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Article · January 2008

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EFFECT OF DIFFERENT CONCENTRATIONS OF ACIDIC OLIVE LEAVES EXTRACT MOUTHRINSE ON PLAQUE, GINGIVITIS AND PERIODONTAL POCKETS ON ADULTS

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Effect of different concentrations of acidic olive leaves extract mouthrinse on plaque, gingivitis and periodontal pockets on adults

ABSTRACT

Aims: The aims of this study were to evaluate the effectiveness of olive leaves extract mouthrinse on the development of plaque, gingivitis, periodontal pockets in different concentrations as pure extract 50%, apple cider vinegar (ACV) extract rinse 50%, ACV + olive leave extract (OLE) 15% in acidic medium, 20% ACV + OLE mouthrinse compared with the ideal standard control mouthrinse chlorhexidine (CHX) 0.2%.

Materials and Methods: Fifty adult volunteers participated in this controlled single blind cross–over study, 10 subjects for each group, one control and four experimental groups with different concentrations of the rinse for 1 minute twice daily during 8 weeks period, 2 weeks interval between each visit for motivation and reinforcement of application of the material.

Three applied indices (plaque and gingival indices by Löe and Silness), CPITN index by Ainamo recorded at baseline, after each experimental period and at the last visit, then examining the biological activity of the material in the laboratory.

Results: It had been found that materials were effective on the three applied indices with significant percentage reduction (for OLE 50%, ACV 50%) at $p < 0.001$ using unpaired Z–test, and at $p < 0.001$, $p < 0.01$ for OLE + ACV 51%, OLE + ACV 20% respectively using unpaired Z–test except for some visits for the two materials. Percentage reductions for the four applied concentrations were greatest for OLE + ACV 20%, OLE + ACV 15%, ACV, OLE respectively for the three applied indices with significant differences at $p \leq 0.05$ using repeated measures analysis of variance and Duncan test.

Conclusion: Using OLE mouthrinse in different acidic concentrations offer benefit in plaque, gingival, periodontal pocket depth reductions, but it is much lower than that of CHX 0.2% mouthrinse.

Key Words: Olive leave extract, apple cider vinegar, mouthrinse, plaque, gingivitis, CPITN.

الخلاصة

إن الهدف من هذه الدراسة هو تقييم تأثير فعالية مستخلص ورق الزيتون الصافي مع مستخلص ورق الزيتون الصافي في وسط حامضي بتراكيز مختلفة المستخدم كغرغرة فم على الصفائح الجرثومية، التهاب اللثة، عمق الجيوب اللثوية لمجموعة من البالغين باستخدام المؤشرات الخاصة بكل حالة مع فحص الفعالية البيولوجية مختبريا.

لقد تبين أن تأثير المحلول ذو التركيز 20% أقوى في منع تكوين الصفائح الجرثومية، التهاب اللثة، عمق الجيوب اللثوية من التراكيز 15% مستخلص ورق الزيتون في وسط حامضي ويليه خل التفاح ذو التركيز 50% ثم ورق الزيتون الصافي 50% لكن بدرجة أقل من غرغرة الكلورهيكسيدين 0,2% الشائع استعمالها.

INTRODUCTION

Chemicals have been used for plaque, gingivitis reduction since 2700 BC when recommendations have included rinsing the mouth with urine from a child.[1] Other recommendations have include beer, wine, vinegar, a rinse seed.[2] Today, with advanced chemical engineering, a number of products are marketed for plaque and gingivitis reduction.[2]

Many remedies and treatments have been ascribed to vinegar over the millennia and in many different cultures, but few have been verifiable using controlled medical trials and several that are effective to some extent have significant risks and side effects. In most cases, alternative treatments are more effective and less risky. Nevertheless, there is verifiable evidence that vinegar is effective for certain conditions like teething and dental infections.[3]

Apple cider vinegar (ACV) has antifungal, antibacterial and antiviral properties primarily coming from the malic acid and acetic acid portion of the vinegar. It acts as a buffer in the body because the acetic acid reacts with base or acid compounds to form an acetate, therefore rendering them chemically bioavailable for the body's utilization.[3] The ACV can reduce the toxicity of certain compounds by converting the toxin into an acetate compound which is less toxic, while ACV in itself is considered alkaline. A chemically pure vinegar (acetic acid) is neither acid nor basic forming as it leaves no ash as the entire portion, when burned evaporates completely.[4] The ACV contains trace elements such as K, Ca, Mg, P, Cl, Na, Cu, Fe, Se, F and vitamins such as C, E, A, B_1, B_2, B_6 provitamin beta–carotene. Potassium is the most important of all minerals that promote cell, tissue growth.[5] All varieties of vinegar contain about 4–7% acetic acid with 5% being the most common amount.[5] The ACV is an effective treasure by introducing the important minerals into the blood

stream, also helps in clotting of blood and healing process.[6, 7]

Olive leave extract (OLE) exhibits important antiviral, a broad–spectrum antimicrobial properties. The most active compound is oleuropein and products form its hydrolysis such as elenolate, a salt derived from the elenolic acid all have attribute to add to the antimicrobial functions.[8] Besides it improves the mouth's periodontal condition,[9] and it takes down dental infections in a matter of hours.[10]

Aims of the Study:

1) To evaluate the effectiveness of pure and mixed OLE with ACV at different concentrations on plaque, gingivitis and periodontal pockets in adults.
2) To evaluate any adverse reaction of this extract.

