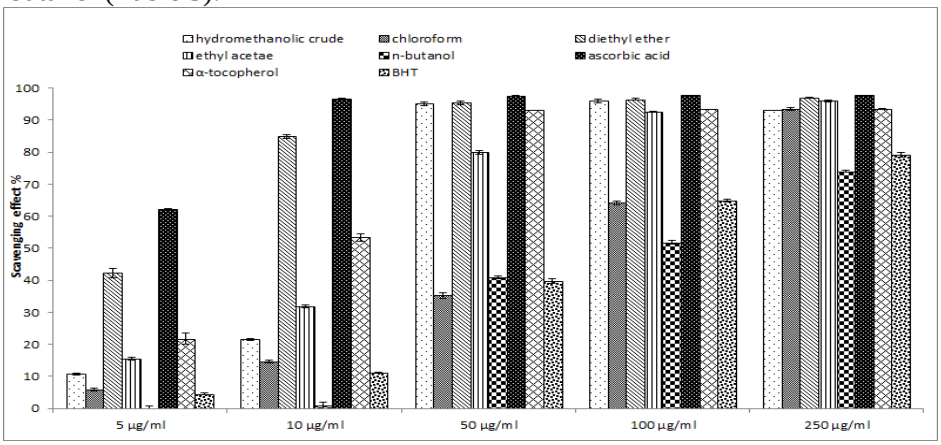

Fig. 1 The DPPH radical scavenging activities of ascorbic acid, α-tocopherol, BHT and extracts of *S. chudaei*. Each value is expressed as mean ± SD (n = 3).

Table 3. Antioxidant activities of extracts from *S. chudaei* and standards measured by different assays

Plant Extracts	DPPH[a,b]	Reducing power[a,c]	β-Carotene / linoleic acid (%)[a]
hydromethanolic extract	19.26±0.25	259±1.52	76.58±1.43
chloroforme fraction	74.23±1.1	821±1.41	73.15±1.68
diethyl ether fraction	6.00±0.1	48.5±0.7	95.08±0.33
ethyl acetate fraction	8.16±0.2	257±1.41	73.81±2.05
n-butanol fraction	88.36±0.5	924±5.03	41.19±6.6
ascorbic acid	4.00±0.1	47±0.28	11.05±1.43
α-tocopherol	9.55±0.07	507±4.16	94.95±0.94
BHT	72.16±0.1	633±11.5	96.92±0.51

[a]Each value is presented as mean ± SD (n = 3)
[b]IC_{50} in µg/ml
[c] Concentration at which the absorbance was 0.5

It seemed that diethyl ether fraction was superior to all extracts tested ($p < 0.05$), with regard to scavenging abilities. When comparing IC_{50} values obtained for standards (BHT: 60 µg/ml and α-tocopherol: 9.55 µg/ml) and both diethyl ether and ethyl acetate fractions, it was found that these fractions showed a strong antioxidant potential. Hydromethanolic crude extract also showed a higher free radical scavenging activity than BHT, while chloroform and n-butanol fractions were less active than the others.

The results obtained in this assay are in agreement with previous investigations on *Salvia* species, although different solvents were used for extraction. Şenol et al. [35] showed that ethyl acetate extracts of *Salvia adenophylla, Salvia cedronella, Salvia hedgeana, Salvia hydrangea, Salvia kronenburgii, Salvia napifolia, Salvia nygdeggrerii, Salvia pachystachys, Salvia pisidica, Salvia potentillifolia, Salvia russelli, and Salvia wiedemannii* exhibited DPPH radical scavenging effect more than 90% at maximum concentration tested of 100 µg/mL. Kamatou et al. [34] also showed that methanolic extracts of *Salvia abicaulis, Salvia runcinata, Salvia aurita* had a good scavenging efficiency with IC_{50} values of 19.9 µg/ml, 19.3 µg/ml and 16.6 µg/ml, respectively.

