Antimicrobial Effects of Methanolic Extract of Salvia Officinalis L, Including MRSA and Multidrug Resistant Acinetobacter Baumannii

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ABSTRACT

Microbial infections due to opportunistic bacteria and fungi in hospitals are becoming serious health problems, particularly in immune-compromised individuals. Moreover, microbes are becoming resistant to presently available drugs. Therefore, there is a need to develop new drugs to treat microbial infections. Salvia officinalis (S. officinalis) has been widely used in Middle East countries and Europe as a food preservative and for treatment of infections. Present study was aimed to investigate activity of S. officinalis against resistant and opportunistic microorganisms. ATCC strains of some bacteria (Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa) were purchased from Micromaster, Saudi Arabia. Methicillin Resistant Staphylococcus aureus (MRSA), Multi-drug resistant Acinetobacter baumannii and some fungi (Candida albicans and Aspergillus flavus), commonly causing serious infections in hospitals and opportunistic infections, were obtained from the Microbiology Department, Prince Abdulaziz Bin Musaad Hospital, Arar, Saudi Arabia. Agar dilution method was used to determine minimum inhibitory concentrations of methanolic extract of S. officinalis against test organisms as recommended by Clinical and Laboratory Standards Institute. The extract showed great activity against Staphylococcus aureus, MRSA and the multidrug resistant Acinetobacter baumanni (MICs 5-10 mg/ml), moderate activity against Candida albicans (MIC 10-20 mg/ml) and weak activity against Gram negative bacilli and Aspergillus flavus (MICs 20-40 mg/ml). To conclude, methanolic extract of S. officinalis possesses good activity against resistant and opportunistic infections: Staphylococcus aureus, MRSA, multidrug resistant Acinrtobacter baumannii and Candida albicans. Further investigations are needed to isolate active components from S. officinalis and develop new drugs.

Key Words: Salvia Officinalis, Methanolic Extract, Opportunistic Infections, MRSA, Multi Drug Resistant Acinetobacter Baumannii

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INTRODUCTION

Microbial infections due to opportunistic bacteria and fungi in hospitals, particularly in immune-compromised individuals, are becoming a serious health problem. Microbes are also becoming resistant to available antimicrobial drugs, thus requiring alternative medications [1]. Moreover, antimicrobial drugs killing the bacteria and

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fungi are likely to damage the human cells. Antimicrobial resistance, toxicity and poor immune-system of the host are the major problems encountered in the management of infectious diseases, and require the development of new, more effective and less toxic drugs.

Salvia officinalis L, (S. officinalis) commonly named as 'Sage' in English, and 'Maramia' in Arabic, has been used since ancient times as herbal tea, flavoring agent in food, a food preservative, and for the treatment of skin and respiratory infections, as well as for the elevation of mood, feeling of wellbeing and improvement of memory in Saudi Arabia, other Middle East countries and Europe [1, 2].

Recently, extracts of S. officinalis and some other species of Salvia were shown to possess antibacterial, antifungal and insecticidal activities, against gram-positive bacteria (such as Staphylococcus aureus, Micrococcus luteus, Bacillus cereus and Bacillus subtilis), gram negative bacteria (such as Escherichia coli, Salmonella enteretidis and Agrobacterium tumefaciens), food-borne and opportunistic fungi (Aspergillus and Fusarium species) as well as insecticidal effects against Spodoptera littoralis larvae and Tribolium castaneum adults [3].

The present study was aimed to further explore the antimicrobial activities of the methanolic extract of S. officinalis. Besides the determination of its activity on representative Gram positive and gram negative bacteria (Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa), which commonly cause opportunistic and serious infections in hospitals, its activity was also investigated against Methicillin Resistant Staphylococcus aureus (MRSA), Multi-drug resistant Acinetobacter baumannii) and some opportunistic fungi (Candida albicans and Aspergillus flavus).

METHODS

Plant

S. officinalis L plant was purchased from the local market, as it is commonly available in Saudi Arabia. Its identification was certified from a botanist at the College of Science, Northern Border University, Arar, Saudi Arabia. Arial parts of these plants were washed with distilled water, and freeze-dried.

