

Comparative Study On Antibacterial Activities Of Two Natural Plants Versus Three different intracanal Medications

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Abstract:

The aim of this study was a trial to find natural agent to be used as intracanal medication to eliminate the uses of chemical medications with their cytotoxic effects. Antibacterial activities of two natural plants (freshly minced garlic extract and fresh lemon solution) were compared with that of three traditional intracanal medications (10% citric acid, 5.25%NaOCl, and CPCP). Mixed root canal flora (collected from necrotic teeth with open cavity, visible sinus tract) and four bacterial strains isolated from infected root canals (α -hemolytic Streptococci, Streptococci pyogenes, Enterococci faecalis and Pseudomonas aeruginosa) were tested. All bacteria were inoculated on blood agar plates as well as inside prepared sterile root canals. On the blood agar surfaces the inhibitory zones were measured around each medication used. pH of each medication was also determined to evaluate its antibacterial properties in relation to its pH value. The results showed that both freshly minced garlic and CPCP represented the statistically significant largest inhibitory zones when compared with other three medications used ($F= 7.3341$ at $p< 0.005$). There was no significant difference between bacterial inhibitory action of freshly minced garlic and CPCP. The vials containing samples collected from root canal medicated with CPCP appeared the clearest media, followed by that medicated with minced garlic and 5.25% NaOCl, respectively. The bacterial inhibitory growth in vials containing the samples medicated with fresh lemon solution and 10% citric acid appeared nearly similar or slightly less than that of 5.25% NaOCl. The sensitivity of bacteria used to fresh lemon solution was similar to that of 10% citric acid. There was no significant difference between lemon solution, 10% citric acid and 5.25% NaOCl solution to inhibit the microorganisms used. The medications were ranked in descending order of its acidity as the following: 10% citric acid(pH 1.68), lemon solution(pH 2.21), CPCP (pH 4.27) and 5.25% NaOCl (pH 12.96). It is difficult to determine the pH of minced garlic as it is in semi-solid form.

Introduction:

Bacteria and their product play an essential role in the pathogenesis of pulpo-periapical disease^{20,33}. The predominant microbial groups frequently isolated from infected root canal are the aerobic and facultative anaerobic organisms such as Streptococci, Enterococci, Staphylococci, Lactobacilli, Pseudomonas, Neisseria and Veillona^{2,4}. These types of organisms frequently isolated more than obligate anaerobic species in developed periapical lesion, during the standard culture technique^{4,37}.

Usually, the streptococci is considered nonpathogenic as it is the most frequently found in the oral flora³⁵, although it was isolated from heart valves after attack of subacute bacterial endocarditis indicating its potential pathogenicity in other environments⁸. Perez et al 1993²⁷, demonstrated streptococcus sanguis penetrating the root dentinal tubules and considered as major cause of endodontic failure.

It was also reported that Enterococci faecalis was pathogenic but of low virulence³⁵. It may play a significant role in clinical condition of flaring-up where it was determined in some of such cases¹¹.

One of the main goals of endodontic treatment is eliminating or maximum reduction of the root canal flora through chemomechanical instrumentation. Numerous medications have been widely used to achieve this goal. NaOCl is the most popular irrigant with excellent tissue dissolving and antibacterial properties³⁵. Siqueira et al 1998³⁰, compared antibacterial action of different concentration of NaOCl, they showed that 4% NaOCl solution provided the largest inhibitory zone than the lower concentration. However it may produce postoperative sequelae of pain, edema and/or hemorrhage⁹. Citric acid and EDTA are chelating agent that react chemically with mineral to chelate the dentin removing the smear layer that generated during instrumentation as well as killing most of the bacterial flora^{30,37}. Citric acid also proved to be more effective than EDTA, killing all bacteria tested³⁷.

For several years, formcresol and phenolic derivatives have been used as effective intracanal medications, creating a desirable action of fixation and disinfection³⁵. The biological test revealed that the formcresol was highly toxic. It diffused beyond the apex within 15 minutes causing periapical irritation or bony sequestrum^{7,36}. Compharated parachlorophenol (CPCP) was also used with less

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irritating and toxic effect on periapical tissue than that of formocresol without sacrificing its antimicrobial action³⁴.

