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Full Length Research Paper

Antibacterial effect of some Egyptian plants on antibiotic-resistant oral bacteria

Magdy A. Abu-Gharbia, El-Sayed M. Soltan, Osman M. El-Maghraby and Walaa M. Abd El-Raheem*

Department of Botany, Faculty of Science, Sohag University, Sohag, Egypt

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The present study was conducted with a view to evaluate the therapeutic potentials of six plants traditionally used in Egypt against multi-drug resistant oral pathogens isolated from infected patients with different oral diseases. All ethanol extracts of tested plants were found to exhibit potential antimicrobial properties against multi-drug resistant isolates but maximum zone of inhibition was observed in ethanol extracts of *Allium sativum* and *Syzygium aromaticum* which showed the minimum inhibitory concentration (MIC) against *Streptococcus mutans* and *Staphylococcus aureus* to be 0.39 mg/ml. The antibacterial activities observed could be due to the presence of some of the secondary metabolites like, alkaloids, anthraquinones, sterols, glycosides, saponins, terpenes and flavonoids which detected in ethanol extracts. The bioautographic results revealed that the maximum zone of inhibition of *Syzygium aromaticum* and *Allium sativum* were observed at R_f 0.64 & 0.82, respectively which indicates that the compound retained at these R_f values having potent antibacterial efficacy against the selected oral pathogens. The findings of present study lend support for the use of them in preparation of toothpastes and mouth rinses containing these antimicrobial agents for the prevention of oral microbial diseases.

Keywords: plant extracts, antibacterial, minimum inhibitory concentration (MIC), phytochemical, bioautography.

INTRODUCTION

It is well known and world-wide accepted that oral health is a reflection of one's general health, affecting the ability of an individual to eat, speak, and contributes significantly to a sense of confidence and well-being (Daniluk *et al*., 2006). The human oral cavity is perhaps the most complex and heterogeneous microbial habitat in the human body. It may act as a reservoir for several pathogens related to systemic infections. Thus, it is no surprise that oral health is being increasingly linked with other conditions such as heart disease, pregnancy, and stroke (Taylor *et al*., 2004).

Several antibacterial agents including, fluorides, chlorhexidine, ampicillin, erythromycin, penicillin, tetracycline, and vancomycin have been used widely in dentistry to inhibit bacterial growth (Allaker and Douglas, 2009). However, excessive use of these chemicals can result in derangements of the oral and intestinal flora and cause side effects such as microorganism susceptibility, vomiting, diarrhoea and tooth staining (Feres *et al*., 2010). These problems necessitate further search for natural antimicrobial agents that are safe for humans and specific for oral pathogens. The fact that some plants have been used traditionally for centuries and modern

*Corresponding Author E-mail: walaa123us@yahoo.com; Tel.: +20934601159; fax: +20934601159

scientific studies have shown the existence of good correlation between the traditional or folkloric application of some of these plants further strengthens the search for pharmacologically active compounds from plants (Egharevba and Kunle, 2010).

It has been well documented that many medicinal plants confer antimicrobial activity towards oral bacteria (Jebashree *et al*., 2011; Smullen *et al*., 2007). The literature survey of the folklore medicine reveals the use of *Syzygium aromaticum* to maintain oral hygene, it is natural analgaesic used as an anodyne for dental emergencies and applied to a cavity in a decayed tooth, also relieves toothache. It also helps to decrease infection in the teeth due to its antiseptic properties (Prashar *et al*., 2006). Other example is *Allium sativum* extract that has been reported to inhibit growth of various Gram-positive and Gram-negative bacteria including: *Micrococcus, Enterobacter, Escherichia*, *Klebsiella, Lactobacilli, Pseudomonas, Salmonella*, *Shigella* and *Proteus* (Martin and Ernst, 2003; Tsao and Yin, 2001).

Once the antimicrobial property of the plant extracts is screened under *in vitro* condition against oral pathogens, *in vivo* trials can be carried out for the treatment of dental caries and other oral diseases by external application on the caries tooth or as a preventive mouthrinses and toothpates.

Therefore, the present study was carried out to further validate the *in vitro* antibacterial potential of six medicinal plants namely: *Cinnamomum cassia* (bark), *Allium sativum* (bulb), *Syzygium aromaticum* (buds), *Punica granatum* (seeds), *Citrus lemoniumn* (fruit) and *Hibiscus sabdariffa* (calyces) against multi-drug resistant (MDR) oral pathogens of *Streptococcus mutans, Enterococcus faecalis*, *Staphylococcus aureus, Actinomyces viscosus, Lactobacillus acidophilus, Nisseria meningitidis, Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Haemophilus parainfluenza* and *Eikenella corrodens* and provide information on the type of secondary metabolites contained in the active plants.