MATERIALS AND METHODS

Preparation of Different Types of the Mouthrinse with Different Concentrations:

One hundred mg of OLE was percolated successively at for 48 hours with 500 ml ethyl alcohol (ETOH); then the extract was condensed under rotary evaporator for use for preparing different concentrations of liquid extract for clinical application in dentistry in the following percentages:

1) Pure OLE 50%.
2) OLE + ACV 15%.
3) OLE + ACV 20%.
4) ACV 50%.

Study Population

A sample of 50 adult volunteers including referrals of Faculty of Dentistry, University of Mosul, Iraq were recruited for this study with ten subjects for each of the five groups, control, OLE 50%, 15% OLE + ACV, 20% OLE + ACV, 50% ACV. Their ages range from 18–40 years and have no history of systemic diseases. All the subjects had merely plaque, gingivitis, pocket depth $\leq$ 3 mm, no attachment loss. The purpose of this study was explained to them and the products being evaluated before entering and participating in this study.

Study Design

The present study had a controlled single blind cross over experimental design. It consisted of 4 experimental periods × 2 weeks interval between each visit

plaque,[11] gingival[12] and periodontal pocket indices[13] were recorded for all subjects at baseline and every 2 weeks interval for 8 weeks period.

The indices were measured by the same clinician at baseline, between each visit and the final visit. The participants were instructed to complete their mouthrinse during the duration of this study.

The four test subject groups were instructed to rinse with 10 ml of 50% of pure OLE, 15% OLE + ACV, 20% OLE + ACV, 50% ACV twice daily for 1 minute, while for control subjects they were instructed to rinse with 0.2% CHX for 1 minute twice daily with instruction not to use the rinse with brushing but independently or proceeded by water if it is used after brushing with instruction (don't brush your teeth right away as that they grind the vinegar into the enamel). The participant compliance was evaluated by finishing the supplied volume of the mouthrinse that was supplied to them. They were also asked to report any adverse reaction experienced during the period of the study. The materials had been examined for their biological activity in the laboratory to clarify their effectiveness to suppress pathogens as a mouthrinse.

Statistical analysis 8included mean, standard deviation between visits using unpaired Z–test at $p < 0.001$ level for comparison of each material with the control rinse because the sample was more than 30, paired t–test used when the sample is 30 or less. The percentage difference for each index was measured at $p \leq 0.05$ using Z–test, while the comparison of different indices with different materials was measured at $p \leq 0.05$ using repeated measures analysis of variance and Duncan test. Analysis of variance was used between more than two different groups while here we have only one group and more than two visits (4 visits), therefore repeated measure is the appropriate test since sample size remains 50. All had been calculated as follow:

$$\text{All} = \left.\begin{array}{l} 2^{nd} - 1^{st} \text{ visit} \\ + 3^{rd} - 1^{st} \text{ visit} \\ 4^{th} - 1^{st} \text{ visit} \end{array}\right\} \div 3$$

RESULTS

Fifty adult volunteers were divided equally into 5 groups, each group comprised 10 individuals using CHX, OLE + ACV 20%, OLE + ACV 15%, ACV 50%, OLE 50% equally.

The first group using CHX rinse was considered as a control group, while the other four groups were considered as test groups, each group complaint of plaque gingivitis, periodontal pocket depth problems.

It had been found significant difference reductions between the fourth visits at $p < 0.001$ using unpaired Z–test between CHX 0.2% and OLE 50% clearly shown in Table (1). Also significant difference reductions between visits at $p < 0.001$ between CHX 0.2% and ACV 50% as shown in Table (2) except for CPITN for the second visit between the two materials with non significant difference at p value = 0.323 according to unpaired Z–test.

Mean and standard deviation between visits for CHX 0.2% and OLE + ACV 15% clearly shows significant reduction differences for the three applied indices at $p < 0.001$ and < 0.05 except for gingival index at the second visit which was not significant at $p= 0.80$, and at the fourth visit at $p= 0.67$ according to unpaired Z–test in Table (3).

While significant differences between visits for CHX 0.2%, OLE + ACV 20% for the applied indices is clearly demonstrated in Table (4) at $p< 0.01$, 0.001 differently except for the second visit of plaque index which was not significant at $p = 0.12$, and the third visit of gingival index at $p = 0.40$ according to unpaired Z–test.

