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Evaluation of Anti Bacterial Activity: Anti adherence, Anti Biofilm and Anti Swarming of the Aquatic Extract of Black Raisins and Vinegar of Black Raisins in Hilla City, Iraq

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Abstract : Background: Raisins are dried grapes, prepared from some varieties of grapes (Vitis *vinifera).* The history of raisin consumption is very old.
Objective: An evaluation of Antibacterial activity of the extracts of black raisins and black raisin vinegar against bacteria Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumonia, Streptococcus feacalis, Streptococcus mutanus, Streptococcus feacalis, Escherichia coli, Salmonella typhi, Moraxallia catarralis, Pseudomonas aeroginosa, Proteus mirabilis, Klebsiella pneumonia, Enterobacter spp. Acinetobacter, Serratias pp and Candida albicance.
Methods: Two black raisins products were used to determine the antibacterial activity of black raisins; crude aquatic extracts of dried black raisins, and the vinegar of dried black raisins. Agar well diffusion method, biofilm inhibition test using tissue culture plate method, adherence and swarming inhibition assays were done for estimation and evaluation of the antibacterial activity of black raisins.
Results: Result showed that both the crude extract of black raisins and the vinegar of black raisins have potential antibacterial activity. The results were determined by measuring the inhibition zone, the inhibition of bacterial motility using swarming assay with all gradual different concentrations of black raisins and the vinegar of black raisins (Pearson correlation =0.9; P value $\leq$ 0.05, the inhibition of bacterial cell adherence to oral epithelial cells, quorum sensing and biofilm formation.
Conclusions: Black Raisins and vinegar of black raisins exhibit marked antimicrobial activity bacteria and fungicidal activity. They can inhibit motility, inhibit biofilm formation and inhibit bacterial cell adherence to oral epithelial cells. Based on the results it can be concluded that they can inhibit bacterial colonization and adherence to teeth and oral cavity, and provide production against different human pathogens and this may have clinical relevance.
Keywords: Black Raisins, biofilm, bacterial adherence, swarming.

Introduction

There are several natural products used as raw materials in the fragrance industry and flavor[37]. Raisins are dried grapes, prepared from some varieties of grapes (Vitis *vinifera).* The history of raisin consumption is very old. The earliest account of raisin use comes from a mention in the bible around 1000 B.C[1].

Raisins are a source of carbohydrates and they contain large amounts of iron, vitamins and minerals [2].

Raisins are usually included in breakfast cereals, dairy, confectionery products and most recently nutritional bars[3,4].

Raisins have been found to contain several chemical compounds that may assist in fighting oral bacteria. Laboratory studies have shown that the extracts of raisins were found to slow the growth of *Streptococcus mutans*, the main bacteria behind tooth decay. Five chemicals in raisins; oleanolic acid, oleanolic aldehyde, betulin, betulinic acid, and 5-(hydroxymethyl)-2-furfural seem to be responsible for slowing the bacteria. In addition, oleanic acid prevents *Streptococcus mutans* from sticking to tooth enamel[5].

Besides being traditional and popular snack food, raisins contain polyphenols, antioxidants, flavonoids and iron that may benefit overall human health. The sweetness of raisins is contributed by mainly glucose and fructose, but not sucrose. It is well documented that sucrose, the main dietary sugar, serves as a substrate for the synthesis of adherent glucans in human dental plaque associated with tooth decay and gum disease.

The various phytochemicals reported in raisins include triterpenes, fatty acids, flavonoids, amino acids, hydroxylcinnamic acids and 5-hydroxymethyl-2-furaldehyde. Although various *in vitro* studies have been performed to investigate the mode of actions of these phytochemicals and their effects on bodily functions, much less attention has been paid to their effects or their activity against bacteria. Therefore the current study aims to evaluate antibacterial activity of black raisins and vinegar of black raisins[5].

Bacterial biofilms are sessile microbial communities attached to a surface by polysaccharides, proteins, and nucleic acids. Biofilms are ubiquitous in natural, medical, and engineering environments. Due to their increased resistance to antimicrobial treatment, biofilms formed by pathogenic bacteria pose serious problems to human health. They cause diseases such as cystic fibrosis, prostatitis, and periodontitis. In contrast, some communal bacteria are crucial for nutrient assimilation and beneficial to the human immune system. While most antibiotics primarily aim to inhibit cell growth, their excessive use may result in bacterial drug resistance. Biofilm inhibitors do not affect cell growth and there is less chance of resistance development. Since biofilms play an important role in bacterial pathogenesis and drug resistance, biofilm inhibitors will help combat infectious diseases[6].