Because of the cytotoxic effect of most of the commercial intracanal medications used, the trend of recent medicine attends to the use biological medication extracted from natural plants to eliminate any cytotoxic activity of chemical agent. Horiba et al 1991¹⁵, made an attempt to use the extracts of Japanese green tea as intracanal medications.

The aim of this study was to evaluate the antibacterial activity of two natural plants (garlic and lemon) against the root canal flora compared with three traditional intracanal medications [10% citric acid, 5.25% sodium hypochloride (NaOCl) and compharated parachlorophenol (CPCP)]

Materials and Methods:

Clinical samples: Under aseptic condition, five samples were initially collected from necrotic tooth with open cavity visible sinus tract. The tooth was isolated with sterile rubber dam, while the operative field was disinfected with 0.3 % iodoethanol. Small notch was performed on the shaft of sterile H-file using sterile bur. Several strokes with this H- file were done against the dentinal wall of infected root canal, to take the bacterial sample. The contaminated working part of each file was separated and suspended in tightly plugged sterile vial containing 1ml brain heart infusion broth (BHBI). Each vial was vortexed well for 1 minute. The vials were transferred to lab and processed within 1 hour.

The suspension was incubated at 37°C for inspecting bacterial growth. Seeding was done from each sample using sterile swabs that were brushed across the blood agar plates. All agar plates were incubated in 37°C for 2 days for growth of aerobic and facultative bacteria.

Four strains of bacteria preisolated from clinical specimens were also used separately ($\alpha\alpha$ -hemolytic Streptococci, Streptococci pyogens, Enterococci faecalis and Speudomonas aeruginosa). A suspension was prepared for each of the four selected strains, equivalent to the turbidity of 0.5 McFarland.

Antimicrobial activity: To examine the antimicrobial activity of the tested medications, the bacterial strains were then reseeded on blood agar plates collectively for mixed root canal flora, and separately for each selected strain. Each blood agar was divided into 5 regions. After spreading the sample, one of five tested medications[fresh minced garlic extract, fresh lemon solution , 10% citric acid , 5.25%NaOCl , and CPCP (Sultan chemists Inc.)] applied in the center of each region. All the blood agar plates were incubated in 37°C for 2 days. After incubation period, each agar plate was examined for bacterial inhibitory action of all tested medications by measuring the visible clear, approximately circular inhibitory zones surrounding each medication in millimeter using transparent ruler. To compensate the irregularity in shape, the diameter was measured four times, each radius was at 45° to the other and calculate the mean of them. The data was statistically analyzed by analysis of variance and t-student tests.

Antibacterial effect inside the root canal: A total of 90 freshly extracted single rooted teeth were decapitated at CEJ. Each root canal was instrumented up to #45 K-file and then autoclaved to ensure the complete sterilization of specimens. The prepared roots were divided into five groups, according to bacteria used [mixed root canal flora (randomly selected from the previously prepared suspensions) α -Hemolytic Streptococci, Streptococci pyogens, Enterococci faecalis and Pseudomonas aeruginosa (that were grown separately in sterile BHIB to make suspensions)]. Each root canal was flooded two times with either of inoculum and maintained in 37°C for 15 minutes to dry the samples. According to medications used, each group was subdivided into 6 subgroups each of 3 infected root canals. The root canals of the first subgroup were condensed with fresh minced garlic extract. Each three root canals of other four subgroups inoculated with bacteria were treated with 20 microns of each liquid tested medication, using digital pipette with disposable tips. While of the sixth subgroup was left empty to serve as control. All treated root canals were covered with sterile pieces of aluminum foil and incubated at 37°C for 2 days. After incubation period, the root canals were wetted with small amount of BHIB. Two sterile paper points were inserted into the treated root canal to absorb the BHIB medium. Few strokes with sterile H-file (#45) were used to scrape off the debris and bacteria potentially adhering to the medicated canal walls. The files with collected dentin debris and absorbant paper points were inoculated in 10ml screw top vial containing 1.5 mg BHIB medium. The vials were vortexed well and incubated at 37°C for 48hs. After incubation period, each vial was examined for the presence or absence of bacteria by turbidity of the medium. 0.1 ml of each tube was plated on the surface of an agar plate and colonies of bacteria

were counted and compared to the control. No attempt was made to determine the types of bacteria remain vital.

pH for all tested medications was recorded to evaluate their antibacterial effect in relation to pH values.

