International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

Efficacy of Garlic Extract as a Surfactant along with the Root Canal Sealer - An Invitro Study

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Abstract: ***Herbal medicines have become more popular due to their high antimicrobial, anti-inflammatory and biocompatible properties. When compared to the commercial root canal medicaments, they are biocompatible and less cytotoxic. The most important factors of root canal sealers are its ability to penetrate into the dentinal tubules thereby forming a physical barrier, thus adding a herbal surfactant improves the penetration. Hence this study was done to evaluate the effect of garlic extract as a surfactant along with the root canal sealer. Methodology: Fresh natural garlic bulbs were obtained blended and pressed with gauze and the filtrate was centrifuged and filtered using disposable syringe filter to obtained raw garlic extract. GROUP I – AH PLUS sealer, GROUP II- AH PLUS sealer + Garlic Extract. Flow test was performed for both the groups, 0.5ml of sealer was placed in the glass slab with a load of 100g on top. The diameters of the compressed disc sealer were measured using graduated scale and contact angle measurement test was done using contact angelometer. The angle between the sealer surface and the liquid was measured. For antimicrobial activity agar diffusion test and modified contact test was performed. Results: Addition of garlic extract as a surfactant with AH Plus sealer has shown better flow and wetting ability compared with AH Plus group alone.***

Keywords: antimicrobial, contact angle, garlic extract

1. Introduction

Herbal medicines and natural phytochemicals are proved to be good alternatives to the synthetic medicines because of their high antimicrobial, anti-inflammatory and biocompatible properties.[1] Numerous medicinal plants have been investigated for the prevention and treatment of various oral diseases. Garlic (Allium sativum) is one of the most widely investigated in the field of medicine since ancient times and proven to have antibacterial, antifungal, and antiviral properties.[2] Garlic has a wide spectrum activity against gram positive and gram negative organisms and also effective against multi drug resistant organisms. This is because when cloves of garlic are crushed or chopped it releases compound allicin by the enzymatic action of alliinase (cysteine sulfoxide lyase) on alliin. This allicin and other sulfur derived compounds of garlic are believed to possess therapeutic effects.[3]

Compared with the commercial root canal medicaments herbal medicines are biocompatible and less cytotoxic. The most important factors of root canal sealers is its ability to penetrate into the dentinal tubules thereby forming a barrier and adding a herbal surfactant that reduces the interfacial tension between two immiscible phases, [4] significantly improving the penetration of the sealer. Hence this study was done to evaluate the effect of garlic extract as a surfactant along with the root canal sealer.

2. Materials and Methodology

This in vitro study was conducted in the department of conservative dentistry and endodontics and department of microbiology at Krishnadevaraya college of dental sciences, Bangalore.

3. Sample Preparation

Fresh natural garlic (A. sativum) was obtained. It was then blended using sterilized mortar and pestle and squeezed using gauze. This extract was centrifuged at 10, 000 rpm for 20 min and then filtered with a 0.45-um sterile disposable filter to obtain raw garlic extract (GE) and stored at −20°C until use.[2]

4. Preparation of Endodontic Sealer

The endodontic sealer used in this study was AH Plus (Dentsply/ De Trey, Konstanz, Germany) Immediately before testing, all materials were prepared according to the manufacturers' instructions 1:1 (paste : paste, by volume) and the garlic extract was added 1:1 ratio by weight and mixed till homogenous consistency was achieved.

Grouping
Group I- AH plus sealer
Group II- AH plus sealer + Garlic extract

a) Flow test
An international standard was used to conduct the flow test: American Dental Association (ADA) specification no. 57.

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According to this specification, the sealers were spatulated until obtaining a homogeneous mixture, and 0.5 mL of each one was dropped on the center of a polished glass slab (100 g). Three minutes later, another glass plate was placed centrally on top of the sealer, followed by a weight giving a total mass of 120 g. Ten minutes after the weight was removed and the maximum and minimum diameters of the compressed discs of sealers were measured using a divider and graduated ruler. Five determinations were carried out and the mean value was calculated.