Bacterial motility can be essential for their colonization and in the subsequent formation of biofilms. Loss of flagellation renders the bacteria less motile or non- motile, which may impair its ability to colonize a surface. Swarming motility is also associated with the expression of virulent genes, the ability to invade human cells and increased resistance to antibiotics. Thus, swarming motility plays an important role in the initiation of catheter-associated urinary tract infection and in the subsequent spread of a biofilm over a catheter surface. For these reasons, the inhibition of *P. mirabilis* swarming is of medical importance[7]. The majority of bacterial pathogens exploit specific adhesion to host cells as their main virulence factor. "A large number of bacterial adhesions with individual receptor specificities have been identified[8]. Many bacterial pathogens are able to express an array of different adhesions. Expression of these adhesions at different phases during infection play the most important role in adhesion based virulence[8].

Antimicrobial agents are anything which works against the life of the microorganism [39]. Medicinal plants extract have been used by human beings since ages due to their therapeutic potential values and has become an important component of health care system. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world.[40]

"The immune responses and Inflammatory are evoked as a host defense against various environmental stimuli. These include external stresses such as infection by pathogens, inhalation of foreign materials like asbestos, exposure to heavy metals, ultraviolet and ionizing radiation as well as internal stresses such as excessive accumulation of metabolites like urate crystals and oxidized lipids, superoxide and nitric oxide, autoimmune responses and cancer" [41 42].

Numerous studies have shown that inhibiting a single adhesion in this coordinated effort can often be enough to make a pathogenic bacterium non-virulent. This has led to the exploration of adhesion activity interruption as a method of bacterial infection treatment. Black raisins contain a lot of antimicrobial compounds; therefore the current study aims to evaluate antimicrobial activity of black raisins and vinegar of black raisins against different pathogens.

Material and Methods: -

Preparation of aquatic extracts:

The dried fruit of black raisins were obtained from the local market in Hila city, Iraq. Preparation of aquatic extracts of black raisins [Aqueous extract was soaked 50 gram of Fruit of black raisins in100 ml distillated water and mixed, then filtration the mixture and the juice sterile by filtration[38].

This extract was considered as the 50% concentration. The extracts used for screening of antimicrobial activity [9].

Preparation black raisins vinegar:

Vinegar was prepared according to the ordinary old classical method for preparing vinegar. The dried fruit was mixed with appropriate quantity of water. It is mother that is taking in oxygen and converting to the wine alcohol then to the vinegar acid. The mixture was left to ferment 40 days. The longer it fermented the more acidic and strong it will become until all the alcohol was consumed. The vinegar was filtered through several layers of cheesecloth into a funnel, and then the end product was good vinegar (due acidity). The vinegar was saved in dry non-reactive container.

Bacterial Isolates

Different fifteen clinical microbial isolates (Gram positive, Gram negative (listed at Table-1) were isolated and identified by using conventional biochemical tests and Api system (Biomeraux, France)[10]. and cultivated in pure culture, at microbiological laboratory/college of Medicine/ Babylon University, Iraq.

In vitro antibacterial activity testing agar well diffusion assay[11]

Loop full growths (0.1ml) from bacterial isolates were inoculated into nutrient broth incubated at 37 ℃ for 18 hours. The bacterial suspensions were diluted with normal saline (standard methods). The turbidity was adjusted and compared with standard tube (McFarland number 0.5) to yield a uniform suspension containing 1.5×10^8 CFU / ml. Cotton swabs were dipped and streaked into adjustment suspension. The entire Mueller-Hinton agar (for all tested bacteria) surface of plates and the plates were left for a set of 5 -15 minutes at room temperature to dry. Media were cut into four wells (5mm diameter) by cork borer and 20µ of the cardamom extracts solutions and oil of cardamom were added (The plates were performed in triplicates). All plates of the tested organisms were then allowed to incubate at 37°C overnight. After 24 h of incubation, each extract was noted for zone of inhibition for all isolates. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm).