RESULTS:

The positive control samples collected from infected root canal exhibited obvious growth of aerobic and facultative anaerobic bacteria (Fig. 1). The means of the diameter of inhibitory zone for all tested medicaments on blood agar and pH of each medication are shown in table (1). All medications used in this study were inhibitory against all bacterial strains, but with some variations in size of inhibitory zones (table 1). The freshly minced garlic and CPCP represented the statistically significant largest average zones of inhibition against all bacteria used, when compared with the other three medications ($F = 7.3341$ at $p<0.005$). Each of fresh lemon solution, 10% citric acid and 5.25% NaOCl was also effective against all bacteria, however they were not more effective than that of freshly minced garlic and CPCP (Fig. 2).

There was not statistically significant difference between inhibitory action of freshly minced garlic and CPCP ($t= 0.2522$ at $p<0.05$). The inhibitory zones produced around other three medications were relatively with similar diameter. 5.25% NaOCl produced the smallest mean inhibitory zone (table 1). There was also insignificant difference between lemon solution versus 10% citric acid ($t= 0.0469$ at $p<0.05$) lemon solution versus 5.25% NaOCl ($t=0.5143$ at $p<0.05$) and 10% citric acid versus 5.25% NaOCl ($t=0.6319$ at $p<0.05$).

The means percent of CFU counts presented in medicated root canals as compared with control group were given in table (2) and Fig. (3). The vials containing samples collected from root canal medicated with CPCP appeared the clearest media, followed by that medicated with minced garlic and 5.25% NaOCl, respectively. The bacterial inhibitory growth in vials containing the samples medicated with fresh lemon solution and 10% citric acid appeared approximately similar or slightly less than that of 5.25% NaOCl.

DISCUSSION:

The most detected bacteria in the present study were aerobes and facultative anaerobes. No attempt was made to detect the obligate anaerobic organisms, where the samples were collected from infected root canals, directly contact with the oral cavity through sinus tract. The environment of such cases was unsuitable for obligate anaerobes. Baumgartner & Falker 1991⁴, also determined higher proportion of facultative anaerobes in open root canals than strict anaerobic bacteria, however, when the cavity sealed the environment became oxidation reduction, the facultative bacteria were increased in number and strict anaerobic bacteria became more pronounced. The microflora of infected root canals usually comprises aerobic and anaerobic bacterial combinations in synergism²¹. With canal opening, some of these bacteria (strict anaerobes) will eliminate and alter such synergism.

Table (1): Representing the means of inhibitory bacterial zones (in centimeters) provided by tested medications.

Bacteria	Intracanal medications				
	Garlic *	Lemon	10% citric	5.25% Naocl	CPCP *
Mixed R.C. flora	2.8	1.4	1.35	1.1	2.3
α -hemolytic Strep.	2.3	1.2	1.2	1.2	2.4
Strep. Pyogens	1.7	1.2	1.2	0.9	1.6
Enterococci Faecalis	2.4	0.8	0.7	0.8	2.4
Pseudomonas aeruginosa	1.1	0.5	0.7	0.6	1.1
Mean value	2.06	1.02	1.03	0.92	1.96
PH value	-	2.21	1.68	12.96	4.27

* Highly significant difference, F value=7.3341at $p< 0.005$.

Table (2) : Representing the mean % of counts in experimental groups as compared with the control.

Bacteria	Intracanal medications					
	Control	Garlic	Lemon	10% citric	5.25% Naocl	CPCP
Mixed R.C. flora	100%	0%	8%	10%	8%	0%
α -hemolytic Strep.	100%	0%	3%	3%	5%	0%
Strep. Pyogens	100%	2%	5%	5%	7%	0%
Enterococci Faecalis	100%	8%	12%	11%	10%	5%
Pseudomonas aeruginosa	100%	5%	20%	18%	15%	2%

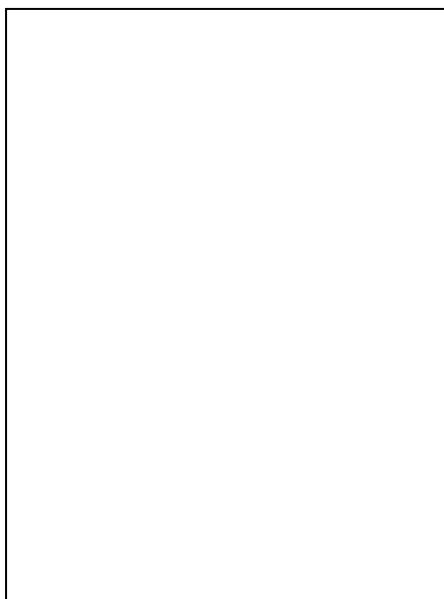


Fig (1): Showed control sample clinically isolated from infected open root canal.