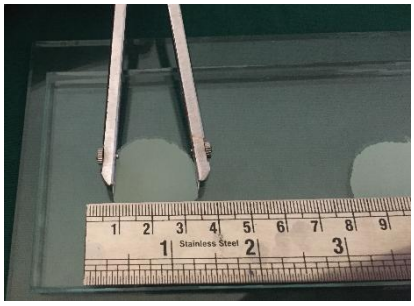


Figure 1: Flow Test

b) Contact Angle Measurement

The sealers were loaded in an insulin syringe, and controlled volume (0.1 mL) of each sealer was dispensed on glass surface through a nozzle. The contact angle was measured 30 minutes after the sealer was dispensed. The measurements were made using a dynamic contact angle analyzer, by imaging the sealer dispensed onto the surface being characterized. The captured images were analyzed using ZEN blue V3.0 software to determine the contact angle.

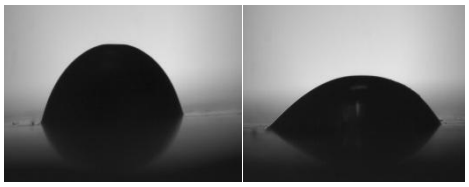


Figure 2: Contact Angle

c) Antimicrobial activity

Grouping of samples:

GROUP I – Saline (negative control)
GROUP II – CHX (positive control)
GROUP III - GE 100%
GROUP IV - GE 75%
GROUP V – AH PLUS
GROUP VI - AH PLUS + GE100%
GROUP VII - AH PLUS + GE75%

Agar diffusion test (ADT)

The antibacterial activity of *Enterococcus faecalis* (ATCC 29212) was evaluated. The microorganisms were cultivated in Brain Heart Infusion – BHI broth at 37°C for 18 h. Then, a bacterial suspension was prepared with 0.85% saline solution to match the turbidity equivalent to 0.5(1.5 x 108 CFU mL-1) McFarland standard tube. Thereafter, seven wells of 6 mm in diameter and 4 mm in depth (one for each material) were made with a punch by removing the agar at equidistant points and then filled immediately with the materials to be evaluated. All plates were maintained at room temperature for 2 h for pre diffusion of the materials and then incubated at 37ºC for 48 h under aerobic condition. The inhibition zones around each one of the wells were then measured using millimeter ruler. The results were recorded in terms of the average diameter of inhibition zone.

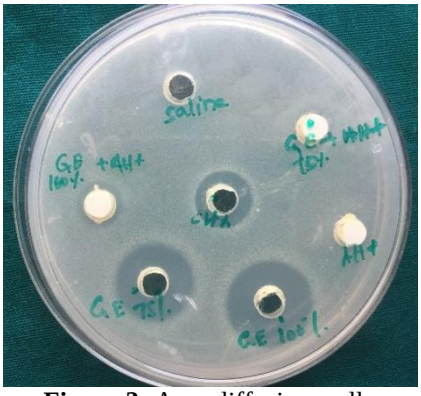


Figure 3: Agar diffusion wells

Modified direct contact test (DCT):

Sealer was mixed according to the manufacturer's instruction and it is coated on to the sterile ependorffs tube using sterile cement carrier and allowed to set for 24hrs. other materials to be evaluated are added to the tubes under aseptic conditions and 1ml of sterile BHI broth was added. 20ul of adjusted culture of E.fecalis was inoculated into the preparation. All the tubes were incubated at 37^{o}C for 24 hrs. after 24 hrs optical density(600nm) of each groups were measured using digital spectrophotometer and colony forming units were calculated according to Mc farland's 0.5 = 0.06 =1.5x10^{8} CFU/ml.