When comparing the five applied materials for the last three visits (difference from the first visit) for CPITN, there was significant reduction between visits at $p \leq 0.05$ using unpaired Z–test with the most significant reduction in the following order: CHX 0.2%, OLE + ACV 20%, OLE + ACV 15%, OLE 50%, acv 50%. This is clearly shown in Table (5), but for plaque index the reduction order was in the following manner: CHX 0.2%, OLE + ACV 15%, OLE + ACV 20%, OLE 50%, ACV 50% as it is shown in Table (6).

For gingival index comparison, the most significant reduction difference was as follow: CHX 0.2%, OLE + ACV 20%, OLE + ACV 15%, ACV 50%, OLE 50% respectively as it is shown in Table (7).

But when comparing the three applied indices for the five different concentrations of the materials for four visits shows that the potent reduction difference was in the following manner: CHX 0.2%, OLE + ACV 20%, OLE + ACV 15%, ACV 50%, OLE 50% with significant difference between ACV 50%, OLE 50% and non significant difference for the rest materials at $p \leq 0.05$ using repeated measures analysis of variance and Duncan test as it is shown in Table (8).

Figure (1) shows reduction in the mean for CPITN, plaque index, gingival index from the first visit for OLE + ACV 20%. The reduction was very sharp for the three indices but it was greatest for gingival index then CPITN which nearly reaches

the same level followed by plaque index, but it doesn't reach zero level, but the reduction for OLE + ACV 15% was greatest for CPITN followed by gingival index, plaque index which doesn't reach zero level at the last visit as it is clearly shown in Figure (2), but for ACV 50%, the manner of reduction was as follow: CPITN, plaque index, gingival index as it is shown in Figure (3).

The manner of reduction for OLE 50% was CPITN, gingival index, plaque index respectively as shown in Figure (4).

When comparing these concentrations with the control rinse, the reduction was greatest for CPITN, gingival index, plaque index respectively which reaches zero level for all indices as it is shown in Figure (5).

Concerning biological activity of the materials, Table (9) shows that the inhibitory effect of OLE on number of Gram positive and negative microorganisms is much lower than that of ACV at different concentrations, and it is higher inhibitory effect for combination of OLE + ACV especially for *Staphylococcus aureus* and *Streptococcus pyogens* bacteria as it is shown in Tables (10) and (11) respectively.

DISCUSSION

No known of interactions between OLE and other pharmaceuticals have been performed.[8] Insufficient evidence regarding controlled studies to evaluate their dental effectiveness led to conduction of this study.

The present study showed that 5 types of mouthrinses resulted in reduction in the mean CPITN, gingival index, plaque index which were significantly lower than the control rinse by the following order: OLE + ACV 20%, OLE + ACV 15%, ACV 50%, OLE 50%. The OLE exhibits important broad spectrum antimicrobial properties. The most active compound is oleuropein and products from its hydrolysis such as elenolate, a salt derived from elenolic acid. All have attributes to the anti–inflammatory, anti–microbial functions,[8, 14–16] that was responsible for reduction in plaque, gingivitis, CPITN indices. In addition to that ACV has antifungal, antiviral, antibacterial properties primarily coming from the malic and acetic acid portions of the vinegar. Also it contains trace elements; the most important of all minerals is K that promotes cell tissue growth and helps in clotting of blood and healing process.[17–19]

The OLE contains natural flavonids and esters that create a structural complex that infectious microorganisms may not readily develop a resistance to.[20] The OLE

has proved that it improves the mouth's periodontal condition.[9, 21–23]

There was no report of any adverse effects by mouth washing with the test or control solutions. Although CHX has proven role in reducing plaque, gingival indices,[24] tooth staining is the major limiting factor for its use in daily practice.[25] This had led to continuous and extensive investigations, seeking alternative agents.

CONCLUSIONS

Based on the results of this investigation, using OLE + ACV 20%, OLE + ACV 15%, ACV 50%, OLE 50% for 1 minute twice daily for 8 weeks could reduce plaque accumulation, gingival inflammation and periodontal pocket depth. Hence, these products could be prescribed as an adjunct to daily oral hygiene measures, but all these concentrations remain lower to CHX mouthrinse which remains the master gold standard material with which all mouthrinses, materials must be compared.

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Table (1): Comparison of CPITN, plaque index and gingival index increase percent age reduction in the second, third and fourth visits between patients using CHX 0.2% and OLE 50%

Parameters	**Visits**	**Percent Increase Reduction From First Visit (Mean ± SD)**		***p*–value***
		CHX 0.2% n= 10	**OLE 50% n= 10**	
CPITN	**Second**	53.86 ± 19.49	10.48 ± 15.68	<0.001
	Third	77.43 ± 15.43	39.7 ± 16.71	<0.001
	Fourth	97.84 ± 3.41	52.23 ± 26.48	<0.001
Plaque Index	**Second**	61.33 ± 21.37	9.26 ± 16.26	<0.001
	Third	85.81 ± 8.38	36.51 ± 20.34	<0.001
	Fourth	98.48 ± 3.23	54.81 ± 17.29	<0.001
Gingival Index	**Second**	48.63 ± 25.6	27.51 ± 20.43	<0.001
	Third	81.50 ± 8.74	29.80 ± 16.93	<0.001
	Fourth	98.48 ± 3.23	47.97 ± 19.69	<0.001

*Statistical analysis according to unpaired Z–test.
CHX: Chlorhexidine; OLE: Olive Leave Extract; SD: Standard Deviation.

