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Antimicrobial activity of aqueous extracts of pomegranate, sumac, sage, anise, hand bull tongue, thyme, cloves, lemon and mint against some food-borne pathogens

Maha A. Mahmood Zainab A. Al- Dhaher Abbas S. AL-Mizraqchi
Dept. Basic sciences / College of Dentistry / University of Baghdad

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Summary

The increasing rate of resistance development for commonly used antibiotics have led to search for newer, more effective, affordable and easily available medicine. Medicinal plants have revived as a consequence of current problems associated with the use of antibiotics. Aqueous extracts of nine plants (pomegranate, sumac, sage, anise, hand bull tongue, thyme, cloves, lemon and mint) were qualitatively and quantitatively examined against twenty microbial isolates, mostly food borne including pathogens. (*E.coli*, *Salmonella typhi* and *Candida albicans*) antimicrobial screening was done by agar diffusion (well diffusion) and minimal inhibitory concentration (MIC) methods. Among the screened plants, cloves were the most inhibitor against isolates of *Escherichia coli*, *Salmonella typhi* and *Candida albicans* followed by the inhibitory effect of sumac against (*E.coli*, *Salmonella typhi* and *Candida albicans*). On the other hand, pomegranate, sage and lemon showed varied inhibitory effect against the tested food borne isolates, whereas the extracts of anise, hand bull tongue, thyme and mint showed no antimicrobial activities against most of the tested isolates. Regarding the inhibition zone and MIC results, the present study certified that *Candida albicans* was the most sensitive pathogen as compared with the other food borne pathogens tested (*Escherichia coli* and *Salmonella typhi*).

Key words:pomegranate,somac,sage,anise,handbulltongue,thyme,lemon,mint.

الفعالية الضد مايكروبية للمستخلصات المائية للرمان ,السماك ,المريمية ,اليانسون ,ورد لسان الثور ,الزعتر ,القرنفل ,الليمون والنعناع ضد بعض الممرضات المجهرية المنتقلة بواسطة الاغذية

مها عادل محمود زينب عبد الجبار الظاهر وعباس صبري المزرقجي

كلية طب الاسنان ,جامعة بغداد ,فرع العلوم الاساسية

الخلاصة

ان ازدياد ظهور السلالات المقاومة للمضادات الحيوية من قبل الاحياء المجهرية الممرضة ادى الى البحث عن مضادات جديدة اكثر فاعلية وتوفرا لذا فان النباتات الطبية كانت البديل نتيجة المشاكل المرتبطة باستخدام المضادات الحيوية .في هذه الدراسة تم اختبار وتقييم المستخلصات المائية لكل من الرمان ,السماك ,المريمية ,اليانسون ,ورد لسان الثور ,الزعتر ,القرنفل ,الليمون والنعناع ضد عشرون عزلة جرثومية اغلبها ممرضات مجهرية منتقلة بواسطة الغذاء (*Escherichia coli*, *Salmonella typhi* and *Candida albicans*). تم قياس الفعالية الضد مايكروبية باستخدام تقنية الانتشار بوسطة ال(اگار) كما تم إجراء اختبار التركيز المثبط الأدنى وقد كان مستخلص القرنفل الأكثر قوة في تثبيط عزلات (*Escherichia coli*, *Salmonella typhi* and *Candida albicans*) يتبعه التأثير التثبيطي لكل من السماك ضد (*Escherichia coli*, *Salmonella typhi* and *Candida albicans*) ومن جهة اخرى الرمان والمريمية والليمون اظهروا اختلافا في مستوى تثبيط العزلات المختلفة المنتقلة بواسطة بينما لم يسجل أي تأثير لكل من مستخلصات الينسون وورد لسان الثور والزعتر والنعناع على أي من العزلات المذكورة انفا كما اشارت الدراسة بالاعتماد على نتائج أقطار التثبيط والتركيز المثبط الأدنى أن الخميرة (*Candida albicans*) كانت الحي المجهري الاكثر حساسية للمستخلصات السابقة الذكر عند مقارنتها بكل من بكتريا (*Escherichia coli*, *Salmonella typhi*).