5. Statistical Analysis

Independent Student t Test was used to compare the mean flow test values (in mm) and contact angle (in degrees) between AH Plus & AH Plus sealer + garlic extract groups. The level of significance [P-Value] was set at P<0.05

6. Result

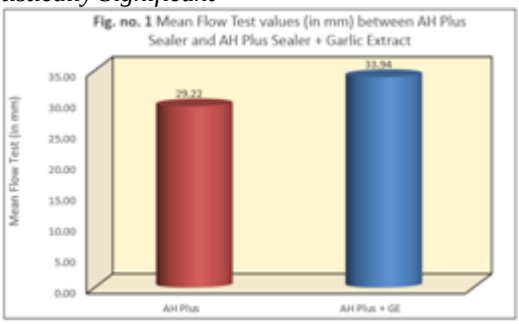
Flow test

Table 1: Comparison of mean flow test values between AH plus sealer and AHplus sealer + Garlic extract

Table no. 1 Comparison of mean Flow Test values (in mm) between AH Plus Sealer and AH Plus Sealer + Garlic Extract using Independent Student t Test

Variable	Sealer	N	Mean	SD	Mean Diff	t	P-Value
Flow Test	AH Plus	5	29.22	1.90	- 4.72	-4.230	0.003*
	AH Plus + GE	5	33.94	1.62			

* - *Statistically Significant*



Graph 1: Comparison of mean flow test values between AH plus sealer and AHplus sealer + Garlic extract

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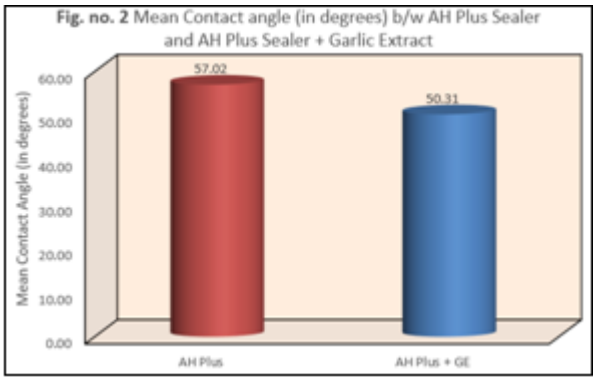
The above table and graph demonstrates the comparison of mean Flow test values (in mm) between AH Plus Sealer and AH Plus Sealer + Garlic Extract. The test results demonstrate that the mean Flow test values for AH Plus Sealer + Garlic Extract is significantly higher [33.94 ± 1.62] as compared to AH Plus Sealer alone [29.22 ± 1.90]. This mean difference in the flow test values between 02 groups was statistically significant at P=0.003.
[Refer Table no. 1 &graph. no. 1]

Contact angle test

Table 2: Mean contact angle between AH Plus Sealer and AH Plus Sealer + Garlic Extract

Comparison of mean Contact angle (in degrees) b/w AH Plus Sealer and AH PlusSealer+ Garlic Extract using Independent Student t Test							
Variable	Sealer	N	Mean	SD	Mean Diff	t	P-Value
Contact Angle	AH Plus	5	57.02	3.65	6.72	2.817	0.02*
	AH Plus+GE	5	50.31	3.88			

* - Statistically Significant



Graph 2: Comparison of mean contact angle (in degrees) between AH Plus Sealer and AH Plus Sealer + Garlic Extract

The above table demonstrates the comparison of mean contact angle (in degrees) between AH Plus Sealer and AH Plus Sealer + Garlic Extract. The test results demonstrate that the mean contact angle in AH sealer group [57.02 ± 3.65] was significantly higher as compared to the AH Plus Sealer + Garlic Extract group [50.31 ± 3.88]. This mean difference in contact angle between 02 groups was statistically significant at P=0.02 [Refer Table no. 2 &graph. no. 2]

Antimicrobial tests:
Agar well diffusion test & Modified direct contact test:

	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI	GROUP VII
INHIBITION ZONES (mm)	0	12	20	17	0	10	9
	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI	GROUP VII
OD (600nm) AFTER 1 DAY	0.643	0.048	0.019	0.022	0.032	0.009	0.012
CFU/ml	$16x10^8$	$1.2x10^8$	$0.45x10^8$	$0.54x10^8$	$0.79x10^8$	$0.22x10^8$	$0.3x10^8$

Table 3& 4: Comparison of antibacterial activity using agar well diffusion method and modified direct contact test.

