RESEARCH ARTICLE

Study of the Antibacterial Activity of Elettaria Cardamomum Extracts on the Growth of Some Gingivitis Inducing Bacteria in Culture Media

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ABSTRACT

The our work was carried out with an objective to appraisal the antibacterial action of cardamom extracted by four different solvents and prepared in a number of concentrations (50, 100 and 200 mg/mL) toward three different pathogenic bacteria responsible mainly for induce gingivitis infection and comparison their action with the action of antibiotics (Ciprofloxacin (5µg), Ampicillin (30µg) in culture media.

The results of phytochemical profiles for different extracts of cardamom showed the presence of alkaloids, glycosides, tannins, and terpenes in all used extracts while flavonoids were present in all extracts except watery extract. Saponins were present only in the ethanolic extract, while phenols were found to be present only in ethanolic and chloroformic extracts.

The antibacterial screening of the different extracts of cardamom and standard antibiotics showed various degrees of zones of inhibition in the culture media depending largely upon the type of plant extract, the concentration of extract in addition to the type of tested bacterial. Almost all the cardamom extracts were found to have significant activity ($p < 0.05$) against all tested bacteria com the pared with a negative control. The highest antibacterial potential was observed for the ethanolic cardamom extract, whereas other cardamom extracts showed closed results in general. At the same time, the current study was recorded that inhibition zones diameter against tested bacteria raised significantly ($p < 0.05$) as the extract concentration raised.

The MIC values of watery, ethanolic, chloroformic, and acetonic extracts of cardamom ranged from 0.624 to 1.248 mg/mL, 0.078 to 0.312mg/mL, 0.624 to 1.248 and 0.312 to 0.624 mg/mL against tested bacteria respectively. While the MBC values ranged from 1.248 to 10 mg/mL, 0.624 to 2.5 mg/mL, 2.5mg/mL to 5 mg/mL and 2.5mg/mL respectively.

Keywords: Antibacterial Activity, Cardamom, Culture Media, Gingivitis.

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INTRODUCTION

Gingivitis is one of the most common complaints in dental practice, and about more than 90% of the population suffers from periodontal disease[1] Recent studies have shown, the prevalence of gingivitis in adults was recorded to exceed 75% and even to approach 100% in some populations[2] gingivitis can be defined as "inflammation process of the gingival tissue without loss of tooth attachment (i.e., periodontal ligament)". Gingivitis is an irritation of the gums. It usually results from exposure to the bacterial plaque that aggregation in the small gaps between the gums and the teeth. These aggregations perhaps tiny and may be microscopic, but the bacteria can produce detrimental chemicals and toxins that induce inflammation of the gums around the teeth. This inflammation process can, over the years, produce deep pockets between the teeth and gums and lead to loss of bone around teeth, an effect otherwise called as periodontitis.[3]

The symptoms of gingivitis represent by a present of swollen gums, mouth sores, bright-red, or purple gums, shiny gums, swollen gums that expel pus, oral odor, gums that become sensible, or maybe agonizing to the touch, gums also become bleed facilely, until with amiable brushing especially when cleaning, gum pockets.[4]

Bacterial biofilms have been appeared to be the primary causative factor at the beginning of gingival inflammation then the destruction of periodontal tissues[5] Evidence suggests that the aggregation of bacterial plaque on a tooth is a direct cause of gingivitis.[6]

Because of a growing failure of chemotherapeutic agents and antibiotics resistance elicited by pathogenic bacterial causative

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agents has to make to the researching of other medicinal plants for more antimicrobial activity, and the plant extracts were found to have potential against microorganisms.[7,8] Natural substances and their active principles, which are employed as a vital source for novel drug material discovery and treatment of pathological conditions, have attracted attention in the last years. Herbs and spices are generally considered safe and proved to be effective against various human and animal ailments.[9] So this fact demands from researchers to detection for new and active antimicrobial components, in particular from plant sources[10,11] Cardamom (*Elettaria* cardamomum *L.)* belongs to Zingiberaceae family, is a perennial herbaceous plant with subterranean rhizomes which produces several leafy shoots and panicles. India considers the largest producer of cardamom in the world with an annual production exceed to the 4000 MT, followed by Nebal 2500 MT and Bhutan 1000 MT[12] the potential therapeutic applications of cardamoms are extensive ranging and involving its use as a stomachic, diuretic agent, abortifacient, anti-microbial, anti-fungus, anti-viral and is benefit in the management of constipation, colic, diarrhea, dyspepsia, vomiting, headache, epilepsy as well as cardio-vascular disturbances.[13] From the previous evidence, the present study was aimed to evaluate the antibacterial activity of different cardamom extracts and concentrations on some gingivitis, causing pathogens in two different *in vitro* screening methods.

