Determination of antimicrobial and antioxidants activity of Salvia przewalskii seed oil against pathogenic bacteria and fungi

M Alsirrag¹ and R Ali²

¹University of Karbala, Karbala, Iraq
²University of Kufa, Najaf, Iraq
Dr_manal@yahoo.com
ryadh.alhabeeb@uokufa.edu.iq

Abstract. In this study antimicrobial and antioxidant effect of methanolic and aqueous extracts of Salvia przewalskii seed oil were tested against four strain of bacteria (Streptococcus bovis, Proteus mirabillis, Enterococcus gallinarum, Escherichia coli) and two strain of fungi (Rhizopus oryzae and Rhizopus stolonifer). Aqueous and methanolic extracts of Salvia przewalskii seed oil were prepared by solvent extraction, the results showed the hydro distillation of seed parts of S. przewalskii in vitro yellow oils in dry weight (0.3% and 0.5% (v/w; ml 50 g) products, antibacterial data showed higher ability toward E.coli (26.67±0.89) compared with standard streptomycin (22.78±0.49) and highest inhibition zone measured 25 mm. as well as methanol extract at (200mg/ml) recorded highest antioxidant ability (86.87±0.76) and DPPH scavenging activity at 20 µl/ml (89.56±0.47).

Key words: Salvia przewalskii, antioxidant, antimicrobial.

1. Introduction
Salvia or sage its large genus includes many type of species about 1000, its has wide uses in Pharmaceutical and many of artificial or commercial industries includes the production of fixed oils and additives substances. Salvia oils isolated by vacuum distillation from the plant’s dried aerial parts haveFeatured biological ability and medicinal properties. its oils contain different type of vital compounds, mainly include polyphenols hydrocarbons, tanines, penzohydrocarbones, camlyphenoles (1).

The fixed oils were derived from upper part of the plant have a high spectrum of applications: antimicrobial, anti-inflammatory, antioxidant, antiviral and anticoagulant.(2,3). An fixed oils is aconcentrated hydrophobic contain volatile aromatic liquids obtained from plant substances . Fixed oils are substances extracted by several ways using steam. Its contain fatty acids and its esters manufactueed by solvent extraction, hot and cold pressing. And it is utilize in perfume , cosmetic and several substances, for flavouring edible and softers , and cleaning materials. (4). Sage is perennial plant included in the Salvia species, belongs to the Labiatea family. It is known as sage and garden sage. Medical and culinary use of this medicinal herbs belong to ancient times. Sage has antimicrobial, antioxidant, antiviral, and immunosuppressive effects, therefore its had medical and aromatic usage is important In addition to this, sage is used traditionally for different purposes, like as preservation of natural and processed food, as a sweetener, for food coloring some of the aromatic compounds of saliva is derived from this genus hase shown higest antibacterial ability, antiflamantry and anti-mutagenic effect of saliva is often related to the presence of active compounds such as: carnosic acid, carnosol, rosmarinic acid and camphor.
(5) the fixed oils of some Salvia species have recently been investigated, showing strong antimicrobial ability. (6) demonstrated that S. chloroleuca oil exhibited moderate to high antimicrobial ability especially for Bacillus subtilis, Staphylococcus epidermidis and S. bovis with inhibition zones of 21, 19, 15 mm Some polyphenolic extracts from Salvia were examined by (7) for antioxidant ability in correlation with their polyphenolic content. Haigh active free radicals present in vital systems derived from tissue metabolic activities (8), more researches revealed that the polyphenolic extracts had highest chelating ability towards DPPH_ and ABTS_+ radicals and protected from hydroxyl and peroxy radical-induced DNA damage.

DPPH method is an antioxidant test deepened on chemical action between ion indicator produces acolorant agent in solvent. This free ion fixed at room conditions, it changed according to an inhibition substances, gave high change colorless in extracts solution. The benefit of this methods provides simple and rapid methods to determinate inhibatory radicals by colorimetry so it can be perfect assay to show different products at experiment time (9). The aime of this study was to evaluate the antioxidant potential and antibacterial capability of the fixed oil extract from Salvia przewalskii seed.

2. Material and methods

Collection of Salvia przewalskii seed
Salvia przewalskii seed were collected from the local market (herbalists) in 2017 at Kerbala city, it was cleaned of impurities and grind and preserved at sterilized bottle in can sealed and it was stored in the refrigerator.

Preparation of fixed oil:
The air –dried and ground leaves collected and was submitted to water 1:3 distillation for 3 hours using a Clevenger - the obtained essential oil was dried over anhydrous barium sulphate and after filtration it was kept in dark -18C in glass vial until use the yield was 2ml /100g dry leaves .(10).

Preparation extract:
Alcoholic extract
(40 g) of dried powder of saliva extracted by (220) ml 80% methanol overnight and filtered using vacuum pump The residue was re extracted 3-4 times with methanol until it was exhausted . Methanol evaporated using vacuum rotary at 50C .The methanol-free extract was dried using lyophilize and keep it at dark condition until use(11).

