Journal of Advanced Medical and Dental Sciences Research

@Society of Scientific Research and Studies ***NLM ID:*** *101716117*

Journal home page: www.jamdsr.com doi: 10.21276/jamdsr Indian Citation Index (ICI) Index Copernicus value = 100.00

(e) ISSN Online: 2321-9599; (p) ISSN Print: 2348-6805

ORIGINAL RESEARCH

Comparative evaluation of antimicrobial efficacy of clove and thyme essential oil against Enterococcus faecalis: An *in vitro* study

Tina Agarwal[1], Ramakrishna Yeluri[2]

[1]Ph.D. Scholar Department of Pediatric and Preventive Dentistry, Teerthanker Mahaveer Dental College and Research Centre, Moradabad, Uttar Pradesh, India

[2]Professor and Head of the Department Department of Pediatric and Preventive Dentistry, Teerthanker Mahaveer Dental College and Research Centre, Moradabad, Uttar Pradesh, India

ABSTRACT

Background: It's since the ancient times that essential oils have been used for the treatment of various types of infectious diseases and E. faecalis being a multidrug resistant microorganism is the main causative factor for the failure of the root canal therapy. So, this study aimed for comparative evaluation between antimicrobial efficacy of thyme and clove essential oil against E. faecalis.

Method: This is an in vitro study in which separation of the aqueous (AqEO) and non aqueous phases (MiEO) was done for both thyme and clove oil in a Sonicator water bath. After this evaluation of antimicrobial efficacy was done by agar diffusion method. Data was obtained by measuring zone of inhibition in millimeters and was analysed by the Statistical Software STATA – 13.

Results: A significant mean difference was found between both thyme and clove MiEO. Zone of inhibition was absent for thyme (AqEO), thus its mean value was scored as zero, for clove (AqEO) mean value was found to be 13.67, and thus a significant mean difference was found between both the groups for (AqEO).

Conclusion: MiEO of both thyme and clove showed better antimicrobial efficacy against E. faecalis as compared to AqEO of thyme and clove.

Keywords: Antimicrobial efficacy, Essential oil, Enterococcus faecalis.

Received: 14, April 2023 Accepted: 18 July, 2023

Corresponding author: Tina Agarwal, Ph.D. Scholar Department of Pediatric and Preventive Dentistry, Teerthanker Mahaveer Dental College and Research Centre, Moradabad, Uttar Pradesh, India

This article may be cited as: Agarwal T, Yeluri R. Comparative evaluation of antimicrobial efficacy of clove and thyme essential oil against Enterococcus faecalis: An *in vitro* study. J Adv Med Dent Scie Res 2023;11(7):28-31

INTRODUCTION

Essential oils, with another name as volatile oils are the sources of natural products[1]. These are complicated mixtures of hydrocarbons and their derivatives, derived from oxygenation of two different isoprenoid pathways[2]. Basically, these are aromatic oily liquids obtained from different plant materials such as buds, seeds, herbs, twigs, wood, fruits, bark, roots, leaves and flowers.[1]

Volatile oils are comprised of different kind of biological efficacies such as antioxidant, antimicrobial, antibacterial, antiviral and insecticidal[3]. Successfully used for treatment of pain associated with chronic illness or associated with some medical procedures[4], also for reducing postoperative nausea[5], for possible symptom relief in cancer[6,7] and even it also helps to treat pediculosis in children[8]. These activities are due to a number of terpenes and terpenoids along with their oxygenated and aromatic compounds[9].

Some of these compounds parts are hydrosoluble and some of them are hydrophobic. Even the terpenes are water-repellent in nature, but some of them can be diluted in water due to their different anatomical morphology and different mixing temperatures. But terpenoids have higher water solubility than terpenes[10].

Antimicrobial efficacy of essential oils has shown an increase in interest in previous years and has been exhibited to be the most effective alternative on those strains which are resistant to multiple drugs.[11]

E. faecalis is that pathogen which can survive in extreme environment and it can vary from hazardous disease to a less severity disease in any individual.[12] It is one of the top three nosocomial infectious pathogen[13] or strain[14] and impervious to presently available drugs posing a true analeptic threat.[15] It colonizes the root canal of teeth and sustain without the support of other bacteria.[16,17] It's is a highly resilient microorganism; it plays a major role in the reinfection of root canals even after the completion of endodontic procedures.[18]

So, in this study, good effect of thyme and clove essential oil has been presented on E. faecalis bacterial strain.

