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Evaluation the Inhibitory Effect of Aloe Vera Aqueous and Alcoholic Extracts on Salivary Streptococcus Mutans In Comparison to Chlorohexidine Gluconate

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Key words
Aloe Vera, dental caries, streptococcus mutans
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Abstract

diabetic properties. The aim of this study (an in vitro study) was to evaluate the effect of Aloe Vera alcoholic and water aqueous extract in different concentration (18%, 28%, 38%, and 48%) on the viable count and inhibition zone of streptococcus mutans and compared with chlorhexidine 2%. In this study the isolation and identification of streptococcus mutans was performed from saliva of individual, aged under 65 years old, these bacteria was isolated, purified and diagnosed according to morphological characteristic features and biochemical test. The effect of Aloe Vera extracts in different concentrations were tested on the isolated species by using agar diffusion technique and the effect of different concentrations of aloe vera on viable count of streptococcus mutans.

Results: In vitro experiments, statistically reduction in the viable count of streptococcus mutans was recorded at all concentration of alcoholic Aloe Vera extract and increase in the concentration lead to increase in the reduction of viable count of streptococcus mutans while in this study showed significant reduction in viable count of streptococcus mutans at higher concentration of water extract of Aloe Vera at 38% and 48%.

The diameter of inhibition zone of streptococcus mutans were highly significant at all concentration of alcoholic extract in comparison to water extract

Introduction

Aloe barbadensis Miller (family Lilaceae) commonly known as Aloe Vera , is easily grown in hot and dry climates and widely distributed in Asia, Africa, and other tropical areas, it consists of two different parts, each of which produce substances with completely different composition and therapeutic properties. The outer green rind includes the vascular bundles, and the inner colorless parenchyma contains the aloe gel. The per cyclic tubules, which are present just beneath the outer green rind of the leaves cells produce a bitter yellow exudates (latex)that has laxative properties[(1)]. The gel is a clear soft moist and thin tester jelly like material and is removal

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from leaves after separation from the inner cellular debris. The gel consist of 99.5 % water and other remaining 0.5-1 % is a solid material consist of a range of compounds including water soluble vitamins , minerals , enzymes, polysaccharide, phenol compounds and organic acids[2,3].The chemical composition of aloe Vera leaves is highly complex and includes vitamins B1,B2,B6,C and beta carotene, enzymes such as amylase, alkaline phosphates, superoxide dismutase, lactic dehydrogenate and Lipase ,saccharine inorganic compounds(calcium, chlorine, copper, potassium, zinc chromium[4]. Aloe Vera has used as a moisturizing agent and for the treatment of skin abrasion, minor burns and irritations. [5, 6 and&7] Aloe Vera has been included in many herbal products for oral use including dentifrices and mouth rinses. Streptococcus mutans is the chief oral pathogens in the etiology of dental caries [8]. Due to their antimicrobial properties, Aloe Vera may be useful in preventing dental caries by acting on the dental pathogen. In recent times, there has been an explosion of interest shown in the use of plants for the treatment of various diseases. Research on the medicinal and therapeutic value of these natural products has also increased. People are aware of ill –effects associated with over prescription of antibiotics. So in the present study we investigated the inhibitory effects of Aloe Vera extracts in different concentrations on most important cariogenic bacteria isolated from patient from patients with periodontitis and dental caries.

Materials and Methods:

This study was conducted in the laboratory of Collage of Dentistry Al Mustansiriya University. Aloe Vera was extracted by the two following methods which were:

a. Water Aloe Vera extract (of aloe Vera) was prepared according to method described by Cowan's (1999) [9]. 100 gm from dried leaves of aloe Vera by was infused in 500 ml of boiling distilled water and, left to cool at room temperature mixing well the solution by magnetic stirrer then filtered the infusion by filter paper then dried the extract at room temperature and kept in closed glass container till used.

b. Alcoholic extract of Aloe Vera

The preparation of alcohol extract was done by weighting 100 gm of dried leaves of Aloe Vera then infused in 500ml of ethanol alcohol in 98% concentration and kept at room temperature for 48 hours with shaking, and then filtered the extracts , kept till used.