The above table demonstrates the comparison of antibacterial activity using agar well diffusion method and modified direct contact test. The results for agar well diffusion method demonstrated the maximum antibacterial activity for group with 100% garlic extract followed by 75% garlic extract, chlorhexidine, AH plus + 100% garlic extract, AH plus + 75% garlic extract. Both saline group and AH plus group presented least antimicrobial activity. For modified direct contact test the maximum antibacterial activity were seen in AH plus + 100% garlic extract followed by AH plus + 75% garlic extract, 100% garlic extract, 75% garlic extract, AH plus only, chlorhexidine, and least for saline.

7. Discussion

A root canal sealer helps to seal irregularities in the root canal wall such as apical ramifications and deltas, as well as the spaces where the primary root filling material fails to reach.[5] A root canal sealer also acts as a binding agent between the root canal walls and the main root filling material. Thus, adequate flow and wetting are the important physicochemical properties of a sealer during root canal obturation. [5]

The adhesion of root canal sealers is mainly influenced by the relative surface free energy (wetting ability) of the intra radicular dentin surface.[6] Hence, the contact angle is a useful indicator regarding the wettability of a liquid. A liquid tends to spread on a solid surface, which is expressed in terms of the formation of contact angle. When the contact angle is less than 90°, the liquid wets the substrate; if it is greater than 90°, it is said to be non-wetting. A zero contact angle represents complete wetting. Low contact angles are generally associated with a better interaction between a solid surface and a liquid.

The main active component of garlic is allicin which destroys the cell wall and cell membrane of root canal bacteria. Allicin has ability to prevent both germination of spores and growth of hyphae.[7] Concentrated garlic extract (95%) contains 34% allicin, 44% total thio sulfinates, and 20% vinyldithiins which is believed to be responsible for antimicrobial activity.

In our study, syringe driven filters are used thereby maintaining the sterility of solution as normal microorganism such as bacteria (bacterial size ranges from 1 µm to about 5 µm) cannot pass through the pores of filters.[7] Despite the fact that addition of antimicrobial to the sealer is desirable, changes in the sealer composition may affect its physical and their chemical properties which in turn may affect the long term stability. [8]

According to ANSI/ADA specifications no 57, root canal sealers should have a flow of minimum 20mm diameter disk. In this study addition of garlic extract to ah plus sealer has increased the flow of the sealer compared with the control group. This might be because of the reduced viscosity due to addition of garlic extract.[8]

In this study the measurements for contact angle were reduced with the addition of the garlic extract. Lower the contact angle, the more hydrophilic the substrate, this in turn helps in the penetration of the sealer into the finer

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anatomical spaces of the root canal system thereby positively affecting its anti microbial action in dentine.

The anti microbial activity of the sealer was assessed by means of direct contact test and agar well diffusion method which provides both quantitative and reproducible approach to simulate the contact of micro organisms with the endodontic sealer, providing information on the bactericidal effect. In this current study maximum zone of inhibition was observed in the group with 100% garlic extract alone followed by 75% garlic extract than the other groups with the addition of garlic extract and AH PLUS sealer. This might be due to the setting nature of the sealer which limits the diffusion of the test material into the agar wells. In modified direct contact test least number of CFU(ie: high antimicrobial activity) were found in the group with 100% garlic extract + AH PLUS followed by 75% garlic extract + AH PLUS and AH PLUS only group when compared to 100% and 75% garlic extract and highest CFU was seen in saline group.

The ANSI/ADA standard requires that the setting time of a sealer shall be within 10% of that stated by the manufacturers. In this study, addition of garlic extracts to AH Plus has delayed the setting reaction and is in agreement with ANSI/ADA standards.

As shown in this study addition of garlic extract to AH Plus sealer has shown better flow, contact angle and antimicrobial activity. However more research has to performed in order to understand the physico mechanical characteristics of the sealer added with garlic extract. The present study had same limitations such as the efficacy of the extracts, which was observed for a short duration of time. Further studies can be undertaken to explore the antimicrobial efficacy of endodontic sealers mixed with herbal extracts for a longer duration of time.

8. Conclusion

Within the limitations of this study, addition of garlic extract to AH PLUS sealer showed increased wetting ability and antimicrobial efficacy when compared to ah plus group alone.

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