METHOD

Enterococcus faecalis MTCC 439 was obtained from Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India.

Armamenterium

Thyme essential oil, Clove essential oil, Amoxicillin antibiotic disc (Hi-Media) as positive control, Distilled water as a negative control, Test microorganism: Enterococcus faecalis (MTCC 439), Mueller Hinton broth, Mueller Hinton agar, Micropipetes, Glass plates, laminar air flow chamber, Sonicator water bath (IGene Labserve Pvt. Ltd. New Delhi). Carbon dioxide incubator (Candle jar) and Petri dishes.

Culturing and sub culturing of test microorganism

Strain of *Enterococcus faecalis* (MTCC 439) was taken and added to 5ml Brain Heart Infusion broth (BHI). Incubation is done at 37°C for 24 hrs. Density of broth was adjusted to 0.5 McFarland opacity standard scales. Procedure was carried out in laminar flow chamber to prevent contamination. Sub-culturing was done by taking microbial colonies from the nutrient broth and uniformly spreading it on Mueller Hinton agar plates.

Preparation of working solutions: By broth micro dilution method[11]

As the volatile oils are hydrophobic in nature, so, the purpose of the study was to derive a homogenous aggregate of micelle comprising of essential oil and water that could be easily diffused in the water-based liquid culture medium. For this sterile water and essential oil (about 2 ml) was mixed in microcentrifuge tubes and the micelles (MiEO) were acquired by sonication at 40 kHz, 20 minutes and at 25°C, in a Sonicator water bath (IGene Labserve Pvt. Ltd. New Delhi). After separation of the aqueous and non aqueous phases (micelles) (MiEO), the recovery of bottom aqueous phase was done and it was further used as the second working solution of essential oil (AqEO).

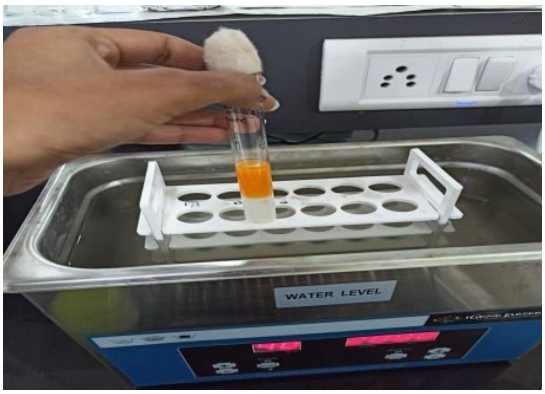
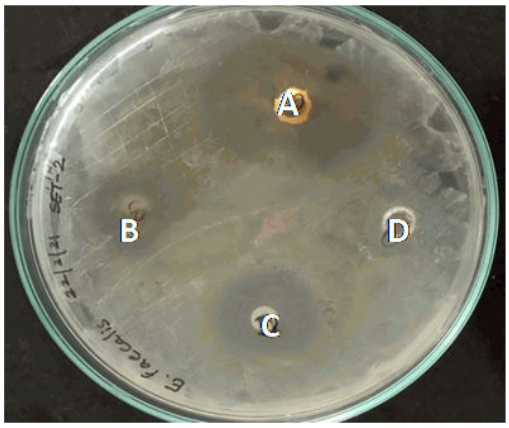


FIGURE 1: Aqueous (AqEO) (bottom) and non aqueous (micelles) (MiEO) (top) in a sonicator water bath (IGene Labserve Pvt. Ltd. New Delhi).

Determination of antimicrobial efficacy by agar diffusion method[19]

Microbial colonies of E. faecalis were picked from the nutrient broth. With the help of swab and lawn technique the E. faecalis colonies were uniformly distributed over agar plates. Open end of 6 mm diameter micropipette was used to punch the holes for the preparation of agar wells. Four agar wells were prepared at equidistant points on each agar plates. Both hydrophobic (MiEO) and hydrophilic (AqEO) parts of Thyme oil were placed separately on agar wells of two agar plates and were labeled as A. Hydrophobic (MiEO) and hydrophilic (AqEO) parts of Clove oil were placed separately on agar wells of two agar plates and were labeled as B. Amoxicillin antibiotic disc (positive control) was labelled as C on both the agar well plates. Distilled water (negative control) was labelled as D on both the agar well plates. Both the agar well plates were kept in carbon dioxide incubator at 37ºC for 24 hrs. After 24hrs, zones of inhibition around each sample were measured in millimeters. The procedure was repeated three times. Zones having wider diameters for a sample were considered to have greater antimicrobial activity against E. faecalis.