Fig(2): Showed the inhibition zones surrounding the medication used, CPCP (P), minced garlic (G), Lemon (L), 10%citric acid(C) and 5.25% Naocl (N) .

The bacterial species used in this study for further antibacterial activity tests, were $\alpha\alpha$ – hemolytic Streptococci, Streptococci pyogens, Enterococci faecalis and Pseudomonas aeruginosa. There were several reasons for this choosing. They were the most commonly bacteria present in infected root canal flora^{22,26,35} and easily infiltrated the dentinal tubules without difficult²⁷. Most of previous experimental studies used these organisms^{2,4,5,22,29,30}, as to be implicated in cases of endodontic failure²⁹. Also this choose was to obtain accurate results on collective groups of organisms including gram+ve aerobes

(Streptococci pyogens and α – hemolytic Streptococci)^{20,35} and more resistant grame+ve and grame -ve facultative anaerobic (Enterococci faecalis and Pseudomonas aeruginosa.) organisms^{15,22,26,35}.

The use of antimicrobial agent is still considered the fundamental principles during endodontic treatment. All bacterial strains were able to penetrate into the dentinal tubules, but to different extents²⁹. Many studies have shown that, despite correct instrumentation, it was impossible to obtain a canal system free of bacteria^{5,24}. It can only remove the bacteria that penetrated to a shallow depth of dentinal tubules, while those localized deep in dentin may remain and subsequently grow and multiply in the presence of percolated tissue fluid causing treatment failure²⁹. So, in conjunction to mechanical preparation, intracanal irrigation and medication have been claimed to be valuable adjunct in root canal disinfection to attain bacteria free spaces^{2,6}.

In the present study, the bacterial inhibition produced by both minced garlic and CPCP was superior to the other three medications used, whether on agar plates or inside the root canals. This result was in agreement with other previous studies^{2,28}.

CPCP was used in liquid form, while during clinical application it is usually used in vapor form applied on cotton pellet placed in pulp chamber. Such method of application has been proved to be less effective than that of liquid form^{10,17,23,31}. It was demonstrated that CPCP became ineffective in root canal within a short period of time^{17,23}, as it lose its germicidal vapor^{31,33}. Barbosa et al 1997², suggested that the effect of CPCP was dose dependent. Accordingly, for vaporization to be antimicrobically effective, the concentration of medication applied on cotton pellet must be 100 to 1000 times than that of medication applied directly³³. Unfortunately it would produce cytotoxic effect, as considered the highly toxic medication^{32,33}.

It was believed that the addition of camphor liquifies the compound, may serve as a vehicle and diluent, reducing the irritating effect of the agent³³. Contrarily, Soekanto et al 1996³², demonstrated that camphor itself had cytotoxic effect and that camphorating process may increase the toxicity of phenolic compound rather than reduce it.

Both minced garlic and lemon solution extracts seem to be biologically compatible materials as they are frequently used during our meals. It has been reported that they have antibacterial action against various bacteria inhabiting the intestines^{16,18}.

Garlic has been used in medicine for centuries, treating variety of diseased including snake bite, rheumatism, abdominal pains, lowering blood pressure, parasites infections, pulmonary condition, and small pox^{16,25}. In previous study ascribed to garlic, it has been reported its antibacterial and antifungal properties^{19,28}. Moore & Atkins 1977²⁵, demonstrated that the garlic extract was more effective against pathogenic yeast life fungi, especially candida albicans that were resistant to the most antibacterial agents. Shalaby 1984²⁸, suggested that garlic had antibacterial properties and can be used as ointment in treatment of skin infection caused by staphylococcus and streptococcus with 100% successful prognosis. When garlic was inoculated in mice infected with sarcoma, the animal remained alive for six months observation period with no evidence of tumor growth¹⁸.