Table (2): Comparison of CPITN, plaque index and gingival index increase percent age reduction in the second, third and fourth visits between patients using CHX 0.2% and ACV 50%

Parameters	**Visits**	**Percent Increase Reduction From First Visit**	***p*–value**

		(Mean ± SD)		
		CHX 0.25 n= 10	**ACV 50% n= 10**	
CPITN	Second	53.86 ± 19.49	53.93 ± 8.98	0.323 (NS)
	Third	77.43 ± 15.43	62.55 ± 10.51	<0.001
	Fourth	97.84 ± 3.41	70.98 ± 11.64	<0.001
Plaque Index	Second	61.33 ± 21.37	5.09 ± 11.84	<0.001
	Third	85.81 ± 8.38	10.73 ± 15.30	<0.001
	Fourth	98.48 ± 3.23	59.60 ± 15.09	<0.001
Gingival Index	Second	48.63 ± 25.6	7.50 ± 11.57	<0.001
	Third	81.50 ± 8.74	13.00 ± 17.43	<0.001
	Fourth	98.48 ± 3.23	39.00 ± 20.09	<0.001

CHX: Chlorhexidine; ACV: Apple cider vinegar; SD: Standard deviation; NS: No significant difference according to unpaired Z–test.

Table (3): Comparison of CPITN, plaque index and gingival index increase percent age reduction in the second, third and fourth visits between patients using CHX 0.2% and OLE and ACV 15%

Parameters	**Visits**	**Percent Increase Reduction From First Visit (Mean ± SD)**		***p*–value**
		CHX 0.25 (n= 10)	**ACV + OLE 15% (n= 10)**	
CPITN	Second	53.86 ± 19.49	44.27 ± 11.81	<0.001
	Third	77.43 ± 15.43	72.23 ± 10.62	<0.001
	Fourth	97.84 ± 3.41	92.54 ± 6.03	<0.001
Plaque Index	Second	61.33 ± 21.37	21.33 ± 13.16	<0.001
	Third	85.81 ± 8.38	44.24 ± 15.35	<0.001
	Fourth	98.48 ± 3.23	82.00 ± 8.98	<0.001
Gingival Index	Second	48.63 ± 25.6	48.70 ± 30.28	0.80 (NS)
	Third	81.50 ± 8.74	67.53 ± 27.75	<0.05
	Fourth	98.48 ± 3.23	95.03 ± 12.02	0.67 (NS)

CHX: Chlorhexidine; ACV: Apple cider vinegar; OLE: Olive leave extract; SD: Standard deviation; NS: No significant difference according to unpaired Z–test.

Table (4): Comparison of CPITN, plaque index and gingival index increase percent age reduction in the second, third and fourth visits between patients using CHX 0.2% and OLE and ACV 20%

Parameters	**Visits**	**Percent Increase Reduction From First Visit (Mean ± SD)**		***p*–value**
		CHX 0.25 (n= 10)	**ACV + OLE 20% (n= 10)**	
CPITN	Second	53.86 ± 19.49	45.60 ± 14.83	<0.01
	Third	77.43 ± 15.43	73.38 ± 8.95	<0.001
	Fourth	97.84 ± 3.41	93.83 ± 5.64	<0.001
Plaque Index	Second	61.33 ± 21.37	58.37 ± 14.16	0.12 (NS)

	Third	85.81 ± 8.38	64.59 ± 14.68	<0.001
	Fourth	98.48 ± 3.23	68.32 ± 12.28	<0.001
Gingival Index	Second	48.63 ± 25.6	39.04 ± 17.26	<0.01
	Third	81.50 ± 8.74	79.71 ± 10.34	0.40 (NS)
	Fourth	98.48 ± 3.23	95.92 ± 5.35	<0.01

CHX: Chlorhexidine; ACV: Apple cider vinegar; OLE: Olive leave extract; SD: Standard deviation; NS: No significant difference according to unpaired Z–test.

