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Biological activity of the aqueous and methanolic *Salvia officinalis* extracts

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Abstract: *Salvia officinalis* is medical plant used since a long time for treatment of bronchitis, cough, mouth inflammation, skin disease and many other diseases. To detect the biological activity of methanolic and aqueous *Salvia officinalis* extracts and compare their effectiveness. Both aqueous and methanolic *Salvia officinalis* extracts was prepared and their antibacterial, antifungal and antioxidant activity was tested .Results were analyzed using SPSS version**.** In vitro antibacterial activity show that the methanolic extract of *Salvia officinalis* was significantly inhibited bacterial isolates than aqueous extract, also most of yeasts affected by extracts with the superiority of methanol extract .It has been shown that the hydrogen peroxide scavenging was greater in the case of methanol extract of *S.officianlis* and lower in the case of aqueous extract this assay carried out with compared to the (Butyalted hydroxyl toluene) BHT as antioxidant stander material.

Keywords: *Salvia officinalis***,** extract**,** antibacterial, antifungal, antioxidant**.**

INTRODUCTION

Salvia genus belongs to Lamiaceae family and Nepetideae subfamily[1].This genus have about 900 species distributed over the world ,*Salvia officinalis* (Sage) one of the most important species[2]. Since a long time Sage tea used for treatment of digestive and circulation disturbance ,cbronchitis ,cough, mouth inflammation ,skin disease and many other diseases[3].c*Salvia officinalis* have many organic compound and essential oil the most important compound that the plant Sage contain are flavonoids , phenolics and sesquitrpenes[4,5]. Sage oil has antibacterial activity attributed to the presence of thujones

the inhibitory activity of the organic compound and the oil of sage against Gram -positive and Gram-negative bacteria and against rang of fungi has demonstrated such as *Candida albicans* ,*C.crusei* ,*C.pseudotropicalis* ,*Torulopsis glabrata* ,*Cryptococcus neoformans* and *Aspergillus flavus* [6,7].The extract of *Salvia officinalis* has antiviral, ,anti-inflammatory , antitumor and antioxidant activity ,this antioxidant action due to Rosmarinic acid and derivatives[8].

MATERIAL AND METHODS

Preparation of plant extracts: 20 gm of the powdered (dry aerial parts) of *Salvia officinalis* were weight in flask percolated with 200 ml methanol this flask was proper sealed with aluminum foil and left for 24 hours. The solution was then filtered using a funnel filled in a filter paper and the methanol extracts collected in Petri dishes and dried at room temperature. The aqueous extract prepare in a similar way with replace the methanol with water [9].

Phytochemical Screening: The preliminary Phytochemical analysis of both methanolic and aqueous extracts carried out using standard procedures to identify the various constituents described by Harborne[10].The tests included Alkaloids test , tannins , flavonoids test, glycosides test , phenols test ,Saponins test ,Carbohydrates test and peptides and free amine groups test.

Antibacterial activity : *In vitro* antibacterial activity were determined for both methanolic and aqueous extracts against many bacterial isolates :*Escherichia coli* , *Staphylococcous aureus* ,*Pseudomonas aeruginosa* ,*Bacillus subtilus* , *Kebsiella ssp* , *Proteus ssp*., a suspension of each isolate was prepared and standardized to a turbidity equivalent to that of 0.5 MacFerland scale (1×10^8 cfu/ml) for bacteria .

The test was performed according to Perez C,Pauli *et al.*[11] by adding 10µl of each microorganism inoculums on the surface of Muller-Hinton agar then sterile cotton swabs used for streaking the suspensions , after drying, two 5 mm diameter pores was made in each plate by using cork porer , 100µl of both extracts was add separately in the pores of plates. All tests were done with duplicate and control plates, the plates were incubated at 37°C for one day after incubation period the diameter of the inhibition zones were evaluated in millimeters.

Antifungal activity: Antifungal bioactivity of extracts were tested against four isolates of yeasts *Candida albicans* ,*C.trpicalis* and *Cryptococcus neoformans* and two isolates of filamentous fungi *Fusarium solani* and *Geotrichum candidum* using Sabouraud Dextrose Agar (SDA) medium for yeast and Potato Dextrose Agar (PDA) for filamentous fungi, the suspensions of yeasts and fungi were prepared and standardized to a turbidity equivalent to that of 0.5 MacFerland scale (1×10^8 cfu/ml) .10µl of each microorganism suspension add on the surface of the media then spread with sterile L-shape glass rod, after drying, two 5mm diameter pores was made in each plate ,100µl of both extracts was add separately in the pores of plates cultures were incubated at 25◦C for 2 days for yeasts and 5 days for filamentous fungi, after incubation period the diameter of the inhibition zones were evaluated in millimeters.