Antibacterial activity assay

The antibacterial activity was determined by agar disc diffusion [10]. Agar plates were inoculated with 0.1 ml broth culture of tested organisms and spread with a sterile L-shaped rod glass spreader. Antibiotic disks of ciprofloxacin were added to the center of agar plate. (The plates were performed in triplicates). All plate of the tested organisms were then allowed to incubate at 37°C for overnight. After 24 h of incubation, each extract was noted for zone of inhibition for all isolates. The diameters of the zone of inhibition were measured by measuring scale in millimeters (mm).

Test microorganisms: -

Eleven-gram positive and gram-negative bacteria were used in this study (Table-1) to determine the antimicrobial activity of black raisins. All bacterial strains were maintained on freshly prepared blood agar. Isolates were identified by species based on the standard biochemical and microbiological methods[10].

Table1: Bacterial isolates used in this study

Gram negative bacteria	Gram positive bacteria
Psudomonas aeroginosa	Staphylococcus aureus
Salmonella typhi	Staphylococcus epidermidis
Proteus mirabilis	Streptococcus pneumoniae
Klebsiella pneumoniae	
Enterobacter spp.	
Acinetobacter	
E. coli	
Serratia spp	

Biofilm Formation Assay

Tissue culture plate method (TCP) assay (also called semi quantitative microtiter plate test (biofilm assay) was widely used and was considered a standard test for detection of biofilm formation[12]. Isolates from fresh agar plates were inoculated in TSB containing 1% glucose and incubated for 18 hours at 37C° and then diluted to a 1:100 ratio with fresh TSB. Individual wells of sterile, polystyrene, 96 well-flat bottom tissue culture plate's wells were filled with 150µl aliquots of the diluted cultures. The broth served as the control to check non-specific binding of media. Each isolate was inoculated in triplicate. The tissue culture plates were incubated for 24 hours at 37°C. After incubation, the contents of each well was gently removed by tapping the plates. The wells were washed four times with phosphate buffer saline (PBS pH 7.2) to remove free-floating 'planktonic' bacteria. Biofilms formed by adherent 'sessile' organisms and plant extracts in plate were fixed by placing in oven at 37C° for 30min. All wells stained with crystal violet (0.1% w/v). Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying. 150µl of acetone/ethanol (20:80, v/v) mixture were added to dissolve bounded crystal violet. The optical density (O.D.) was recorded at 630 nm and the results were interpreted according to the table -2. This method was repeated and modified by adding garlic extracts in stage 2 to inhibit the biofilm formation by black raisins extracts.

Bacterial Adhesion Assay

The ability of bacteria to adhere to oral epithelial cells is one of important virulence of bacterial properties. A bacterial adhesion assay was performed by preparing the bacterial broth and incubating it for 72 hrs. Dilution of bacterial broth was prepared using phosphate buffer saline (PBS). The solution was adjusted to a bacterial concentration 10^{8} (CFU/ cm3). Oral epithelial cells were collected by swabbing the epithelial layers of oral cavity using cotton swabs. They were then transferred directly into sterile tubes contain PBS (PH 7). The epithelial cells were then washed using PBS and centrifuged (5000 rpm for 10 minute) three times. Epithelial cells were filtrated using filter paper and placed on cover slide by pressing the cover on surface of filter paper then lifted to be dry. The cover slides were placed on sterile glass plate. 5ml of previously prepared bacterial broth and extracts were added. The plates containing the epithelial cells and bacterial broth were incubated for 1hr at 37c. The un-adherent bacteria was removed by washing the cover slides with PBS. The epithelial cells were fixed by ethanol for 15 minutes, stained with giemsa stain (30%) for 20 minutes and then washed by DW and lifted to air dry. The cover slides were placed on glass slides in an inverted position, and then tested under light microscope [13, 14]. This method was repeated and modified by adding the garlic extracts in stage 3 to inhibit the biofilm formation by black raisins extracts. The results were interpreted as shown in table-3.

Table 2: Classification of bacterial adherence by TCP method

Mean OD values	Adherence	Biofilm formation
<0.120	Non	None / Weak
0.120 – 0.240	Moderately	Moderate
>0.240	Strong	High

Table 3: Classification of bacterial adherence and biofilm formation by TCP method [15]**.**

Mean of OD value at 630nm	Adherence	Biofilm formation
<0.120	Non	Non
0.120-0.240	Moderately	Moderate
>0.240	Strong	High

Swarming inhibition by plant extract:

The method of Iwalokun, 2004 [16], which contain the following steps was used: Bacterial isolates were cultured on nutrient agar incubated at 37^{o}C for 24 hrs as a control. Plant extract was added separately in concentration of (1%, 2%, 3%) respectively and incubated at 37^{o}C for 24 hrs. Few drops of 90% ethanol was added to petri dishes, covered and cultured with bacterial isolate and incubated at 37 ^{o}C for 24 hrs. Anti swarming activity of plant extract was determined based on the swarming diameter.

