ISSN: 0067-2904
GIF: 0.851

Antimicrobial and Antibiofilm Activity of Mango Seeds Extract

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Abstract

Mango fruit is one of the most nutritionally rich fruits with unique flavor, this fruit belonged to family of *Anacardiaceae* and it is an excellent source of vitamins specially vitamin A, carotene pigments and potassium. In this study the antimicrobial activity of mango seeds extract has been investigated against gram positive bacteria (*Staphylococcus aureus* and *Bacillus spp.)* and gram negative bacteria (*Pseudomonas aeruginosa* and *E. coli*) and yeast *Candida albicans* by well diffusion method in nutrient agar and the results were expressed as the diameter of bacterial inhibition zones surrounding the wells, and the antibiofilm of its extracts was observed against *Staphylococcus aureus*. The seeds extractions prepared by two solvents: 85% ethanol and 85% acetone, also the minimum inhibition concentration values (MIC) was determined for the two extracts. Also the compounds of the mango seeds extracts were identified by using gas chromatography-mass spectrometry (GC-MS). The results showed that the ethanol mango seeds extraction more efficient than acetone extraction as the inhibition zones by ethanol extract ranging between 15mm – 27mm while by acetone extract between 12mm–25mm, the MIC for two solvents extracts was approximately at the concentration 125 µg/ml and the antibiofilm test showed the ethanol extract inhibited the bacterial adhesion on glass tube and that cause biofilm detachments on it in compare with control tubes more than acetone extract which showed low effect.

Keywords: antimicrobial activity, antibiofilm activity, well diffusion method, minimum inhibition concentration, gas chromatography-mass.

الفعالية الضدمايكروبية والمضادة للاغشية الحيوية لمستخلص بذور المانجا

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الخلاصة

المانجا من الفواكه الغنية من الناحية الغذائية ذات طعم مميز، وتعود هذه الفاكهة لعائلة *Anacardiaceae* وهي مصدر ممتاز للفيتامينات خصوصا فيتامين A ،صبغة الكاروتين والبوتاسيوم. في هذه الدراسة تم التحقق من فعالية مستخلص بذور المانجا ضد البكتيريا الموجبة لصبغة كرام (*Bacillus* and *Staphylococcus aureus*) والسالبة لصبغة كرام (*E. coli* and *Pseudomonas aeruginosa*) وخميرة (*Candida albicans*) بطريقة الانتشار بالحفر في الوسط الغذائي الصلب وظهرت النتائج كأقطار تثبيط البكتريا في المناطق المحيطة بالحفر، كما لوحظ التأثير المضاد للاغشية الحيوية للمستخلص ضد بكتريا *Staphylococcus aureus*. تم تحضير المستخلص للبذور بواسطة مذيبين 85%ايثانول و85%اسيتون ، كذلك تم تحديد قيم التركيز الادنى للتثبيط MIC للمستخلصات. تم تشخيص المركبات لمستخلص بذور المانجا باستعمال جهاز GC–MS. اظهرت النتائج ان مستخلص الميثانول لبذور المانجا اكثر كفاءة من مستخلص الاسيتون حيث ان مناطق التثبيط لمستخلص الايثانول يتراوح بين 15ملم–27ملم بينما في مستخلص الاسيتون يتراوح بين 12ملم–25ملم. ان التركيز الادنى للتثبيط للمستخلص بالمذيبين كان