MATERIALS AND METHODS

Plant materials collection

Plant materials of six plants (*C. cassia*, *A. sativum*, *S. aromaticum*, *P. granatum*, *C. lemoniumn* and *H. sabdariffa*) purchased from a well-known local market of Sohag district, Egypt. Plants were authenticated by a taxonomist at the Botany Department, Faculty of Science, Sohag University, Egypt.

Preparation of ethanol extracts (Hedge et al., 2009)

The air-dried, of powdered plant parts (100 g) were extracted by maceration with ethanol (95%) for 3 days. After that the ethanol layer was decanted off. The process was repeated thrice. The solvent from the total extract was filtered, evaporated using rotary vacuum evaporator at 50°C and stored at -4°C till further uses.

Bacterial cultures and growth conditions

The pathogenic bacteria were isolated from the oral cavities of 140 patients who attended the Dental Clinic, Department of Dentistry, Sohag University Hospital, Sohag District for treatments from different oral and dental diseases. Table 1 showed the antibiotic resistance profiles of the oral pathogens. The test strains were maintained on nutrient agar slants at 4° C and subcultured on to nutrient broth for 24h prior to testing. These bacteria served as test pathogens for antibacterial activity assay.

Antibacterial susceptibility test

Primarily antibacterial activities of the ethanol extracts were investigated by the agar well diffusion method (Jeyaseelan and Jashothan, 2012). The ethanol extracts were serially diluted by DMSO (10%) from 25 to 200 mg/ml. Cups with 8mm diameter were made in Muller Hinton agar (MHA) plates (Merck, Germany) and streaked by the microorganism saline suspension from overnight bacterial agar culture with turbidity equal to a 0.5 Mc Farland (Mahboubi *et al*., 2014). 100 µliters of each dilution were added in each cup and solvent as negative control was done. The plates were incubated at 37°C for 24 hours.

Minimum inhibitory concentration (MIC)

The MIC of the extracts was determined by macro broth dilution method (Chakraborty *et al*., 2011). Briefly the ethanol extracts were serially diluted and dilutions were added to each of the tubes containing Muller Hinton Broth (Merck, Germany) with the final inoculums count of 5×10^5 CFU/ml. After 24 h incubation at 37 °C, the test tubes were evaluated for possible growth. The lowest concentration which inhibits the visible growth of bacteria in liquid medium is defined as MIC.

Minimum bactericidal concentration (MBC)

50 µliter from each tube with no visible growth in MIC test was collected, sub cultured in MHA plates and incubated for 24 hours at 37°C. MBC was defined as the lowest concentration with no growth of bacteria (Chakraborty *et al*., 2011).

Table 1. Antibiotic resistance profile of bacterial isolates used

Antibiotics	Test bacteria										
	S.m	E.f	S.a	A.v	L.a	N.m	P.a	E.c	K.p	H.p	E.d
P	R	R	R	R	S	R	R	R	R	R	R
AMX	S	S	R	R	R	R	R	R	R	R	R
GN	R	R	S	R	R	S	R	R	S	R	S
S	R	R	S	R	R	S	R	R	R	R	S
TC	R	R	R	R	R	R	R	S	R	R	R
ER	R	R	S	R	R	R	R	R	R	S	R
CPR	S	S	S	S	S	S	S	S	S	S	S
CHR	S	R	S	R	R	S	S	R	R	R	S
M	R	R	R	R	R	R	R	R	R	R	R
CF	R	R	R	R	R	S	R	R	R	S	R
CL	S	S	S	S	S	R	R	R	R	R	R

P, penicillin; AMX, amoxicillin; GN, gentamycin; S, streptomycin; TC, tetracycline; ER, erythromycin; CPR, ciprofloxacin; CHR, chloramphenicol; M, metronidazole; CF, cefotaxime; CL, clindamycin; S.m, *S.mutans*; E.f, *E.faecalis*; S.a, *S.aureus*; A.v, *A.viscosus*; L.a, *L.acidophilus*; N.m, *N.meningitidis*; E.c, *E.coli*; K.p, *K.pneumoniae*; P.a, *P.aeruginosa*; H.p, *H.parainfluenza*; *E.c*, *E.corrodens*; R, Resistant; S, Sensitive

Qualitative preliminary phytochemical analysis of various plants

The preliminary phytochemical studies were performed for testing the different phytoconstituents present in the six plant extracts as per the standard procedures **(**Khandelwal, 2010; Shah and Seth, 2010).