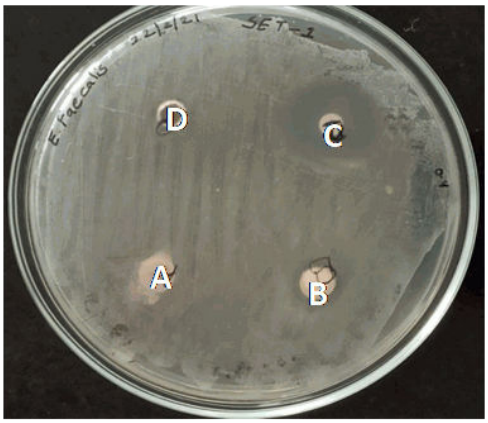


FIGURE 2 (A):

A: Thyme essential oil (Micelles part)

B: Clove essential oil (Micelles part)

C. Amoxicillin antibiotic disc (positive control)

D. Distilled water (negative control)

FIGURE 2 (B):

A: Thyme essential oil (Aqueous part)

B: Clove essential oil (Aqueous part)

C. Amoxicillin antibiotic disc (positive control)

D. Distilled water (negative control)

Figure 2: Zone of inhibition of Thyme and Clove (MiEO and AqEO) with amoxicillin antibiotic disc (positive control) and Distilled water (negative control)

Statistical analysis

The Statistical Software STATA – 13 was used for the statistical analysis. Mean and standard deviation was estimated for both different essential oils by Independent T-Test. The mean values were compared by descriptive statistics and one-way Analysis of Variance (ANOVA) then appropriately followed by Post-hoc tukey test for multiple comparisons between essential oils. In the present study, the level of significance was set at p = 0.05 and any p – value less than 0.05 is considered as significant.

RESULTS

Data was collected by measuring zone of inhibition diameters in millimeters. Mean zone of inhibition values between thyme MiEO and AqEO (Table 1) and clove MiEO and AqEO (table 2) showed significant difference between the two phases. Mean value for thyme MiEO was found to be 30.67, for clove MiEO 20.33 (Table 3), thus a significant mean difference was present between both the groups for MiEO (Table 4, 5). No zone of inhibition was found for thyme (AqEO), thus its mean value was scored as zero, for clove (AqEO) mean value was found to be 13.67 (Table 6), thus a significant mean difference was present between both the groups for (AqEO) (Table 7,8).

Table 1: Comparison of mean zone of inhibition between Thyme MiEO and Thyme AqEO using Independent T-Test

Group	Mean	SD	t-value	p-value	95% Confidence Interval	
					Lower Limit	Upper Limit
MiEO	30.67	0.577	92.000	<0.001 (S)	29.741	31.592
AqEO	0	0				

S = Significant

Table 2: Comparison of mean zone of inhibition between Clove MiEO and Clove AqEO using Independent T-Test

Group	Mean	SD	t-value	p-value	95% Confidence Interval	
					Lower Limit	Upper Limit
MiEO	20.33	0.577	14.142	<0.001(S)	5.358	7.975
AqEO	13.67	0.577				

S = Significant

Table 3: Descriptive Statistics for comparison of mean values for zone of inhibition after 24 hours of Micelles Phase

Group	N	Mean	SD	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Thyme	3	30.67	0.577	29.23	32.10
Clove	3	20.33	0.577	18.90	21.77
Amoxicillin	3	24.33	0.577	22.90	25.77
Distilled Water	3	0	0	0	0

Table 4: One-way Analysis of Variance (ANOVA) of zone of inhibition after 24 hours of Micelles Phase

	Sum of Squares	Degree of freedom (df)	Mean Square	F	P-value
Between Groups	1581.667	3	527.222	2108.889	<0.001
Within Groups	2.000	8	0.250		
Total	1583.667	11			

Table 5: Tukey's Post Hoc Test for multiple comparisons of zone of inhibition after 24 hrs of Micelles Phase