Isolation of mutans streptococci:

Stimulated salivary samples were collected from52 patient aged (under 56years) of both sexes subject are healthy subjects who didn't suffer from a systemic diseases. Information about each subjects, name, age, gender, frequency of smoking, wearing of fixed or removable prosthetic appliances, taking any antiseptic mouth washing, taking any broad spectrum antibiotic therapy, having a medical history that revealed any disease or medical condition were recorded. Each individual was asked to chew a piece of Arabic chewing gum (0.5) for five minutes to stimulate salivary collection was much possible then saliva was collected under suitable condition, samples were homogenized by vortex mixer for two minutes then ten-fold dilution was performed by transferring 0.1 ml of saliva to 0.9 ml of phosphate buffer saline (PH 0.7)from dilution $10^{-3}$of

salivary samples ,0.1 was taken and spread in duplicate on the Mitis salivarius Bacitracin agar Media, the plates were incubated an aerobically using candle for 48 hours at 37C°and then aerobically for 24 hr at room temp.(10)

Sensitivity of streptococcus mutans to different concentrations of water and alcohol Aloe Vera and 0.2%chlorohexidine cluconate

Sensitive of streptococcus mutans to different concentrations of Aloe Vera, chlorohexidine. Different concentration of water and ethanol Aloe Vera in addition to chlorohexidine gluconate 0.2 % were used in this experiment. They were as follow:

Water Aloe Vera 18%, 28%,38%,48%),(alcoholic Aloe Vera 18%,28%,38,48%). A volume of 25 ml of muller Hinton agar was poured into sterile glass petridishes, left at room temperature for 24 hr to each plate. 0.1ml of Msincolums was spread, left for 20 minute at room temperature then wells of equal size and depth were prepared about, each well was filled with 0.2 ml of the test agents. plates well left at room temperature for one hour then incubated an aerobically for 24 hr. at 37C°; zone of inhibition was measured a cross the diameter of each well.

Effect of alcoholic and water extract and chlorohexidine on viability counts of streptococcus mutans

Different concentration of Aloe Vera were prepared Brain heart infusion broth (PH7.0) were prepared and distributed in test tubes by 8.9 ml in each one. One of the test agents was added to each tube. After that 0.1 ml of bacteria in columns was added to each tube 0.1ml was transferred to 0.9 ml of sterile phosphate buffer saline PH 7.0 and a tenfold dilution was performed from dilution 10^{-3} 0.1 ml was taken and spread in duplicate on MSB agar plate, the plates then incubated an aerobically at $37C^0$ for 48 hr. then colony forming per unit per milliliter was counted.

Statistical analysis:-is performed using SPSS for calculation of the statistical parameter. Mean and standard deviation .ANOVAs test applied for calculating the significances between the different variables, accepted ≤ at 0.05.

RESULT:

Table - 1- shown that the age of the patients ranged from 8 years to 56 years with a mean of 29.8(SD ±13.5). About one third (n=19, 36.5%) of the cases was in the age group of (20-29) years. This was followed by the cases in the age group of (30-39) years (n=13, 25%).

Regarding gender, about 57.7% of the patients were females (n=30), and 42.3% were males.

The result in table 2 showed a high statistical significant difference between two extracts types at all concentrations especially .at 38% and 48%conc.

($P < 0.05$), where the mean count of streptococcus mutans by1 using the Aqueous type is more than alcoholic one at all concentration which revealed less sensitivity of bacteria to this type of extract.

The result in table 3 showed that count of streptococcus mutans was tested in vitro. A high statistical difference was found on performing the ANOVA test among the three groups ($P < 0.05$) ,the result revealed the microorganism is less sensitive to Aqueous type than alcoholic and the latter is less effective than chlorohexidine at all concentration .

A high statically significant difference was found on performing the dunnetti test
(P <0.05) when we considered the chlorhexidine extract as control group where we found the control group is more effective than other two types but more effective than Aqueous one at all concentrations as seen in table 4 and fig 1.
The results of agar well diffusion method were revealed that the sensitivity of MS to different concentrations of Aloe Vera (Aqueous and alcoholic extract) in vitro, where the diameter of inhibition zone of MS were found statistical significant increases by using alcoholic extract in compares to Aqueous extract in all examined concentration(mean of alcoholic extract 18%=5.627,28%=6.22.38%=7.09.48% =7.95 compared to aqueous extract 3.47,3.90,4.19,4.61mm)respectively (P <0.05),as well as the mean diameter of inhibition zone of MS increased as the concentration of Aloe Vera extract increased in both types of extracts. As seen in Table- 5 and fig -2
Table 6 illustrate a high statistical significant results by performing the ANOVA test among the three extract types, which revealed highly significant differences among the three groups for all concentrations(P<0.05) where the mean diameter of inhibition zone induced by chlorhexidine extract 2% is more than alcoholic and aqueous extracts for all concentrations.
When performing the dunnetti test for compares of two extract types with control group (chx2%) ,the result revealed a high statically different between the two types of extract with control group for all concentrations, where the mean diameter of inhibition zone by CHX is more than both types for all concentrations.(P <0.05)
As seen in table -7.
figure 3-demonstrate the result of compares of two extract types with the control group(chlorhexidine),the result revealed that the control group is more effective in all concentration than two extract types with high statistical difference where the P < 0.05.