Results

The screening of antimicrobial activity of aqueous extracts of dry black raisins and vinegar of black raisins against Gram positive, gram negative bacteria and yeast was carried out using the well diffusion agar test and the results were shown in Table-4 and Figure-1(A and B).

Table-4: Antimicrobial activity of aqueous extract of black raisins and vinegar of black raisins against Gram positive, gram negative bacteria and yeast

Microbial Isolates	Mean of Inhibition Zone (mm) Aqueous Extract of Black Raisins ± (SD/ SEM)*	Mean of Inhibition Zone (mm) by Vinegar of Black Raisins
Staphylococcus aureus	18 ± (1.15/0.66)	19.67±(1.52/0.88)
Staphylococcus epidermidis	10.67±(1.155/0,66)	20.33±(0.57/0.33)
Streptococcus pneumonia	16.33±(1.52/0.88)	20.33±(0.57/0.33)
Pseudomonas aeroginosa	25.33±(0.577/0.33)	17.33±(6.42/3.71)
Proteus mirabilis	12.33±(0.577/0.33)	30.33±(0.57/0.33)
Acinetobacter	26.33±(0.577/0.33)	20.67±(0.57/0.33)
Klebsiella pneumonia	21±(1/0.57)	19.70±(1.57/0.90)
Enterobacter	15±(0/0)	40.33±(0.57/0.33)
Escherichia coli	23.33±(0.57/0.33)	20±(0/0)
Serratia spp	15±(1/0.57)	12±(2/1.15)
Salmonella typhi	15±(1/0.57)	30.33±(2.51/1.45)
Candida albican	12.3315±(1/0.57)(0.57/0.33)	30.67±(1.55/0.66)