Table (5): Differences of CPITN among second, third and fourth visits of patients using different materials

Material Types	**Percent Increase Reduction From First Visit (n= 50) Mean ± SD**		
	Second Visit	**Third Visit**	**Fourth Visit**
CHX 0.2%	53.86 ± 19.49 [a]	77.43 ± 15.43 [b]	97.84 ± 3.41 [c]
ACV 50%	10.48 ± 15.68 [a]	39.7 ± 16.71 [b]	52.32 ± 26.48 [c]
OLE 50%	53.93 ± 8.98 [a]	62.55 ± 10.51 [b]	70.98 ± 11.64 [c]
OLE + ACV 15%	44.27 ± 11.81 [a]	72.23 ± 10.62 [b]	92.54 ± 6.03 [c]
OLE + ACV 20%	45.60 ± 14.83 [a]	73.38 ± 8.95 [b]	93.83 ± 5.64 [c]

CHX: Chlorhexidine; ACV: Apple cider vinegar; OLE: Olive leave extract; SD: Standard deviation.
Means with different letters horizontally have significant difference at $p \leq 0.05$ using paired Z–test.

Table (6): Differences of plaque index among second, third and fourth visits of patients using different materials

Material Types	**Percent Increase Reduction From First Visit (n= 50) Mean ± SD**		
	Second Visit	**Third Visit**	**Fourth Visit**
CHX 0.2%	61.33 ± 21.37 [a]	85.81 ± 8.38 [b]	98.48 ± 3.23 [c]
ACV 50%	9.26 ± 16.26 [a]	36.51 ± 20.34 [b]	54.81 ± 17.29 [c]
OLE 50%	5.09 ± 11.84 [a]	10.73 ± 15.30 [b]	59.60 ± 15.09 [c]
OLE + ACV 15%	21.33 ± 13.16 [a]	44.24 ± 15.35 [a]	82.00 ± 8.98 [a]
OLE + ACV 20%	58.37 ± 14.16 [a]	64.95 ± 14.68 [b]	68.32 ± 13.28 [b]

CHX: Chlorhexidine; ACV: Apple cider vinegar; OLE: Olive leave extract; SD: Standard deviation.
Means with different letters horizontally have significant difference at $p \leq 0.05$ using paired Z–test.

Table (7): Differences of gingival index among second, third and fourth visits of patients using different materials

Material Types	Percent Increase Reduction From First Visit (n= 50) Mean ± SD		
	Second Visit	Third Visit	Fourth Visit
CHX 0.2%	48.63 ± 25.6 [a]	81.50 ± 8.74 [b]	98.48 ± 3.23 [c]
ACV 50%	27.51 ± 20.43 [a]	29.80 ± 16.93 [a]	47.97 ± 19.69 [b]
OLE 50%	7.50 ± 11.57 [a]	13.00 ± 17.43 [b]	39.00 ± 20.09 [c]
OLE + ACV 15%	45.70 ± 30.28 [a]	97.53 ± 27.75 [b]	95.03 ± 12.02 [c]
OLE + ACV 20%	46.04 ± 17.26 [a]	79.71 ± 10.34 [b]	95.92 ± 5.35 [c]

CHX: Chlorhexidine; ACV: Apple cider vinegar; OLE: Olive leave extract; SD: Standard deviation.
Means with different letters horizontally have significant difference at $p \leq 0.05$ using paired Z–test.

Table (8): Comparison of CPITN, plaque index and gingival index among patients using different materials for measurements of four visits

Material Types	Increase Reduction From First Visit (n= 50) Mean ± SD		
	Second Visit	Third Visit	Fourth Visit
CHX 0.2%	76.38 ± 12.78 [a]	81.87 ± 10.99 [a]	76.2 ± 12.52 [a]
ACV 50%	34.14 ± 19.62 [d]	33.53 ± 17.96 [d]	35.09 ± 19.02 [b]
OLE 50%	62.49 ± 10.38 [c]	25.14 ± 14.08 [e]	19.38 ± 16.36 [c]
OLE + ACV 15%	69.68 ± 9.49 [b]	49.19 ± 12.49 [c]	70.42 ± 23.35 [a]
OLE + ACV 20%	70.94 ± 9.81 [b]	63.88 ± 14.04 [b]	71.56 ± 10.98 [a]

CHX: Chlorhexidine; ACV: Apple cider vinegar; OLE: Olive leave extract; SD: Standard deviation.
Means with different letters vertically have significant difference at $p \leq 0.05$ repeated measures analysis of variance and Duncan test.

Table (9): Inhibitory effect of olive leave extract 50% on number of Gram positive and negative bacteria
(Diameter of the inhibitory cycle is measured in mm)

Bacteriological Rate	Olive Leave Extract Concentration							Control	
	1%	1.6%	3.12%	6.25%	12.5%	25%	50%	Can	Amp
Staph. aureus	-	-	-	-	8	10	20	16	14
Strept. pyogens	-	-	-	-	-	12	16	15	13
Pseudo. aeruginosa	-	-	-	-	-	-	10	16	18
Kleb. pneumonia	-	-	-	-	12	14	18	18	20

Staph.: Staphylococcus; Strept.: Streptococcus; Pseudo.: Pseudomonas; Kleb.: Klebsiella.
Can: Canamycin; Amp: Ampicillin.

