Plant Archives Vol. 20, Supplement 1, 2020 pp. 2684-2687 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ISOLATION AND IDENTIFICATION OF BACTERIAL SPECIES ASSOCIATED WITH DENTAL CARIES AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ALCOHOLIC EXTRACTS FOR *SUAEDA AEGYPTIACA* AND *CITRUS SINENSIS* PLANTS

Ghosoon Al-Kanaany
Department of Biology, College of Science, University of Basrah, Iraq.
Email : gh_fadhel@yahoo.com

Abstract

Studies were carried out to identify 32 bacteria isolated from dental caries belong to the genera : *Escherichia*, *Pseudomonas*, *Citrobacter*, *Sphingobacterium*, *Staphylococcus*, *Leuconostoc*, *Acinetobacter*, *Ochrobactrum*, *Klebsiella*, *Enterococcus*, *Yersinia*, *Chromobacterium* and 18 isolates were unidentified by Vitek$_2$ compact system. Antibacterial activity of *Suaeda aegyptiaca* and *Citrus sinensis* plants was investigated. Peels orange(*Citrus sinensis)* were extracted with hot water, and then its antimicrobial activity against Gram-negative and Gram-positive bacteria was examined, data demonstrate effect was more against the bacteria S*phingobacterium thalpophilum* with zone of inhibition (30 mm at 500mg/ml) concentration whereas aqueous extract of peels orange revealed no effect on the bacterial species (*Leuconostoc mesenteroides*, *Ochrobactrum anthropc*, *Escherichia coli*). On the other hand methanol extracts of peels orange had effected on one species only(*Citrobacter freundii*).The study also indicated that aqueous extract of *Suaeda aegyptiac*a plant do not effect on all bacterial species tested while methanol extract of *Suaeda aegyptiac*a revealed activity on the species (*Klebsiella pneumoniae, Pseudomonas oryzihabitans*, *Staphylococcus vitulinus*).

Keywords**:** *Citrus*, *Suaeda*, antimicrobial activity, Vitek 2 compact system

Introduction

Dental caries acknowledged as tooth decompose, a persistent disease is rare among human and is one of the most problems in the world at present. Demolition of calcified layer was produced by acids that product from the fermentation of bacteria to carbohydrates particularly sucrose. Dental caries caused by diverse factors such as dynamics within the plaque group, nature of the tooth enamel, diet, fluoride, physiology, host and pH caries is also associated with poor cleaning of the mouth (Yadav and Prakash, 2017).

Medicinal Plants

Medicinal plants comprise a different types of plants used in herbalism and several plants have activity of medicinal. These medicinal plants regard as a rich resources of components which can be used in drug development and synthesis. Several plants regard as significant source of nutrition and others plants used as therapeutic, which regard as significant source for active components which are used in toothpaste and aspirin. (Hassan, 2012).

Plants as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. According to the World Health Organization plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world drugs are of natural product origin. Pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants to produce more cost effective remedies that are affordable to the population.

Medicinal plants are a source for a wide variety of natural antioxidants and are used for the treatment of diseases during the world. Some of these properties are antimicrobial, anti- diabetic, anti-cancer, immunomodulatory, anti-atherosclerosis (Shirzad *et al.*, 2012), At present, due to advantageous effects of antioxidants in prevention of diseases and the treatment, there has been important interest in finding normal antioxidants from plant origin. In many countries in Europe, herbal medicines are either completely accredited as medicines with efficiency proven by clinical experiments. In United States and Iran, many herbal products are regarded as dietary complement and hence are not needed to meet the standards for drug.

Citrus sinensis plant

Citrus is the most economically significant fruit yield in the world. *Citrus* fruit is grown all over the world in more countries(Abouzar and Nezhad, 2016). *Citrus* fruit has been recognized as an significant food and integrated as part of our daily diet, playing key roles in health promotion and in supplying nutrients and energy. Orange trees are commonly cultivated in tropical and subtropical climates for its delicious juice and value of medicinal. *Citrus senensis* peels have many medicinal characters and are widely used against different diseases, such as, cancer, colic, carmunative, diuretic, immuno – enhancing, immune system and skin ,tonic to digestive system, stomachic. It is also helping to fight viral and bacterial infections and used to treat and prevent colds, vitamin deficiencies, scurvy and flu (Hussain *et al.*, 2015).

