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Phytochemical analysis and antibacterial activity of some medicinal plants against methicillin-resistant *Staphylococcus aureues*

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Abstract
The objective of this study is, first to report the prevalence of methicillin resistant *Staphylococcus aureues* (MRSA) infections and their antibiotic susceptibility patterns in Teaching and Emergency Hospital in Erbil city, and second to screen four medicinal plants: *Eucalyptus globulus, Anethum graveolens, Prosopis farcta* and *Ficus carica* (both aqueous extract and ethanolic extract) for potential antibacterial activity against MRSA isolates , then screening the phytochemical properties of these plants. Out of total 110 strains of *Staphylococcus aureues* isolated from different clinical specimens, 15 (13.6%) were found to be methicillin resistant. All MRSA strains were found to be resistant to penicillin and gentamycin while 86.66% were resistant to cephalothin ,doxycyclin and tobramycin. However, no strains were resistant to vancomycin. Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values were performed using micro broth dilution method. The MIC/MBC of aqueous and ethanolic extracts of *E. globulus* against MRSA isolates were 25/12.5 mg/ml and 6.25/3.125 mg/ml respectively, while of *P. farcta* were 100/25 mg/ml and 25/12.5 mg/ml respectively. The results also revealed that extracts of *F. carcia* were inactive against MRSA. Preliminary phytochemical analysis revealed the presence of tannins and phenols in almost all the crude extracts followed by alkaloids and resins, while the glycosides was not detected in any extracts of the four plants tested.

التحليل الكيمياوي و النشاط المضاد للبكتريا لبعض النباتات الطبية ضد المكورات العنقودية المقاومة للمثيسيلين

سهيلة نافع داروغه

المستخلص
تضمنت هذه الدراسة تسجيل نسبة انتشار المكورات العنقودية المقاومة للمثيسيلين ومدى حساسيتها للمضادات الحيوية المختلفة في المستشفى التعليمي و الطواريء في محافظة اربيل اولا، ودراسة تأثير أربعة انواع من مستخلصات النباتات الطبية اليوكالبتوس، الشبنت، التين و الخرنوب (المستخلص المائي والمستخلص الكحول الاثيلي) كمضادات حيوية مختلفة ضد المكورات العنقودية المقاومة للمثيسيلين(MRSA)، ومن ثم تحديد المكونات الكيمياوية لهذه النباتات. في هذه الدراسة تم عزل ١١٠ عزلة للمكورات العنقودية الذهبية من مجموع ٥٩٧ عينة ماخوذة من نماذج سريرية مختلفة وان ١٥ (١٣،٦%) من هذه العزلات هي المكورات العنقودية المقاومة للمثيسيلين . كل العزلات المقاومة للمثيسيلين كانت مقاومة ١٠٠% للبنسيلين وجينتامايسين بينما ٨٦,٦٦% من هذه العزلات اظهرت مقاومة للمضادات سيفالوثين، دوكسي سايكلين و توبرومايسين بينما كل العزلات كانت حساسة للمضاد الجيوي فانكومايسين.تم تحديد التركيز المثبط الادنى (MIC) و التركيز القاتل الادنى (MBC)، حيث اظهرت النتائج ان MIC/MBC للمستخلصين المائي و الكحولي لنبات اليوكالبتوس ضد المكورات العنقودية المقاومة للمثيسيلين كانت (١٢،٥/٢٥) ملغم/مل و (٦،٢٥ /٣،١٢٥) ملغم/مل على التوالي بينما لنبات الخرنوب MIC/MBC كانت (١٠٠ /٢٥) ملغم/مل و (٢٥ / ١٢،٥) ملغم/ مل على التوالي بينما اشارت النتائج بان نبات التين بمستخلصيها المائي و الكحولي لم يكن فعالا ضد المكورات العنقودية المقاومة للمثيسيلين. واشار التحليل الكيمياوي للمستخلصات المائية والكحولية لهذه النباتات وجود التانيين و الفينول في معظم النباتات وبعدها القواعد والراتنجات وعدم وجود مركب الكلايكوسيد في أي مستخلص نباتي.