Fractionation of the most active extracts on silica gel column

Different solvent mixtures were used in elution systems i.e., hexane, ethyl acetate and methanol. The concentrated ethanol extract of 100 g was fractionated by column chromatography on silica gel (60-120 mesh, Merck, Germany). The fractions were collected and subjected for further analysis.

Thin layer chromatography of the column fractions

TLC of the fractions was performed on a precoated silica gel aluminum plate (10 x 20 cm, silica gel 60 F_{254} TLC plate, Merck). The collected fractions from column chromatography were applied on base line of different TLC plate by using capillary and placed at 45° angle in the development chamber containing mobile phase. The R_f values of the standard and the fractions were determined as described by (El-Olemyl *et al*., 1994) using the following formula:

R_f = Distance travelled by solute / Distance travelled by solvent

Determination the most bioactive fractions by bioautography agar overlay

The developed TLC plates were thinly overlaid onto molten nutrient agar inoculated with an overnight culture of standard *S. aureus*. The plates were incubated in a dark and humid chamber at overnight at 37°C. Subsequently, the bioautogram was sprayed with an aqueous solution of 2, 3, 5-triphenyl tetrazolium chloride and further incubated for at 37°C for 4 h. Microbial growth inhibition appeared as clear zones against a pink background. The R_f values of the spots showing inhibition were determined. The R_f of the inhibition zones on plate B was compared with the R_f of reference chromatogram (plate A) as well as R_f of the spots on plate C. The experiment was repeated twice.

RESULTS AND DISCUSSION

Resistance profiling of the microbial strains (eleven) was done by using eleven different antibiotics belonging to different classes (Table 1). All bacterial strains were found to be multidrug resistant as they were showing resistance to more than five antibiotics. Data indicated that ciprofloxacin was the most effective one against all of the bacterial isolates. In dentistry, emergence of multidrug resistance in human pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin using them as mouth rinse or toothpaste has advantages as decreasing apparent side effects, decreasing poisonous effects on

Table 2. Antibacterial activity of medicinal plants determined by agar well diffusion method