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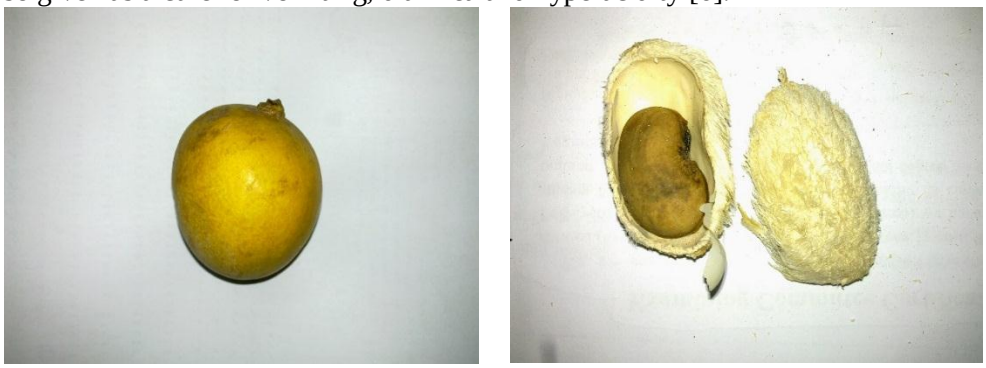
تقريبا 125 مايكروغرام/مل والفحص المضاد للاغشية الحيوية اوضح ان مستخلص الايثانول ثبط التصاق البكتريا على زجاج الانابيب وهذا سبب عدم التصاق الغشاء الحيوي على الزجاج مقارنة مع انابيب السيطرة اكثر من ما في مسخلص الاسيتون الذي كان قليل التأثير.

Introduction

Mango is one of the most important tropical fruits in the world. During processing of mango, by-products such as peel and kernel (seed) are generated. The seed takes up about 17-22% of the fruit [1]. The shape of mango seed is a single flat oblong seed that can be fibrous or hairy on the surface, the inside seed coat 1 - 2 mm thick is a thin lining covering a single embryo, 4 - 7 cm long, 3 - 4 cm wide, and 1 cm thick. Mango seed consists of a tenacious coat enclosing the kernel [2] Figure-1. Barreto *et al*. [3] reported that the extract of different parts of mango possess several biological and pharmacological properties. Alcoholic extract of mango seeds showed anti-inflammatory activity in acute, sub-acute and chronic cases [4]. Sowmiya *et al*. [5] mentioned the antibacterial activity for mango seeds ethanolic extract, the extract showed good activity against pathogenic bacteria such as: *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebisella pneumoniae and Streptococcus pyogenes.* Phenolic, tannins and flavonoids compounds in mango seed are found to be responsible for antimicrobial property. They acts on microorganisms by inhibiting extracellular microbial growth and by avoiding oxidative phosphorylation. Also mango seed kernel oil used to kill abdominal worm and so given as a cure for vomiting, diarrhea and hyperacidity [6].



A B

Figure 1- A- Mango fruit B- Mango seed (Imaged by author)

Materials and Methods:

Bacterial and Fungal isolates

The bacterial isolates were obtained from central Lab. in department of Biology / College of Science/ University of Baghdad, the used bacteria were:
Gram-positive: *Staphylococcus aureus* and *Bacillus spp.*, Gram-negative: *Escherichia coli* and *Pseudomonas aeruginosa* and the yeast: *Candida albicans.*

Preparation and Extraction of Mango Seed

The mango seeds were collected manually from mango fruits, washed to get rid of any adhering fruit flesh and then air-dried. The seeds were grinded by using grinder till became powder and preserved for analyses. After that, the extraction solvents required (85% ethanol and 85% acetone) were prepared to the extraction process. For preparing extracts we prepare 1:10 (w:v) weight the powder and dissolved in ethanol acetone (85%) then used vortex to mix them for approximately 5min, and so could prepare any concentration we need in the same way. and leave the mixture of extraction at the refrigerator for 24hrs. then the extracts were centrifuged in cooling centrifuge for about 15-20 min. then taken the supernatant and stored the extracts in 4°C until utilization for the next procedures [7].

Antimicrobial Activity for Mango Seed Extracts (ethanol extract and acetone extract)

The antimicrobial activity of various mango seed extracts was tested by agar diffusion method. The plates containing nutrient agar was spread with 0.1 ml of all the isolates, with cork-borer making 8mm wells in the agar then these wells filled with 0.1 ml of various seed extracts. The plates inoculated with different isolates were incubated at 37°C for 24hrs after that results observed as measuring the diameter of inhibition zones (clear zone without growth around the colony) [8].