Citrus belongs to the family Rutaceae and is found in tropical and subtropical areas in South east Asia. The *Citrus* peels consist of high amount of phenolic compounds including some flavonoid compounds. *Citrus* peel extracts and essential oils are acknowledged to exhibit different biological activities such as antioxidant activities and antimicrobial .The peels of *Citrus* fruits are rich sources of coumarins, b and g-sitosterol, flavonoid glycosides, glycosides and volatile oils .They also contain fibers, polyphenols and especially vitamin C.

Taxonomy of *Citrus sinensis*

Domain	:	Eukarya
Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Sapindales
Family	:	Rutaceae
Genus	:	*Citrus*
Species	:	sinensis

***Suaeda aegyptiaca* plant**

This herb is widespread in Europe, Canary Islands, Mediterranean region, Australia, Asia, Argentina, northeast coast of North America. Many of which are adapted to live in saline soil and live in salt marches or arid saline soil.

The species distributed throughout Arabia in saline habitats especially on coasts and it is grow in rather different plant communities and even as weed in irrigated gardens and fields (Waisel, 1994).

Taxonomy of *Suaeda aegyptiaca* :

Domain	:	Eukaryota
Kingdom	:	Plantae
Phylum	:	Tracheophta
Order	:	Caryophylleles
Family	:	Chenopodiaceae
Genus	:	Suaeda
Species	:	*aegyptiaca*

The present study aims at isolation, characterization of bacterial species are associated with dental caries and evaluates antimicrobial activity of peels *Citrus sinensi*s and *Suaeda aegyptiac*a plants extraction.

Materials and methods Collection of samples

60 samples were collected from patients (for both sexes females and males with different ages)suffering from dental caries from Al-Basrah general hospital by using sterile cotton swabs and dissolved into 2ml brain heart infusion broth media after then transported to the lab and incubated at 37°C for 48 hrs.

Collection of plants

Suaeda aegyptiaca plant samples were collected from garden College of Science, University of Basrah and peels of *Citrus sinensis* collected from local markets in Basrah province. Aerial parts of this plant were cleaned with running water after then with sterile distilled water, and air dried at room temperature (25°C) for three days, then samples were grounded into powder by electrical sterilized mixer grinder. The powdered parts were kept in plastic bags at 4°C until use.

Identification of bacterial isolates

All the isolates were inoculated on nutrient, MacConkey and blood agar plates. The streaked plates were incubated at 37°C for 24 hours. Identification of isolates were done based on colony morphology later Gram stained. Identification with the Vitek-2 system was performed with ID-GN, ID-GP cards, according to the manufacturer's instructions.

Preparation of plants extracts

Water extract of plant was prepared by weighting 20g of plant powder in conical flask, and 400 ml of distilled water was added then mixed with hot plate and magnetic stirrer for six hours. The mixture was filtered through filter paper (Wattman No. 1) and the filtered extract was concentrated with rotary evaporator, then the aqueous extract left at room temperature to remove any excess water.

Methanolic extract of plant was prepared by weighting 20g of plant powder was mixed with 400ml of methanol by successive continuous hot percolation using Soxhlet extractor for 8 hours at 60ºC. The solution was evaporated to dryness in a rotary evaporator (Farjana *et al.*, 2014).

Antimicrobial activities assays

The antibacterial activities of the plants extracts were evaluated by agar well diffusion method. Tested bacterial genera were grown in nutrient broth to agree with (0.5) McFarland standards to be inoculated on Muller-Hinton agar plate by sterile cotton swabs. The plates were dried for (15-20 min) After inoculation, and the wells were perforated using sterile cork borers. Once wells were formed, they were filled with 100 µl of each plant extract (methanol, water) that dissolved in dimethyl sulphoxide (DMSO), where each plant extract was prepared with different concentrations (100, 200, 300, 400, 500) mg/ml. The plates were incubated for37°C at 24 hours to allow plants extracts to diffuse through the agar media to form zones of inhibition. The diameters of the zone of inhibition for different plants extracts against different bacteria were measured in millimeter. An agar well (6 mm) showing no zone of inhibition was considered as no antimicrobial activity. All experiments were done in triplicate and the mean values were used (Farjana *et al.*, 2014).

Results

Identification of bacterial isolates

Among 60 clinical samples were collected from patients suffering from dental caries from Al-Basrah general hospital in Basrah province,50 isolates characterized by using conventional methods, whereas the isolates exhibited differential hemolysis pattern on blood agar and some of isolates ferment lactose when grown on MacConkey media.