Water extract:
100g of salvia ground samples were extracted by soaking in cold distilled water (1:4 w/v) and homogenized for 3 min at 3000 rpm, The mixture was filtered through cheesecloth and centrifuged for 15 min at 2000 rpm ,The supernatant was filtered through Whatman No1 and dried by lyophilized and kept in dark at 4°C.(12)

The antioxidant evaluation tests of Salvia extract

1- DPPH Scavenging experiment:
The free radical scavenging of various extracts was performed according to (13) 1.5 ml of (0.2 mM) solution of DPPH mixed with 3ml of each concentration (5-200 mg/ml) for the each solvent extract and (5-20µl/mol) for the fixed oil .the absorbent of DPPH reagent was read by spectrophotometer at 517nm after 15 min of keeping in incubator at 25C.

Inhibition percent (IP) % = (A_{blank} - A_{sample} / A_{blank}) × 100.

2- Test of ABTS :
Antioxidant by this method measured by using 2ABTS test free radical ABTS dissoleved in distilled cold water reached 7 mM concentrate after this added of 1.45mM sodium persulfate the mixture kept
at room condition overnight in the away from light before used , the radical cation was added to 0.01M PBS(phosphate buffer saline PH7,until to gave absorbant 0.70 nm then the test substance was added to 100 withABTS solution to make volume to 1 ml after that absorbance was readed spectrophotometry quickly with 1 min after added of sample this was made in triplicate and controls contain 990 µl of buffer solution to replace ABTS(14) ,The Trolox chemical was added as indicator (2 to10 µM). This test were calculated according to the capability of ion to scavenge hydrogen ion, and the percent of supernatant ABTS measured at study object from four separate testing solvents.(15).

Test microorganisms :
All the microorganisms which includes Streptococcus bovis, Proteus mirabilis, Enterococcus gallinarum, Escherichia coli and two strain of fungi Rhizopus oryzae and Rhizopus stolonifer were isolated from stools of patients from Allhusainii hospitals and purified on selective media as MacConkey Agar, and XLD Agar also bacterial isolate were diagnosed by API 20E of Enterobacteraceae family . It have been obtained from the laboratories of the Department of Health Surveillance at a holy city of Karbala. Strains of bacteria have been grown on nutrient agar (Hi-media) at 37°C used for 24 hour.

Antimicrobial experiment:
The antimicrobial ability was tested by the standard disc seperation way(16). Inocula of Streptococcus bovis, Proteus mirabilis, Enterococcus gallinarum, Escherichia coli plates (Petri dish ) and each sample had been added to a concentration of tested oil (4% , 2%, v/v in ethylene glycol). Zones of an activation were measured
The standard of the antibiotic streptomycin was 2 mg/ml. samples read were done with triplicate.
Antifungal activity assay (Disc diffusion technique)
Preparation of inocula
7 days old cultures grown on Czapec dox agar media at room temperature were selected. Culture plates were flooded with 0.45% sterile saline solution and inoculum turbidity was adjusted to 0.5 McFarland standards according to CLSI document M2-A8. Antifungal activity assay (Disc diffusion technique) Disc diffusion method was performed according to CLSI M44-A document. 100µl of the inoculum was seeded on the plates containing CDA medium. The plates were allowed to dry for 2-5 minutes. 100µl of the extract was loaded to the sterile discs of 6mm diameter and placed on the test plates. The plates were incubated at 37°C for 72- 96 hrs. The diameter of the inhibition zones were measured in mm ,Discs loaded with solvents without extract served as negative control and standard fungicides served as positive control. All the tests were performed in triplicates (17).

3.Statistical Analysis
The results were expressed as mean values, standard deviations and analyzed for variance (SAS). Significant differences between control and treatments were determined using Duncan’s multiple range test at a level of P 0.05. Statistical analysis was performed using the statistical analysis system(18).

4.Results and Decision
Table (1) showed the of antimicrobial ability of the tested oil from seeds of S. przewalskii. each concentrations of sample appeared antibacterial action toward Streptococcus bovis, Proteus mirabilis, Enterococcus gallinarum, Escherichia coli as well as antifungal ability toward Rhizopus oryzae and R. stolonifera the samples 4(%) concentration showed higher antibacterial ability against E.coli and comprised with the lower sample concentration (2%) and it corresponded with literature data in (19).
The standard antibiotic streptomycin recorded high degree activity against all tested pathogenic microorganisms. This study recorded that tested oil of S. przewalskii appeared significant antibacterial
and antifungal ability and it can be used as antimicrobial inhibitor, at same study on some of pathogenic microorganism found that inhibitory zone of methanolic extract of \( C. molmol \) showed highest antibacterial ability towards each of \( Strepfracoccus bovis \) and \( Escherichia coli \) (20).