Minimum Inhibition Concentration (MIC)

Seven concentrations for the solvents extract were prepared (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml and 15.62 µg/ml). Extracts samples were pipetted into glass tubes containing nutrient broth, the final volume was 2ml (solvent extract and nutrient broth) in each tube. The tube was mixed by using vortex mixture before the previous steps were repeated in order to obtain another dilute sample. Then, 0.1ml from the overnight inoculum for each isolates were added (the isolates were calibrated with McFarland tube 10^8 cell/ml). The tubes were incubated at 37°C for 24hrs. the first concentration of component that did not permit any visible growth of the inoculated test organisms in broth culture (clear) after series of growth in tubes (turbid tubes) was regarded as minimum inhibitory concentration (MIC) in each case [9].

Identification of the Mango Seed Extracts Compounds using GC-MS

Gas chromatography – mass spectrometry was used for identification of the mango seed extract by using apparatus type Shimadzu GCMS-QP2010Ultra, the injection volume was 5µl.

Antibiofilm activity of various mango seeds extracts

This test performed to detect the ability of the extract to effect on or prevent the bacteria *S. aureus* to adhere on smooth surface (glass tube). This test was performed by cultivating the tested bacteria in glass tube contain 2ml nutrient broth, all tubes were incubated at 37°C for 24hrs, after incubations the tubes content removed then 2ml of 125 µg/ml mango seed extract by ethanol solvent added to one tube and 2ml of 125 µg/ml mango seed extract by acetone added to another tube the tubes incubated at 37°C for 24hrs (control tube made by nutrient broth and bacteria only) after that the tubes content were removed and the tubes stained by adding 2ml of 0.1% crystal violate for approximately 1-5min. then washed the tubes and see the effect of the extractions on the film that present on the wall of glass tubes in compare with the control tube. The result arranged as medium or weak effect of extract on biofilm production and [10, 11].

Results and Discussions:

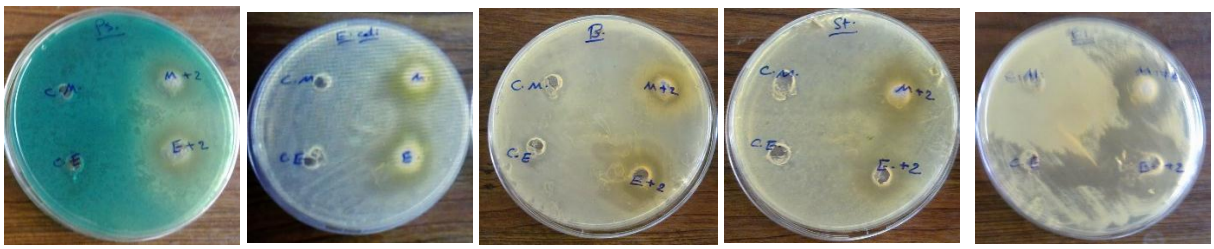
Antimicrobial activity for mango seed extracts (ethanol extract and acetone extract)

The antimicrobial activity of 100mg/ml mango seeds extract by both solvents ethanol and acetone (85%) were performed by agar diffusion method, the results of these activities of each solvents were expressed in terms of diameter (mm) of the inhibition zone as in the Table-1. The results showed the ethanol extract was active slightly more than acetone extract on most microbial growth which give highest effect on bacteria *P. aeruginosa* and *S. aureus* 20mm in other hand acetone extract effect was 17mm, 18mm respectively and on fungi (yeast) *C. albicans* the result was 27mm for ethanol extract in compare with 25mm for acetone extract (Figure-2).

Table 1- Antimicrobial activity (inhibition zones mm) of mango seed extract for both ethanol and acetone solvents by using well diffusion method in agar plate.