Discussion:

There has been a recent resurgence of interest in the use of natural products and their potential antibacterial effect could be use in the prevention and treatment of oral diseases. Dental caries is continuous to be the most common oral disease in the world and its prevalence continues to rise in the developing countries. Dental caries is caused by acidogenic species of bacteria, mainly streptococcus mutans., lactobacillus and Actinomyces These oral bacteria species metabolize sucrose to lactic and other organic acids in dental plaque produced on surfaces of the tooth and dissolve calcium phosphate in the enamel, consequently giving rise to dental caries. Aloe Vera was selected for their inhibitory effect on Streptococcus Mutans as it is strongly associated with the inhibition of dental caries [(11).]
In this study Aloe Vera extract were used. The antimicrobial effect of Aloe Vera has been attributed to the plants anthraquinones: Aloe emodin, aloetic acid, aloin anthracine,anthranal,barbalion, chrysophanic acid, ethereal oil, ester of cinnamonic acid, isobarbalion and resistanol.[(12)]In randomized controlled clinical trial, an Aloe Vera containing tooth paste showed significant improvement in plaque and gingival dental scores as well as in microbiological counts[(13)]. Fani and Kohanteb concluded that Aloe Vera gel at optimum concentration could be used as antiseptic for prevention of

dental caries and periodontal diseases. [14]

Sensitivity of MS to different concentration of Aloe Vera aqueous and alcoholic extracts in comparison to chlorhexidine gluconate water by using agar well diffusion method had been tested in this study. the results showed that the Aloe Vera extracts were able to inhibit the growth of MS, these findings were in coincidence with Sandera et al.,2005;[15]Bupesh et al[16];Al Bayati, 2009[17]; Fani and Kohanteb2012[14].

The diameter of inhibition zone of streptococcus mutans were found statistical significant increases by using alcoholic extract in compares to Aqueous extract in all examined concentration, as well as the mean diameter of inhibition zone of MS increased as the concentration of Aloe Vera extract increased in both types of extracts.

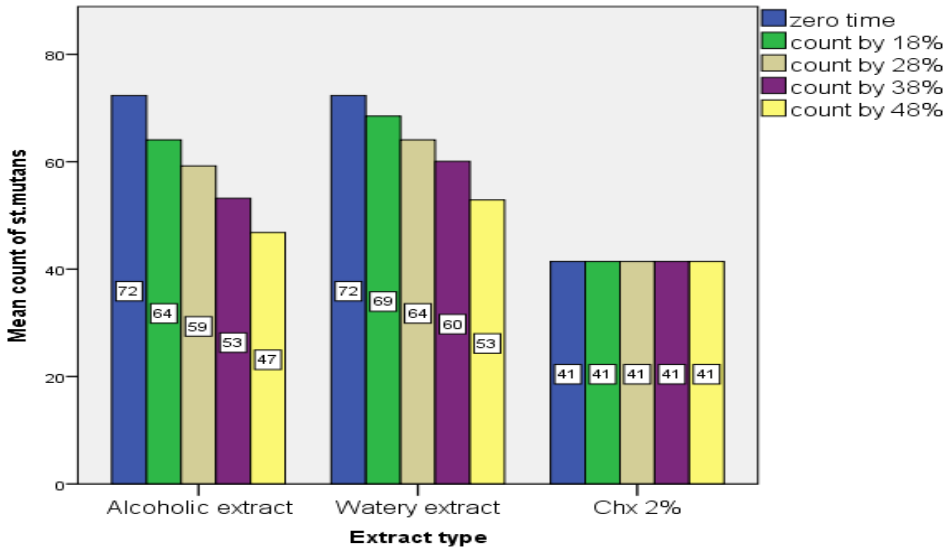
Where the mean diameter of inhibition zone induced by chlorohexidine extract 2% is more than alcoholic and aqueous extracts for all concentrations. This study also investigated the effect of Aloe Vera extracts and chlorohexidine on the viability counts of MS, in vitro. The result revealed the microorganism is less sensitive to aqueous type than alcoholic and the latter is less effective than