Group		Mean Difference (* Significant)	P-value	95% Confidence Interval	
				Lower Bound	Upper Bound
Thyme	Clove	10.333*	<0.001	9.03	11.64
	Amoxicillin	6.333*	<0.001	5.03	7.64
	Distilled Water	30.667*	<0.001	29.36	31.97
Clove	Amoxicillin	-4.000*	<0.001	-5.31	-2.69
	Distilled Water	20.333*	<0.001	19.03	21.64
Amoxicillin	Distilled Water	24.333*	<0.001	23.03	25.64

Table 6: Descriptive Statistics for comparison of mean for zone of inhibition after 24 hrs of Aqueous Phase (AqEO):

Group	N	Mean	SD	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Thyme	3	0	0	0	0
Clove	3	13.67	0.577	12.23	15.10
Amoxicillin	3	24.33	0.577	22.90	25.77
Distilled Water	3	0	0	0	0

Table 7: One-way Analysis of Variance (ANOVA) of comparison of zone of inhibition after 24 hrs of Aqueous Phase (AqEO):

	Sum of Squares	Degree of freedom (df)	Mean Square	F	P-value
Between Groups	1253.667	3	417.889	2507.333	<0.001
Within Groups	1.333	8	.167		
Total	1255	11			<0.001

Table 8: Tukey's Post Hoc Test for multiple comparisons of comparison of zone of inhibition after 24 hrs of Aqueous Phase (AqEO):

Group		Mean Difference (* Significant)	P-value	95% Confidence Interval	
				Lower Bound	Upper Bound
Thyme	Clove	-13.667*	<0.001	-14.73	-12.60
	Amoxicillin	-24.333*	<0.001	-25.40	-23.27
	Distilled Water	0	1.000	-1.07	1.07
Clove	Amoxicillin	-10.667*	<0.001	-11.73	-9.60
	Distilled Water	13.667*	<0.001	12.60	14.73
Amoxicillin	Distilled Water	24.333*	<0.001	23.27	25.40

DISCUSSION

Thyme: Another name as Thymus vulgaris is a shrub. It has life span of more than two years (perennial). Its leaves are full of fragrance and are a mixture of green and gray color. It belongs to Family: Lamiaceae. Its main origin is from Europe (Southern part) and from Countries which are at border areas of Mediterranean, but, now it is easily available all over the globe specifically in areas which comes under temperate climates or moderate rainfalls.[20] It has anti-inflammatory, antioxidant, antibacterial, antifungal, analgesic, antispasmodic, antiseptic and antitumor properties.[21]

Clove: Another name as Syzygium aromaticum. As the name says it is an aromatic plant which belongs to Family: Myrtaceae and basically it's a native of Indonesia.[22] It has different properties such as antibacterial, antifungal, analgesic, antiviral, anti-inflammatory, antiplasmodial, anticancer, antioxidant, anti-ulcerogenic, anti-mutagenic and anti-genotoxic.[23] Eugenol is the major component of clove oil, and is acquired from the species named as *Eugenia caryophyllata.*[24] Eugenol also comes under classification of Food and Drug Administration (FDA) of United States as 'Generally Recognized As Safe (GRAS)'.[25]

According to the study results, the thyme MiEO showed better antimicrobial activity against E. faecalis as compared to clove MiEO. This could be in accordance with the study conducted by Liu F et al (2020)[26] in which they have shown that thyme oil with different concentrations can show an effective antibiofilm and germicidal activity against E. faecalis. On the other hand, clove AqEO showed some antimicrobial activity against E. faecalis, however there was no antimicrobial activity was present for thyme AqEO against E. faecalis. This result could be half contradictory according to the study conducted by Nagy-Bota MC (2021)[27] in which AqEOs of both thyme and clove showed good antibacterial effect against E. faecalis.

In this study 30 mcg Amoxicillin disc was used as positive control which showed good antibacterial activity against E. faecalis which is in accordance with the Garlapati R et al (2016) who conducted a study for the evaluation of the antibacterial ability of 30mcg Augmentin disc against *E.faecalis* and concluded that 30mcg Augmentin showed maximum antimicrobial activity against *E.faecalis* among other samples[28].