4.2. *Reducing Power*

Reducing power is generally associated with the presence of reductones, which exert antioxidant action by breaking the free radical chain through donating a hydrogen atom [36]. In this assay, Fe^{+3}/ferricyanide complex [$FeCl_3/K_3Fe(CN)_6$] is reduced to the ferrous form by antioxidants and can be monitored by measuring the formation of navy blue color at 700 nm [37]. As shown in Fig. **2**, The reducing power of plant extracts increased with an increased in extracts concentration. At a very low extract concentrations (0.025 mg/mL) the absorbance at 700 nm varied from 0.304 (diethyl ether fraction) to 0.024 (n-butanol fraction).

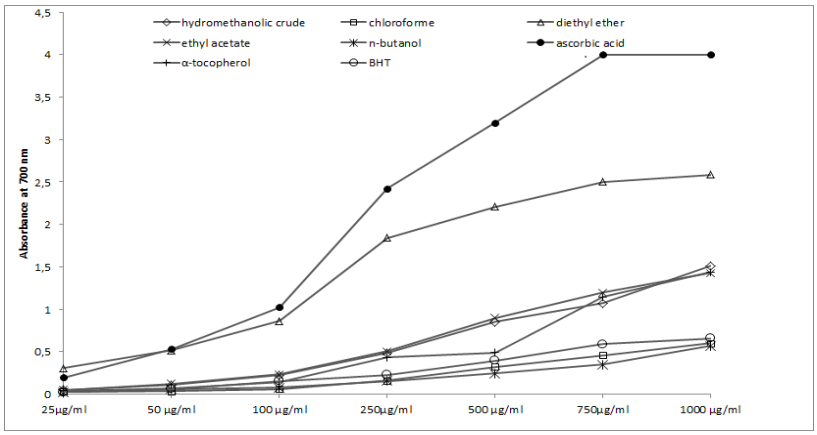

Fig. 2 Reducing powers of various concentrations of hydromethanolic crude extract and its fractions from *S. chudaei*. Each value is expressed as mean ± SD (n = 3).

At concentration of 1 mg/ml the reducing power values measured for each extract varied from 2.585 (diethyl ether fraction) to 0.569 (n-butanol fraction). According to table 3, diethyl ether fraction had the strongest ability to reduce Fe (III) compared to other extracts and synthetic antioxidants BHT and α-tocopherol and showed no significant difference with ascorbic acid ($P>0.05$). In this assay, IC_{50} values were in the order: diethyl ether < hydromethanolic crude = ethyl acetate < chloroforme < n-butanol. Our results are in accordance with the previous published data showing the high reductive capability of *Salvia* species extracts [2,3].

4.3. *β-Carotene-linoleic Acid Bleaching Assay*

In this model system, β-carotene undergoes rapid discoloration in the absence of an antioxidant, which results in a reduction in absorbance of the test solution with reaction time. This is due to the oxidation of linoleic acid that generates free radicals that attack the highly unsaturated β-carotene molecules in an effort to reacquire a hydrogen atom. When this reaction occurs the β-carotene molecule loses its conjugation and, as a consequence, the characteristic orange color disappears. The presence of antioxidant avoids the destruction of the β-carotene conjugate system and the orange color is maintained [38].

As shown in Fig.3, all of the extracts were able to reduce the rate of degradation of β-carotene by scavenging linoleate-derived free radicals. The absorbance of the control at 470 nm decreased to a minimal value of 0.196 after 120 min, while those of the extracts were still between 0.224 and 0.636. The percentages of antioxidant activities of the hydro-methanolic extract and its fractions are given in table 3. The results showed that diethyl ether fraction had prominent effects on the inhibition of linoleic acid oxidation (95.08%), and their activity did not differ from the activity of α-tocopherol ($p>0.05$). The hierarchy of the extracts was: diethyl ether>hydromethanolic extract>chloroforme>ethyl acetate>n-butanol. Esmaeili and Sonboli [3] found that mean antioxidant activity of methanolic extract from aerial parts of *S. brachyantha* was 69.45%. Ebrahimabadi et al. [5] have also reported that leaves of *S. eremophila* show strong antioxidant activity (72.42 %). These data are very similar to antioxidant activities of methanolic extract reported in this study.