Antioxidant assay: Antioxidant assay carried out by hydrogen peroxide scavenging activity assay in this assay hydrogen peroxide scavenging of extracts can be determined using replacement titration methodology .1ml of 0.1 mM H_2O_2 and 0.1 ml of different concentrations of the extracts are mixed together.2drops of 3% ammonium molybdate ,10ml of 2M sulphuric acid and 7ml of 1.8 M potassium iodide are added to the reaction mixture . The mixed solution is then titrated with

5.09 mM NaS_2O_3.Appearance of yellow colour is marked as the end point of the reaction .control was reaction mixture with out of extract.

% of scavenging of hydrogen peroxide is calculated as follows:

% Inhibition =(V0-V1) /V0 × 100

V0=volume of NaS_2O_3 solution used to titrate the control

V1= volume of NaS_2O_3 solution used to titrate the extract mixture[12.]

Cytotoxicity test: This test was carried out for prepared extracts against human fresh blood according to Nair *et al.*[13] Different concentrations of extracts were prepared (10, 50,100 and 200 ppm), then 100µl of each concentration was added to each tube of human blood solution. The tubes were left at room temperature and formation of turbidity of blood solution was tested after 15, 30 and 60 min. as an incubation for the cytotoxicity of extracts.

RESULTS

Phytochemical Screening: The phytochemical analysis show that methanol and aqueous extracts of *Salvia officinalis* have several compounds, **Table (1).**

Table 1: Phytochemical analysis of methanolic and aqueous extracts of *Salvia officinalis*

Test / Extract	Alkaloids	tannins	flavonoids	Carbohydrates	Glycosides	Phenols	Saponins	peptides and free amine groups
Aqueous Extracts	+	+	+	–	+	+	+	+
methanol Extracts	+	+	–	+	+	+	+	–

Antibacterial activity : In vitro antibacterial activity show that the methanolic extract of *Salvia officinalis* was more effective than aqueous extract with significantly high difference ,the isolates of *Klebsiella* spp and *Bacillus subtilus* were best inhibited isolates with inhibition zones 31mm and 29 mm respectively **Fig.(1)** and **(2).**

Antifungal activity: Most of yeasts affected by extracts with the superiority of methanol extract ,while the filamentous fungi were resistance to aqueous extract and sensitive to methanol extract especially *Fuasrium solani* whit inhibition zone about 20mm **Fig.(3)**