MATERIALS AND METHODS

Plant Materials

Seeds of cardamom (*Elettaria* cardamomum) were obtained from the Al-Diwanyia local markets, Then the studied plant was identified in our physiology and pharmacology department.

Preparation and Plant Extracts

The crude plant was cleaned carefully and air-dried at 25°C. The plant ground to a fine powder by using an electrical blender, then approximately one hundred grams of the prepared powder samples were processed with soxhlet apparatus at 60°C with water, ethanol, chloroform, and acetone each one alone and the extraction process lasted for 72 hours. After that, the extracts obtained were filtered through Whatman No4 filter paper and concentrated to dryness in rotary vacuum evaporator at 70° C until all solvent had been removed to produce pure extract material. The application of the following formula calculated the yield of extraction: yield of extraction = Mass of dry extract/ Mass of crude material × 100. Several concentrations used the recipient for each solvent extract (50, 100, 200 mg/mL).

Phytochemical Characteristics of Plant Extracts

The various extracts of cardamom were used for preliminary screening for detection of the presence of secondary metabolites by using standard procedures which include: alkaloids, flavonoids, saponins, glycosides, tannins, terpenes, phenols were carried out according to, WC Evans, *et al.* and E Baron, *et al.*[14,15]

Test microorganisms

The organisms of *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli were* obtained from patients who attended Specialized Dentistry Center in Al-Diweyia province. Identification of the above-mentioned bacteria was done based on cultural characteristics, microscopically examination, growth on selective media, and standard biochemical tests. After complete identification, all prepared bacteria were subculture on Nutrient agar slants and stored at 4°C until required for our study.[16]

Assay of Antibacterial Activity

The antibacterial action of all cardamom extracts was investigated by employed the agar well diffusion assay. Briefly, 15 mL of nutrient agar was seeded with 1.0 mL of standardized agar cultures of the tested bacteria (adjusted to 1.0 x 10^7 CFU/ ML) by decanted the broth cultures into sterile Petri dishes, mixing with nutrient agar, rotating pleasantly to assure the equal distribution of the bacteria after that permit to the media to ossify on a plane surface. Three equal wells on the agar media with 6 mm diameters were performed through the use of a sterile pasture pipette, and the cut agar discs were taken away by the use of sterilized forceps. Each hole was filled up with 100 µL from each concentration of plant extracts, a lonely in a petri dish. The plates were left to stand for at least one hour for pre-diffusion of the extract to occur then incubated at 37°C for 24 hours after incubation period, complete the plates were gathered and examined for growth inhibition zone. Besides, the antibacterial activity of the cardamom extracts compared with negative Control and Ciprofloxacin (5µg), Ampacillin (30µg). Each experiment was carried out in triplicate and the mean zone of inhibition diameter was recorded with caliper.[17]

Determination of MIC and MBC values

Minimum inhibitory concentration (MIC) of cardamom extracts against tested bacteria strains was determined by the broth dilution method using serially diluted (2-fold) plant extracts according to the NCCLS.[7] For this purpose, serial tubes were prepared with Mueller-Hinton broth to various concentrations lie between 6.25 to 0.781 mg/mL from each cardamom extract. Bacteria inocula were setting to contain approximately 10^5 CFU/mL. The test plates were incubated at 37°C for 18 hours. The MIC was represented as the lowest concentration of extract that inhibits the growth of the tested micro-organisms. MBC was determined by plating to count the contents of wells that showed no visible growth of bacteria on Mueller- Hinton plates and incubating at 28°C for 18 hours. The lowest concentration that prevented any single bacterial colony formation on a solid medium was represented as the MBC.[17]