Pathogen	Plant ethanol extracts															Cp
	H. sabdariffa					*A. sativum*					*C. lemoniumn*					
	25	50	100	150	200	25	50	100	150	200	25	50	100	150	200	
S. m	11± 0.40	15± 0.10	27± 0.61	30± 0.35	33± 0.10	20± 0.03	25± 0.11	32± 0.61	36± 0.40	41± 0.05	14± 0.10	20± 0.14	27± 0.35	31± 0.10	34± 0.17	27± 0.37
E. f	10± 0.05	15± 0.37	20± 0.37	25± 0.13	31± 0.03	16± 0.10	20± 0.17	25± 0.19	32± 0.10	36± 0.19	10± 0.10	15± 0.05	21± 0.14	27± 0.10	32± 0.16	24± 0.19
S. a	12± 0.05	18± 0.61	25± 0.03	34± 0.37	36± 0.05	25± 0.10	33± 0.16	37± 0.61	40± 0.05	42± 0.61	18± 0.05	26± 0.19	32± 0.18	36± 0.05	39± 0.10	34± 0.37
A. v	11± 0.37	15± 0.11	20± 0.40	27± 0.05	30± 0.40	20± 0.11	27± 0.05	32± 0.68	37± 0.35	40± 0.61	10± 0.37	15± 0.61	21± 0.37	27± 0.12	29± 0.03	27± 0.61
L. a	9±0. 35	14± 0.35	21± 0.37	25± 0.61	29± 0.35	17± 0.11	21± 0.37	27± 0.03	32± 0.11	38± 0.37	9±0. 11	15± 0.11	19± 0.35	25± 0.05	27± 0.37	25± 0.15
N. m	18± 0.15	24± 0.40	30± 0.35	35± 0.37	39± 0.40	18± 0.03	24± 0.15	30± 0.61	34± 0.15	36± 0.17	13± 0.15	19± 0.11	30± 0.05	35± 0.05	39± 0.35	24± 0.19
P. a	23± 0.37	29± 0.11	35± 0.03	40± 0.13	43± 0.11	14± 0.61	17± 0.35	22± 0.68	26± 0.40	32± 0.19	13± 0.12	21± 0.19	29± 0.35	35± 0.12	38± 0.61	17± 0.05
E. c	16± 0.61	22± 0.37	28± 0.61	36± 0.40	41± 0.37	16± 0.10	20± 0.37	29± 0.15	34± 0.10	39± 0.40	14± 0.10	20± 0.14	27± 0.12	34± 0.35	39± 0.10	22± 0.37
K. p	19± 0.68	25± 0.11	30± 0.35	34± 0.27	39± 0.27	15± 0.37	20± 0.21	26± 0.26	30± 0.03	34± 0.29	12± 0.27	19± 0.05	27± 0.27	33± 0.27	39± 0.13	18± 0.27
H. p	17± 0.68	24± 0.25	30± 0.11	36± 0.11	40± 0.61	19± 0.15	26± 0.25	31± 0.20	35± 0.06	38± 0.29	15± 0.40	20± 0.68	27± 0.44	32± 0.15	37± 0.68	25± 0.35
E. d	12± 0.03	19± 0.27	28± 0.27	32± 0.37	39± 0.40	18± 0.35	23± 0.40	28± 0.15	33± 0.27	37± 0.11	17± 0.03	21± 0.37	29± 0.03	33± 0.05	37± 0.11	27± 0.15
Continue																
S. m	13± 0.05	18± 0.44	24± 0.37	30± 0.10	33± 0.44	15± 0.61	24± 0.10	30± 0.44	35± 0.10	40± 0.10	20± 0.61	27± 0.10	33± 0.61	37± 0.44	41± 0.61	27± 0.37
E. f	9±0. 19	13± 0.10	22± 0.05	26± 0.05	29± 0.13	12± 0.61	19± 0.19	26± 0.19	30± 0.10	37± 0.61	16± 0.05	24± 0.44	30± 0.28	33± 0.37	37± 0.61	24± 0.19
S. a	16± 0.05	22± 0.05	27± 0.10	35± 0.17	40± 0.37	14± 0.05	19± 0.19	28± 0.44	37± 0.05	41± 0.05	26± 0.05	30± 0.61	36± 0.61	40± 0.28	43± 0.19	34± 0.37
A. v	11± 0.61	17± 0.61	23± 0.37	27± 0.19	31± 0.13	14± 0.61	20± 0.19	25± 0.61	34± 0.13	38± 0.37	22± 0.05	27± 0.05	31± 0.19	37± 0.44	41± 0.05	27± 0.61
L. a	10± 0.05	15± 0.44	22± 0.61	26± 0.05	29± 0.19	11± 0.37	15± 0.13	23± 0.19	29± 0.37	35± 0.10	19± 0.13	24± 0.10	29± 0.05	32± 0.37	39± 0.13	25± 0.15
N. m	12± 0.37	17± 0.10	23± 0.19	28± 0.14	32± 0.61	11± 0.19	17± 0.05	23± 0.44	28± 0.19	34± 0.19	20± 0.28	26± 0.05	29± 0.19	32± 0.28	38± 0.19	24± 0.19
P. a	9±0. 19	13± 0.05	17± 0.61	20± 0.10	24± 0.19	9±0. 05	14± 0.19	20± 0.10	25± 0.61	30± 0.19	17± 0.61	21± 0.37	25± 0.61	30± 0.44	34± 0.13	17± 0.05
E. c	10± 0.37	15± 0.44	21± 0.13	26± 0.19	30± 0.13	9±0. 05	15± 0.19	22± 0.61	27± 0.44	32± 0.44	18± 0.05	22± 0.05	29± 0.28	33± 0.19	37± 0.05	22± 0.37
K. p	16± 0.10	22± 0.05	28± 0.10	35± 0.37	39± 0.10	10± 0.61	14± 0.19	19± 0.15	25± 0.19	30± 0.37	19± 0.13	24± 0.28	29± 0.19	34± 0.05	36± 0.28	18± 0.27
H. p	10± 0.10	15± 0.10	22± 0.44	27± 0.10	30± 0.61	13± 0.44	20± 0.10	26± 0.44	31± 0.19	37± 0.28	22± 0.05	27± 0.05	31± 0.28	36± 0.44	40± 0.05	25± 0.35
E. d	11± 0.05	18± 0.05	24± 0.19	29± 0.05	32± 0.61	13± 0.37	19± 0.10	26± 0.19	32± 0.05	35± 0.05	20± 0.37	28± 0.13	33± 0.61	39± 0.05	41± 0.13	27± 0.15

Zone of Inhibition, including the diameter of the well (8 mm); mean ± SD value of three independent experiments, CP: Ciprofloxacin (5µg/ml), S.m, *S.mutans*; E.f, *E.faecalis*; S.a, *S.aureus*; A.v, *A.viscosus*; L.a, *L.acidophilus*; N.m, *N.meningitidis*; E.c, *E.coli*; K.p, *K.pneumoniae*; P.a, *P.aeruginosa*; H.p, *H.parainfluenza*; *E.c*, *E.corrodens*

tissues, and also they are more economical than chemical materials (Dahiya and Purkayastha, 2012; Akinyele *et al*., 2014).