CONCLUSION

- Both Micelle (MiEO) phase of thyme and clove showed significant antimicrobial efficacy against E. feacalis, but thyme MiEO was found to be more effective against E. faecalis than clove MiEO.
- Aqueous (AqEO) phase of clove showed a significant antimicrobial efficacy against E. feacalis, however, thyme AqEO showed no antimicrobial activity against E. faecalis.
- Thus, the study concluded that MiEO of both thyme and clove showed better antimicrobial efficacy against E. faecalis as compared to AqEO of thyme and clove.

However, though the essential oils are safe, effective, pocket friendly and free of side effects, there is requirement of more extensive in vitro and in vivo studies on larger samples to evaluate its antimicrobial efficacy.

REFERENCES

1. Irshad M, Subhani MA, Ali S, Hussain A. Biological Importance of Essential Oils. In: El-Shemy HA, editor. Essential Oils - Oils of Nature. London: Intech Open 2020: 1-14. doi: http://dx.doi.org/10.5772/intechopen.87198.
2. Sharifi-Rad J, Sureda A, Tenore GC, Daglia M, Sharifi-Rad M, Valussi M et al. Biological Activities of Essential Oils: From Plant Chemoecology to Traditional Healing Systems. Molecules 2017; 22(1): 70. doi: 10.3390/molecules22010070. PMID: 28045446; PMCID: PMC6155610.
3. Agarwal T, Yeluri R. Herbal Formulations in Dentistry: Review. EJMCM 2023; 10(1): 1888-1897.
4. Mahboubi M. Mentha spicata as natural analgesia for treatment of pain in osteoarthritis patients. Complement Ther Clin Pract 2017; 26: 1-4. doi: 10.1016/j.ctcp.2016.11.001. Epub 2016 Nov 2. PMID: 28107842.