Statistical analysis

SPSS software program (version 23.0) was used for statistical analysis of antibacterial results of different plant extracts. Data were recorded as mean ± standard error for means. The comparison among the groups was carried out by the use of one-way analysis of variance (ANOVA) followed by least significant differences (LSD) test with a p-value ≤ 0.05 was considered significant.[18]

RESULTS

The percentage yields of the different extracts of cardamom used in this study (watery, ethanolic, chloroformic, acetonic extracts) were 12, 31, 17, and 19% w/w respectively. The study of preliminary phytochemical analysis tests to different extracts of the seeds of cardamom are as shown in Table 1. The results revealed the presence of alkaloids, glycosides, tannins and terpenes in all used extracts, while flavonoids were present in all extracts except watery extract. Saponins were present only in the ethanolic extract, while phenols were found to be present only in ethanolic and chloroform extracts.

The antibacterial screening of different extracts prepared from seeds of cardamom plant compared with standard antibiotics as positive control were done by agar well diffusion method and serial microdilution against three commonly induced gingivitis bacteria. The different extracts and conventional antibiotics showed various degrees of zones of inhibition in the culture media depending mainly on the bacterial species, type of solvents used for the extraction process in addition to the concentration of extract.

Ethanolic extract of cardamom showed highly significant activity ($p < 0.05$) toward all tested bacteria compared with negative control and other extracts, and this depends on the concentration of extract as recorded by their inhibition zones (Table 2). *Staphylococcus aureus* gave inhibition zone of (15.33 ± 0.55) mm at 50 mg/mL, whereas *Streptococcus mutans* and *E.coli* gave inhibition zones of (11.3 ± 0.83, 10.18 ± 0.9) mm respectively. Arise at 200 mg/mL to the (31.38 ± 0.78, 24.32 ± 0.48, 19.32 ± 0.44) mm respectively (Figure 1 and 4).

Other extracts (watery, chloroformic and acetonic) gave closed results in general. *Staphylococcus aureus* showed

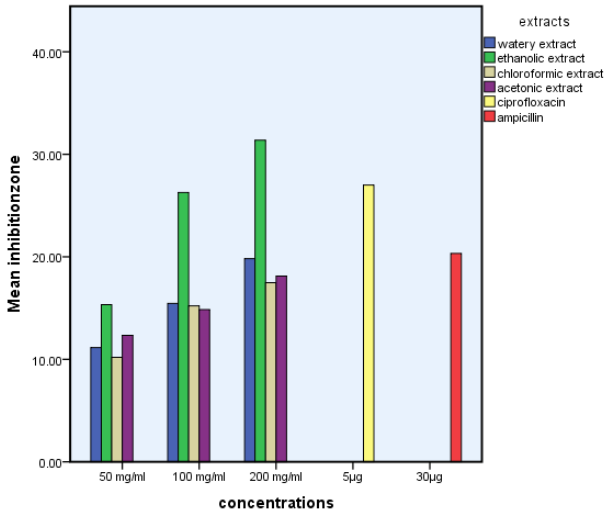

Figure 1: Antibacterial effects different extracts of cardamom compared with some antibiotics against *Staphylococcus aureus* growth in culture media

Table 1: Phytochemical screening of different extracts of cardamom

	phytoconstituents						
Type of extract	*alkaloids*	*flavonoids*	*saponins*	*glycosides*	*tannins*	*terpenes*	*phenols*
Watery extract	+	_	_	+	+	+	_
ethanolic extract	+	+	+	+	+	+	+
Chloroformic extract	+	+	_	+	+	+	+
acetonic extract	+	+	_	+	+	+	_

+ positive result _ negative result

Table 2: The antibacterial effects of different extracts of cardamom compared with some antibiotics against *Staphylococcus aureus* growth in culture media