Preparation of Methanolic Extract

100 grams of dried and powdered plant was macerated in 1000 ml of methanol (100 g/ 1000 ml) for one week at room temperature (about 25°C). The extract was then filtered using a filter funnel and the excess methanol evaporated under reduced pressure in a rotary evaporator. Once evaporated, the extract remained as a dry residue, which was stored in the refrigerator at 4°C. Immediately before use for the antimicrobial assay, the extract was suspended in DMSO to a final concentration of 400mg/ml. Fresh suspension of the extract in DMSO was used for the estimation of its activity against each microorganism.

Microorganisms

The ATCC strains (Numbers given in Table-1) of representative gram positive and gram negative bacteria (Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa) were purchased from Micromaster Saudi Arabia. MRSA, multi-drug resistant Acinetobacter baumannii and opportunistic fungi (Candida albicans and Aspergillus flavus) were obtained from the Microbiology Department of Prince Abdulaziz Bin Musaad Hospitl, Arar, Saudi Arabia.

Table 1. Antibacterial activity of Methanolic extract of S. officinalis (Maramia)

S.No	Name of Bacterial ATCC Strains	Methanol Extract of S. officinalis (mg/ml)				
		40	20	10	5	2.5
1	Staphylococcus aureus ATCC 254996	S	S	S	S	R
2	Staphylococcus aureus (MRSA)	S	S	S	S	R
3	Acinetobacter baumanni (MDRO)	S	S	S	S	I
5	Klebsiella pneumonia ATCC 700603	S	S	R	R	R
6	Pseudomonas aeruginosa ATCC 254992	S	S	R	R	R
7	Escherichia coli ATCC 25922	S	R	R	R	R

Growth media

Muller Hinton Agar (MHA) was used for bacteria and Sabouraud Dextrose agar (SDA) for fungi, and purchased from Micromaster, Saudi Arabia.

Minimum inhibitory concentration (MIC) value determination assay

The agar dilution method was used for the determination of the minimum inhibitory concentration (MIC) of the methanolic extract of S. officinalis against the standard (ATCC) strains of Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, as well as MRSA, Multi-drug resistant Acinetobacter baumannii, Candida albicans, and

Aspergillus flavus) as recommended by the Clinical and Laboratory Standards Institute [4].

2 ml of stock solution of the extract (800 mg/2ml DMSO) was added to 18 ml of MHA (For bacteria) and 18 ml of SDA (For fungi), then serially diluted to obtain final concentrations of 40, 20, 10, 5, and 2.5 mg/ml of extract in sterile petri plates. 2ml of DMSO was added to 18 ml of MHA (For bacteria) and 18 ml of SDA (For fungi), without S. officinalis extract, and then serially diluted to obtain concentrations of 10, 5, 2. and 1.25% DMSO in sterile petri plates as positive controls. The standard inoculum of 1µl for bacteria and of 0.5 MacFarland (10^5 CFU) for fungi, were spot inoculated in the test and control petri plates. All the plates were incubated aerobically for 12-24 hours at 37°C for bacteria, and at 25°C for fungi. The antimicrobial activity of the methanolic extract of S. officinalis was determined as Minimal Inhibitory Concentration (MIC) value, and was defined as the lowest concentration of the extract completely inhibiting the growth of the microorganism.

RESULTS AND DISCUSSION

Methanolic extract of S. officinalis had great activity against some bacteria, like Staphylococcus aureu, MRSA, and multi drug resistant Acinetobacter baumannii, (MIC of 5-10mg/ml of the extract); and there was a significant growth of these bacteria in corresponding DMSO controls. However, in the case of Enterococcus faecalis, there was also no growth in DMSO controls, i.e. were highly sensitive to DMSO. Because of that, the effect of S. officinalis against Enterococcus faecalis could not be determined. The extract had moderate activity for Klebsiella pneumonia & Pseudomonas aeruginosa (MIC of 20mg/ml); and there was a significant growth in corresponding DMSO controls, whereas, Escherichia coli had low sensitivity to the extract as well as DMSO (MIC of 40mg/ml for the extract and no growth in the corresponding concentrations of DMSO, 10%), (Table-1 and Fig. 1-3).