Fig 1-. Mean count of MS by three types of extracts at diff

chlorohexidine at all concentration. On the other hand, chlorohexidine 0.2% considered effective in reduction of viable counts of MS as numerous studies confirmed this effectiveness in vitro (Featherstone,2006[18];Al-Bazaz,2010[19].

Conclusion:

Based on results of this study, Aloe Vera extracts demonstrated antibacterial effect against streptococcus mutans. This inhibition was more evident with alcoholic extract than water extract and more effect with high concentration of Aloe Vera extract. On other hand the viable count of streptococcus mutans decreasing with increase the concentration and with alcohol extract more than water extract. So the Aloe Vera extract could be a good medicinal herbal agent against streptococcus mutans.



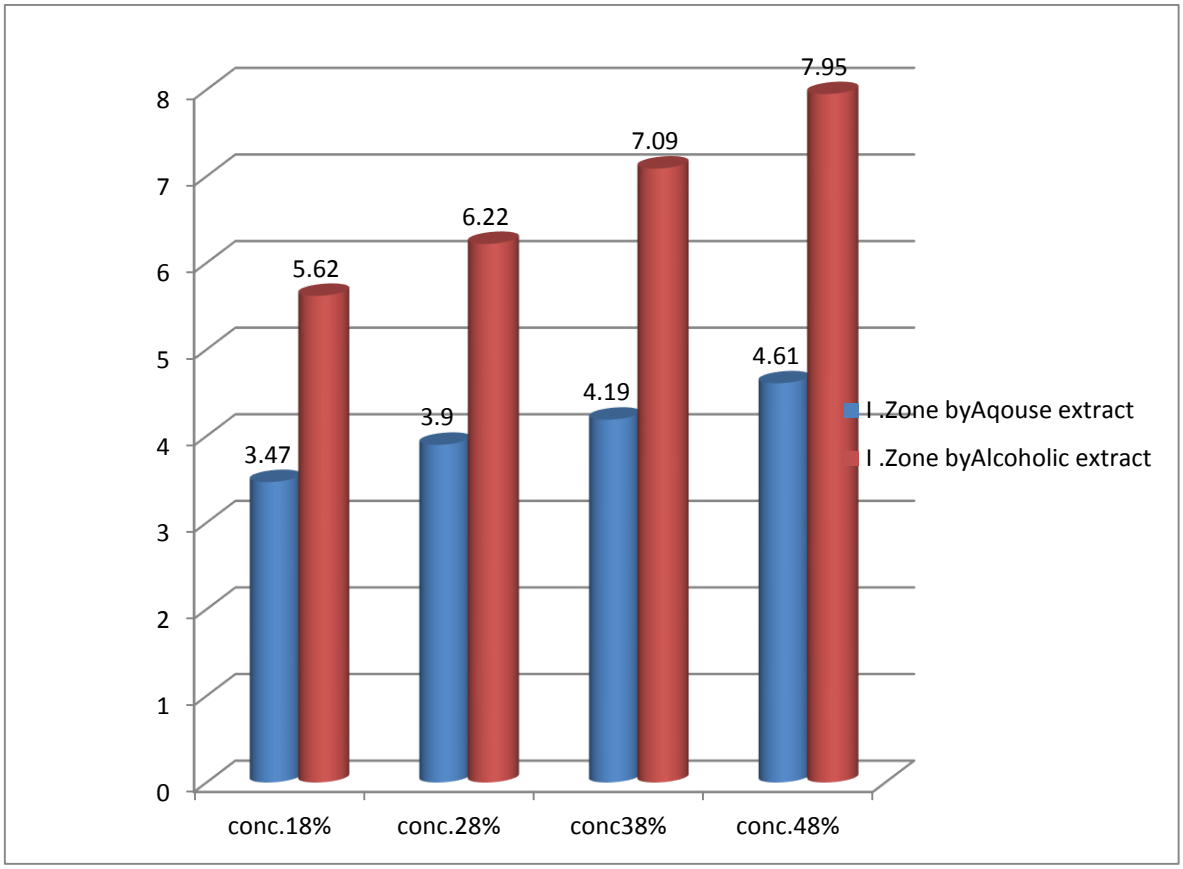


Figure 2-Mean of inhibition zone by different concentrations of Aloe -Vera of both extract