Sample	*P. aeruginosa*	*E.coli*	*Bacillus* spp.	*S. aureus*	*Candida albicans*
Mango seeds ethanol extract	20mm	18mm	15mm	20mm	27mm
Mango seeds acetone extract	17mm	18mm	12mm	18mm	25mm
Control ethanol (85%)	_____	_____	_____	_____	_____
Control acetone (85%)	_____	_____	_____	_____	_____

*Note: ____ means no inhibitions zone



1 2 3 4 5

Figure 2- Antimicrobial activity for mango seeds ethanol and acetone extracts

(1- *P. aeruginosa* 2- *E. coli* 3- *Bacillus* spp. 4- *S. aureus* 5- *Candida albicans*)

The wells: CM = Control acetone, CE = Control ethanol, M or M+2 = acetone extract, E or E+2 = ethanol extract

Abdullah [12] found that the ethanolic extract of mango kernel has the best antibacterial activity (18mm) for *B. subtilis* followed by acetone extract (17mm), methanol extract (17mm) and distilled water (14mm); since the control tetracycline has shown the highest inhibition 28 mm on *B. subtilis*, therefore, it is essential to compare the capability of the extract with the best standard of inhibition activity [7]. Antibacterial activities of methanol and ethanol extracts of mango seed showed inhibitions to 25 types of tested organisms with variable inhibition zones. Except one organism (*Rhodococcus equi*), no resistance among the tested strains was shown. The mean zone of inhibition produced ranged between 5 mm for *Nocardia farcinica* and 18 mm *Mycobacterium smegmatis* which showed the highest zone of inhibition. *Candida albicans* and *Aspergilllus niger* were both inhibited by the extracts 10mm and 9mm respectively. The methanol and ethanol extracts of mango seed showed good inhibitory effects against almost all tested strains [13].

Minimum Inhibition Concentration (MIC)

Different concentration of the mango seeds of both ethanol extract and acetone extract (85%) were performed (1000, 500, 250, 125, 62.5, 31.25 and 15.62) µg/ml. The results showed that the MIC (the concentration of first clear tube appear after serial turbid tubes considered as MIC tube) for all tested microorganisms was 125 µg/ml in mango seeds ethanol extract and also for acetone extract the MIC was 125 µg/ml for the tested isolates except *P. aeruginosa* the MIC was 250 µg/ml and *Bacillus* spp. MIC was 62.5 µg/ml.

Minimum inhibitory concentration (MIC) refers to the lowest concentration of the antimicrobial agent which is required for the inhibition of visible growth of the tested microorganism [14]. The antimicrobial activity of an extract is usually quantified by determining the MIC values which could be used as a guide for the treatment of most infections, The MIC values of *Mangifera indica* seed extract were 156 µg/ml and 625 µg/ ml respectively against *Micrococcus flavus*. The seed showed MIC values 78 µg/ ml and 1250 µg/ml against *Proteus morganii* and *Klebsiella pneumoniae* respectively [15]. Alternatively to synthetic compounds, natural compound obtained from some plants are rich in phenolic compounds and can enhance overall quality through decrease in lipid oxidation and microbial growth [16].

Identification of the mango seed extracts compounds using GC-MS

To determined the components of mango seeds in ethanol and acetone extraction the gas chromatography-mass was conducted, the results of this analysis shown in the following Tables (-2,-3) and Figures (-3,-4).

Table 2- GC-MS analysis showing the compounds present in ethanolic extract of mango seeds