Type of extract	*Concentration (mg/mL)*	*Zone of inhibition against bacteria organism (mm)*
Watery extract	50 mg/mL	11.14 ± 0.58^{a}
	100 mg/mL	15.46 ± 1.21^{b}
	200 mg/mL	19.82 ± 0.94^{c}
Ethanolic extract	50 mg/mL	15.33 ± 0.55^{b}
	100 mg/mL	26.28 ± 0.92^{d}
	200 mg/mL	31.38 ± 0.78^{e}
Chloroformic extract	50 mg/mL	10.19 ± 0.68^{f}
	100 mg/mL	15.22 ± 1.33^{b}
	200 mg/mL	17.48 ± 1.6^{g}
Acetonic extract	50 mg/mL	12.33 ± 0.9^{a}
	100 mg/mL	14.86 ± 0.67^{b}
	200 mg/mL	18.12 ± 1.08^{c}
Negative control	water	0 ± 0^{h}
	Diluted ethanol (10%)	7.12 ± 0.44^{i}
	Diluted chloroform (10%)	0 ± 0^{h}
	Diluted acetone (10%)	7.8 ± 0.92^{i}
Positive control (standard reference antibiotics)	Ciprofloxacin (5µg)	27 ± 0.78^{d}
	Ampicillin (30µg)	20.33 ± 0.91^{c}

Table 3: The antibacterial effects of different extracts of cardamom compared with some antibiotics against *Streptococcus mutans* growth in culture media

Type of extract	*Concentration (mg/mL)*	*Zone of inhibition against bacteria organism (mm)*
Watery extract	50 mg/mL	10.1 ± 0.12^{a}
	100 mg/mL	14.12 ± 0.28^{b}
	200 mg/mL	17.88 ± 0.74^{ce}
Ethanolic extract	50 mg/mL	11.3 ± 0.82^{a}
	100 mg/mL	18.11 ± 0.86^{c}
	200 mg/mL	24.32 ± 0.48^{d}
Chloroformic extract	50 mg/mL	11.12 ± 0.19^{a}
	100 mg/mL	16.22 ± 1.08^{e}
	200 mg/mL	20.1 ± 0.33^{f}
Acetonic extract	50 mg/mL	10.33 ± 0.66^{a}
	100 mg/mL	13.9 ± 0.61^{b}
	200 mg/mL	15.48 ± 0.55^{be}
Negative control	water	0 ± 0^{g}
	Diluted ethanol (10%)	0 ± 0^{g}
	Diluted chloroform (10%)	0 ± 0^{g}
	Diluted acetone (10%)	0 ± 0^{g}
Positive control (standard reference antibiotic)	Ciprofloxacin (5µg)	24 ± 0.28^{d}
	Ampicillin (30µg)	17.33 ± 0.17^{ce}

inhibition zones of (11.14 ± 0.58, 10.19 ± 0.68, 12.33 ± 0.9) mm at 50 mg/mL respectively. Whereas arise at 200 mg/mL to the (19.82 ± 0.24, 17.48 ± 1.6, 18.12 ± 1.08) mm respectively. *Streptococcus mutans* showed inhibition zones of (10.1 ± 0.12, 11.12 ± 0.19, 10.33 ± 0.66) mm at 50mg/ml respectively. Whereas arise at 200mg/mL to the (17.88 ± 0.74, 20.1 ± 0.33, 15.48 ± 0.55) mm respectively (Table 3, Figure 2) *E.coli* showed inhibition zones of (11.18 ± 0.58, 9.9 ± 0.11, 9.32 ± 0.84) mm at 50mg/ml respectively. Whereas arise at 200 mg/mL to the (16.88 ± 1.22, 14.56 ± 0.72, 15.1 ± 0.22) mm respectively (Table 4, Figure 3 and 5).

