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Evaluation of Antibacterial Activities of Aqueous and Methanolic Extracts of *Areca catechu* against Some Opportunistic Oral Bacteria

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The aqueous and methanolic extracts of *Areca catechu* seeds (known as areca nut: a component of a betel quid, where their chewing habitis a popular cultural tradition in some South East Asian countries used as an oral cleanser) have shown some antimicrobial potentials against some oral bacteria which are capable of causing opportunistic infections in the mouth. The objective **of this study is to detect the antibacterial activities of aqueous and methanolic extracts of *Areca catechu* against *Escherichia coli* and *Staphylococcus aureus*.Manystudies showed different antibacterial activity, depending on the *Areca catechu* extracts method, concentration, and the bacterium under test. The aqueous and methanolic extracts were added to growth medium of *Escherichia coli* or *Staphylococcus aureus*. The antibacterial activities of the attained fractions were assessed using agar-well diffusion test. In this study we have observed that the Methanolic extract inhibited the growth of *Escherichia coli* or *Staphylococcus aureus*, while aqueous extract was ineffective.** In conclusion **the methanolic extract of the *Areca catechu* seeds "areca nut" can be a potential source for developing antibacterial agent against *E. coli* and *S. aureus* which are commonly found on human oral cavity and responsible for some oral infections.**

Keywords: *Areca catechu,* Areca nut, *Staphylococcus aureus, Escherichia coli*, MIC, MBC.

Many plants and plants products have been used as medicines since the begging of the human civilization, they are very common as a cultural tradition especially in some countries in South East Asia. These plants are known to contain large selections of chemical substances with therapeutic properties that can be used in the treatment of some human diseases. (Amudhan *et al*., 2012; Hossain *et al*., 2017). Many studies have mentioned the multifaceted highlights of Areca ctachu as antibacterial activity against some oral bacteria (Chatragadda *et al*., 2017)(Bobby Cyriac *et al*., 2012) also as antioxidant and anti-inflammatory, hepatoprotective (Pithayanukul *et al*., 2009), it also found to show a significant antimicrobial activities (Lalitha *et al*., 2010). It has been reported by (IARC, 2004) that areca nuts are the fourth most commonly used psychoactive substance in the world (Hernandez *et al*., 2017). In some areas of Saudi Arabia, women used the *Areca catechu* plant seed (Areca nut) as a mouth fragrant (says, 2017).

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In the present study the antibacterial activities of aqueous and methanolic extracts of *Areca catechu* against *Escherichia coli* and *Staphylococcus aureus* were tested.

Areca catechu

Areca catechu palm is a stem tall slender, single-trunked palm that has the ability to grow up to 30 meters, the common practice of the plant is by the use of the husk of Areca nut as herbal "chewing sticks" instead of tooth brushes to maintain oral health and hygiene, the seeds are well known for masticatory seed and are used in medicine as well (Jose *et al*., 2011; Joseph and Singh, 2008). The Areca nut is the seed of the Areca catechu that grows mostly in the tropical Pacific, Asia and parts of east Africa. The habit of chewing the Areca nut for its stimulating properties has been used in about one tenth of the world's population making it one of the most consumed psycho active substance (Lingappa *et al*., 2011). Dried form of the Areca nut was claimed to strengthen gums, sweeten the breath, eliminate bad taste and act as dentifrices (Shwetha HR *et al*., 2017). It has been reported that the main components of Areca are polyphenols, fat polysaccharides and protein. Also the nuts contain alkaloids arecoline, tannic acid which was suggested that it can suppress bacteria in the mouth (Al-Bayati, 2016). It has been reported that Areca nut exerts a direct antimicrobial effect against oral bacteria including *Streptococcus mutans*, *Streptococcus salivarius*, *Candida Albicans* and *Fusiform nucleatum* (Bobby Cyriac *et al*., 2012; Shwetha HR *et al*., 2017)

Presence of Staphylococcus aureus and Escherichia coli in the oral cavity

The oral cavity is considered as a home to microbial communities with important implications for human health and disease, it contains more than 300 known species of bacteria(Bik *et al*., 2010; Wilson Melanie J *et al*., 1997). Some oral infections are caused by at least in part by *Staphylococcus aureus* e.g., angular cheilitis, periodontitis, and staphylococcus mucositis(G McCormack *et al*., 2015; Smith *et al*., 2003; SMITH *et al*., 2001). Furthermore, the presence of *E. coli* is well documented to be located in the intestines, although a small amount of the bacteria is found in the mouth(Kenneth Todar, 2012; Ryan Mac, 2017).

Antibacterial activity of Areca catechu

Many researches have reviewed the antimicrobial activity of the Areca nut, as in aqueous/methanolic or as in hot/cold extracts of the leaves, seeds and green husks from different regions of the world, and they showed a broad spectrum antimicrobial activity against Gram positive and Gram –negative bacteria (Bhat

Table 1. Antibacterial activity of aqueous extract of *Areca catechu* seeds by agar-diffusion method at different concentrations

Bacteria	Concentrations (mg)			
	0.5	1	2	4
Staphylococcus aureus	0.0* ±0. 0	0.0±0.0	0.0±0. 0	0.0±0.0
Escherichia coli	0.0±0.0	0.0±0.0	0.0±0.00	0.0±0.0

* Inhibition zones in mm (Means of triplicate)

Table 2. Antibacterial activity of methanolic extract of Areca catechu seeds by agar-diffusion method at different concentrations

Bacteria	Concentrations (mg)			
	0.5	1	2	4
Staphylococcus aureus	2* ±0.0	3±0. 5	3.5±0.25	5±0.5
Escherichia coli	0.0±0.0	0.0±0.0	1.5±0.25	3±0.25

* Inhibition zones in mm (Means of triplicate)

Sarpangala *et al*., 2017; Negi and Dave, 2010; Pahadia *et al*., 2013; Rahman *et al*., 2014; Reena R Nelson Anthikat, 2009)

MATERIALS AND METHODS

Bacterial Isolates

The bacterial isolates of *Escherichia coli* and *Staphylococcus aureus* were obtained from King Khalid UniversityHospital (KKUH), King Saud University. Riyadh, Saudi Arabia.