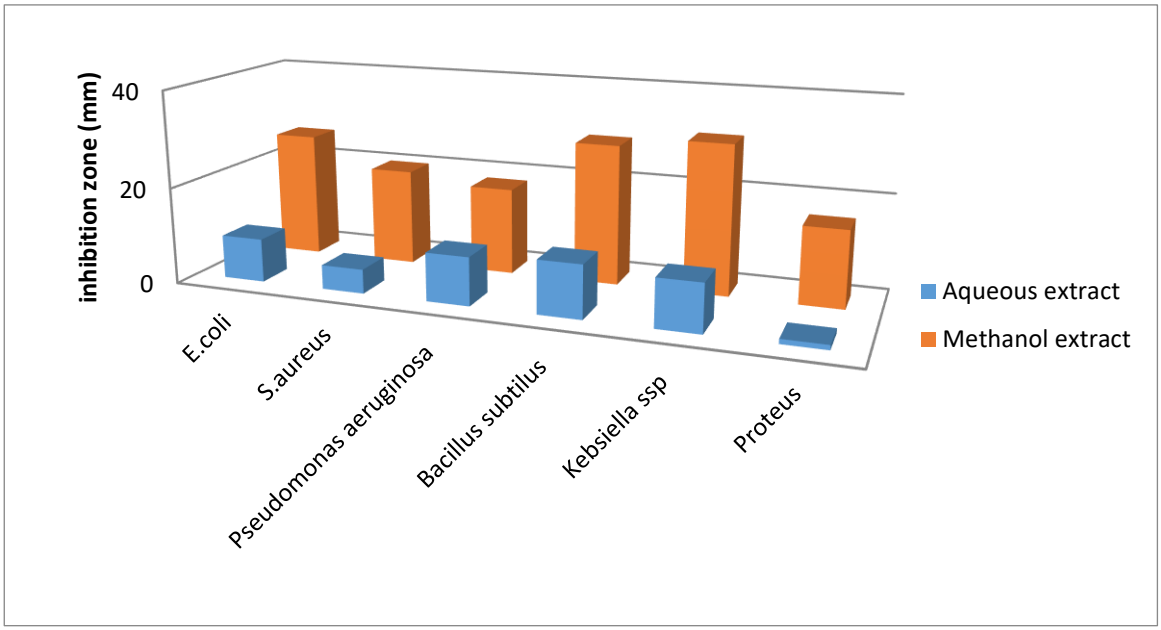

Fig.1: Antibacterial activity of extracts

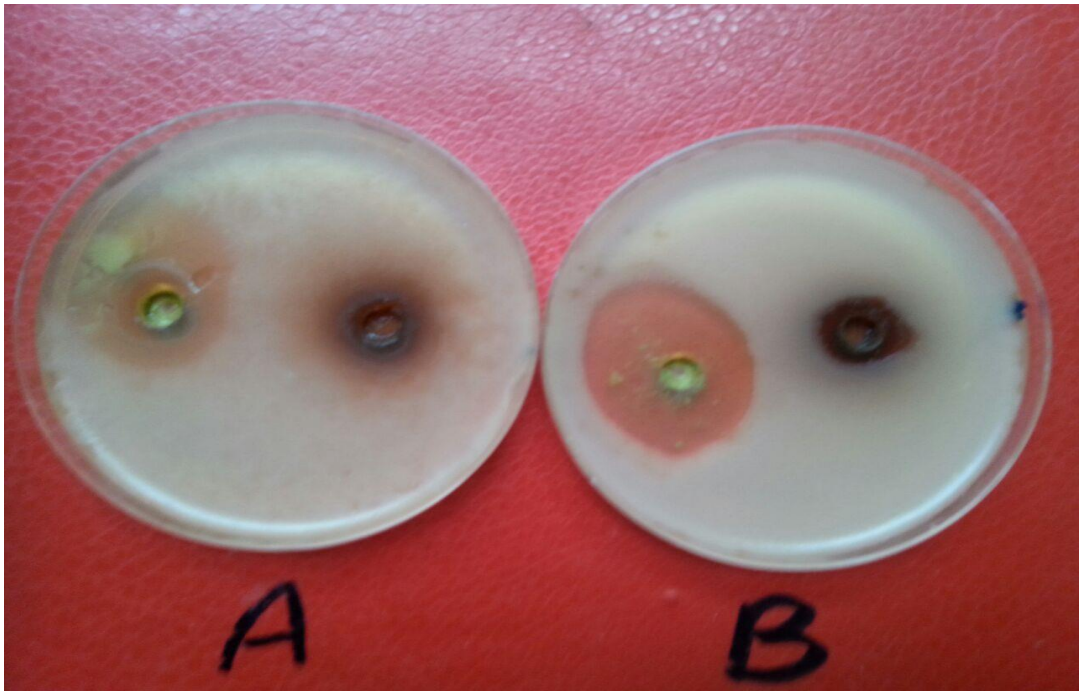

Fig.2: Antibacterial activity of extracts

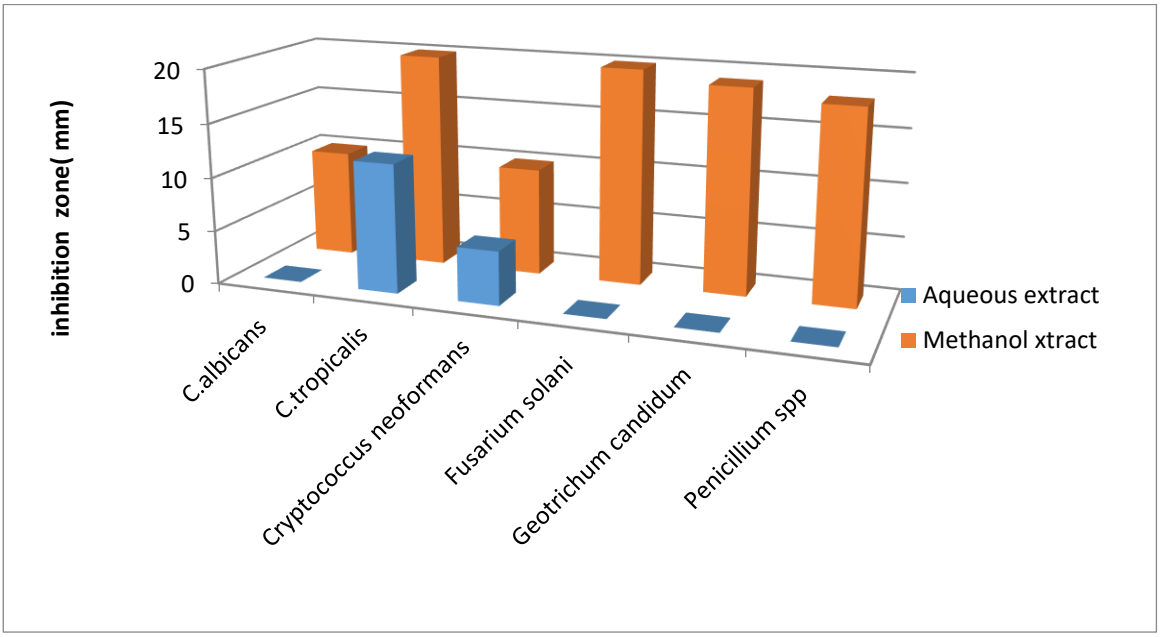

Fig.3: Antifungal activity of extracts

Antioxidant assay: Antioxidant assay carried out by hydrogen peroxide scavenging activity assay in this assay hydrogen peroxide scavenging of extracts can be determined using replacement titration methodology .It has been shown that the hydrogen peroxide scavenging was greater in the case of methanolic extract of *S.officianlis* and lower in the case of aqueous extract this assay carried out with compared to the (Butyalted hydroxyl toluene) BHT as antioxidant stander material **Table (2).**

Table 2: Antioxidant activity of extracts

Conc. / Extract	% Hydrogen peroxide scavenging			
	20mg/ml	50mg/ml	100mg/ml	200mg/ml
Aqueous extract	0	7.1	14	22.8
Methanol extract	7	14.2	21.4	28.5
BHT	11.4	15.7	22.8	35.2

Cytotoxicity test: The cytotoxic activity of *Salvia officinalis* extracts were assessed by using different concentrations of human blood solution the result showed no turbidity formation after 15, 30 and 60 min from adding 100µl of the studied extracts to all concentrations of human blood solution, indicated no cytotoxic effect of both methanol and aqueous extracts.

DISCUSSION

Salvia officianlis is the most valuable species in terms of biologically active principles content with compared to other species of salvia genus[14]. Phytochemical screening of *Salvia officianlis* extracts (methanol and aqueous) show presence of many chemical compounds like phenols,glycosides,tannins ,alkaloids ,saponins and carbohydrates, some of these compounds have been associated to antibacterial activity and thus have curative properties against pathogens[15].In current study both methanol and aqueous extract have antibacterial activity with superiority of methanol extract. The bacterial isolate *Bacillus subtilus* and *Klebsiella* ssp were the most effected by methanol extract[16] have suggested that the plant metabolites promote bacterial lysis and damaged of the cell membrane .