Evaluation of antibacterial activity

Six plant extracts were screened for potential

Table 3. Minimum Inhibitory Concentration (mg/ml) of plant extracts oral pathogens

	Plant extracts					
Pathogen	***H. sabdariffa***	***A. sativum***	***C. lemoniumn***	***P. granatum***	***C. cassia***	***S. aromaticum***
S. m	12.5	0.39	12.5	12.5	6.25	0.39
E. f	25	1.56	25	25	12.5	1.56
S. a	6.25	0.39	6.25	3.125	6.25	0.39
A. v	25	1.56	6.25	12.5	6.25	1.56
L. a	25	1.56	12.5	25	25	1.56
N. m	12.5	1.56	6.25	12.5	25	0.78
P. a	3.125	3.125	6.25	25	25	3.125
E. c	6.25	1.56	3.125	25	25	1.56
K. p	6.25	3.125	6.25	3.125	25	3.125
H. p	12.5	1.56	12.5	25	12.5	0.78
E. d	6.25	1.56	6.25	25	6.25	1.56

S.m, *S.mutans*; E.f, *E.faecalis*; S.a, *S.aureus*; A.v, *A.viscosus*; L.a, *L.acidophilus*; N.m, *N.meningitidis*; E.c, *E.coli*; K.p, *K.pneumoniae*; P.a, *P.aeruginosa*; H.p, *H.parainfluenza*; *E.c*, *E.corrodens*

Table 4. Minimum bactericidal concentration (mg/ml) of plant extracts against oral pathogens

	Plant extracts					
Pathogen	***H. sabdariffa***	***A. sativum***	***C. lemoniumn***	***P. granatum***	***C. cassia***	***S. aromaticum***
S. m	25	0.39	25	25	6.25	0.39
E. f	50	1.56	50	50	25	1.56
S. a	12.5	0.39	12.5	6.25	12.5	0.39
A. v	50	1.56	12.5	25	25	3.125
L. a	50	1.56	25	25	50	1.56
N. m	25	1.56	12.5	25	25	0.78
P. a	6.25	3.125	12.5	50	50	3.125
E. c	12.5	1.56	6.25	25	50	1.56
K. p	25	3.125	12.5	6.25	50	3.125
H. p	25	1.56	25	50	12.5	0.78
E. d	25	1.56	25	50	12.5	1.56

S.m, *S.mutans*; E.f, *E.faecalis*; S.a, *S.aureus*; A.v, *A.viscosus*; L.a, *L.acidophilus*; N.m, *N.meningitidis*; E.c, *E.coli*; K.p, *K.pneumoniae*; P.a, *P.aeruginosa*; H.p, *H.parainfluenza*; *E.c*, *E.corrodens*

antibacterial activity against MDR oral bacteria. The diameter of inhibition zones are shown in Table 2. The results of antimicrobial susceptibility assay are encouraging as all of the tested plants exhibited potential antimicrobial properties against MDR pathogens even under low concentrations thus minimizing the possible toxic effects. Also, the inhibitory efficacy of the extracts was dose-dependent as the diameter of the growth inhibition zone was directly proportional to the concentration of the extracts.

By comparing the antimicrobial activity of different concentrations of the six plant extracts with the most effective antibiotic (ciprofloxacin), the results demonstrated that the inhibitory effect of all extracts at concentrations of 200 and 150 mg/ml was equal to or greater than the inhibitory effect of standard ciprofloxacin against the selected microorganisms. This has clearly indicated that antibiotic resistance does not interfere with the antimicrobial action of plant extracts and these extracts might have different modes of action on test organisms.

Moreover, the ethanol extracts of *S. aromaticum* and *A. sativum* emerged as the most potent agent against the tested bacteria in which the diameter of zone of growth inhibition varied between 25 and 36 mm (in *S. aromaticum* extract), 25 and 37 mm (in *A. sativum* extract) at concentration of 100 mg/ml. Reviewed studies reported the antimicrobial activity of the selected medicinal plants against test pathogens (Biswas *et al*., 2014, Abramovic *et al*., 2012).