Plant material and preparation of extracts (aqueous and methanolic)

Fifty grams of *Areca catechu* seeds were cut into small pieces and soaked in sterilized distilled water 1:3 (w/v) for 24 hours. Then, the mixture was homogenized in a house hold blender for one minute at full speed. The homogenate was then filtered through double layer of cheese cloth and centrifuged at 3000xg for 10 minutes at 4°C. The supernatant was then filtered through sterile filter (0.22 μm) and kept at -20°C for later use. The supernatant is referred to as aqueous extract. The same protocol was used for methanolic extraction but the sample was soaked in 95% methanol. After centrifugation, methanol was evaporated using a steam of air at room temperature. The sample was then re-suspended to its original volume with distilled water. This was filtered through sterile filter (0.22 μm) and kept at -20°C for later use. The supernatant is referred to as methanolic extract (Janakat *et al*., 2004)

Agar well-diffusion test

The antibacterial activity was tested by agar well diffusion method. *Escherichia coli* or/ and *Staphylococcus aureus* were spread onto the surface of LB agar with sterile swab (0.1 ml containg10^6 cell/ml). Six mm diameter wells were punched into the agar and filled with 0.1ml of the aqueous extract or protein fractions that showed antibacterial activity. Then the plates were incubated overnight at 37°C. All experiments were carried out in triplicates. After 24 hours of incubation inhibition zones were measured. Control wells were filled with sterilized distilled water (Perez C *et al*., 1990).

Determination of minimal inhibitory concentration (MIC) minimal bactericidal concentration (MBC)

The MIC is defined as the lowest concentration (mg/ml) of the extract resulting in clear broth media, while minimum bactericidal concentration (MBC) is defined as the lowest concentration (mg/ml) of the extract resulting in no growth of bacteria after culture on agar media. In this study the (MIC) and (MBC) were determined using broth dilution method (Viksø Nielsen *et al*., 1997).

The broth media dilution method was used to determine the MIC of the extract of *Areca catechu* seeds; the final concentration of the extract was 0.5–40 mg/ml in brain heart infusion broth; all of different concentrations were inoculated with 100 µl of strains and incubated at 37° C for 24 hour. All tests were performed in triplicate.

The bacteria used in this study were tested for their sensitivity to aqueous and methanolic extracts of *Areca catechu* seeds.

RESULTS AND DISCUSSION

In this study, the antibacterial properties of the Areca nut extracts were firstly determined by agar-diffusion method and was screened for its

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous extract of *Areca catechu* seeds

Bacteria	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus aureus	25	33
Escherichia coli	17	39

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanolic extract of *Areca catechu* seeds

Bacteria	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus aureus	5	17
Escherichia coli	7	21

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration

antimicrobial properties against *Escherichia coli* and *Staphylococcus* aureus. Tables 1and 2 show the possibility forming of the diameter of the inhibition zones resulting from exposure of the bacteria to the aqueous and methanolic extracts of Areca nut. The inhibition of bacteria in this study was directly related to the concentration of extracts, where inhibition zones increasing with increasing extract concentration (0.5-4mg) (Bobby Cyriac *et al*., 2012; Joseph and Singh, 2008; Pahadia *et al*., 2013; Rahman *et al*., 2014; Reena R Nelson Anthikat, 2009). *Staphylococcus aureus* was the most readily inhibited bacterium(Joseph and Singh, 2008; Rahman *et al*., 2014) followed by *Escherichia coli* (Bik *et al*., 2010) unlike the results of *E. coli* showed a complete resistance to the extract.

The methanolic extracts in this study were hardly to inhibit the bacteria. The results are similar to those presented by (Bobby Cyriac *et al*., 2012), while the inhibition of bacteria growth reported here for *Areca catechu* extracts are greater than those reported by (Chatragadda *et al*., 2017) using *Staphylococcus aureus* and *Escherichia coli.*

The MICs for the aqueous and methanolic extracts of Areca nut varied with the bacteria used (Table 3-4). The lowest observed MICs were for both *Escherichia coli* and *Staphylococcus aureus* (1mg/ml) in case of aqueous extract while *Escherichia coli* and *Staphylococcus aureus* showed MICs of 6 and 5 mg/ml respectively in case of methanolic extract, which is much higher than 0.1 mg/ml as in (Chatragadda *et al*., 2017).

The minimum bactericidal concentration (MBC) of methanol extracts of *Escherichia coli* was 36 mg/ml compared with values of 29 mg/ml for the other bacteria.

Conclusion and Recommendations

This study concluded thatthe methanolic extract of the Areca nut contains a potent antibacterial agent that is protein in nature and may be used in the treatment of some oral infections.

Although that this present study supports the use of natural products for medication as antibacterial agents found in plant extract, which exhibits antibacterial activity to some opportunistic oral bacteria, we have some reservations towards the side effects that needs to be studied and evaluated more thoroughly. The results highlight the traditional use of "Areca nut" and some scientific validation of the claimed biological antimicrobial activity *in vivo*.

Further clinical trials are required to determine the efficacy of *Areca catechu* seeds extract on *Escherichia coli* or/and *Staphylococcus aureus*.

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