**Antibacterial Efficiency of Sage (*Salvia officinalis*) Extracts on Respiratory Infections**

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**Abstract**

 The study included the use of ethanolic extract of Sage (*Salvia officinalis* L) leaves at concentrations (0.1) and (0.5) mg/ml, to study the antibacterial activity of active compounds in the sage plant against common bacteria that caused the respiratory infections, gram negative bacteria (*Pseudomonas arigenossa*) and gram positive bacteria (*Staphylococcus aureus*) using agar well diffusion method.

The results of the study showed the both concentrations of ethanolic extract of Sage have significant antibacterial activity against these strains of bacteria, due to its high contained forms of active compounds, essential oils and flavonoids.

**Key words:** *Salvia officinalis*, Respiratory infections, Antibacterial activity.

**Introduction:**

Respiratory tract infections (RTIs) are common and frequent diseases, they present one of the major complaints in children and adolescents (Bellanti, 1997). Respiratory tract infections (RTIs), mainly involving the upper airways, are common in children and their recurrence constitutes a demanding challenge for the pediatricians (Couriel, 2002).

Acute infections of the airways continuously play an important role world-wide. The impact of these diseases is shown by their high incidence; substantial morbidity and potential consequences; tendency of over diagnosis, especially in streptococcal pharyngitis and acute otitis media; the associated overuse and misuse of antibiotics; and contribution to high healthcare costs and indirect social costs (Bauchner *et al.*, 1999; Ballow, 2008).

The high incidence of RTIs in young infants and preschool children are explained by increased exposure to respiratory pathogens at home or in child care centers; environmental factors; or defects in the immune system (Owayed *et al.*, 2000; Arden *et al.*, 2006).

Crowded conditions, like those in day-care settings, favor the colonization and spread of pathogens causing RTIs. Also environmental risk factors include passive smoking, exposure to pollutants and absence of breast-feeding (Lodha *et al.*, 2002).

Medical plants are world widely used and many of they were provided their efficiency in treatment many diseases, for these is become an interest in studying them in order to provide a scientific explanation for their beneficial effect (Pushparaj *et al.*, 2000; Alarcon-Aguilar *et al.*, 2002; Hosseinzadeh *et al.*, 2002; Kameswararao *et al.*, 2003; Singh *et al.*, 2007).

 *Salvia officinalis* L. (common sage, garden sage or Dalmatian sage) is a medicinal and aromatic plant of the Lamiaceae (= Labiatae) family, native to Mediterranean countries, which today is cultivated all over the world (Lima, 2006). The botanical name of sage is a clear reference to the important curative properties of the plant: the genus name Salvia comes from the Latin salvāre meaning “to save” or “to heal” and officinalis means medicinal (Dweck, 2000; Miura *et al.*, 2002).

*S. officinalis* is the specie of the genus Salvia with the highest EO production (Giannouli & Kintzios, 2000), additionally, many other active compounds that gives it it's medicinal and aromatic properties and makes it a rich source of bioactive compounds (Giannouli & Kintzios, 2000; Dweck, 2000; Barnes *et al.*, 2002; Lima, 2006).

Common sage, since ancient times, has been an ingredient in perfumes, a flavoring in a variety of food preparations, and a medicinal plant used in folk medicine for the treatment of a variety of ailments (Malamas & Marselo, 1992), where many studies mentioned that sage have many of biological activities, such as antioxidant, antibacterial, hyperglycemic and anti-inflammatory activities (Cherevatyî *et al*., 1980; Baricevic *et al*., 2001; Alarcon-Aguilar *et al*., 2002; Lima, 2006).

Also other studies, conducted on Sage extracts and their EO, have shown it's hypotensive properties, anti-spasmodic effect and central nervous system-depressant activities (Newall *et al*., 1996). addition to therapeutic effects for metabolic and endocrine diseases (Istudor, 2001).

The essential oil extracted from *S. officinalis* has antibacterial activity due to the presence of 1, 8-cineol and an antifungal substance (pereira *et al.*, 2004). The plant is reported to have a wide range of biological activities, such as antibacterial, fungistatic and virustatic effects (Sivropoulou *et al.*, 1997; Miladinovic, 2000; Lawless, 2002; Glamoclija *et al.*, 2006; Horiuchi *et al.*, 2007).

The antimicrobial properties as well as the tannins based astringent activities of *S. officinalis* benefit the reduction in plaque growth, the inhibition of gingival inflammation and have positive effects on caries prophylaxis (Willershausen *et al.*, 1991).

Some constituents of the plant, such as the triterpenes oleanolic and ursolic acids or the diterpene carnosol, were shown to present anti-inflammatory properties or related biological activities (Baricevic *et al.*, 2001).

In light of the recent emergence of bacteria which are resistant to multiple antimicrobial drugs, posing a challenge for the treatment of infections, the need to discover new antimicrobial substances for use in combating such microorganisms becomes patent (Pereira *et al.*, 2004).

Recently, antimicrobial activity of *S. officinalis* (Sage) leaves was showed against vancomycin-resistant enterococci (Horiuchi *et al.*, 2007).

Horiuchi *et al.* (2007) isolated the effective compound from the extract and identified it as carnosol, one of diterpenoids. Carnosol showed a weak antimicrobial activity and greatly reduced the MICs of various aminoglycosides (potentiated the antimicrobial activity of aminoglycosides) and some other types of antimicrobial agents in VRE.

extract on growth of gram negative bacteria (*Pseudomonas aeruginosa*) and gram positive bacteria (*Staphylococcus aureus*) was evaluated.