The positive control ciprofloxacin (5 µg) showed a respective mean of inhibition zones of 27 ± 0.78, 24 ± 0.28 and 25.5 ± 0.24 mm toward the growth of *staphylococcus aureus*, *streptococcus mutans*, and *E.coli*, respectively. ampicillin antibiotic disc (30µg) showed a respective mean of inhibition zone of 20.33 ± 0.91, 17.33 ± 0.17 and 18.28 ± 0.14 mm toward the growth of *staphylococcus aureus*, *streptococcus mutans* and *E.coli* respectively (Table 2-4). The negative control (water, diluted ethanol, diluted chloroform, diluted acetone) did not show any inhibition zone on tested bacteria except the limited effect of diluted ethanol and acetone on the growth of *S.aureus* that gave inhibition zone of 7.12 ± 0.44 and 7.8 ± 0.92 mm respectively (Table 2-4)

The MIC and MBC of the different cardamom extracts are shown in Table 5. The ethanolic seeds extract showed MIC and MBC values at 0.078-0.624, 0.312-1.248 and 0.312-2.5 mg/mL against *S.aureus*, *Streptococcus mutans* and *E.coli* respectively.

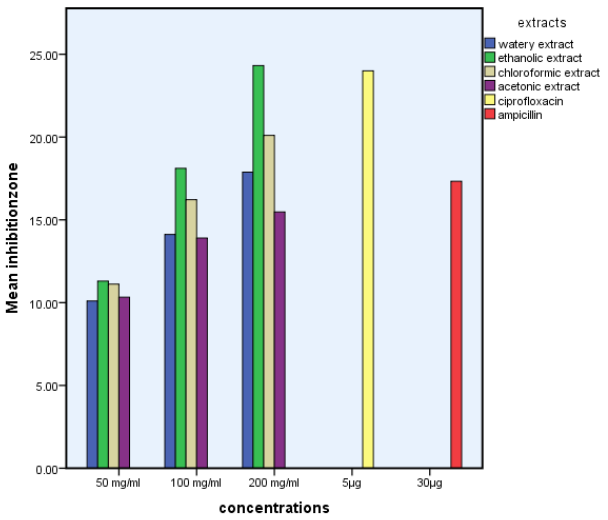

Figure 2: Antibacterial effects different extracts of cardamom compared with some antibiotics against *Streptococcus mutans* growth in culture media.

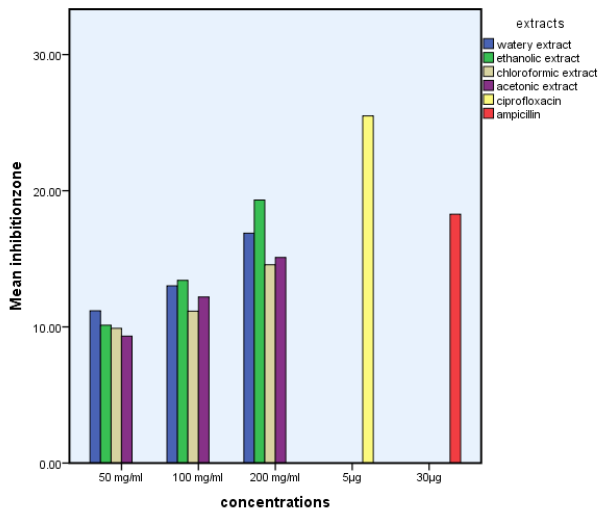

Figure 3: Antibacterial effects different extracts of cardamom compared with some antibiotics against *Escherichia coli* growth in culture media.

Table 4: The antibacterial effects of different extracts of cardamom compared with antibiotics against *Escherichia coli* growth in culture media

Type of extract	*Concentration (mg/mL)*	*Zone of inhibition against bacteria organism (mm)*
Watery extract	50 mg/mL	11.18 ± 0.58^{a}
	100 mg/mL	13.02 ± 0.78^{b}
	200 mg/mL	16.88 ± 1.22^{c}
Ethanolic extract	50 mg/mL	10.12 ± 0.9^{a}
	100 mg/mL	13.42 ± 0.81^{b}
	200 mg/mL	19.32 ± 0.44^{d}
Chloroformic extract	50 mg/mL	9.9 ± 0.11^{a}
	100 mg/mL	11.16 ± 0.62^{a}
	200 mg/mL	14.56 ± 0.72^{b}
acetonic extract	50 mg/mL	9.32 ± 0.84^{a}
	100 mg/mL	$12..2 \pm 0.6^{ab}$
	200 mg/mL	15.1 ± 0.22^{c}
Negative control	water	0 ± 0^{e}
	Diluted ethanol (10%)	0 ± 0^{e}
	Diluted chloroform (10%)	0 ± 0^{e}
	Diluted acetone (10%)	0 ± 0^{e}
Positive control (standard reference antibiotic)	Ciprofloxacin (5µg)	25.5 ± 0.24^{f}
	Ampicillin (30µg)	18.28 ± 0.14^{d}

Table 5: The values of MIC and MBC (mg/mL) for different extracts of cardamom against tested pathogenic bacteria.