Peak Report TIC

Peak	R.Time	Area	Area%	Height	Height%	Name
1	6.44	601434	9.25	80287	5.20	Silane, dimethoxydimethyl-
2	6.81	365002	5.61	50257	3.26	2-Propanone, 1-hydroxy-
3	7.72	109577	1.68	21349	1.38	Acetic acid, (acetyloxy)-
4	8.12	251432	3.87	29641	1.92	2-Propenoic acid, ethenyl ester
5	8.62	900921	13.85	191419	12.40	Acetic acid
6	8.74	211593	3.25	73085	4.74	Propanoic acid, 2-oxo-, methyl ester
7	8.85	102861	1.58	34241	2.22	Furfural
8	9.30	130340	2.00	28082	1.82	Formic acid
9	9.54	35545	0.55	12265	0.79	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
10	10.15	174483	2.68	39689	2.57	2,3-Butanediol, [R-(R*,R*)]-
11	10.35	64984	1.00	15714	1.02	2-Cyclopentene-1,4-dione
12	11.29	259055	3.98	75345	4.88	2-Furanmethanol
13	12.86	123991	1.91	35869	2.32	1,2-Cyclopentanedione
14	15.54	25187	0.39	8847	0.57	6-Methyl-2-pyrazinylmethanol
15	15.92	82734	1.27	12210	0.79	Methyl 2-furoate
16	16.16	69447	1.07	13664	0.89	2,5-Dimethyl-4-hydroxy-3(2H)-furanone
17	16.73	83124	1.28	26295	1.70	2-Propanone, 1,3-dihydroxy-
18	16.81	132648	2.04	39624	2.57	Cyclopropyl carbinol
19	17.02	65439	1.01	21221	1.38	Hexan-2,4-dione, enol
20	17.45	83592	1.29	27191	1.76	Cyclopentane, 1-acetyl-1,2-epoxy-
21	17.58	161513	2.48	49018	3.18	2-Hydroxy-gamma-butyrolactone
22	18.57	404760	6.22	114265	7.40	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-meth
23	18.87	815465	12.54	201569	13.06	Glycerin
24	19.09	53626	0.82	14372	0.93	Butanoic acid, 3-oxo-, 1-methylpropyl ester
25	20.51	422528	6.50	152151	9.86	Dodecanoic acid
26	20.61	285769	4.39	97991	6.35	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
27	22.85	212746	3.27	35552	2.30	Tetradecanoic acid
28	26.20	273687	4.21	41985	2.72	n-Hexadecanoic acid
		6503483	100.00	1543198	100.00	

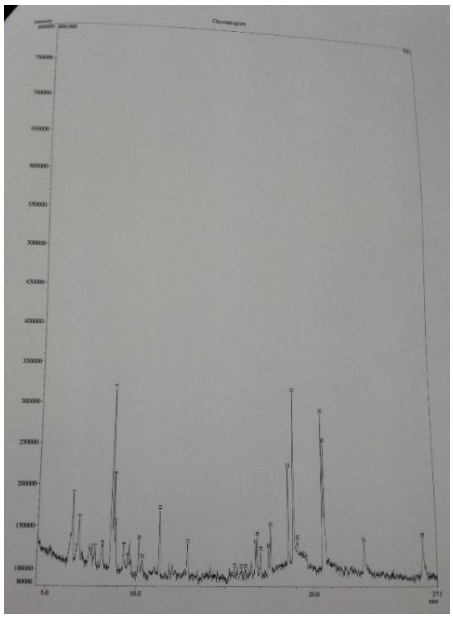


Figure 3- GC-MS analysis showing the peaks of possible compounds present in ethanolic extract of mango seeds sample

Table 3- GC-MS analysis showing the compounds present in acetone extract of mango seeds