Introduction:

Food-borne diseases result from eating food contaminated with bacteria or their toxins or pathogens such as parasites or viruses. Typical symptoms include vomiting, diarrhea, and abdominal cramps (1). The most common bacteria causing food-borne illness are *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp., *Salmonella typhimurium*, *Listeria monocytogenes*, *Clostridium botulinum*, *Vibrio vulnificus*, *Vibrio parahaemolyticus* and others (2). The epidemiology of food borne diseases is changing and reports from different parts of the world indicate that the resistant strains of food borne pathogens have emerged as public health problem(3). The bacterial contamination of food has led to use the plant extracts as antimicrobial additives to control the growth of food borne bacteria (4) *Punica granatum Linn*. *(Pomegranate)*, has long been esteemed as food and medicine, and is a diet in convalescence after diarrhea. It is also used for the treatment of Gastro-Intestinal (GI) diseases. *pomegranate* is considered as an anti parasitic agent, a "blood tonic," and to heal aphthae, diarrhoea, and ulcers (5). *Rhus coriaria* (anacardiaceae) commonly known as *sumac* (also spelled as *sumach*) is a wild bush that grows in all Mediterranean areas. Phytochemicals in *Rhus coriaria* are being used as antibacterial, antidiarrhoea, antidysenteric, antihepatoxic, antiseptic, antispasmodic and antiviral (6). *Salvia officinalis* common name *sage*, it is a popular home remedy. The herb has been recommended for almost every illness or problem by one herbalist or another. Perhaps the most frequently cited effects of *Sage* are its antihydrotic (antiperspiration), antibiotic, astringent, antispasmodic, and hypoglycemic properties. Each of these effects has received some experimental support. Sages spasmolytic quality is useful to quiet nervousness, remedy digestive disturbances, and treat respiratory problems. It also has antimicrobial activities especially against food borne pathogens (7). *Anise (Pimpinella anisum L., Apiaceae)* is an annual herb that is used as an appetizer, tranquillizer and diuretic drug in folk medicine. It is also used to treat dyspeptic complaints and catarrh of the respiratory tract, and as mild expectorants. It was also reported that extracts from anise fruits have therapeutic effects on several conditions, such as gynecological and neurological disorders (8). Hand bull tongue *(Borago officinalis) (Boraginaceae)* a plant with a variety of medicinal properties, a potential which has lately been endorsed by the discovery of high levels of gamma linoleic acid in the seeds. It is useful in many disorders since it is a good remedy for colds and flu especially when it affects the lungs. The leaves of Borago are reportedly used as diuretic, demulecent, emollient and expectorant (9). Thyme, a member of the lamiaceae family, is an aromatic and medicinal plant. It is volatile phenolic oil has been reported to be among the top 10 essential oils, showing antibacterial, antimycotic, anti oxidative, natural food preservative and mammalian age delaying properties (10). Cloves are the aromatic dried flower buds of a tree in the family Myrtaceae. It has a wide range of medicinal properties; even it is now commonly used in Western medicine. It has also been used in folklore treatment of insect bites, gastroenteritis and intestinal parasites (11). Lemon was reported to be inhibitory against some molds (12). In addition, the antibacterial activity of essential oils from lemon was reported against various Gram-positive and Gram-negative bacteria (13). Mint is an herb used for curing several common ailements, essential oils of some Mentha species have been reported to have antibacterial and antifungal properties (14). This work was conducted to investigate the antimicrobial activity of nine different aqueous plant extracts: pomegranate (*Punica granatumLinn*), sumac (*R.coriaria*), sage (*Salvia officinalis*), *anise* (*Pimpinella anisum*), hand bull tongue (*Borago officinalis*), thyme (thymus vulgaris), cloves (*Eugenia caryophyllata*), lemon (*Citrus limonium*) and mint (Mentha requienii) respectively against three food borne microbial pathogens (*E.coli*, *Salmonella typhi* and *Candida albicans*).