Introduction

Staphylococcus aureues is one of the most significant human pathogen responsible for nosocomaila, community and hospital acquired infections. It can cause a range of infectious disease from mild conditions, such as soft tissue infections, to severe life threatening debiliation such as endocarditic to death (1, 2). Methicillin-resistant strains of staphylococci were identified immediately upon the introduction of methicillin into clinical practice (3). The first outbreak caused by MRSA occurred in a British hospital in the early 1960s and emerged in the United States in the mild 1980s (1, 4). MRSA strains have become frequently resistant to multiple other antimicrobial agents and many investigators have reported an increase in the incidence of MRSA during recent years (5, 6, 7). Interest in plants as source of antimicrobial agents is growing. This is because of that the plant-derived medicines have been part of traditional health care in most parts of the world and because of the side effects and the resistance that pathogenic organisms build against antibiotic and there has been an alarming increase in the incidence of new and reemerging infectious diseases (8, 9), and because the antimicrobial properties of medicinal plants are well documented in different parts of the world (10, 11, 12). *Eucalyptus* species belong to the family Myrtaceae. It is a large genus of aromatic trees which are distributed widely throughout the world and are

famous for their rapid growth (13, 14). The oils of the *Eucalyptus* plants are used frequently as a remedy for cold and cough. They are used in pharmaceuticals such as cough syrups, lozenges, nasal drops and mouthwash. *Eucalyptus* is an ingredient in over-the-counter pharmaceuticals as temporary relief of minor aches and pains of muscles and has been employed in steam inhalation treatments and treatment of wounds (15). Aqueous and organic extracts of the leaves of *Eucalyptus* species were investigated and the results show anti-inflammatory, antifungal, antibacterial activity against some gram positive and negative bacteria and may also provide potent antiseptic properties (12, 13, 16). *Anethum graveolens*, also known as shapt or dill-weed, it belongs to the family Umbelliferae (Apiaceae). It is an annual erect glabrous herb growing in the Mediterranean region, Europe, Central Southern Asia. The leaves are alternate, 2-3 pinnately compound, the flowers are complete and yellow. The plant is used both medicinally and as an aromatic herb and spice and cookery. Dill has been used traditionally for gastrointestinal ailments such as flatulence, indigestion, stomachache colic and to tract intestinal gas (17). Some pharmacological effects of the volatile oil as well as aqueous extract of the aerial parts of *Anethum graveolens* have been reported such as antibacterial activity, antifungal, insecticidal, antihyper- cholesterololaemic, antioxidant activity, anti-inflammatory effect (18, 19, 20). The genus *Prosopis* belongs to the family Leguminosea (subfamily Mimosoidae) comprises almost 50 species, 25 of which are on the list of federal noxious weeds. *Prosopis farcta* is a small prickly spiny trees or shrubs. In its native range, it is wide spread and a weed of wheat and cotton fields, invading by root suckers. It is native to Northern Africa, south Western Asia, United States, Iraq, Kuwait, Iran and Turkey (14,21). The antimicrobial activity of extracts from *Prosopis* spp. was investigated and the results show antifungal, antibacterial activity against some gram positive and negative bacteria, antidiarrheic, antiparasitic (22, 23, 24). *Ficus* is a genus of about 850 species of woody trees, shrubs, or small tree in the family

Moraceae. They are native to Southwest Asia and the Eastern Mediterranean region (from Greece to Pakistan). The fruit of most species are edible though they are usually of only local economic importance or eaten as bush food (17, 25).Various parts of this plant like bark, leaves, tender shoots, fruits, seeds and latex are medicinally important. *Ficus* spp. has been applied in folk medicine for cough, sore throat, burning, diarrhea, toothache and microbial infections (11). Various pharmacological actions such as antifungal, antibacterial, antioxidant and anti-inflammatory have been described for *Ficus* spp. (26, 27, 28). The aim of this study is to determine the prevalence of MRSA infection and their susceptibility pattern to various antimicrobial agents and to screen four medicinal plants for their potential antibacterial activity of Erbil Province on clinical isolates of MRSA and then screening of these plants for their phytochemical compounds.