Determination of MIC

The MIC values of different plant extracts against the oral pathogens were found in the range of 0.39-25 mg/ml (Table 3). The ethanol extracts of *S. aromaticum* and *A. sativum* showed the lowest MIC (0.39 mg/ml) against *S.*

Table 5. Phytochemical screening of ethanol extract of tested medicinal plants

Plant spp.	A	Sa	T	St	F	An	G	Rs	P
H. sabdariffa	+	+	+	+	+	+	++	-	-
A. sativum	+	+++	-	++	+++	-	++	+	+
C. lemoniumn	-	-	+	++	+	-	+++	+	+
P. granatum	+	-	+	-	+	-	-		+
C. cassia	-	-	-	+++	-	-	-	-	-
S. aromaticum	-	-	+	+++	+	+	-	-	+++

Note: Highly (+++), moderately (++), slightly (+), and absent (-), A, Alkaloid; Sa, Saponin; T, Tannins; St, Steroids; F, Flavonoids; An, Anthraquinone; G, Cardic glycosides; Rs, Reducing sugar; P, Phenolic compounds

mutans and *S. aureus*. This indicates the highest sensitivity of the two isolates for these active extracts among the six extracts tested in this study.

Determination of MBC

The MBC values obtained in this study (Table 4) ranged from 0.39 to 50 mg/ml. The data obtained through MIC revealed the extracts of *S. aromaticum* and *A. sativum* showed the same values of MIC and MBC against most organisms. This indicated that these two extracts contain bactericidal agent/s while, the extracts of other plants have MIC values less than the corresponding MBCs. This may imply that at MIC concentrations, the growth of these susceptible bacteria was only inhibited and that the organisms were not completely killed (bacteristatic effect).

Phytochemical analysis

The phytochemical constituents of the selected plants investigated are summarized in Table 5. Analysis of the extracts revealed the presence of flavonoids, sterols, saponins, tannins, basic alkaloids, phenolic compounds, anthraquinone, cardic glycosides and reducing sugar.

So, these plants have important phytochemicals may be responsible for their medicinal properties and antimicrobial efficiency. Results of phytochemical analyses are in agreement with the reports of other workers (Adonu *et al*., 2013; Dahham *et al*., 2010).

TLC profiling

The last step was to carry out a preliminary separation procedure to simplify the complex crude extracts of the most highly active plants *S. aromaticum* and *A. sativum* by column chromatographic method and then to verify the antimicrobial activity of the fractions. The chromatographic separation gave several fractions which were collected based on colour differences. TLC profiling of the fractions were then determined. TLC is a simple, quick, and inexpensive procedure that gives the researcher a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound. In this study, TLC profiling of the collected fractions gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals gives different R_f values in different solvent system, but the solvent combination hexane: ethyl acetate (5:5 v/v) was found to be the most promising solvent which showed maximum spots among all solvent systems that maximum numbers of spots (six) of *S. aromaticum* and (eight) of *A. sativum* were observed in this system.

Bioautography assay

To locate the major active constituents responsible for antimicrobial activity against the most sensitive test strains (*S. aureus*), TLC-bioautography was performed. As for the extract of *S. aromaticum*, the compound which retained at R_f value 0.64 shows maximum zone of inhibition against *S. aureus* corresponds to the spots representing phenolic compounds on spraying with on the reference plate while, as the extract of *A. sativum*, the compound which retained at R_f value 0.82 shows maximum zone of inhibition against *S. aureus* corresponds to the spots representing flavonoids on spraying with on the reference plate. This result suggests that the antimicrobial activity present in *S. aromaticum* and *A. sativum* extracts may be due to the presence of phenolic compounds and flavonoids, respectively. These findings corroborated with previous observations (Rana *et al*., 2011; Gupta *et al*., 2009) who reported that the inhibitory activity of *S. aromaticum* is due to the presence of several constituents, mainly eugenol; a notable phenolic compounds and other observations (Ross *et al*., 2000) who reported that the antibacterial efficacy of *A. sativum* is due to allicin (allyl 2-propene thiosulfinate); a notable flavonoid.

CONCLUSION

Ethanolic crude extracts of *S. aromaticum* and *A. sativum* have great potential as antimicrobial agents against MDR oral pathogens. This result should therefore be considered in the treatment strategy of oral diseases. Further studies to characterize the ethanol extracts of these medicinal plants and their usefulness as source of new components in mouthwashes and toothpastes must be performed.

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