***SD / SEM: Std. Deviation / Std. Error of Mean**

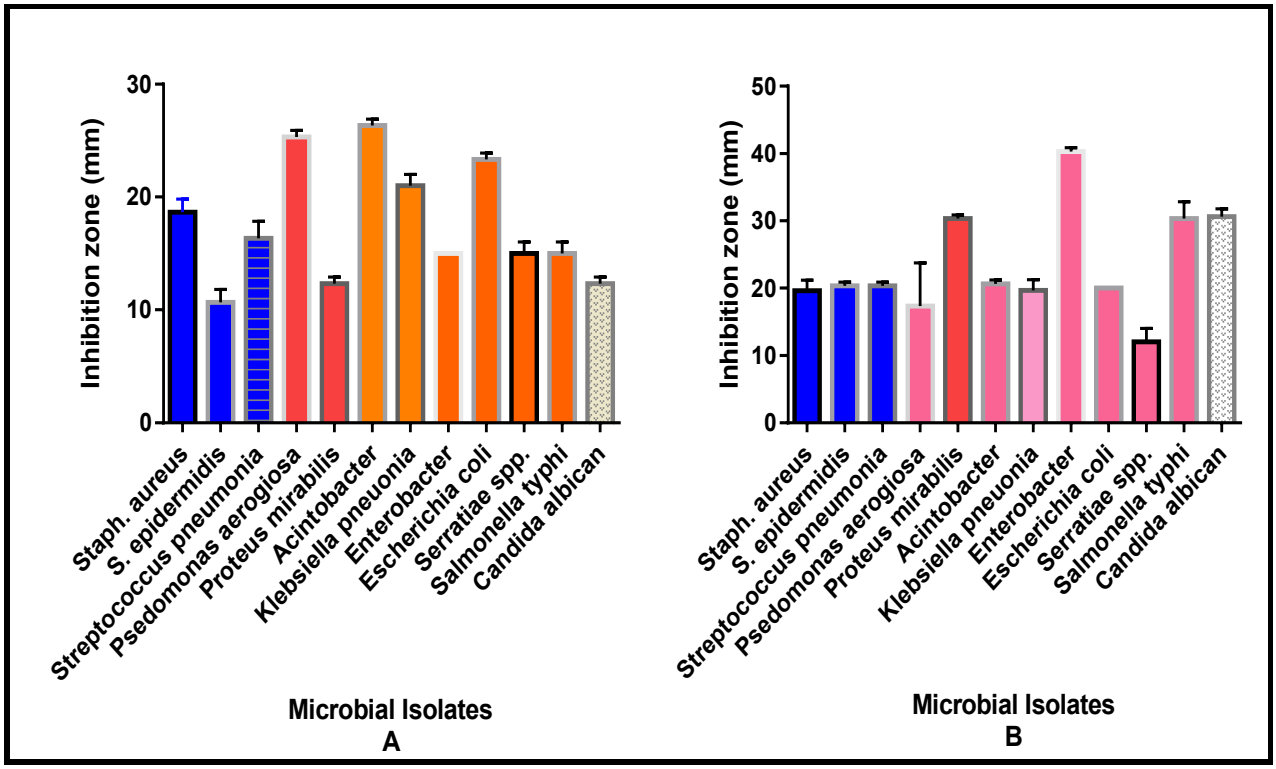


Figure (1): Antimicrobial activity of aquatic extract of black raisins (A) and vinegar of black raisins (B) against Gram positive, gram negative bacteria and yeast.

Anti-biofilm and anti-adherence activity of aqueous extract and vinegar of black raisins against Gram negative bacteria and the results were shown in table-4.The results showed that both aquatic extract and vinegar of black raisins have moderate to high inhibition for biofilm formation and or bacterial adherence (table-5).

Table 5: Anti biofilm and anti-adherence activity of aqueous extract and vinegar of black raisins against Gram negative bacteria.

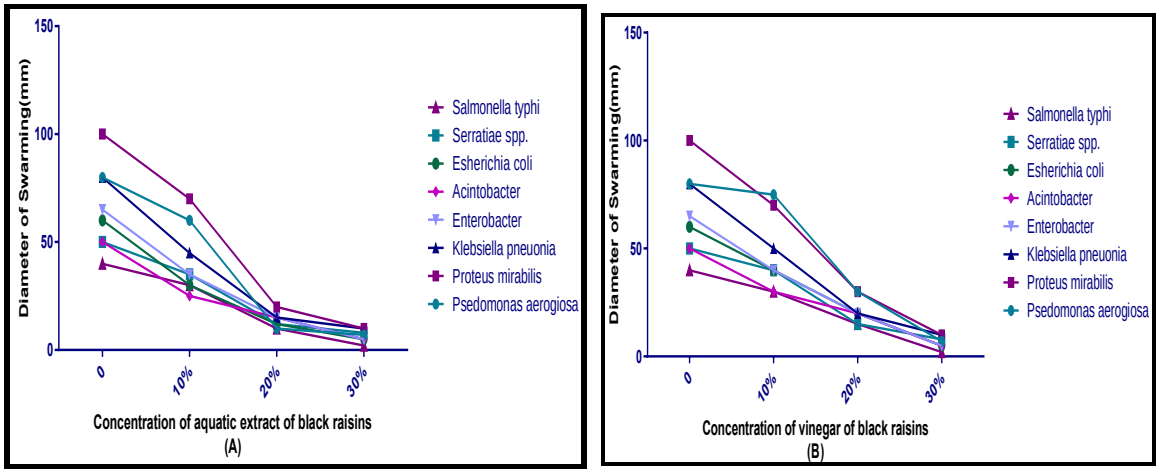
Bacteria	Biofilm formation		Adherence	
	aquatic extract	vinegar	aquatic extract	vinegar
Salmonella typhi	*Moderately	High	Moderately	Moderately
Pseudomonas aeruginosa	Moderately	High	Moderately	Moderately
Proteus mirabilis	Moderately	High	Moderately	High
Klebsiella pneumonia	**High	High	High	High
Enterobacter spp.	Moderately	High	Moderately	High
Acinetobacter	Moderately	Moderately	Moderately	High
E. coli	High	Moderately	High	Moderately
Serratia spp	High	Moderately	High	Moderately

*Moderately (0.120-0.240)**High (>0.240)

The result showed that there is a reversible correlation between both aquatic extract of black raisins and vinegar of black raisins against Gram negative bacteria with the concentrations used in this study. (r=0.9), table -6. (Figure 2 and Figure 3)

Table 6: Anti-swarming activity of aquatic extract of black raisins and vinegar of black raisins against Gram negative bacteria