Materials and methods

Clinical samples and antibiotic susceptibility test

Five hundred ninety seven clinical specimens like wound, burn, urine, vaginal swab, pus and other samples (blood, CSF and throat swab) were collected from patients attending Central Laboratory and Internal Laboratory of Teaching Hospital and Emergency Hospital in Erbil city for various bacteriological examination. Standared isolation procedures were applied to all the samples. Identification of *Staph. aureues* was confirmed by standard techniques based on colonial and microscopic morphology, biochemical activities and coagulase test (29). Antibiotic sensitivity testing was performed on Mueller-Hinton agar with 24 hours incubation at 37 C by the standard disk diffusions method. All *Staph. aureues* isolated were tested for methicillin resistant by using Oxacillin (5µg/disc). A zone of inhibition of less than 10 mm or any discernible growth within the zone of inhibition was taken as indicative of methicillin resistance. Susceptibility of isolated MRSA strains to vancomycin (30µg), clindamycin (2µg), penicillin (10U), erythromycin (15µg), gentamycin (10µg), azithromycin (15µg), norfloxacin (10µg), chloroamphenicol (30µg), cephalothin (30µg), doxycyclin (30µg), ceftriaxone (30µg), tobromycin (10µg) and rifampin (5µg) (30).

Plant material

The four plant samples used in this study were collected from Erbil Province in Kurdistan Region and identified in the Department of Biology, College of Science Education, University of Salahaddin. Table (1) shows the botanical name, family and plant part used.

Table (1):- Profile pf plant used.

Botanical name	Family	Plant part used
Eucalyptus globulus	Myrtaceae	Leaves
Ficus carica	Moraceae	Leaves
Anethum graveolens	Umbelliferae	Stems and leaves
Prosopis farcta	Leguminosea	Fruits (pods)

value 25mg/ml and 6.25mg/ml were obtained for aqueous and ethanol extracts of *E. globulus* respectively, while the corresponding MBC values are 12.5mg/ml and 3.125mg/ml. The MIC and MBC values of 25mg/ml and 12.5mg/ml were recorded for ethanol extract and 100mg/ml and 25mg/ml for aqueous extract for *P. farcta* respectively. Also MIC and MBC values of 50mg/ml and 25mg/ml and 12.5mg/ml and 12.5mg/ml were obtained for aqueous and ethanol extract of *A. graveolens* respectively. Only extracts of *F. carica* were inactive against MRSA. The results of the preliminary phytochemical analysis are shown in Table (5). Phenols and tannins were present in almost all the crude plant extracts (aqueous and ethanol) followed by alkaloids and resins, and flavonoids and saponins were found in lesser amounts. The component glycoside was not detected in the crude extracts of the four plants tested in both aqueous and ethanolic extracts.

Table (2): Number and percentage of isolated MRSA and MSSA from different clinical samples.

Clinical samples	Number of samples	*Staph. aureues*		MRSA		MSSA	
		No.	%	No.	%	No.	%
Wound	80	42	52.5	8	7.3	34	30.9
Burn	117	22	18.8	3	2.7	19	17.3
Urine	327	27	8.3	2	1.8	25	22.7
Vaginal swab	18	6	33.3	1	0.9	5	4.5
Pus	15	7	47.7	1	0.9	6	5.5
Others	40	6	15.0	0	0.0	6	5.5
Total	597	110	18.42	15	13.6	95	86.4

Table (3): Antimicrobial sensitivity of MRSA isolates.

Drug	Disc potency (μg)	Sensitive		Resistant	
		No.	%	No.	%
Vancomycin	30	15	100	0	0.0
Clindamycin	2	5	33.3	10	66.66
Penicillin	10 U	0	0.0	15	100
Oxacillin	5	0	0.0	15	100
Erythromycin	15	5	33.33	10	66.66
Gentamycin	10	0	0.0	15	100
Azithromycin	15	6	40	9	60
Norfloxacin	10	10	66.66	5	33.33
Chloramphenical	30	8	53.33	7	46.66
Cephalothin	30	2	13.33	13	86.66

Doxycyclin	30	2	13.33	13	86.66
Ceftriaxone	30	3	20	12	80
Tobramycin	10	2	13.33	13	86.66
Rifampin	5	9	60	6	40

Table (4): Minimum inhibitory and bactericidal concentration of plant extracts on MRSA.

Plant testet	Plant extracts			
	Aqueous extract		Ethanol extract	
	MIC	MBC	MIC	MBC
E. globulus	25	12.5	6.25	3.125
A. graveolens	50	25	12.5	12.5
F. carica	-	-	-	-
P. farcta	100	25	25	12.5

Table (5): Phytochemical analysis of four crude plant extracts.