Peak Report TIC

Peak	R.Time	Area	Area%	Height	Height%	Name
1	6.40	36284	0.15	12779	0.32	1,4-Dioxin, 2,3-dihydro-
2	6.73	509467	2.14	77775	1.92	2-Propanone, 1-hydroxy-
3	6.92	36209	0.15	13130	0.32	Methanamine, N-hydroxy-N-methyl-
4	7.57	10609712	44.48	1056847	26.10	2-Pentanone, 4-hydroxy-4-methyl-
5	8.10	406530	1.70	65746	1.62	2,2'-Bioxirane
6	8.59	1623268	6.81	378408	9.35	Acetic acid
7	8.72	306714	1.29	108141	2.67	Propanoic acid, 2-oxo-, methyl ester
8	8.82	330799	1.39	90233	2.23	Furfural
9	9.29	273373	1.15	63116	1.56	Formic acid
10	9.53	126715	0.53	39668	0.98	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
11	9.70	87783	0.37	20829	0.51	2,3-Butanediol, [R-(R*,R*)]-
12	10.14	195628	0.82	51422	1.27	2,3-Butanediol, [R-(R*,R*)]-
13	10.35	123373	0.52	24287	0.60	2-Cyclopentene-1,4-dione
14	10.62	32210	0.14	12030	0.30	1,3-Dioxolane-4-methanol, 2,2-dimethyl-, (S)-
15	11.28	526762	2.21	150670	3.72	2-Furanmethanol
16	12.00	58238	0.24	15143	0.37	2,5-Furandione, dihydro-3-methylene-
17	12.71	39661	0.17	12268	0.30	2(5H)-Furanone
18	12.86	183489	0.77	52558	1.30	1,2-Cyclopentanedione
19	13.41	52842	0.22	17165	0.42	2-Methylene cyclopentanol
20	14.15	102791	0.43	33310	0.82	2,5-Dimethyl-4-hydroxy-3(2H)-furanone
21	14.32	146090	0.61	12049	0.30	Propanoic acid, 2-methyl-, methyl ester
22	15.54	36150	0.15	12965	0.32	6-Methyl-2-pyrazinylmethanol
23	15.91	63532	0.27	20038	0.49	3-Furancarboxylic acid, methyl ester
24	16.15	89132	0.37	24992	0.62	2,5-Dimethyl-4-hydroxy-3(2H)-furanone
25	16.72	160075	0.67	55437	1.37	2-Propanone, 1,3-dihydroxy-
26	16.81	161770	0.68	56269	1.39	Butanal
27	17.01	213552	0.90	49879	1.23	Hexan-2,4-dione, enol
28	17.45	219650	0.92	67375	1.66	Maltol
29	17.58	209081	0.88	65319	1.61	2-Hydroxy-gamma-butyrolactone
30	18.57	841643	3.53	251608	6.21	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
31	18.88	1402759	5.88	358370	8.85	Glycerin
32	19.10	87856	0.37	23523	0.58	4-Acetamidobutyric acid
33	20.51	169743	0.71	65872	1.63	Dodecanoic acid
34	20.61	1172648	4.92	314265	7.76	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
35	21.08	466275	1.95	33033	0.82	Octadecanoic acid
36	22.16	1476729	6.19	146348	3.61	9-Octadecenoic acid, (E)-
37	22.84	80185	0.34	22667	0.56	Octadecanoic acid
38	24.10	369500	1.55	37358	0.92	9,12-Octadecadienoic acid (Z,Z)-
39	26.19	822874	3.45	135979	3.36	n-Hexadecanoic acid
		23851092	100.00	4048871	100.00	

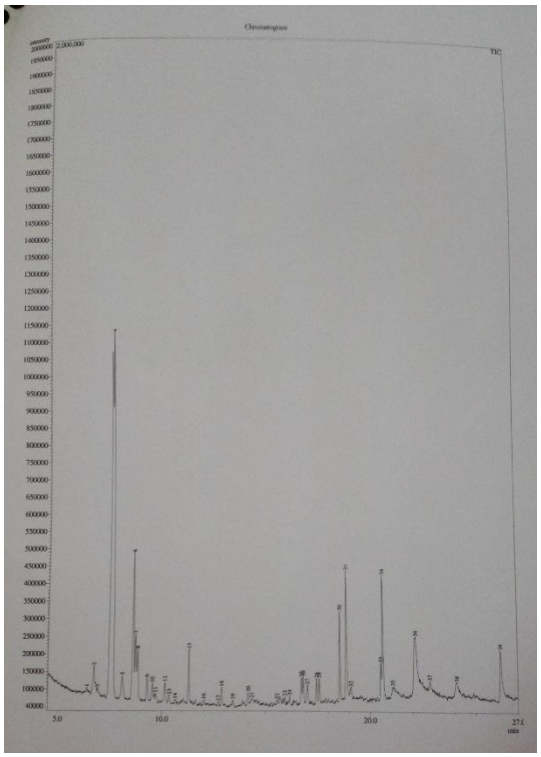


Figure 4- GC-MS analysis showing the peaks of possible compounds present in acetone extract of mango seeds sample