	Type of tested bacteria					
	S.aureus		*S.mutans*		*E.coli*	
Type of extract	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
Watery extract	0.624	5	0.624	1.248	1.248	10
Ethanolic extract	0.078	0.624	0.312	1.248	0.312	2.5
Chloroformic extract	0.624	2.5	0.624	2.5	1.248	5
Acetonic extract	0.312	2.5	0.312	2.5	0.624	5

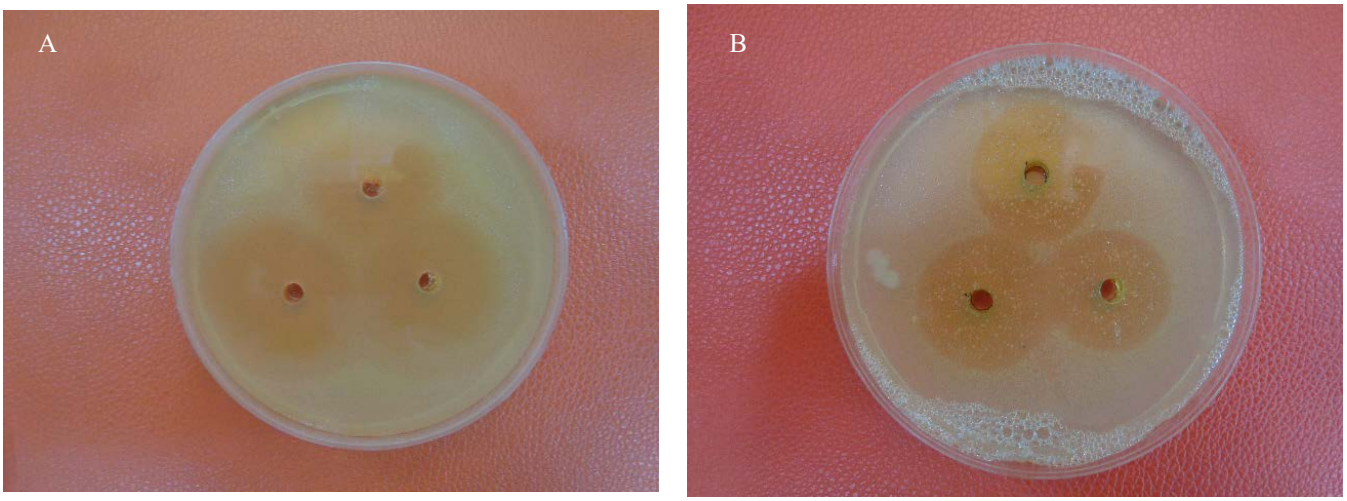

Figure 4: [A]: ethanolic extract of cardamom (200 mg/mL) against *staphylococcus aureus* in culture media [B] : ethanolic extract of cardamom (200mg/ml) against *streptococcus mutans*

Whereas the watery seeds extract showed 0.624-5, 1.248-10, and 1.248-10 mg/mL, respectively. The chloroformic seed extract showed MIC and MBC values at 0.624-2.5, 0.624-2.5 and 1.248-5 mg/ml against the growth of *S.aureus*, *Streptococcus mutans* and *E.coli* respectively. Whereas the acetonic seeds extract showed 0.312-2.5, 0.312-2.5 and 0.624-5 mg/ml respectively.