The results showed some isolates were Gram negative and other isolates were Gram positive when stained with Gram stain. The identification results with $Vitek_2$ compact system were grouped in Table 1. Among 50 bacterial isolates, 32 isolates identified to genera *(Escherichia*, *Pseudomonas*, *Citrobacter*, *Sphingobacterium*, *Staphylococcus*, *Leuconostoc*, *Acinetobacter*, *Ochrobactrum*, *Klebsiella*, *Enterococcus*, *Yersinia*, *Chromobacterium*), 18 isola tes were non identified. The results demonstrated that the bacteria isolates characterized at species level by $Vitek_2$ compact system was divided into four groups based upon the probability of accurate identification as follows:14 isolates with probability of accurate identification (96-99%), 9 isolates with (93 - 95%), b6 isolates with good(89- 92%),3 isolates with (85-88%) .

Table 1: Bacterial species identified by Vitek 2 compact system

No. Isolate	Bacterial species	Numbers
1	*Escherichia coli*	8
2	*Pseudomonas oryzihabitans*	4
3	*Citrobacter freundii*	2
4	*Sphingobacterium Thalpophilum*	2
5	*Staphylococcus lentus*	2
6	*Staphylococcus vitulinus*	2
7	*Staphylococcus auricularis*	2
8	*Staphylococcus haemolyticus*	1
9	*Leuconostoc mesenteroides*	2
10	*Acinetobacter baumannii complex*	2
11	*Ochrobactrum anthropic*	1
12	*Klebsiella pneumoniae*	1
13	*Enterococcus faecium*	1
14	*Yersinia ruckeri*	1
15	*Chromobacterium violaceum*	1

Antibacterial activity of aqueous and methanolic extracts of *Suaeda aegyptiaca* and *Citrus sinensis* plants on some bacterial species.

The present study showed the antimicrobial activity of peels oranges plant aqueous extract against some of bacterial isolates which obtained from dental caries for both Gram positive and Gram negative bacteria, whereas S*phingobacterium thalpophilum* had the highest microbial sensitivity (zone of inhibition 30 mm with concentration 500 mg/ml of peels oranges aqueous extract) and this inhibitory effect increased with increasing aqueous extract concentration, Fig. 1. Aqueous extract of peels oranges revealed different inhibition zones against other bacterial isolates and this study demonstrated that this aqueous extract do not revealed any effect on the isolates (*Leuconostoc mesenteroides*, *Ochrobactrum anthropic*, *Escherichia coli*). methanol extracts of peels oranges effected on one isolate only (*Citrobacter freundii*) and do not effect on the other bacterial isolates as show in Table 2, Fig. 2.

The results showed that aqueous extract of *Suaeda aegyptiaca* plant do not effect on all bacterial isolates tested while methanol extract of *Suaeda aegyptiaca* revealed activity on the isolates (*Klebsiella pneumoniae, Pseudomonas oryzihabitans*, *Staphylococcus vitulinus)* with different inhibition zones whereas *Klebsiella pneumoniae* had the highest inhibition zone 15mm in 500mg/ml concentration as show in Table 3, Fig. 3.

Table 2: Inhibition zone dimeter (mm) of aqueous and methanol extracts of *Citrus sinensis* plants against some bacterial species

Bacterial species	100mg/ml		200mg/ml		300mg/ml		400mg/ml		500mg/ml	
	aqueous	methanol	aqueous	methanol	Aqueous	methanol	aqueous	methanol	aqueous	methanol
A	0	0	7	0	8	0	9	0	14	**0**
B	0	**0**	9	**0**	9	0	10	0	10	**0**
C	11	0	15	0	20	0	28	0	30	**0**
D	10	0	11	0	15	0	15	0	20	**0**
E	7	0	7	0	10	0	11	0	12	**0**
F	0	0	0	13	0	20	0	20	0	22
G	0	0	0	0	0	0	0	0	0	**0**
H	0	0	0	0	0	0	0	0	0	**0**
I	0	0	8	0	8	0	10	0	14	**0**
J	0	0	0	0	0	0	0	0	0	**0**

Table 3 : Inhibition zone dimeter(mm) of aqueous and methanol extracts of *Suaeda aegyptiaca* plants against some bacterial species

Bacterial species	100mg/ml		200mg/ml		300mg/ml		400mg/ml		500mg/ml	
	aqueous	methanol	aqueous	Methanol	aqueous	methanol	aqueous	methanol	aqueous	methanol
A	0	0	0	0	0	**11**	0	**12**	0	12
B	0	10	0	10	0	10	0	10	0	**11**
C	0	0	0	0	0	0	0	0	0	**0**
D	0	0	0	10	0	11	0	15	0	**15**
E	0	0	0	0	0	0	0	0	0	**0**
F	0	0	0	0	0	0	0	0	0	**0**
G	0	0	0	0	0	0	0	0	0	**0**
H	0	0	0	0	0	0	0	0	0	**0**
I	0	0	0	0	0	0	0	0	0	**0**
J	0	0	0	0	0	0	0	0	0	**0**