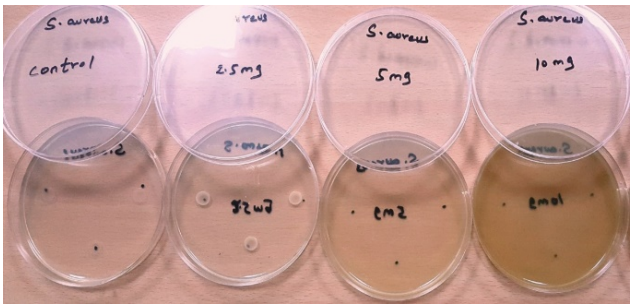

Fig. 1. Growth of Staphylococcus aureus in different concentrations of methanolic extract of S. officinalis in Muller Hinton Agar, showing MIC of 5mg/ml

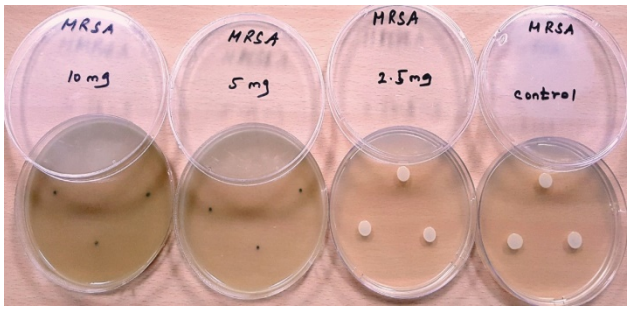

Fig. 2. Growth of MRSA in different concentrations of methanolic extract of S. officinalis in Muller Hinton Agar, showing MIC of 5mg/ml

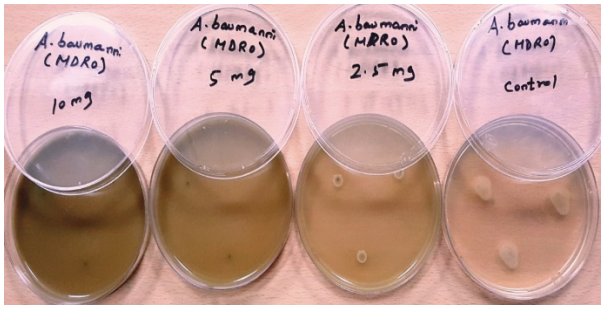

Fig. 3. Growth of Acinetobacte baumannii in different concentrations of methanolic extract of S. officinalis in Muller Hinton Agar, showing MIC of 5mg/ml

Regarding fungi, Candida. albicans were relatively more sensitive to the methanolic extract of S. officinalis (MIC of 20mg/ml) than Aspergillus flavus (Almost complete to around 50% inhibition of the growth with 40 to 5mg/ml of the extract (Table-2 and Fig. 4 & 5).

Table 2. Antifungal Activity of Methanolic Extract of S. officinalis (Maramia)

S.No	Name of Fungal ATCC Strains	Methanol Extract of Salvia officinalis (mg/ml)				
		40	20	10	5	2.5
1	Candida albicans ATCC	S	S	I	R	R
2	Aspergillus flavus	S	I	I	I	R

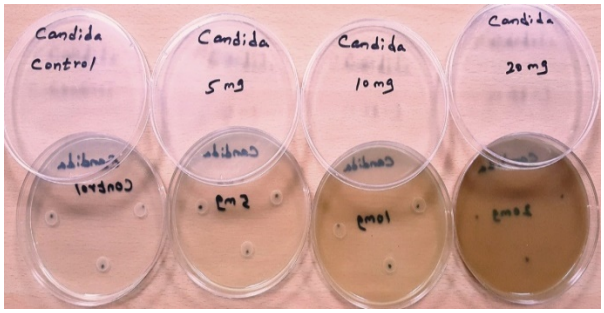

Fig. 4. Growth of Candida albicans in different concentrations of methanolic extract of S. officinalis in Sabouraud Dextrose Agar, showing MIC of 20 mg/ml

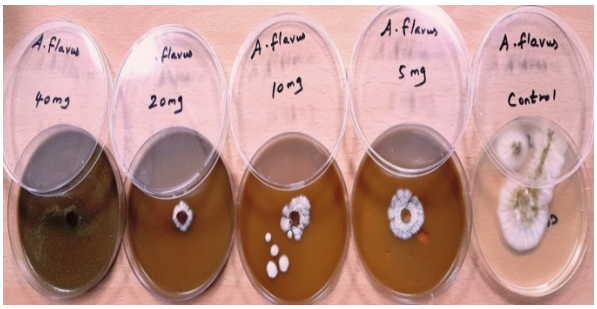

Fig. 5. Growth of Aspergillus flavus in different concentrations of methanolic extract of S. officinalis in Sabouraud Dextrose, showing almost complete to 50% inhibition of the growth with 40 to 5mg/ml of the extract