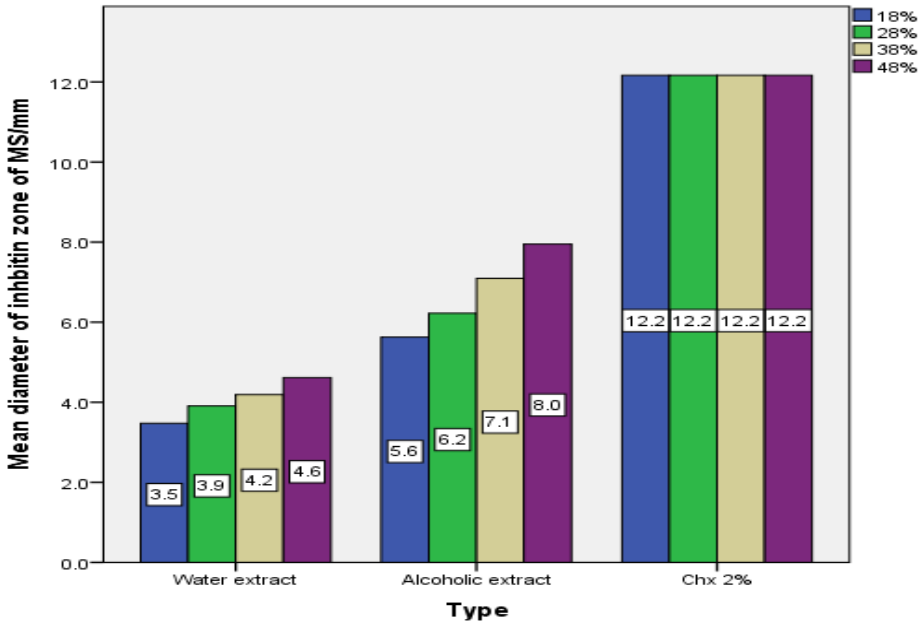


Fig. 2-, Mean diameter of inhibition zone by two types of Aloe Vera extract at different concentrations and CHX.

Table -1- Descriptive criteria of study sample[s] variables

		NO.	%
Age	<10	4	7.7
	10-19	10	19.2
	20-29	19	36.5
	30-39	13	25.0
	≥40	6	11.5
Gender	Female	30	57.7
	Male	22	42.3
	Total	52	100.0

Table 2 Mean count of MS for two extract types at different concentrations

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CONC,	Type of extract	NO.	Mean count of MS	Std. Deviation	T TEST	P VALUE
zero time	Alcoholic ex	52	72.35	9.381	0	NS
	Aqueous ex	52	72.35	9.381		
18%	Alcoholic ex	52	64.08	7.896	-2.730	0.007
	Aqueous ex	52	68.50	8.610		
28%	Alcoholic ex	52	59.23	8.300	-2.877	0.005
	Aqueous ex	52	64.08	8.869		
38%	Alcoholic ex	52	53.19	6.808	-4.821	0,001
	Aqueous ex	52	60.08	7.725		
48%	Alcoholic ex	52	46.81	6.218	-4.500	0.001
	Aqueous ex	52	52.88	7.495		

NS=Non- significant

Table 3-Mean count of M.S by different .extract types and concentrat

Concentrations/extract type		NO.	Mean count of St..mutans	Std. Deviation	95% Confidence Interval for Mean		F TEST	P-VALUE
					Lower Bound	Upper Bound		
zero time	**Alcoholic ex**	52	72.35	9.381	69.73	74.96	218.235	HS
	Aqueous ex	52	72.35	9.381	69.73	74.96		
	Chx	52	41.42	7.201	39.42	43.43		
count by 18%	**Alcoholic ex**	52	64.08	7.896	61.88	66.28	174.757	HS
	Aqueous ex	52	68.50	8.610	66.10	70.90		
	Chx	52	41.42	7.201	39.42	43.43		
count by 28%	**Alcoholic ex**	52	59.23	8.300	56.92	61.54	111.325	HS
	Aqueous ex	52	64.08	8.869	61.61	66.55		
	Chx	52	41.42	7.201	39.42	43.43		
count by 38%	**Alcoholic ex**	52	53.19	6.808	51.30	55.09	87.914	HS
	Aqueous ex	52	60.08	7.725	57.93	62.23		
	Chx	52	41.42	7.201	39.42	43.43		
count by 48%	**Alcoholic ex**	52	46.81	6.218	45.08	48.54	34.964	HS
	Aqueous ex	52	52.88	7.495	50.80	54.97		
	Chx	52	41.42	7.201	39.42	43.43		