The results showed that the ethanol extract of mango seeds contains 28 compounds and the highest% of compounds between them are: Glycerin 13.06%, acetic acid 12.40%, dodecanoic acid 9.86%,4H-Pyran-4-one,2,3-dihydroxy-6-meth7.40%,2-Furancarboxaldehyde,5-(hydroxymethyl)-6.35%, Silan, dimethoxydimethyl- 5.20%, 2-Furanmethanol 4.88% and Propanoic acid, 2-oxo-,methyl ester 4.74%; While the acetone extract contain 39 compounds and the highest% of compounds between them are: 2-Pentanone, 4-hydroxy-4-methyl- 26.10%, Acetic acid 9.35%, 2-Furancarboxaldehyde, 5-(hydroxymethyl)- 7.76% and 4H-Pyran-4-one, 2,3-dihydro-3,5dihydroxy-6-methyl- 6.21% and other compounds in both extracts. Mango seed kernel consist of many compound some of them are crude protein, oil, ash, crude fiber, and carbohydrate [1], The mango seed reported to be a good source of phenolic compounds [17] including microelements like selenium, copper and zinc, in addition, the extract of mango seeds contains phenolic compounds, tannins and flavonoids which found to be responsible for antimicrobial property. They effects on microorganisms by inhibiting extracellular microbial; growth and by avoiding oxidative phosphorylation [6]. Mango seed kernels contained an amount of phenolic compounds, unsaponifiable matter, lipids and a low amount of crude protein, and the quality of protein was good because it was rich in all essential amino acids with highest values of leucine, valine and lysine. Eight phenolic compounds were reported, tannin and vanillin were in highest amounts. Unsaponifiable matter showed the occurrence of high amounts of squaline followed by sterols and tocopherols. Stearic acid was the main saturated fatty acid, while oleic acid was the major unsaturated fatty acid in all lipid classes. Also phospholipid had a high amount of palmitic, linoleic and linolenic acids [18]. According to mango variety, mango seed kernels contain on a dry weight average 77% carbohydrate, 11% fat, 6.0% protein, 2.0% crude fiber and 2.0% ash. Mango seed kernels were shown to be a good source of polyphenols, β-sitosterol, tocopherols and phytosterols as campesterol, the specific bioactive component that identified in the ethanolic extract of mango kerenal by GC-MS and responsible for antibacterial activity as phenol is 2,4-bis (1,1-dimethylethyl) in 0.92% concentration [7].

Antibiofilm activity of various mango seeds extracts

The effect of mango seed extract for both ethanolic extract and acetone extract was conducted against *S. aureus* to study the ability of these extracts to prevent the production of biofilm on glass tube from this bacteria, the result was observed qualitatively by appearance of thin film adherence on the wall of glass tubes as showing in the Figure-5.

The results obtaind from Figure-5 made clear that in compare with control tubes the mango seed ethanolic extract has a good ability to affect bacterial biofilm and prevent its adnerence on the walls and bottom of glass tubes, while the mango seed acetone extract have medium ability to effect on biofilm adherence on glass tubes walls and bottom at the same condition with ethanol extract. Geethashri *et al*. [19] found in there study on antimicrobial activity of mango extracts on a variety of pathogens, its antibiofilm activity against *S. aureus*. The ethanolic extract of Thai mango (*Mangifera indica* L. cv. 'Fahlun', Anacardiaceae) seed kernels (MSKE) contains a relatively high phenolic content of pentagalloylglucopyranose (PGG) (61.28%) and relatively smaller amounts of gallic acid (GA) (0.44%) and methyl gallate (MG) (0.68%) [20]. Mango seed extract and its principles have been pharmacologically documented to have anti–inflammatory, antioxidant, anti-tyrosinase, and hepatoprotective activities [21]. The alcoholic extract from mango seed kernel extract and its phenolic principles (gallic acid, methyl gallate and pentagalloylglucopyranose) demonstrated potent antibacterial activity *in vitro* against *Staphylococcus aureus* [22]. Prashant *et al*. [23] observed that the chewing some materials of mango and neem tree extract is a good way to cleaning teeth and believed to possess medicinal properties by its antimicrobial effects on the microorganisms *Streptococcus mutans* , *Streptococcus salivarius* , *Streptococcus mitis* , and *Streptococcus sanguis* which are involved in the development of dental caries. Also identify to be an inexpensive, simple, and effective method of preventing and controlling dental caries.