5. Bikmoradi A, Khaleghverdi M, Seddighi I, Moradkhani S, Soltanian A, Cheraghi F. Effect of inhalation aromatherapy with lavender essence on pain associated with intravenous catheter insertion in preschool children: A quasi-experimental study. Complement Ther Clin Pract 2017; 28: 85-91. doi: 10.1016/j.ctcp.2017.05.008. Epub 2017 May 24. PMID: 28779942.
6. Shin ES, Seo KH, Lee SH, Jang JE, Jung YM, Kim MJ, Yeon JY. Massage with or without aromatherapy for symptom relief in people with cancer. Cochrane Database Syst Rev. 2016; (6):CD009873. doi: 10.1002/14651858.CD009873.pub3. PMID: 27258432.
7. Ho, S.S.M.; Kwong, A.N.L.;Wan, K.W.S.; Ho, R.M.L.; Chow, K.M. Experiences of aromatherapy massage among adult female cancer patients: A qualitative study. J Clin Nurs 2017; 26: 4519–4526. https://doi.org/10.1111/jocn.13784
8. Greive, K.A.; Barnes, T.M. The efficacy of Australian essential oils for the treatment of head lice infestation in children: A randomised controlled trial. Australas J Dermatol 2018; 59: e99–e105. https://doi.org/10.1111/ajd.12626.
9. Tongnuanchan P, Benjakul S. Essential oils: extraction, bioactivities, and their uses for food preservation. J Food Sci 2014; 79(7): R1231-49. doi: 10.1111/1750-3841.12492. Epub 2014 Jun 2. PMID: 24888440.
10. Martins MAR, Silva LP, Ferreira O, Schroder B, Coutinho JAP, Pinho SP. Terpenes solubility in water and their environmental distribution. J Mol Liq 2017; 241: 996–1002. https://doi.org/10.1016/j.molliq.2017.06.099.
11. Man A, Santacroce L, Jacob R, Mare A, Man L. Antimicrobial Activity of Six Essential Oils Against a Group of Human Pathogens: A Comparative Study. Pathogens. 2019; 8(1):15. PMID: 30696051; PMCID: PMC6471180.
12. Kayaoglu G and Orstavik D. Virulence Factors of *Enterococcus Faecalis*: Relationship to Endodontic Disease. Crit Rev Oral Biol Med 2004; 15(5):308-320. doi: 10.1177/154411130401500506. PMID: 15470268.
13. Kapadia SP, Pudakalkatti PS, Shivanaikar S. Detection of antimicrobial activity of banana peel (*Musa paradisiaca* L.) on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*: An *in vitro* study. Contemp Clin Dent 2015; 6(4): 496-499. doi: 10.4103/0976-237X.169864. PMID: 26681854; PMCID: PMC4678547.
14. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in combined medical-surgical intensive care units in the United States. Infect Control Hosp Epidemiol 2000; 21(8): 510-515. doi: 10.1086/501795. PMID: 10968716.
15. Wisplinghoff H, Seifert H, Tallent SM, Bischoff T, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in pediatric patients in United States hospitals: epidemiology, clinical features and susceptibilities. Pediatr Infect Dis J 2003; 22(8): 686-691. doi: 10.1097/01.inf.0000078159.53132.40. PMID: 12913767.
16. Sundqvist G. Associations between microbial species in dental root canal infections. Oral Microbiol Immunol 1992; 7(5): 257-262. doi: 10.1111/j.1399-302x.1992.tb00584.x. PMID: 1494447.
17. Fabricius L, Dahlen G, Holm SE, Moller AJ. Influence of combinations of oral bacteria on periapical tissues of monkeys. Scand J Dent Res 1982; 90(3): 200-206.
18. Kaur A, Shah N, Logani A, Mishra N. Biotoxicity of commonly used root canal sealers: A meta-analysis. J Conserv Dent 2015; 18(2): 83–88.
19. Navit S, Jaiswal N, Khan SA, Malhotra S, Sharma A, Mukesh et al. Antimicrobial Efficacy of Contemporary Obturating Materials used in Primary Teeth- An In-vitro Study. J Clin Diagn Res 2016; 10(9): ZC09-ZC12.
20. Singletary, Keith PhD. Thyme: History, Applications, and Overview of Potential Health Benefits. Nutrition Today 2016; 51(1): 40-49. doi: 10.1097/NT.0000000000000139.
21. Nagoor Meeran MF, Javed H, Al Taee H, Azimullah S, Ojha SK. Pharmacological Properties and Molecular Mechanisms of Thymol: Prospects for Its Therapeutic Potential and Pharmaceutical Development. Front Pharmacol 2017; 8: 380. doi: 10.3389/fphar.2017.00380.
22. Cortés-Rojas DF, de Souza CR, Oliveira WP. Clove (Syzygium aromaticum): a precious spice. Asian Pac J Trop Biomed 2014; 4(2):90-96. doi: 10.1016/S2221-1691(14)60215-X.
23. Kamatou GP, Vermaak I, Viljoen AM. Eugenol--from the remote Maluku Islands to the international market place: a review of a remarkable and versatile molecule. Molecules 2012; 17(6): 6953-6981. doi: 10.3390/molecules17066953.
24. Zheng GQ, Kenney PM, Lam LKT. Sesquiterpenes from clove (*Eugenia caryophyllata*). J Nat Prod 1992; *55*: 999–1003. doi: 10.1021/np50085a029.
25. Friedman M. Chemistry, Antimicrobial Mechanisms, and Antibiotic Activities of Cinnamaldehyde against Pathogenic Bacteria in Animal Feeds and Human Foods. J Agric Food Chem 2017; 65(48):10406-10423. doi: 10.1021/acs.jafc.7b04344. Epub 2017 Nov 20. PMID: 29155570.
26. Liu F, Jin P, Gong H, Sun Z, Du L, Wang D. Antibacterial and antibiofilm activities of thyme oil against foodborne multiple antibiotics-resistant Enterococcus faecalis. Poult Sci 2020; 99(10): 5127-5136. doi: 10.1016/j.psj.2020.06.067. Epub 2020 Jul 24. PMID: 32988551; PMCID: PMC7598324.
27. Nagy-Bota MC, Man A, Santacroce L, Brinzaniuc K, Pap Z, Pacurar M et al. Essential Oils as Alternatives for Root-Canal Treatment and Infection Control against *Enterococcus faecalis*—A Preliminary Study. Appl Sci 2021; 11(4):1422. https://doi.org/10.3390/app11041422
28. Garlapati R, Venigalla BS, Surakanti JR, Thumu J, Chennamaneni KC, Kalluru RS. Comparison of the Antimicrobial Efficacy of Two Antibiotics Sparfloxacin and Augmentin as Experimental Root Canal Irrigating Solutions against Enterococcus faecalis - An Invitro Study. J Clin Diagn Res. 2016; 10(3):ZC57-60.