Table (10): Inhibitory effect of different concentrations of apple cider vinegar on number of Gram positive and negative bacteria
(Diameter of the inhibitory cycle is measured in mm)

Bacteriological Rate	Different Concentrations of Apple Cider Vinegar							Control	
	1%	1.6%	3.12%	6.25%	12.5%	25%	50%	Can	Amp
Staph. aureus	-	-	-	6	11	20	25	16	14
Strept. pyogens	-	-	-	-	8	16	20	15	13
Pseudo. aeruginosa	-	-	-	9	12	18	20	16	18
Kleb. pneumonia	-	-	-	-	-	9	15	18	20

Staph.: Staphylococcus; Strept.: Streptococcus; Pseudo.: Pseudomonas; Kleb.: Klebsiella.
Can: Canamycin; Amp: Ampicillin.

Table (11): Inhibitory effect for combination of olive leave extract and apple cider vinegar on number of Gram positive and negative bacteria
(Diameter of the inhibitory cycle is measured in mm)

Bacteriological Rate	Concentrations of Olive Leave Extract + Apple Cider Vinegar							Control	
	1%	1.6%	3.12%	6.25%	12.5%	25%	50%	Can	Amp
Staph. aureus	-	-	-	-	10	16	22	16	14
Strept. pyogens	-	-	-	8	13	18	21	15	13
Pseudo. aeruginosa	-	-	-	-	6	10	13	16	18
Kleb. pneumonia	-	-	-	-	8	12	16	18	20

Staph.: Staphylococcus; Strept.: Streptococcus; Pseudo.: Pseudomonas; Kleb.: Klebsiella.
Can: Canamycin; Amp: Ampicillin.

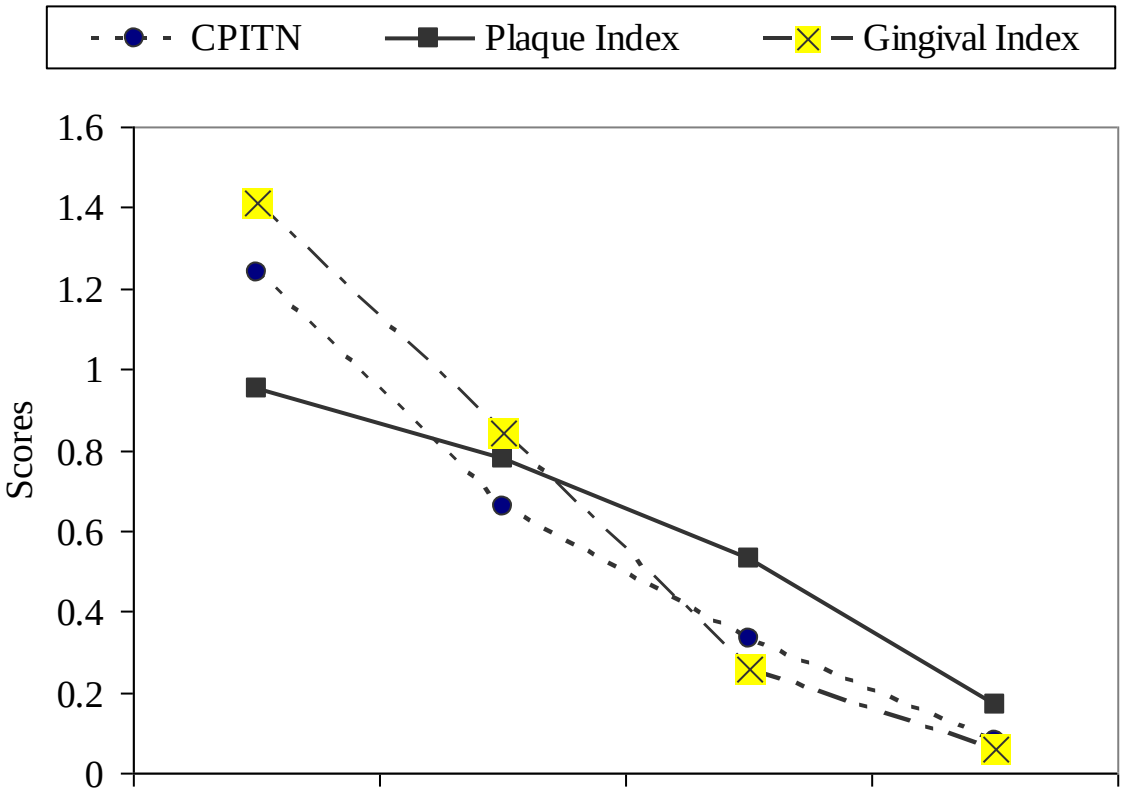

Figure (1): Mean reduction for CPITN, plaque index and gingival index from the first visit for olive leave extract + apple cider vinegar 20%