Gram Negative Bacterial isolates	**Swarming Diameter (mm) using different concentrations of aquatic extract of black raisins or vinegar of black raisins.**						
	0	**10%**		**20%**		**30%**	
	aquatic extract of black raisins or vinegar of black raisins	**aquatic extract of black raisins**	**vinegar of black raisins**	**aquatic extract of black raisins**	**vinegar of black raisins**	**aquatic extract of black raisins**	**vinegar of black raisins**
Pseudomonas aeroginosa	80	60	75	10	30	7	7
Proteus mirabilis	100	70	70	20	30	10	10
Klebsiella pneumoniae	0	0	0	0	0	0	0
Enterobacter	0	0	0	0	0	0	0
Acinetobacter	50	25	30	15	20	5	5
Escherichia coli	60	30	40	12	20	5	5
Serratia sp	50	35	40	12	15	8	8
Salmonella typhi	40	30	30	10	15	2	2



Pearson correlation =0.9; P value ≤ 0.05

Figure-2: Anti swarming activity of aqueous extract of black raisins (A) and vinegar of black raisins (B) against Gram negative bacteria.

Discussion

The results of the present work show that the aqueous extracts of black raisins and the vinegar of black raisins inhibit wide range of gram positive and gram negative bacteria (Figure-1 and Figure-2). There are potential antimicrobial compounds identified in raisins which can inhibit tooth decay bacteria which include oleanic acid, oleanic aldehyde/ Triterpenoids inhibit *Streptococcus mutans* and *Porphyromonas gingivalis* [5], Several triterpenoid compounds isolated from a methanol extract of Thompson seedless raisins were found to inhibit bacteria associated with dental caries and periodontal disease [5]. These compounds are found on the skin of the grape and the raisin surface.

The active component of black raisins; Catechin/ Flavonoids, Gallic acid/ Phenolic acid, Protocatechuic acid can inhibit *E. coli* [18]. Flavonoids inhibit *Bacillus*, *Shigella*, *Salmonella*, *Vibrio*, and *E. coli* species [19]. Ferulic acid/ Phenolic acid inhibit *E. coli* and *Salmonella spp.*[20]. Protocatechuic acid inhibits *E. coli* spp.[21].

A second broad class of antimicrobial compounds found in raisins includes the Maillard non- enzymatic browning reaction products (MRP). Their concentrations increase as drying occurs and they are directly responsible for the color of naturally dried and processed raisins [22]. Some of these compounds were also identified as having antimicrobial activity [20, 21].

Another factor in the antimicrobial activity of these compounds is their action against different classes of bacteria. Flavonoids and triterpenoids have been reported to be antimicrobial against both Gram-positive (Listeria) and Gram-negative (*E. coli and Salmonella*) species. The phenolic acids identified in raisins were observed to be antimicrobial agent against only Gram-negative species (Table 4). This is in agreement with published reports that conclude that the mode of action of phenolic acids against Gram-negative bacteria occurs by destabilization of the outer membrane. Both Gram-positive and Gram-negative pathogens were inactivated when inoculated on intact raisins and raisin products. This suggests that bacteria are exposed to several antimicrobial compounds on and within raisins [22].

Results of this study show that the black raisins and their vinegar decrease the adherent growth of bacterial isolates on glass plate. There was previous observation of the effect of phenolic at decreasing of adherent growth by Klemm and Schembri (2002)[23]. Microbial adhesion is considered the first step in the sequence of events leading to colonization. The ability to adhere is weakened by exposure to sub lethal doses of antibacterial agents [24]. Some studies referred to the effect of polyphenol compounds on the enzymatic activity of glucosyltransferase, which is the essential virulence factor that allows the colonization of bacteria and adherence [24]. Both the black raisins and the vinegar of black raisins exhibited reduction in biofilm.