A=*Pseudomonas oryzihabitans*, B= *Staphylococcus vitulinus*, C= *Sphingobacterium Thalpophilum*, D= *Klebsiella pneumoniae, E= Enterococcus faecium, F= Citrobacter freundii*, G= *Leuconostoc mesenteroides*, H= *Ochrobactrum anthropic*, I= *Chromobacterium violaceum*, J=*Escherichia coli*

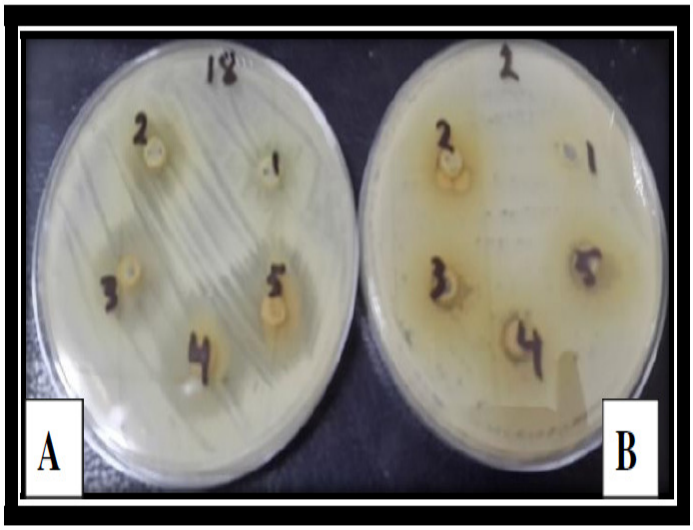

Fig. 1: Effect of aqueous extract of *Citrus sinensis* plant on *A:* Sphingobacterium Thalpophilum*, B:* Pseudomonas oryzihabitans

Fig. 2: Effect of methanol extract of *Citrus sinensis* plant on *C:* Citrobacter freundii

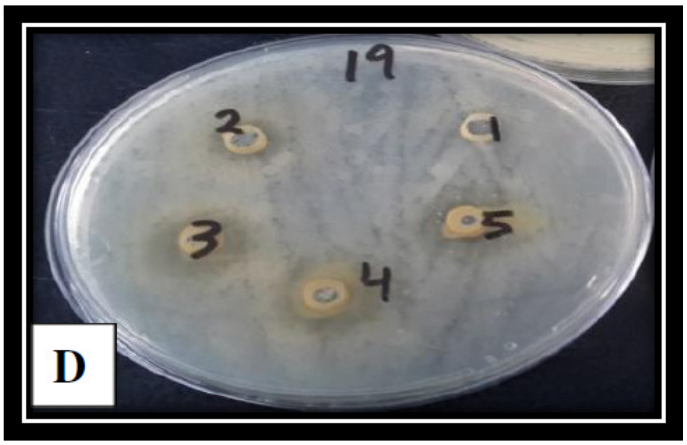

Fig. 3: Effect of methanol extract of *Suaeda aegyptiaca* plant on *D:* Klebsiella pneumoniae

Discussion

Oral diseases sources major health problems in the worldwide. In the recent study the bacterial isolates isolated from dental caries identified with Vitek$_2$ compact system after identified by conventional methods because Vitek$_2$ compact system is an option which proven to reduce turn around times and improve reproducibility and the quality of microbiology results, the Vitek$_2$ system have several advantages that may be of clinical interest for routine testing of gram-positive and gram-negative isolated from the clinical samples: Rapid identification (during three hours), a high level of automation and taxonomically updated databases (Wrenn, 2015). In the present study the Vitek-2 system identified 32 bacterial isolates out of total 50 belong to the genera (*Escherichia*, *Pseudomonas*, *Citrobacter*, *Sphingobacterium, Staphylococcus*, *Leuconostoc*, *Acinetobacter*, *Ochrobactrum*, *Klebsiella*, *Enterococcus*, *Yersinia*, *Chromobacterium*), and 18 isolates remained unidentified this results comparable to those of (Dina *et al.*, 2014) who identified 165 strains out of total 170. In recent years much researches have emerged to use of herbal medicines to treat infections, since herbal medicines are stronger antibacterial activity and fewer side effects than chemical drugs (Saeedi, 2017). Natural extracts obtained from plants can influence microbial formation. From the generalized data is revealed that the greater zone of inhibition exhibited by hot aqueous extract of peel orange (*Citrus sinensis*) against *Sphingobacterium thalpophilum* while hot methanolic extract of peel orange do not effect against all isolates exception *Citrobacter freundii* this inhibition effect may be related to its active compounds that includes saponins, tannins, essential oils, phenolic compounds and flavonoids and others this results do not agree with study of (Hussain *et al.*, 2015), who explained that the alcoholic extracts more effective against pathogens than the aqueous extracts of peels orange (*Citrus sinensis*).