Materials and Methods

Twenty isolates belonging to three pathogenic organisms which are *E.coli* (9 isolates), *Salmonella typhi* (5 isolates), *Candida albicans* (6 isolates) isolated from clinical samples were obtained from Central Teaching Lab. of Baghdad Hospital and used to study the antimicrobial activity of aqueous extracts of nine plant materials that were pomegranate, sumac, sage, *anise*, hand bull tongue, thyme, cloves, lemon and mint against each of the twenty isolates. Aqueous extracts of these plants were prepared by adding 100 gm of dried powder plants in 1000 ml of boiled distilled water, and left for one hour at room temperature. Agitation of the suspension with stirrer had been done alternatively and then the infusions was filtered by filter paper and residue discarded, then the extracts left to dry in glass Petri dishes at 45ºc and the resulted powder kept in tightly closed glass container in a refrigerator (4 ºc) until used. Freshly pomegranate and lemon, prepared water extracts of their fruits were prepared by using juicer machine. After that, different concentrations were prepared from each plant extract to be used for further experiments. Well diffusion method (agar diffusion technique) was used for assessment of antimicrobial activity of all subjected extracts (15). All bacterial and yeast isolates were cultivated in Mueller Hinton Broth and Yeast Malt Broth, respectively at 37 ºc for 24 hours. Petri plates containing 20ml of Mueller Hinton agar medium were inoculated with a 24 hour culture of the isolates under investigation, wells (6mm diameter) were punched in the agar and filled with 50 µl of each crude extract. Six concentrations (700mg/ml, 600mg/ml, 500mg/ml, 400mg/ml, 300mg/ml and 200mg/ml) of each extract were prepared and tested against only isolates that exhibited inhibition zone more than 6mm in diameter against the initial crude extract. In contrast, all isolates that were showed resistance to the initial crude extract were excluded from the later experiments. All inoculated plates were incubated at 37 ºc for 24 hours (for bacteria) or 48 hours (for yeast), the antimicrobial activity was assessed by measuring the diameter of the inhibition zone. Minimum inhibitory concentrations (MIC) of the extracts were determined by preparing various concentrations (25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml). Equal volume of the extracts and nutrient broth were mixed in the test tube. Specifically 0.1ml of standardized inoculums of 1 to 2 $\times 10^8$ cfu/ml was added to each tube. The tubes were incubated aerobically at 37 ºc for 24 hours. Two control tubes were maintained for each test batch (positive control and negative control). MIC was determined as the lowest concentration of the extracts permitting no visible growth (no turbidity) in comparison with the control tubes. All experiments were accomplished in five replicate and the results reported are averages. Results from the experiments were analyzed by using the SPSS 13.5 statistical Package (SPSS Ltd., Working, UK). ANOVA test (one way) was used to express the antimicrobial differences of various plant extracts against bacterial isolates.

Results

Results of well diffusion technique are summarized in tables (1and2). The results in (table 1) showed that anise, hand bull tongue, thyme and mint have no inhibitory effect on the tested isolates since their were no inhibition zones for stock solutions so they were excluded from the experiment.

Table-1 Antimicrobial activity of stock solutions of nine aqueous plant extract against *E.coli, Salmonella typhi* and *Candida albicans*.

Presence of inhibition zones (mm)	Microbial pathogen					
	E.coli N=9		*Salmonella typhi* N=5		*Candida albicans* N=6	
pomegranate	+	9/9	+	5/5	+	6/6
sumac	+	9/9	+	5/5	+	6/6
sage	+	9/9	+	5/5	+	6/6
anise	-	-	-	-	-	-
hand bull tongue	-	-	-	-	-	-
thyme	-	-	-	-	-	-
cloves	+	9/9	+	5/5	+	6/6
lemon	+	9/9	+	5/5	+	6/6
mint	-	-	-	-	-	-

+ = diameter of inhibition zone > 6mm, - = no inhibition, * Values in parenthesis indicate number of isolates out of total isolates tested., ** Stock concentration is 800mg/ml