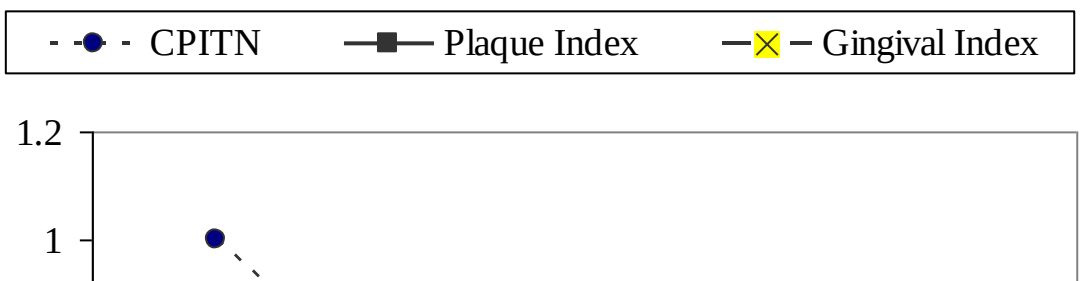

Figure (2): Mean reduction for CPITN, plaque index and gingival index from the first visit for olive leave extract + apple cider vinegar 15%

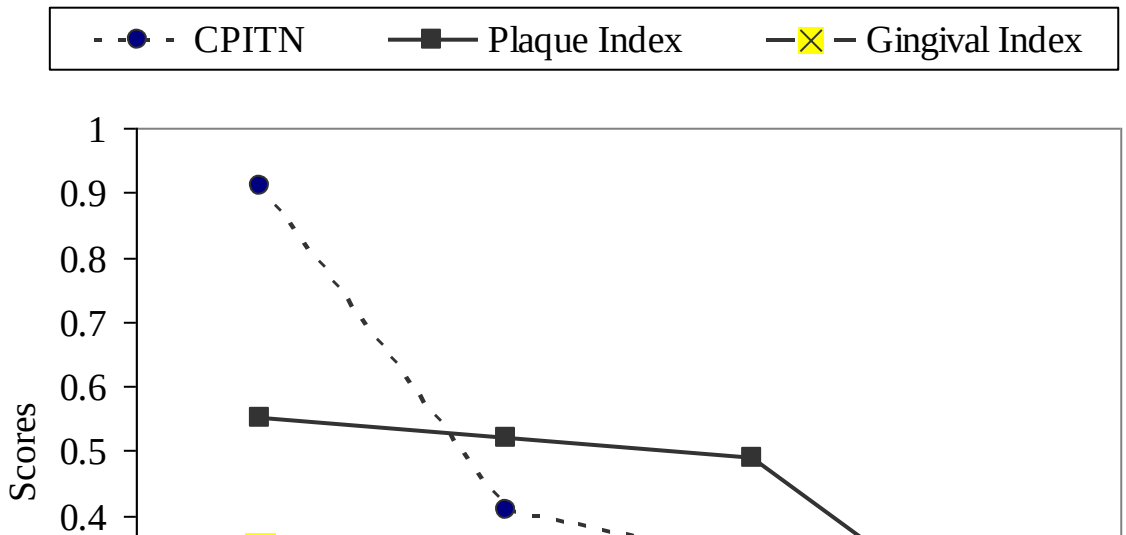

Figure (3): Mean reduction for CPITN, plaque index and gingival index from the first visit for apple cider vinegar 50%