The similar letters denote to statistical non-significant differences whereas the different letters denote to significant differences at ($p < 0.05$)

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DISCUSSION

Chemotherapeutic agents have been vastly employed in

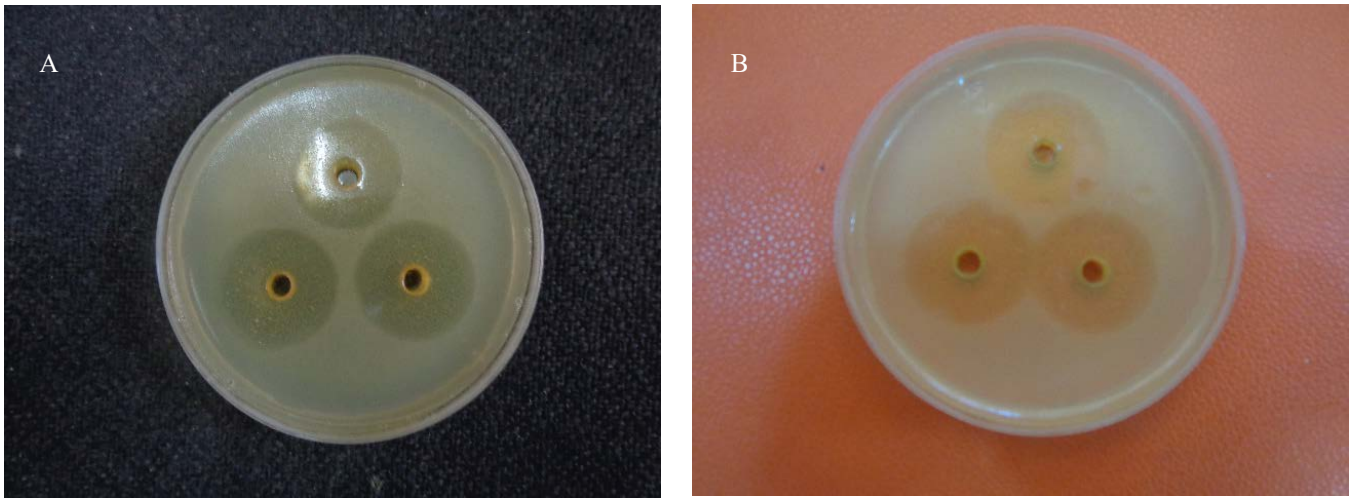

Figure 5: [A]: Chloroformic extract of cardamom (200 mg/ml) against *staphylococcus aureus* in culture media [B]: ethanolic extract of cardamom (200mg/ml) against *Streptococcus mutans.*

human being and veterinary field for more than 70 years ago, with great advantages to both human and animal health. The development of resistance becomes a growing problem all around the world, especially in developing countries, due to the indiscriminate use of antibiotics. So the physicians are left with very limited choice of antibacterial agents to treat various infections caused by potentially pathogenic organisms. Due to present this resistance, it has now become essential to research of novel and innovative antibacterial agents. So among more active sources of novel drugs are plants that have long been investigated due to plants, in general, contain many bioactive ingredients that can be interest in therapeutic as well as their low toxicity compared with chemical drugs. So the present work aimed to investigate the possibility of the use of certain preparation made from cardamom extract as active ingredients for the treatment of gingivitis infection caused by pathogenic bacteria through the testing its activity in the culture media.

The therapeutic characteristics of most medicinal plants and herbs, in general, are maybe return to the presence of varied secondary metabolites like alkaloids, tannins, flavonoids, phenols, and others, which can be synthesized in any part of the plant body. So the preliminary phytoconstituents screening tests may be with beneficial aspects in detecting chemical constituents in the plant material that may help to their quantitative estimation and also in locating the source of the pharmacologically active chemical compound.[19]

The results of the preliminary phytochemical analysis to the various extracts of the cardamom seeds by the use of a different solvent in the present study revealed the presence of alkaloids, glycosides, tannins, and terpenes in the all extracts used in this work. Flavonoids were completely absent in watery extract, but other extracts exhibited a positive result. Whereas saponins were present only in the ethanolic extract, phenols, on the other hands, were found to be present in the ethanolic and chloroform extracts only (Table 1). According to these results, the presence of various phytoconstituents in the fourth different extracts that prepared and used in the present study as flavonoids, alkaloids, and tannins may be responsible for the antibacterial activities demonstrated by the cardamom extract in the culture media. Some of the phytochemical compounds detected in this study as glycosides, saponins, tannins, flavonoids, terpenoids, and alkaloids have variously been reported to have antimicrobial activity.[20] These secondary metabolites exert antibacterial activity via different mechanisms. Flavonoids are hydroxylated phenolic compounds and known to be synthesized by different plants in response to microbial infection and appear to have antimicrobial action which attributed to their ability to formation complex with extracellular and soluble proteins as well as to formation complex with bacterial cell walls.[21] Antimicrobial characteristics of saponins are returned to its ability to produce leakage of proteins, and certain enzymes from the bacterial cell.[22] In addition Tannins phytoconstituents have been reported to form irreversible complexes with proline-rich protein.[23]