While the other subjected concentrations (700mg/ml, 600mg/ml, 500mg/ml, 400mg/ml, 300mg/ml and 200mg/ml) of pomegranate, sumac, sage, cloves and lemon extracts had showed inhibition zones more than 6mm in diameter against the stock solution, the average diameter of inhibition zones of *pomegranate* ranges from (9-12) mm, (9-17) mm, (9-15) mm, against *E. coli*, *Salmonella typhi* and *Candida albicans* respectively (table 2). Additionally, the same table demonstrate the inhibition zones of sumac against *E.coli*, *Salmonella typhi*, and *Candida albicans* were (10-16) mm, (10-19) mm, and (17-22) mm respectively (table 2), while the average diameter of inhibition zones of *sage* extract ranges from (8-13) mm, (10-16) mm and (11-16) mm against *E.coli*, *Salmonella typhi* and *Candida albicans* respectively. The inhibition zones were ranged from 12-23, 7-10 and 20-29 mm for *E.coli*, *Salmonella typhi* and *Candida albicans* respectively when cloves extract was subjected while the inhibition zones were ranged from 8-14, 8-12, 9-15 mm against *E.coli*, *Salmonella typhi* and *Candida albicans* respectively when lemon juice was subjected. The results showed that cloves were the strongest antimicrobial activity (23mm inhibition zone, ($p<0.01$) against *E.coli* followed by sumac (16 mm) in comparison with the other extracts (pomegranate, sage and lemon) which showed inhibition zones that ranged from (12-14mm) for the stock solutions. The largest diameter of inhibition zone (19 mm) for sumac extract was observed against *Salmonella typhi* at concentration 700mg/ml ($p<0.05$), whereas sage and pomegranate are ranked secondly(16 and 17 mm) in inhibition followed by a weak effect of lemon (12mm) and cloves (10 mm) respectively. Cloves proved to be the most inhibitor plant against *Candida albicans* as largest inhibition zones were recorded in different concentrations ($p<0.01$). Aqueous extract of sumac was the following most inhibitor extract (17-22mm inhibit zone) against tested yeast whereas initials extracts of pomegranate, lemon, and sage have a less inhibitory effect on this pathogen as their inhibition zones ranged from 15-16mm. The results showed that the diameter of inhibition zone was proportionally increased according to the concentration of extract ($p<0.05$, $p<0.01$). According to the type of plant extract, there is no significant

differences ($p<0.05$) in inhibition zones of both pomegranate and lemon extracts which are have relatively similar effect on the three tested pathogen (*E. coli*, *Salmonella typhi* and *Candida albicans*), different concentration of cloves, sumac and sage revealed various inhibitory effect with a significant differences ($p<0.05$, $p<0.01$) on the former pathogens as *Candida albicans* seems to be the most sensitive pathogen among them.

The results of MIC for each plant extract against food-related microorganisms are recorded in (table 3). Sumac showed stronger antimicrobial activity against the studied microorganisms in comparison with other extracts. It exhibited MIC values ranged from 200-400mg/ml for most of the tested isolates. However, the tested microorganisms *E.coli*(9 isolates), *Salmonella typhi* (5 isolates) and *Candida albicans* (4 isolates) were resistant to lemon extracts at the concentrations tested(table3). Cloves were the most effective extract against *Candida albicans* (MIC 50-200mg/ml) followed by sumac and Pomegranate (MIC 200-400mg/ml) respectively, whereas the other plant extracts showed varied and lowest inhibitory effect against Gram-negative bacteria (*E.coli* and *Salmonella typhi*).

Table 2:- Antimicrobial activity of plant aqueous extracts against *Salmonella typhi*, *E.coli* and *Candida albicans*.

Extract name	Extract concentration mg/ml	mean inhibition zone (mm)		
		E.coli N=9	*Salmonella typhi* N=5	*Candida albicans* N=6
Pomegranate	700	12	17	15
	600	11	16	13
	500	10	14	11.5
	400	10	13.5	10
	300	9	9.5	10
	200	0	9	9
Sumac	700	16	19	22
	600	14	17.5	20
	500	14	17	19
	400	13	16	18
	300	12	11	17
	200	10	10	17
Sage	700	13	16	16
	600	11	14	14.5
	500	10	13	14
	400	8	12	13
	300	0	12	12
	200	0	10	11
Cloves	700	23	10	29
	600	21	9	25
	500	21	8	25
	400	17	7	24
	300	15	7	22
	200	12	0	20
Lemon	700	14	12	15
	600	12	11	14
	500	11.5	9.5	12
	400	11	9	11
	300	9	8	10.5
	200	8	8	9

Table 3:- the minimal inhibitory concentration (MIC) mg/ml of aqueous treats of (Pomegranate, Sumac, Sage, Cloves, Lemon) against (*E.coli, Salmonella typhi* and *Candida albicans*).