**Materials and Methods**

**Collection and Classification of the plant**

Sage were obtained from Baghdad, Iraq and the plant samples were identified in botany laboratory, department of biology, Sciences faculty / university of Kufa.

The leaves of plant were collected and dried, then the dried plant samples were ground well into a fine powder and stored in darkish bags for using. The treatment was conducted at the laboratory conditions (Plant searches laboratory / Sciences faculty / university of Kufa).

**Preparation of Sage leaves extract**

**Preparation of alcoholic extract:**

The extraction process was conducted according to Hajzadeh and coworkers (2011) by (30g) of dried plant powder was packed in a filter paper type Watman (No.1) and extracted in a soxhlet apparatus using (450 ml) ethanol (90%) at (60 C°) for (13h). After extraction, the extract was filtered and concentrated by rotary evaporator, then dried by oven at (45 C°). The dry material was collected in closed bag and maintained at (4 C°) until use.

The dry extract was dissolved in water to prepare the concentrations (0.1) and (0.5) mg/ml of extract.

In order to prepare sage discs, blank discs were dipped into different sage dilutions and kept refrigerated for (24 h). Bacterial suspensions were prepared by inoculating (2-5) bacterial colonies into test tubes filled with physiologic saline and incubating at (37 C°) for (3-5 min). After incubation, their opacities were compared with that of (0.5) Mac Farland's to ensure that the amount of tested bacteria was proper.

In well diffusion method, after inoculating of the bacterial strains on culture media, holes were filled with antibiotic or sage solutions in separate culture media. Following on from that, they were incubated at (37 C°) for (18-24 h).

In disc diffusion method, culture media were loaded with antibiotic or sage containing discs. Annular zone diameters were measured following (18-24 h) of incubation (Shirazi *et al.*, 2008).

**MIC of Sage leaves ethanol**

Minimal Inhibitory Concentration (MIC) for Sage leaves ethanol extract by broth microdilution procedure according to the method of Abdul-Rahman (2002).

**Results**

Sage leaves ethanol extract shows activity against two strain of bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus* at (0.1 and 0.5 mg/ml) concentration of extract.

Where the results of table (1) shows the ethanolic extract of Sage have significant antibacterial activity against *Pseudomonas aeruginosa* at (0.1 and 0.5 mg/ml) concentration of extract with inhibition zone diameter (15±0.2 and 20±0.26 mm) respectively, with corresponding MIC value at the concentration 2 and 4 mg\ml inhibition the growth of *Pseudomonas aeruginosa* as show in table (2).

As well as, the results of the same table show the Sage extract have significant antibacterial activity against *Staphylococcus aureus* at (0.1 and 0.5 mg/ml) concentration of extract with inhibition zone diameter (16±0.22 and 20±0.3 mm) respectively, with corresponding MIC value at the concentration 4 mg\ml inhibition the growth of *Staphylococcus* *aureus* as show in table (2).

***Table (1)*** *The inhibition zone diameter of ethanolic extract of Sage*

|  |  |  |
| --- | --- | --- |
| **Microorganism** | **Inhibition zone diameter** (mm) | **MIC value** (mg/ml) |
| C1 = 0.1 mg/ml | C2 = 0.5 mg/ml |
| *Pseudomonas aeruginosa* | 15±0.2 | 20±0.26 | 80 |
| *Staphylococcus aureus* | 16±0.22 | 20±0.3 | 90 |

***Table (2)* The MIC of Sage leaves ethanol**

|  |  |
| --- | --- |
|  **MIC****Types of bacteria**  | **% of isolate inhibited MIC (Mg/ml)** |
| **0.5** | **1** | **2** | **4** |
| *Pseudomonas aeruginosa* | **+** | **+** | **-** | **-** |
| *Staphylococcus* *aureus* | **+** | **+** |  | **+** |

 + = Growth.

* =No growth

**Discussion**

The Sage plant expresses antibacterial activity against two strains of bacteria. From the results, the ethanolicextract was active, the activity of the plant as showed in the literature review may be due to phenolic acids (example, salvin and salvin monomethyl ether) previously isolated from sage, especially against *Staphylococcus aureus* (B.H.M.A., 1983).

The obtained results were in agreement with the finding of Zhi-He and Hiroyuki, (1996) who were found that sage leaves extract at 0.2% concentration was gave strong activity against *Staphylococcus aureus* (Zhi-He & Hiroyuki 1996), in the same time Rogério *et al*., (2004) found that the essential oils of *Salvia* *officinalis* was inhibited 83.3% of *Pseudomonas* spp. (Rogério *et al.*, 2004), while Gislene *et al.,* (2000) were found that the collected sage in Brazil was inactive against *Staphylococcus aureus* (Gislene *et al.*, 2000).

In the present study, sage extract exhibited activity against *P. aeroginosa* and *Staphylococcus aureus* that is similar to the results of a study carried out by Hammer *et al.* (1999) and Pereira *et al.* (2004) were observed samples were susceptible to essential oil extracted from sage (Hammer *et al.*,1999; Pereira *et al.*,2004).

 The thujone and camphor that the main active constituents of sage oil have antimicrobial activity against *Staphylococcus* and *Pseudomonas* spp. (Dragan *et al.*,2004).

Finally, we concluded that antibacterial activities of sage provide hope that it can form the basis for an alternative therapeutic agent in the management of respiratory tract infections (RTIs).

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