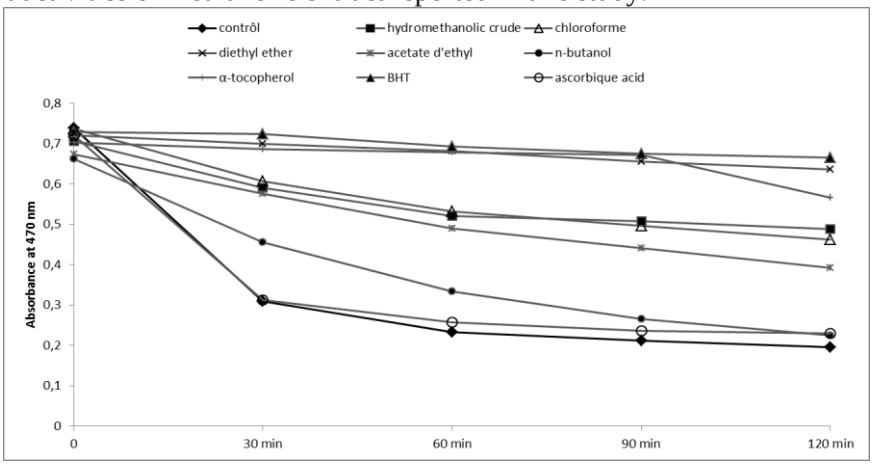

Fig. 3 Inhibition of bleaching of β-carotene–linoleic acid emulsion by the extracts of *S. chudaei*. Each value is expressed as mean ± SD (n = 3).

5. *Correlation between Antioxidant Capacity and Total Phenolic Contents*

Phenolic compounds are considered to be among the most active natural antioxidants because they can donate electrons to radicals and break the radical chains [39]. Some studies demonstrated a relationship between antioxidant activity and the quantity of phenolic substances [39, 40]. In this study, there was a moderate (R^2 = 0.51) linear correlation between IC_{50} of radical scavenging activity and total phenolic contents. Significant correlation between the total phenolic contents and the antioxidant activity was also observed for the β-carotene-linoleic acid assay (R^2=0.66). In addition, the reducing power of the extracts tended to increase with the content of phenolics (R^2 = 0.63). This Finding indicates the significant contribution of phenolics to the antioxidant activity observed in the plant studied. Several reports have shown the relationship between total phenolic contents and antioxidant activity in some *Salvia* species [8, 9, 34].

6. *Antimicrobial Activity*

The antimicrobial activity of *S. chudaei* extracts was evaluated against a panel of 10 microorganisms and their potency were assessed qualitatively and quantitatively by the presence or absence of inhibition zones, zone diameters and MIC values. The results are given in table 4.

The tested extracts showed different inhibitory activities and MIC for each microorganism. The most interesting activity was obtained from diethyl ether fraction with MIC values ranging between 0.195 to 12.5 mg/ml. Gram positive bacteria were the most sensitive. The most sensible one was *B. subtilis* being inhibited by all the extracts (MIC values ranged from 0,390 to 12.5 mg/ml). Concerning Gram negative bacteria, only the diethyl ether fraction was able to inhibit the growth of *P. aeruginosa*, *E.coli* and *Enterobacter cloacae* at concentration (12.5, 0.781 and 6.25 mg/mL, respectively) . On the other hands, all extracts exhibited excellent activity against *Candida albicans* with the MIC value ranged from 0.195 to 0.781 mg/ml. Although this is the first report on the antimicrobial activity of *S. chudaei*, some other members of the *Salvia* genus have been subjected to antimicrobial activity evaluation [5,6, 20, 41]. Since many plant phenolics have been found to be responsible for several biological properties, including antimicrobial properties [42-44], it was expected that a considerable antimicrobial activity of this plant species would be related to its phenolic compounds.

7. *Cytotoxic activity*

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties [45]. Based on the results, the hydromethanolic extract of *Salvia chudaei* has showed good toxic to brine shrimp nauplii, with LC_{50} of 25.61 µg/ml. In addition, the degree of lethality was found to be directly proportional to the concentration of the extract (Fig. 4). The observed lethality of this plant extract to brine shrimps indicated the presence of potent cytotoxic and probably antitumor components. According to Meyer et al. [45], crude plant extract is considered as toxic (active) if it has an LC_{50} value of less than 1000 µg/ml.