Garlic extract appeared to consist of several antimicrobial agents that responsible for bacterial inhibitory growth seen in the present study. Among them, there were dially disulfide, dially trisulfide, allicin and different phenolic acids that responsible for the fungicidal and bactericidal activities of garlic^{12,16,19}. At the beginning of this study, different form of garlic extract were primarily tested to select the suitable one that having antibacterial action. Garlic oil, aqueous garlic solution and freshly minced garlic were tested. Freshly minced garlic was the only form that obtained the desirable effect. This result may be attributed to its volatile effective agent that quickly evaporates and the material become ineffective.

The present result indicated that the fresh lemon solution, 10% citric acid and 5.25% NaOCl had antibacterial effect against all tested bacteria, whether on mixed root canal flora or individual bacterial strains, approximately similar to each other but less effective than that obtained by freshly minced garlic extract or CPCP. Concerning with NaOCl, Siqueira et al 1998³⁰, showed that 4% NaOCl exhibited a statistically significant larger average zone of bacterial inhibition than that produced by citric acid. They suggested that the disinfecting effect of NaOCl depended on the concentration of undissociated hypochlorous acid in solution that exerts its effects by oxidative action on sulfhydryl groups of bacterial enzymes.

Regarding citric acid and lemon solution, they also had antibacterial effects under the condition of the present study. This finding corroborates those of other studies^{30,37}. Yamaguchi et al 1996³⁷, showed that Citric acid (at pH 1.1, 1.3 and 1.6) was effective as irrigating solution for root canal having antibacterial effect against all facultative and obligate anaerobes used. While Ando 1985¹, reported that citric acid and EDTA solution showed less antibacterial action against aerobes and anaerobes strain than those reported by Yamaguchi et al 1996³⁷. Garrett et al 1978¹³, suggested that citric acid was effective on diseased root surface to remove bacterial toxic product such as endotoxin, when used as irrigant during mechanical debridement of deep periodontal pockets. Because the primary function of citric acid is removing the smear layer, particularly contaminated layer, the antibacterial properties must be considered an additional advantage as it can penetrate easily through open dentinal tubules killing bacteria inside³⁰.

The pH of medication plays a major concern on inhibitory growth of bacteria. The lower pH (more acidic) is the higher bacterial inhibitory growth, as well as the more demineralizing effect on tooth structures^{14,21}. Although 10% citric acid (ph 1.68) and lemon solution (pH 2.21) are more acidic than CPCP (pH 4.27), the antibacterial activity of the CPCP is higher . It may be attributed to the effect of camphor itself. Yamaguchi et al 1996³⁷, showed that the amount of calcium removal by 0.5, 1 and 2M (of pH 1.6,1.3 and 1.1 respectively) citric acid at 60 min coincided approximately with taken 0.5M (pH 7.6) EDTA at 120 min. Baumgartner and Mader 1984³, found that the citric acid followed by NaOCl was effective for dissolving inorganic and organic debris. Also the inhibitory effect of 5.25% NaOCl may attributed to be strong alkaline agent (pH 12.96). Lemon solution (pH 2.21) is considered natural source of citric acid (pH 1.68) with lower acidity. As citric acid is the chemical product, it may have some irritating effect than that of natural lemon solution that is usually used daily during our nutrition. So lemon solution can be preferable as root canal disinfectant than citric acid, as they produce similar bacterial inhibitory growth, while the former is lower acidic with lower tooth destructive effect than citric acid. It needs further investigation to know how to eliminate its demineralizing action by natural way, after obtaining its desirable effect of bacterial inhibitory growth. Concerning minced garlic, it is difficult to determine its pH as to be in semi-solid form.

The present result revealed that Enterococcus faecalis and Pseudomonas aeruginosa were less sensitive to all medications used than other tested bacteria, particularly inside the root canals. This result was in agreement with other studies^{26,35}. Noda et al 1999²⁶, suggested that Enterococcus was difficult to remove from infected root canals, as it had a strong ability to resist many antibiotic and disinfectants. Siqueira et al 1998³⁰, advocated that, in clinical condition, bacteria inside root canal may show a different susceptibility to irrigant solutions as interaction with other factors such as organic material, tissue fluids and dentin can influence the antibacterial effectiveness of irrigants. On the other hand, the mixed flora was more sensitive to all tested medications. This observation can be described by synergistic process based on interaction of multiple organism of mixed infection²¹. It was suggested that the low oxygen environment necessary for anaerobic growth will not be obtained by inhibiting aerobic organisms. Thereby their will be no growth of anaerobic organisms.