The carrier of antibacterial activity of the sage oil and extract were alpha-thujone and comphor what is according to literature data[17].

Also extracts have antifungal activity against both yeasts and moulds with superiority of methanol extract, specially *Candida tropicalis* and *Fusarium solani* [18] Showed antifungal activity of the *Salvia officianlis* extract against *C.albicans* and the mould Aspergillus *niger* while ther was no activity against the yeast *Saccharomyces cervisiae* .

The antioxidant activities of *Salvia officianlis* has been widely demonstrated [19,20] .The result of antioxidant activity of methanol extract too. It is Know that sage species are characterized by type polyphenolic compounds that have high antioxidant activity like rosmorinic acid and caffeic acid [21,22].

Regression analysis of the relationship between percentage of hydrogen peroxide scavenging and plant extract concentration (Log value) showed that there was significant correlation between extracts concentration and antioxidant activity, this antioxidant activity was attributed to phenolic compounds and flavonoid[6].

Cytotoxicity test indicated no cytotoxic effect of both methanol and aqueous extracts in all concentrations ,so that the dried leaves of *Salvia officianlis* used as herbal tea , food flavoring,in cosmetics , raw material in medicine ,perfumery and food- industry[14]

The cytotoxicity result gives impression of the possibility to use *Salvia officianlis* extract as anti-infectious drug because there is no cytotoxic effect against human blood solution.

CONCLUSION

Salvia officianlis is medical plant used for treatment of many diseases. Both aqueous and methanolic *Salvia officinalis* extracts weres significantly inhibited bacterial and yeasts isolates with the superiority of methanol extract, It has been shown that the hydrogen peroxide scavenging was greater in the case of methanol extract of *S.officianlis* and lower in the case of aqueous extract this assay carried out with compared to the (Butyalted hydroxyl toluene) BHT as antioxidant stander material.

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