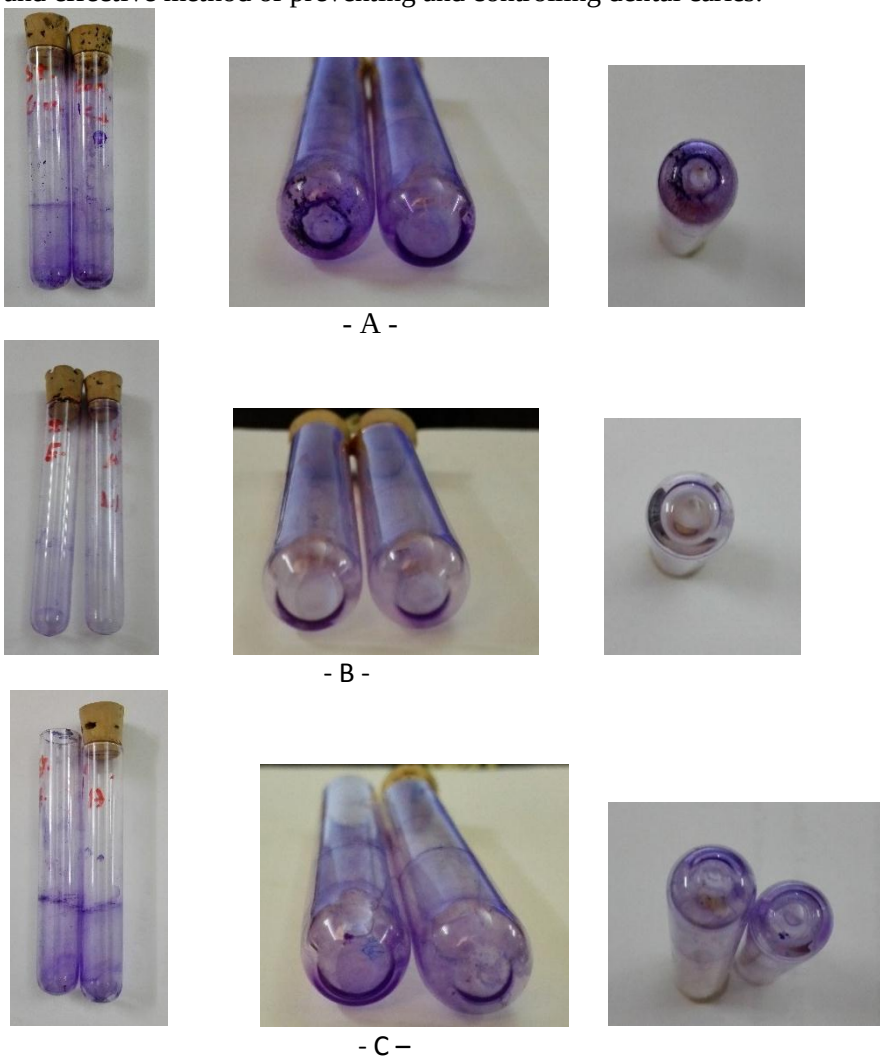


Figure 5- The ability of bacteria *S. aureus* to adhere on glass tube

- **A.** The control tubes (bacteria growth for 24hr. at 37°C only without treated with mango extracts)
- **B.** Tubes treated with ethanol mango seed extract after bacterial growth for 24hr. at 37°C
- **C.** Tubes treated with acetone mango seed extract after bacterial growth for 24hr. at 37°C

Conclusion:

Plant and fruits derived products or materials have been used in health care for treatments of a variety of infectious diseases and industry. In this study we observed that the extractions of mango seeds bioactive compounds by ethanol solvent is more efficient than extraction by acetone solvent, and ethanol considered to be safe and nontoxic in bio treatments. The mango seed extract consist many compounds such as phenolic and some acids that could be affect many microorganisms: bacteria and fungi (yeast) and give an potent effect on microbial growth as well as bacterial production of harmful materials like biofilm production that cause many medical and industrial problems, so the use of some part of plants and extracted the active compound from it can solved these problems and could be more safety than chemical materials that used in many industry, medical fields and pharmaceutical industries.

Acknowledgments:

Special thanks to all the staff of central Lab. in department of Biology / College of Science/ University of Baghdad for providing the microbial isolates and all the laboratory facilities in this research.

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