Phytochemical component	Extracts	Screened plants			
		E. globulus	***A. graveolens***	***P. farcta***	***F. carica***
Alkaloids	Aqueous	+	+	-	+
	Ethanol	-	-	+	+
Tannins	Aqueous	+	+	+	-
	Ethanol	+	+	+	-
Glycosides	Aqueous	-	-	-	-
	Ethanol	-	-	-	-
Flavonoids	Aqueous	+	-	-	-
	Ethanol	-	-	+	-
Saponins	Aqueous	-	+	-	+
	Ethanol	-	-	-	+
Phenols	Aqueous	+	+	+	+
	Ethanol	+	+	+	+
Resins	Aqueous	-	-	+	-
	Ethanol	+	+	+	+

Discussion

The prevalence rate of MRSA infection in the present study was found to be 13.6%, which is in accordance with other reports (5, 33). On the contrary, some of the reports show an alarmingly high incidence of MRSA infection. Rijal and coworker demonstrate an increase in methicillin resistant *Staphylococcus aureues* (56.14%) in patients visiting Western Regional Hospital in Pokhara (34), and Vidhani and colleagues found 51.6% of MRSA in Orthopaedics and Burn Units of LN Hospital, New Delhi (6). The SENTRY antimicrobial surveillance programme found that the prevalence of MRSA in hospital between 1997 and 1999 were very high in the countries like in Japan (71.6%), Singapore (62.9%), Taiwan (61.1%), Portugal (54.4%) and Australia (23.6%) (35). All MRSA isolates encountered in this study were completely resistant to antibiotics such as penicillin, gentamycin and oxacillin. A similar result was noted for penicillin and oxacillin among MRSA strains from India, Nepal, Medagascor and Saudi Arabia (36, 37, 38, 39). All MRSA isolates in this study were completely sensitive to vancomycin. A similar result was obtained for vancomycin in previous reports (7, 38). *Staphylococcus aureues* is a versatile human pathogen, it was strongly considered as a major cause of nosocomial infection. In recent years, the prevalence of MRSA has increased worldwide as it is evident from many surveillance studies (35, 40, 41). However, infection with MRSA varies widely from one geographic location to another, from hospital to hospital and over time (42, 43). Recently, several studies have shown that the MRSA have started to gain resistance to many widely used antibiotics including β-lactams, glycopeptides, aminoglycosides, tetracyclin, and miscellaneous group, and this is probably due to the indiscriminate and empirical use of these drugs (2, 44). Recently, only few antimicrobial agent including vancomycin and teicoplanin are still effective against MRSA, thus, this pathogen can cause serious infection in various body systems in patients. Due to the appearance of increasing in antibiotic resistance, many researchers have investigated alternative approaches to treat staphylococcal infection. Natural products including plant extracts have been extensively studied (45). The results of the present study indicated that three out of four medicinal plants (E. *globulus*, *A. graveolens* and *P. farcta*) were active against hospital strains of MRSA. Findings in the present study supported and agree with the observations of some other researches about the same medicinal plants in their antibacterial activity against MRSA and MSSA (12, 18, 46). The crude extracts of *E. globulus* was strongly active against MRSA strains with ethanol extracts than water extract (MIC value was 6.25mg/ml and MBC was 3.125mg/ml). The MIC values of the crude extracts (both aqueous and ethanol) obtained in this study were bacteriostatic at higher concentration and bacteriocidal at lower concentration. The results also indicated that *F. carica* was inactive against MRSA isolates, similar resultus recorded by other research (11). It is quite possible that this plant which was ineffective in this study do not possess antibiotic properties or the plant extract may have contained antibacterial constituent, just not in sufficient concentrations, so as to be effective. The crude extracts of four plants studied were found to contain one or more of the following compounds: tannins, alkaloids, saponines. Phenols, flavonoids and resins. Other investigators (11, 19, 47) have reported the presence of these compounds in members of the families Myrtaceae, Moraceae, Leguminosea and Umbelliferae, to which the plants used in the present study belong. The inhibitory effects of these medicinal plants on the microorganisms may therefore, be due to the presence of above phytochemical components (16). Different plants possess different constituents and in different concentrations, which accounts for differential antimicrobial effects, as also suggested earlier (8). The mechanisms thought to be responsible for these phytochemicals against microorganisms vary and depend on above compounds. Their mechanism of actions may include enzyme inhibition by the oxidized compounds and act as a source of stable free radical and often leading to inactivation of the protein and loss of function. They have the ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls and disrupt microbial membranes (47, 50). However, search for new antibacterial agents should be continued by screening many other plant families. The antimicrobial and phytochemical studies would provide valuable information to the media of the world knowledge.

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