The results of antibacterial action of different cardamom extracts exhibited varying degrees of antimicrobial effectiveness against tested bacteria which done by agar well diffusion method and serial micro-diluted method in test tubes and the results indicated that antibacterial activity of this plant was dependent mainly on the extract concentrations and type of solvent use as well as the bacteria evaluated. In general, the cardamom extracts inhibited the growth of both the gram-positive and negative bacterial test isolates. This gives an indicator of a possible broad-spectrum mode of action for the different plant extracts. The antimicrobial activity found in the plant extracts have been attributed to some of the secondary metabolites that provide its presence in the plant extracts as flavonoids, tannins and terpenes[24] the results of our study are in agreement with study of Goyal and his colleagues who showed that the different extracts prepared from cardamom were effective significantly against the growth of six bacterial strains including both gram positive and negative bacteria[25] also the acetonic, methanolic, and ethanolic extracts of *A.subulatum* reported antibacterial activity against

two pathogenic bacteria responsible for inducing dental caries including *streptococcus mutans* and *staphylococcus aureus* in culture media.[26] methanolic seed extract of cardamom also showed antibacterial inhibitory activity against 10 human pathogenic bacteria including both gram-positive and negative bacteria and were found highest active on *Salmonella typhi andStreptococcus-β-haemolytica*[27] the antibacterial activity of cardamom essential oil was also recorded by Patil and Kamble who revealed that this oil besides ten spice essential oils have varying inhibition zones against four G^+ and eight G^- bacteria of spoilage and health significance[28] whereas Revati and his colleagues recorded that cardamom ethanolic extracts did not showed an antibacterial effect on most enterococci clinical isolates in culture media.[29]

The ethanolic extract of cardamom was an exhibit to have comparatively higher antibacterial effectiveness than other organic and watery extracts tested in the present study. At the same time, other organic and watery extracts were found to be a significant inhibitory effect toward all the tested bacteria compared with negative control, which not shown any inhibitory action. It is apparent that the ethanolic cardamom extract was generally more potent than the other organic and watery extracts and this result might be probably related to the presence of biologically active principles in the plant that dissolved more readily in and were best extracted by a less polar solvent (ethanol) than water. This finding is in the same line with many literatures that documented by many researchers that reported the differences in the activities of extracts obtained from the same morphological part of a plant using different solvents. For instance, the ethanolic extract of the dry fruits of *Elettaria cardamomium* were more potent than the other organic and aqueous extract against number of tested pathogenic bacteria.[25] Similar results showing that the alcoholic extract having the best antimicrobial activity is also recorded by Preethi and his colleagues *in two different plant : Leucas aspera, Holarrhena antidysenterica***.**[30] Seyydnejad and his colleagues also studied the effects of different alcoholic viz. ethanol and methanol for antimicrobial activity and showed that this difference in the activity between different alcoholic extract is returned to the difference between extract active substances in this two extract.[31]

In the present investigation the MIC values of the most cardamom extracts prepared in this study were lower than the MBC values (Table 5) this suggesting that the lower concentrations of different plant extracts were bacteriostatic whereas at higher concentrations the extracts become with bactericidal action.[32]

In conclusion, of the present finding, cardamom extracts contain a potential antibacterial active ingredient that could be of considerable use for the development of novel pharmaceutical industries as a therapy against gingivitis infections. The ethanol, chloroform, acetone, and watery extracts of seeds of cardamom have a significant inhibitory effect toward screened pathogenic bacteria, especially gram-positive bacteria.

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