HS=highly significant

Table 4- Mean differences in counts of MS by two types of extracts and control group at different concentrations.

Conc.	(I) type of ex.	(J) type control	Mean Difference (I-J)	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
zero time	Alcoholic extract	Chx	30.923*	.000	27.11	34.74
	Aqueous ex	Chx	30.923*	.000	27.11	34.74
18%	Alcoholic extract	Chx	22.654*	.000	19.18	26.12
	Aqueous ex	Chx	27.077*	.000	23.61	30.55
28%	Alcoholic extract	Chx	17.808*	.000	14.24	21.38
	Aqueous ex	Chx	22.654*	.000	19.08	26.22
38%	Alcoholic extract	Chx	11.769*	.000	8.59	14.95
	Aqueous ex	Chx	18.654*	.000	15.48	21.83
48%	Alcoholic extract	Chx	5.385*	.000	2.32	8.45
	Aqueous ex	Chx	11.462*	.000	8.40	14.52

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Table- 5- Mean of inhibition zone of st.mutansby effect of different concentrations for two types of extracts.

	Type of extract	No.	Mean of inhibition zone/mm	Std. Deviation	T test	P value
18%	Aqueous ex	52	3.47	0.751	-12.166	HS
	Alcoholic ex.	52	5.62	1.031		
28%	Aqueous ex	52	3.90	.816	-10.78	HS
	Alcoholic ex.	52	6.22	1.315		
38%	Aqueous ex	52	4.192	0.838	-12.39	HS
	Alcoholic ex.	52	7.096	1.467		
48%	Aqueous ex	52	4.615	0.732	-14.13	HS
	Alcoholic ex.	52	7.950	1.5353		

Table-6-Mean diameter of inhibition zone of MS of three types of extract of different concentration

Conc/types		No.	Mean of inhibition zone/mm	Std. Deviation	95% Confidence Interval for Mean		F TEST	p-value
					Lower Bound	Upper Bound		
18%	Water extract	52	3.47	0.75	3.26	3.68	128.85	HS
	Alcoholic extract	52	5.62	1.03	5.34	5.91		
28%	Water extract	52	3.90	0.81	3.68	4.13	146.89	HS
	Alcoholic extract	52	6.22	1.31	5.85	6.58		
38%	Water extract	52	4.19	0.83	3.95	4.42	168.13	HS
	Alcoholic extract	52	7.09	1.46	6.68	7.50		
48%	Water extract	52	4.61	0.732	4.41	4.81	198.94	HS
	Alcoholic extract	52	7.95	1.53	7.52	8.37		
	Chx 2%	52	12.16	3.79	11.10	13.22		

Table 7-Mean difference of diameter of inhibition zone induced by two types of extract in compares to Chx2 %(control group).

Multiple Comparisons						
Concentrations	(I) Types of extracts	(J) Type (Control)	Mean Difference (I-J) of Inhibition zone	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
18%	Aqueous extract	Chx	-8.6923*	0.001	-9.706	-7.679
	Alcoholic extract	Chx	-6.5385*	0.001	-7.552	-5.525
28%	Aqueous extract	Chx	-8.2577*	0.001	-9.295	-7.220
	Alcoholic extract	Chx	-5.9423*	0.001	-6.980	-4.905
38%	Aqueous extract	Chx	-7.9731*	0.001	-9.024	-6.922
	Alcoholic extract	Chx	-5.0692*	.0001	-6.121	-4.018
48%	Aqueous extract	Chx	-7.5500*	0.001	-8.602	-6.498
	Alcoholic extract	Chx	-4.2154*	0.001	-5.268	-3.163
*. The mean difference is significant at the 0.05 level.						
a. Dunnett t-tests treat one group as a control, and compare all other groups against it.						

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