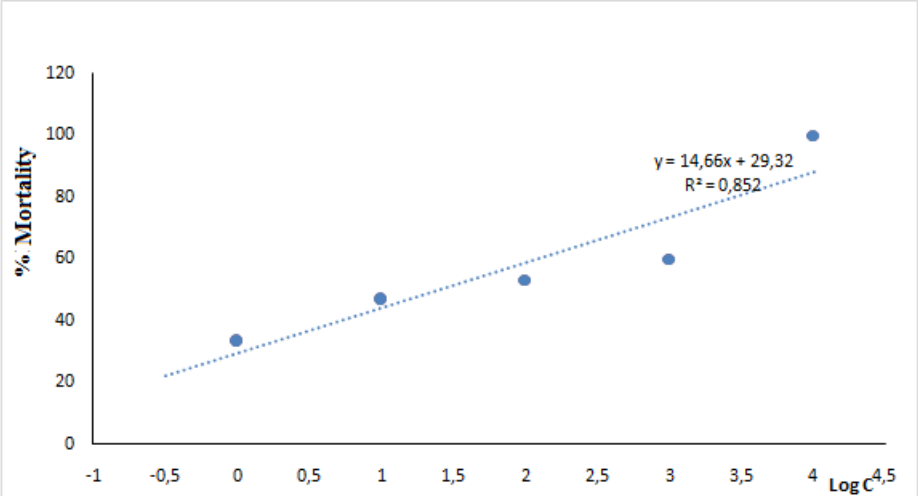

Fig. 4 Determination of LC_{50} of hydromethanolic extract of *Salvia chudaei* against brine shrimp nauplii.

Table 4. Antimicrobial activities of hydro-methanolic crude extract and its fractions from *S. chudaei* against tested microbial strains.

	hydro-methanolic crude extract		chloroform fraction		Diethyl ether fraction		ethyl acetate fraction		n-butanol fraction		Positive controls[C]	
Test microorganisms	DD[a]	MIC[b]	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC
Gram-negative bacteria												
Pseudomonas aeruginosa	-	-	-	-	8.33±1.25	12.5	-	-	-	-	24.16±0.76	0.024
Escherichia coli	-	-	-	-	10.66±1.52	0.781	-	-	-	-	29±1.00	0.024
Klebsiella pneumonia	-	-	-	-	-	-	-	-	-	-	13.33±0.57	0.097
Salmonella enterica	-	-	-	-	-	-	-	-	-	-	19.33±0.57	0.048
Enterobacter cloacae	-	-	-	-	9.16±1.04	6.25	-	-	-	-	20±0.00	0.048
Gram-positive bacteria												
Bacillus subtilis	14.16±0.76	0.390	10.5±0.5	0.781	13.33±1.25	0.390	9.00±1.00	1.56	6.66±0.28	12.5	36±1.00	0.006
Staphylococcus aureus	-	-	-	-	7.50±0.86	12.5	-	-	7.16±0.76	12.5	32±1.00	0.012
Staphylococcus epidermidis	-	-	-	-	8.33±1.15	6.25	-	-	-	-	42.66±0.57	0.003
Listeria monocytogenes	12.66±1.6	3.12	-	-	9.16±0.28	6.25	-	-	7.5±0.5	12.5	34.33±1.15	0.012
Yeasts												
Candida albicans	12.66±0.76	0.390	12.5±0.5	0.390	14.16±0.28	0.195	9.33±1.15	0.781	14±1.00	0.195	33	0.125

A dash (–) indicate no antimicrobial activity
a: Inhibition zone in diameter (mm) around the impregnated discs and each value is presented as mean ± SD (n = 3)
b: Minimal inhibition concentrations; values given as mg/ml
c: positive controls: levofloxacin for bacteria, nystatin for yeast

Conclusion

From the foregoing, it may be concluded that the extracts of *Salvia chudaei* demonstrated potent antioxidant, antimicrobial and cytotoxic activities. Higher levels of total phenolics of plant are probably responsible from the biological activities observed. This finding candidates the plant as a good case for more in-depth studies and we wish our future research lead to the identification of biologically active molecules present in its extracts.

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