pathogen	MIC mg/ml																									
	Pomegranate conc. mg/ml					Sumac conc. mg/ml					Sage conc. mg/ml					Cloves conc. mg/ml					Lemon conc. mg/ml				
	25	50	100	200	400	25	50	100	200	400	25	50	100	200	400	25	50	100	200	400	25	50	100	200	400
E.coli N=9	-	-	-	-	1/9	-	-	-	1/9	3/9	-	-	-	-	-	-	-	1/9	1/9	4/9	-	-	-	-	-
Salmonella Typhi N=5	-	-	-	-	1/5	-	-	-	1/5	2/5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Candida Albicans N=6	-	-	-	2/6	3/6	-	-	-	3/6	3/6	-	-	-	-	2/6	-	-	-	-	-	-	-	-	-	-

N= number of tested isolates

Discussion

Recently, different reports were published showing the antimicrobial activities of medicinal plants. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of health care. Most of the examined plants in this study showed varied inhibitory activity against the tested food borne isolates (*E. coli*, *Salmonella typhi* and *Candida albicans*). The current study revealed that the cloves were the most effective extract against *E.coli* and *Candida albicans* isolates, followed by sumac whereas other plants like sage, lemon, and pomegranate exhibited notable antibacterial activities towards all examined isolates with different potentialities. On the other hand, sumac showed a good antimicrobial activity against *Salmonella typhi* isolates followed by Pomegranate and sage respectively. The result of the present work is in agreement with that of Mahoud,(16) who were certified that cloves were among the potent plant with antifungal and antiyeast activity. Furthermore, BRR and Mahmoud (17) showed the inhibitory effect against *Candida albicans*. This may due to the presence of 14 biologically active compounds in cloves extract (18). The current study indicates the existence of antimicrobial activity of the crude extract of *R.Coriaria* (sumac). This was in accordance with the results of Abu-Shanab (19) who also reported that *R.Coriaria* has strong antimicrobial activity. Similar observations were also shown by (20). This activity may be attributed to different content of compounds founds in this plant like ellagic acid, gallic acid, isoquercitrin, myrecitrin, myricetin, quercitin, quercitrin and tanic acid (10). Sage exhibited good antimicrobial activity against *E.coli*, *Salmonella typhi* and *Candida albicans*. This is interpreted by the presence of plant volatiles compounds which appear to accumulate in the microbial cell membrane causing the leakage of ions, enzymes, and metabolites outside the cells (21). *Punica granatum* (pomegranate) also revealed a good antimicrobial activity against all the tested isolates. These results were in agreement with the results of(22)who found that extracts of *Punica granatum* has strong antimicrobial activity. Pradeep (23) found that different extracts of *Punica granatum* give good antibacterial activity against different bacterial strains. This may be due to the presence of metabolic toxins or broad spectrum antibiotic compounds in this plant (24&25). Another study reported that *Punica granatum* contains large amount of tannins (25%) and the antibacterial activity may be indicating presence of some secondary metabolites (26). According to antimicrobial potentialities, lemon was the weakest one in comparison with the other extracts. Furthermore, no sufficient references could be obtained about the antimicrobial activity of lemon grass (7). The present investigation demonstrated that the increasing of extract concentration led to increase inactivation of cells in all isolates tested but in a range differs according to type of extract and bacterial spp. In contrast, no antimicrobial activity were shown for anise, thyme and mint against all the studied isolates in this study. The results are in harmony to those reported by (27). In addition, Sofia (28) showed negligible antibacterial activity of mint against potent food borne pathogens namely *E.coli*. The variations in inhibitory potency of the tested extracts may be explained by the fact that antimicrobial activity may be affected by type and composition of the plant used in the extract, amount used and type of microorganism, pH value and temperature of the environment. From the microbial sensitivity side of view, *Candida albicans* was the most sensitive pathogen to the examined plant extracts, whereas both of *E.coli* and *Salmonella typhi*. Proved to be the most resistant among the tested food borne isolates. This may due to the presence of outer membrane barriers in Gram-negative bacteria (29). The present in vitro study

demonstrated that microbial growth of potent food borne pathogens (*E.coli*, *Salmonella typhi* and *Candida albicans*) can be inhibited by plant extracts but at different levels which are clearly varied according to type of plant and extract concentration as extracts of cloves, sumac, sage, pomegranate and lemon exhibited a varied antimicrobial activities against the selected isolates used in this study. Whereas anise, hand bull tongue, thyme and mint did not exhibit any inhibitory effects against the selected isolates.

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