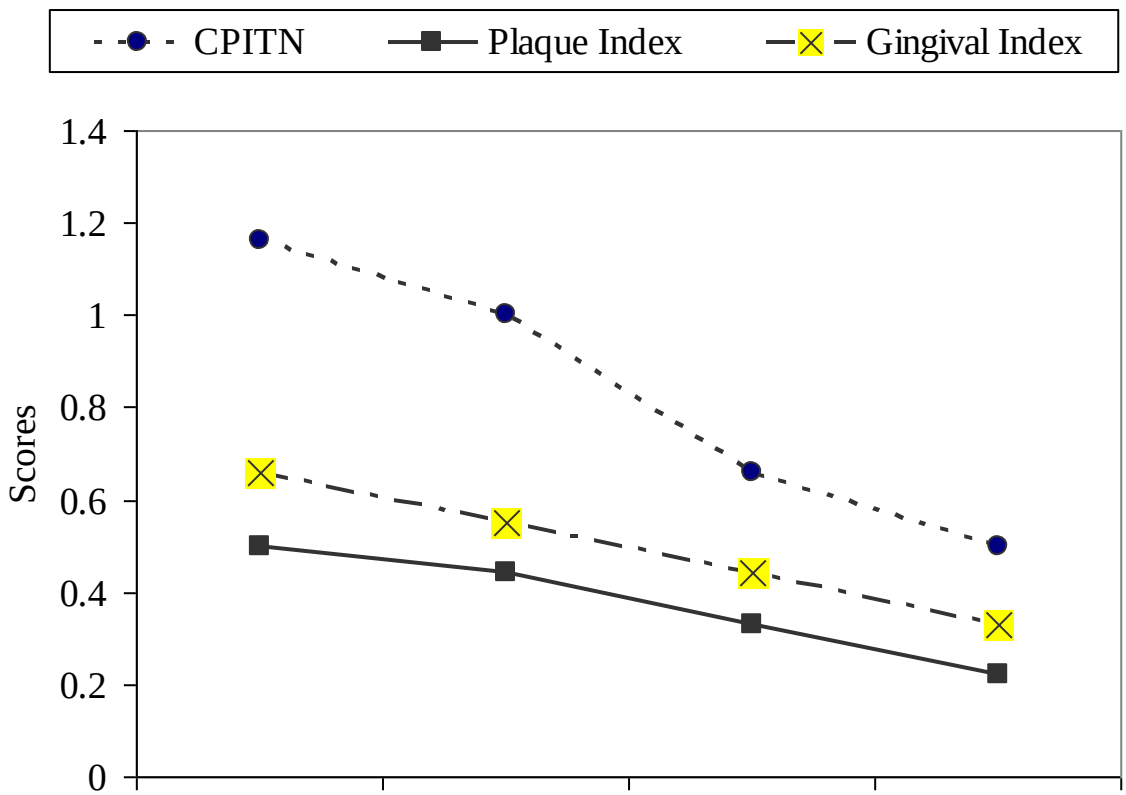

Figure (4): Mean reduction for CPITN, plaque index and gingival index from the first visit for olive leave extract 50%

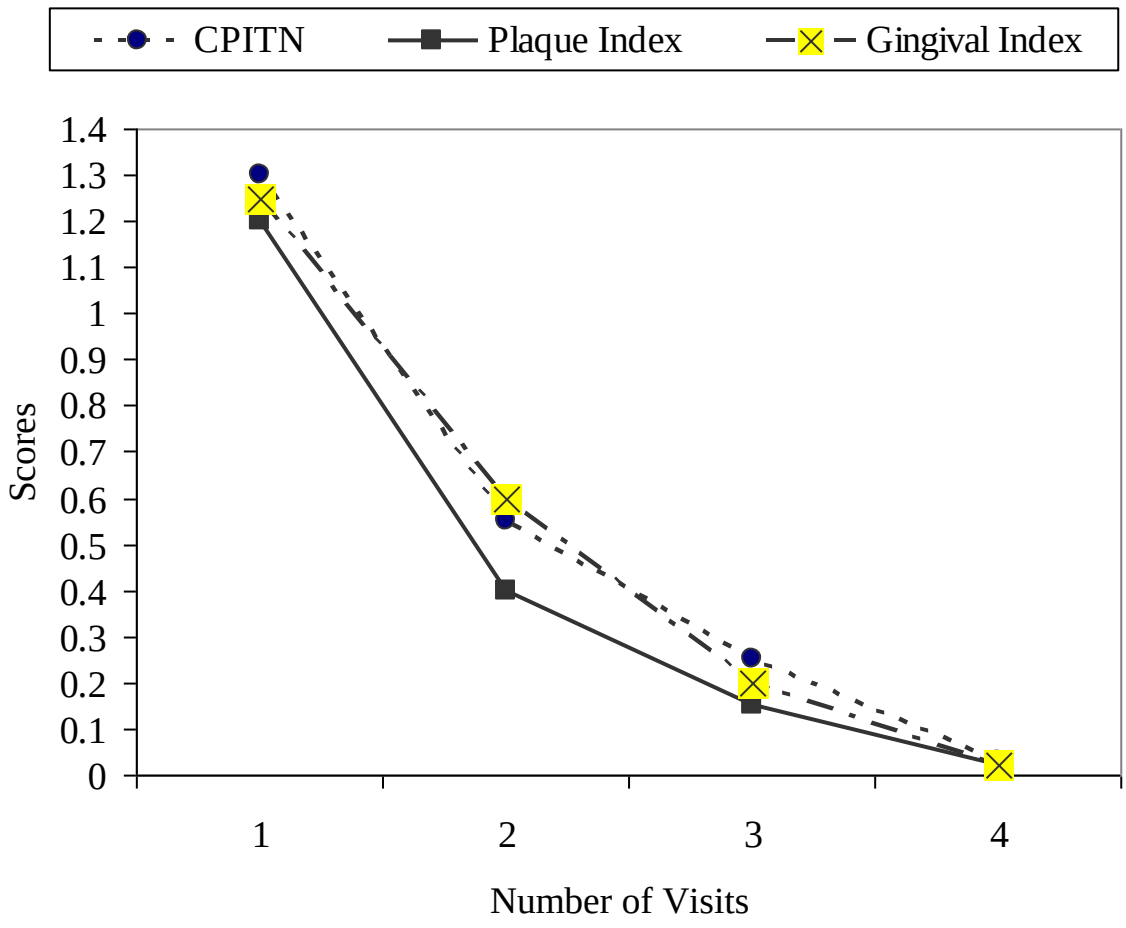

Figure (5): Mean reduction for CPITN, plaque index and gingival index from the first visit for Chlorhexidine 0.2%