Result showed that the epithelial cell adherence inhibition for all selected bacterial isolates are high, (table-4). There are specific attachment mechanisms of bacteria to the host cell or tissue surface. E. *coli* has Type-I fimbriae, P-pili (pap) for adherence to intestinal epithelium, urethral epithelium and upper urinary tract. *Pseudomonas*, *Vibrio* and *Neisseria* possess Type IV pili that contain a protein subunit with a methylated amino acid, often phenylalanine, at or near its amino terminus. These "N-methylphenylalaninepili" have been established as virulence determinants in pathogenesis of *Pseudomonas aeruginosa* in lung infections of cystic fibrosis patients. These type of fimbriae and receptor in *Neisseria gonorrhoeae* is thought to be an oligosaccharide. Type IV pili are the tcp (toxin coregulatedpili) fimbriae used in attachment of *Vibrio cholerae* to the gastrointestinal epithelium [25, 26]. The treatment of bacteria with aquatic extract of black raisins and vinegar of black raisins show significant inhibition of bacterial adherence to the epithelial cells so one can conclude that aquatic extract of black raisins and vinegar of black raisins can prevent pathogenic *E coli* from causing diarrhea, urethritis and pyelonephritis.

The antibacterial activity of vinegar was also evaluated by swarming assay. The result shows that both black raisin products shows high anti-swarming activity except *K. pneumonia* and *Enterobacter* when different concentrations are used. *Proteus mirabilis* has the ability to promote the infection during swarming because the highly motile swarming cells could migrate through the urinary tract and cause many infections [27].The swarming phenomena has been studied in different genera *Serratia spp*., *Salmonella spp*., *Pseudomonas* spp., *E. coli* and *P. mirabilis, Klebsiella pneumonia, Enterobacter, Acintobacter*. There the present work can strongly confirm the ability of black raisins to inhibit all UTI isolated pathogens[28].

Swarm cell differentiation and swarming behavior are the results of complex sensory transduction and global control mechanisms. *Proteus mirabilis* swarming requires the sensing and integration of a variety of

environmental, cell-to-cell, and intracellular signals and involves regulated expression of gene networks leading to morphological and physiological changes[30]. The signals regulating swarming and the pathways for signal transduction are still poorly understood. In this paper, we present evidence that compounds in black raisin and black raisin vinegar serve as environmental cues to stop *P. mirabilis* and other gram negative bacteria from swarming.

The result shows the effects of crude extract of black raisins and the vinegar of black raisins on the growth and adherence of pathogenic bacteria onto human buccal epithelial cells was investigated. Black raisins and the vinegar of black raisins completely inhibited the growth and adherence of pathogenic bacteria onto the buccal epithelial cells. A lot of studies have shown that the activity of the compounds within the black raisins such as triterpenes, betulin, oleanolic and betulinic acids have anti-cavity and anti-gum disease properties [31]. Pathogenic biofilms have been associated with persistent infections due to their high resistance to antimicrobial agents, while commensal biofilms often fortify the host's immune system. Hence, controlling biofilm formation of both pathogenic bacteria and commensal bacteria is important in bacterium-related diseases.

Recently, several other flavonoids showed the ability to inhibit the biofilm formation of *Streptococcus mutans* [32], *Aeromonas hydrophila* [33], and *Escherichia coli* O157:H7 [34]. Additionally, a transcriptome analysis demonstrated that apple polyphenols, including phloretin, possessed anti-inflammatory effects against inflammatory bowel diseases (IBDs) in vitro[35].

Other studies demonstrated for the first time that phloretin, a natural flavonoid, is a nontoxic inhibitor of enterohemorrhagic *E. coli* O157:H7 biofilms but does not harm commensal *E. coli* K-12 biofilms. Also, most importantly, our results confirmed that phloretin shows anti-inflammatory properties in both the *in vitro* and *in vivo* inflammatory colitis models. The effect of phloretin was noticeably more pronounced than that of the conventional IBD drug 5-ASA. Taken together, our results show that the triple biological activity of phloretin that may make it useful as a lead compound for biofilm inhibitors of *E. coli* O157:H7 as well as an antioxidant compound and may have beneficial effects on IBD[36].

Most of the test microorganisms are food born pathogen; therefore, black raisins and its vinegar are a hurdle component in certain processed foods and exert a protective effect against foodborne pathogens when contaminated foods are ingested.

On the basis of the experimental results and discussion, it can be postulated that the aqueous of black raisins and vinegar of black raisins possesses the potent antimicrobial properties, inhibit biofilm formation, inhibit adherence and inhibit swarming. These extracts are active against Gram positive and Gram negative bacteria and they can inhibit the groups of bacteria which cause dental carries, diarrhea and urinary tract infection. With this in mind, the consumption of black raisins is suggested because juice may inhibit adhesion of many bacteria and can prevent diseases caused by bacteria. This research may open the door for further exploration of orally administered vaccines which exploit bacterial adhesins.

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