### Table (1) a Zone inhibition of \( saliva \) przewalskii seeds (water extract)

| Microorganism type       | Conc 1:50 (2%) | Conc 1%25(4%) | Streptomycin (2 mg/ml) | Zone diameter (mm) |
|--------------------------|----------------|---------------|------------------------|-------------------|
| Streptococcus bovis      | 20.33±0.56     | 22.67±0.63    | 22.43±0.45             | 22                |
| Proteus mirabilis        | 19.76±0.67     | 15.90±0.87    | 23.76±0.43             | 19                |
| Enterococcus gallinarum, | 18.98±0.54     | 14.87±0.78    | 29.90±0.41             | 21                |
| Escherichia coli         | 25.98±0.56     | 26.67±0.89    | 22.78±0.49             | 25                |
| Rhizopus oryzae          | 23.76±0.78     | 22.99±0.76    | 23.87±0.45             | 19                |
| Rhizopus stolonifera     | 19.76±0.81     | 18.64±0.76    | 24.78±0.51             | 23                |
| Streptomycin             |                |               |                        | 22                |

### Table (1) b Zone inhibition of \( saliva \) przewalskii seeds (methanol extract)

| Microorganism type       | Conc 1:50 (2%) | Conc 1%25(4%) | Streptomycin (2 mg/ml) | Zone diameter (mm) |
|--------------------------|----------------|---------------|------------------------|-------------------|
| Streptococcus bovis      | 22.33±0.53     | 22.27±0.63    | 22.43±0.45             | 22                |
| Proteus mirabilis        | 19.76±0.57     | 13.90±0.89    | 23.76±0.43             | 19                |
| Enterococcus gallinarum, | 17.98±0.54     | 14.87±0.58    | 29.80±0.41             | 22                |
| Escherichia coli         | 25.98±0.76     | 26.55±0.69    | 22.78±0.39             | 23                |
| Rhizopus oryzae          | 20.76±0.79     | 21.96±0.76    | 21.85±0.35             | 20                |
| Rhizopus stolonifera     | 18.76±0.51     | 19.44±0.77    | 23.48±0.51             | 22                |
| Streptomycin             |                |               |                        | 22                |

In the DPPH ion test alleyways high effect radical ha been on laboratory scale used in the examination of the antioxidant capability of active substance as biological supernatant by the capability of component to play as free ion and ion inhibitor after that we can estimate the antioxidant ability (21) Table 2 appeared the range data of ion inhibition capacities to each seed supernatants of the scavenging ion.

At this experimental degrees of samples, all tested solvents appeared a minimum - activates and inhibition capability at different added . soluble and alcohol supernatant of saliva recorded high activity as well as its inhibition capacities saline fixed oil showed lowest antioxidant ability compared with BHA the commercial standard . (Table 2) recorded highest results showed that the free ion ability (%) recorded increased significantly with adding of the solvents from 2.5 to 20 \( \mu \)mol saliva fixed samples and the testing added about 100 \( \mu \)g/mol, the methanol extract showed the highest (86.87±0.76) scavenging ability compared to other extracts and BHA another study revealed that there are many solution from \( Salvia euphratica \),
Salvia sclarea had antioxidant ability and the commercial BHA concentration from 18.8±1.21 to 23.4±0.97 μg/ml (23), this results agreed with (18) that fixed oil extracted from species Salvia sclarea had component of phenolic compound it has ability as antioxidant ability as well as results agreed with (23) that found (Salvia officinalis L.) methanolic solution had higher antioxidant ability measured by DPPH scavenging ability. At same time results in this study showed antifungal ability of extract towered each Rhizopus stolonifera and Rhizopus oryzae this agreed with (24,25,26) its founded that fixed oil of saliva sclarea had antifungal activity. Parameters at table (4) showed the ability of different extract to reduced ABTS and the highest value recorded it 43.56mg/MM for methanol extract with 100mg/ml conc. This result compatible with (27) it found that highest value of antioxidant ability recoded 36.42 at ABTS methods.

**Table (2):** DPPH ion capacities range of solutions extracts

| Conc  | Extraction with alcohol solvent | Extraction with water solvent | BHA  |
|-------|---------------------------------|------------------------------|------|
| 5     | 44±0.24                         | 48±0.29                      | 50.78±0.78 |
| 20    | 44.67±0.65                      | 51.72±0.32                   | 65.45±0.32 |
| 25    | 48.87±0.45                      | 55.76±0.46                   | 69.31±0.41 |
| 50    | 50.76±0.36                      | 64.89±0.61                   | 73.21±0.34 |
| 100   | 55.76±0.78                      | 79.32±0.53                   | 80.32±0.32 |
| 125   | 67.90±0.45                      | 80.21±0.54                   | 85.34±0.43 |
| 150   | 78.90±0.67                      | 84.89±0.61                   | 86.90±0.74 |
| 200   | 81.42±0.51                      | 86.87±0.76                   |      |

**Table (3):** DPPH ion capacity (%) of saliva sp. testing oil

| Conc  | DPPH ion capacities (%) |
|-------|--------------------------|
| 2.5   | 29.67±0.34               |
| 10    | 43.67±0.56               |
| 15    | 67.89±0.57               |
| 20    | 89.56±0.47               |

**Table (4) Determination of TEAC test**

| Sample                | TEAC value for DPPH |
|-----------------------|---------------------|
| Water extraction 50mg/ml | 22.65               |
| Water extraction 100mg/ml | 25.74              |
| Mthanol extraction 50mg/ml | 33.12              |
| Mthanol extraction 100mg/ml | 43.54              |

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