Inhibition zone of all extracts against pathogens in this study increased with increase in concentration which is in agreement with previous studies (Hussain *et al.*, 2015) also results obtained showed the aqueous extract of *Suaeda aegyptiaca* do not effect on all bacterial isolates were tested this study does not similar to those obtained by (Al-Mawla, 2007) who reported antibacterial activity of aqueous extract of *S. aegyptiaca* was examined against four pathogenic bacteria: *Staphylococcus aureus, Escherichia coli, Salmonella typhi and Shigella desynterial*. While methanolic extract of *Suaeda aegyptiaca* revealed activity on the isolates (*Klebsiella pneumoniae, Pseudomonas oryzihabitans*, *Staphylococcus vitulinus*), this results agreement with study (Al-Mujammaae, 2005), who showed that ethanolic extract had inhibitory effect on *Staphylococcus aureus, Staphylococcus epidermidis* and *Klebseilla pneumoniae*. Chemical analysis showed that *S. aegyptiaca* contains different active compounds: phenols, alkaloids and terpens.

Conclusions

In the present study confirmed the anti- microbial potential of *Citrus sinensis* peel and *Suaeda aegyptiaca* extracts plants against bacteria isolated from dental caries tested .whereas the data demonstrate that the effect of oranges peels aqueous extract was more against the bacteria S*phingobacterium thalpophilum.*

References

Yadav, K. and Prakash, S. (2017). Dental caries: A microbiological approach. J. Clin. Infect. Dis. Pract., 2: 118.

Hassan, B.A. (2012). Medicinal plants (Importance and Uses). Pharmaceut. Anal. Acta., 3: e139.

Shirzad, H.; Taji, F.; Pourgheysari, B.; Raisi, S. and Rafieian-Kopaei, M. (2012) .Comparison of antitumour activities of heated and raw garlic extracts on fibrosarcoma in mice. J Babol Univ Med Sci., 14: 77–83.

Abouzari, A. and Nezhad, N.M. (2016). The investigation of *Citrus* fruit quality popular characteristic and breeding. Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis., 64: 725-740.

Hussain, K.A.; Tarakji, B.; Kandy, B.P.P.; John, J.; Mathews, J.; Ramphul, V.; Darshan, D.D. (2015). Antimicrobial effects of *Citrus sinensis* peel extracts against periodontopathic bacteria: An *in vitro* study. Rocz Panstw Zakl Hig., 66: 173-178.

Waisel, Y. (1994). Biology of halophytes. Academic press, London. 395.

Farjana, A.; Zerin, N. and Kabir, M.S. (2014). Antimicrobial activity of medicinal plant leaf extracts against pathogenic bacteria. Asian Pac J Trop Dis.,4: S920-S923.

Wrenn, C. (2015). Introduction of the Vitek 2 Compact and Implementation of Eucast guidelines in a microbiology department. Masters dissertation. Dublin: Royal College of Surgeons in Ireland, 121.

Dina, M.; Atef, D.M.; Rania, A. and Ghonaim, M.D. (2014). The role of Vitek-2 automated system in identification and susceptibility testing of Gram negative rods in an intensive care unit patients in Egypt. Med. J. Cairo Univ., 82: 357-362.

Saeedi, M.; Yeganegi, M.; Alizadeh, B.B.; Vasiee, A.R. and Tabatabaei, Y.F. (2017). Antimicrobial effects of Leek (*Allium ampeloprasum* L. subsp .iranicum)extract on some food-borne pathogens *in vitro*. JFST., 14: 73-82.

Al-Mawla, R.H. (2007). Extraction of some active materials in the aerial part of *Shannginia aegyptiaca* and study their Biological activity. M.Sc. Thesis. College of Sciences, University of Al-Anbar.

Al-Mujammaa'e, R.M.D. (2005). Effect of *Suaeda aegyptiaca* extracts on some microorganisms *In vivo* and *In vitro*. Master of Science in Biotechnology, College of Science, Al-Nahrain University, 111.