From this study we can conclude that fresh minced garlic and fresh lemon solution may be useful as intracanal medications, as they inhibited the growth of all bacteria used. While it need further investigation to evaluate their biological behaviors on periapical tissues.

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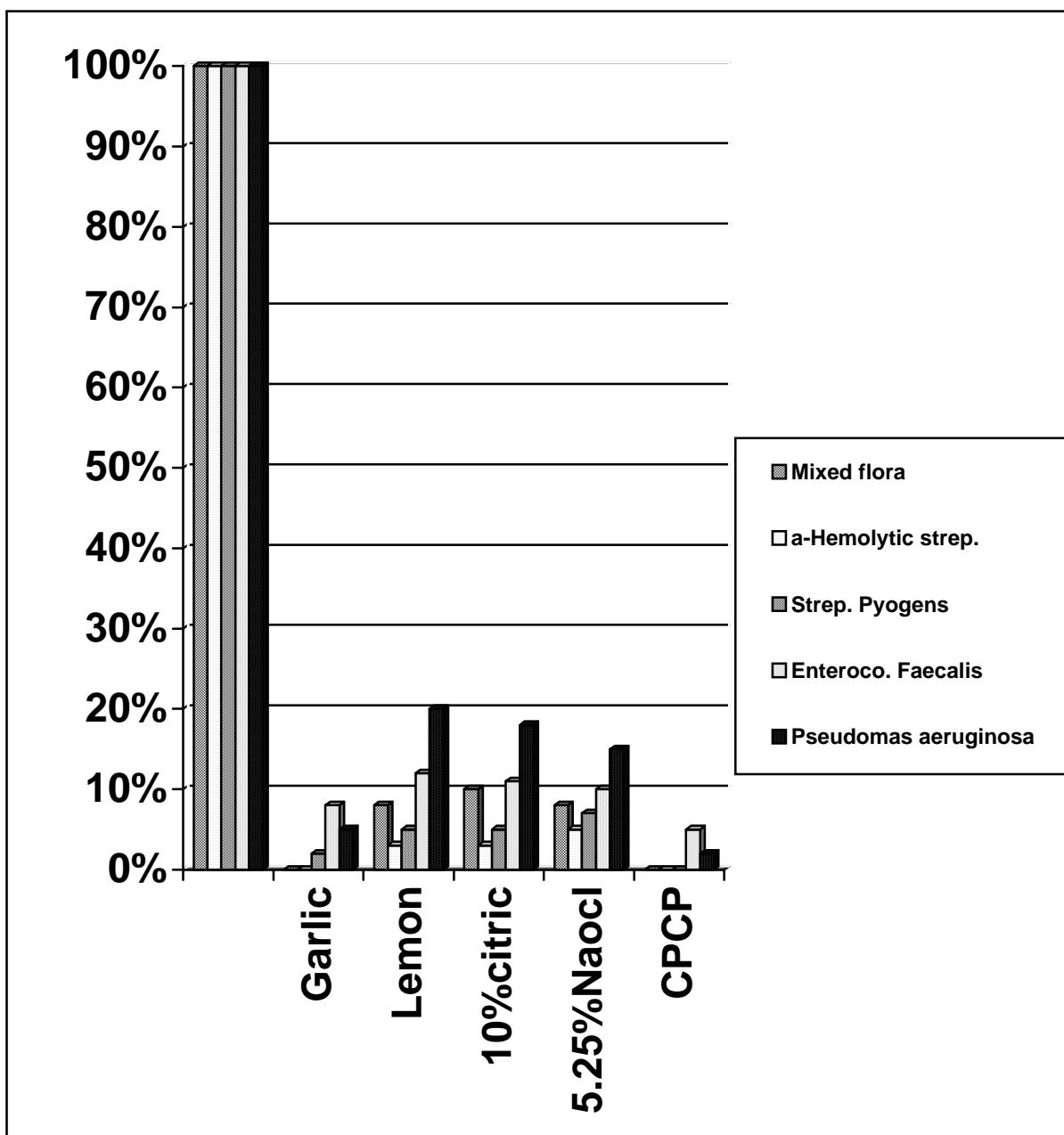


Fig. (3): Histogram representing the mean % of counted organisms in all experimental groups as compared with control.

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