DMSO which is commonly used as a solvent in phytochemical studies, has been shown to possess antimicrobial activity and may interfere with the determination of MICs of plant extracts. Variable effects of DMSO against different microorganisms are reported in the literature. In general, 10-15% DMSO is reported to inhibit the growth of most organisms [5, 6], 1% showed no effect [7], whereas, between 2-8 gave variable results for different microorganisms [8].

In the present study, DMSO was used as a solvent for the methanolic extract of S. officinalis and as active control in the antimicrobial assays. DMSO 5% and below did not affect the growth of the organisms tested, except that DMSO 10 to 1.25% completely inhibited the growth of Enterococcus faecalis and partially inhibited Aspergillus flavus. Therefore, MIC values of the extract of S officinalis between 2.5 to 20% observed for other microorganisms tested in the present study are least likely to be affected by the presence of DMSO. In a recent study also, Staphylococcus aureus was reported to be relatively more resistant and Enterococcus faecalis more sensitive to DMSO [9].

Staphylococcus aureus, gram positive cocci, reside normally in the upper respiratory tract, skin and intestinal mucosa. They can become pathogenic and may cause serious infections, like bacteremia, pneumonia, endocarditis and brain abscess, etc. Within days, they become resistant to commonly used drugs [10-12]. The results of the present study revealed that the methanolilc extract of S. officinalis had promising activity against MRSA, which are becoming resistant to even Vancomycin, termed as vancomycin-resistant Staphylococcus aureus [13].

In an earlier study also Staphylococcus aureus was shown to be more sensitive to essential oil of S. officinalis (MIC 0.6 mg/ml) than gram negative bacilli, like Salmonella and E. coli (MICs 5 and 10 mg/ml, respectively). Their results also show that the essential oil was more effective than methanolic extract used in the present study, MICs for Staphylococcus aureus and MRSA were 5mg/ml and Escherichia coli 40 mg/ml[3].

Acinetobacter baumannii is a Gram-negative bacillus, an emergent opportunistic pathogen and prone to cause infections of respiratory tract, urinary tract, skin and eyes. Recently, it has been designated as a “red alert” human pathogen, because of its extensive antibiotic resistance [14]. The methanolilc extract of S. officinalis was also very effective against multi-drug resistant A. baumanni. We could not find any study in the literature to compare the results of the extract against Acinetobacter baumannii.

Candida infections are the most frequently encountered nosocomial fungal infections [15]. In the present study, the methanolic extract of S. officinalis demonstrated a significant activity against Candida albicans. Essential oils of S. officinalis have also been demonstrated to inhibit Candida albicans [16].

Aspergillus flavus is commonly found in the air and is the most common cause of superficial infection. Aflatoxins released by Aspergillus flavus are the most potent hepatocarcinogenic natural compounds [17]. In the present study the methanolic extract of S. officinalis also demonstrated a moderate activity against Aspergillus flavus. In a previous study, food isolate of Aspergillus flavus was reported to be more resistant to essential oil of S. officinalis as compared to standard Aspergillus niger [3].

The present study has disclosed the potential of S. officinalis as a natural source for the production of new antibacterial and antifungal drugs. Other plants’ extracts and their active principles have also been shown to possess antimicrobial activity. For example, various extracts of Nigella sativa and its active component, e.g. thymoquinone, were shown to be active against resistant Streptococci, Staphylococci and Pseudomonas species, as well as some opportunistic fungi and dermatophytes [18-20].

CONCLUSIONS

The methanolic extract of S. officinalis showed good activity against Staphylococcus aureus, MRSA, and the multidrug resistant Acinetobacter baumanni; moderate activity against Candida albicans and weak activity against some gram negative bacilli and Aspergillus flavus. Further researches are needed for the isolation of active principles from S. officinalis extracts and determination of their antimicrobial activities, which could lead to development of new drugs against resistant bacteria, like MRSA, and multidrug resistant A. Baumanni.

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