IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 21, Issue 9 Ser.2 (September. 2022), PP 25-31
www.iosrjournals.org

POTENCY OF OKRA FRUITS (*Abelmoschus esculentus*) AS ANTIBACTERIAL AGENT AGAINST *Enterococcusfaecalis* ATCC®29212™ (In Vitro)

Filia Dana Tyasingsih[1], AtikaResti Fitri[1], Ika Astrina[1], AldytaEmirsyalRianto Chaniago[2]

[1]*Department of Oral Biology, Faculty of Dentistry, Universitas Sumatera Utara,Indonesia)filiadana19@gmail.com, atikaresti.fitri@usu.ac.id, ika_a65@yahoo.com*
[2]*Undergraduate Student, Faculty of Dentistry, Universitas Sumatera Utara*.

ABSTRACT
Introduction: *Pulp infection is mainly induced by anaerobic bacteria. Enterococcus faecalis(E. faecalis) has been investigated to cause the failure of root canal treatment. The long-term use of the chemical antibacterial agent, including chlorhexidine, may lead to bacterial resistance. Okra fruits (Abelmochus esculentus) containing various active compounds such as flavonoids, saponins, steroids, alkaloids, tanninshave been known to have antibacterial properties. Therefore, this study aimed to investigate the effect of okra fruits extract against E. faecalisATCC® 29212™.*
Methods:*As many as 3 kilograms of okra fruits were dried and extracted by using 12 liters ethanol solvent. The extract was diluted in Mueller Hinton Broth (MHB) into 7 different groups:I (extract of okra10%); II (extract of ocra 15%); III (extract of ocra 20%); IV (extract of ocra 25%); V (positive control I, Chlorhexidine 2%); VI (positive control II, Vancomycin); and VII (negative control, DMSO), with 6 samples each and then compare the change of turbidity with 0.5 Mc. Farland. After serial dilution, It was dropped on Mueller Hinton Agar (MHA) and the number of bacterial colonies was counted in CFU/ml.A dilution test was used to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). A diffusion test method was performed to measure the formation of the inhibition zone surrounding the disk paper. One way ANOVA was used to determine the significant difference among groups (p<0,05).*
Results:*It showed that the okra fruit extracts with concentrations of 10%, 15%, 20%, 25% were not found to have the MIC and MBC valuesagainst E. faecalis ATCC®29212™. Furthermore, the concentrations of extract were increased to 30%, 40%, 50%, and 60%. The extract of okra could inhibit the growth of E. faecalis at a concentration of 50% (MIC), while eradicating the bacteria at a concentration of 60% (MBC). In the diffusion method, the extract of okra fruits 40%, 50%, 60% inhibited E. faecalis colonies with the mean value of inhibition zone were 9,5±0,3; 13,0±0,7; 15,4±0,3 respectively. Otherwise, there was no inhibition zone found following the treatment of the extract at the concentration of 30%.*
Conclusion:*Our study concludes that the extract of okra fruits has an antibacterial effect against E. faecalis with the MIC values at a concentration of 50% and the MBC values at a concentration of 60%.*
Keywords: *Okra Fruit Extract, Antibacterial Agent, Enterococcus faecalis.*

Date of Submission: 26-08-2022 Date of Acceptance: 09-09-2022

I. Introduction

Pulp infection is a disease that occurs in the root canals of teeth, with 85%-95% of cases caused by anaerobic bacteria. *Enterococcus faecalis*(*E. faecalis*) are frequently found in root canals with necrotic pulp.[1]Various factors lead to *E. faecalis being* involved in root canal infection; bacterial resistance and virulence; the ability to compete with other microorganisms (bacteria invading into the dentinal tubules); and the ability to survive in low nutritional conditions. The high resistance of *E. faecalis* is relatively caused by its capabilityof survivalin an alkalic environment.[2, 3]Therefore, root canal treatment is performed to eliminate the infection which aims to relieve pain, as well asto control sepsis from the pulp and surrounding periapical tissue.[3] Root canal treatment consists of pulp chamber preparation, pulp tissue extirpation, root canal irrigation, medication, and root canal filling. Reduction or elimination of necrotic tissue in the root canal is requiredto establish the healing process through cleaning and shaping the root canal.[4]The step of cleaning is carried out by removing vital and necrotic pulp tissue, as well as eliminating microorganisms.[5]

However, some cases are commonly found to have unsuccessful root canal therapyinduced by the development of microorganisms during root canal treatment. The residual necrotic tissue in the root canal is the major cause of treatment failure.[3, 5]In addition, the environment with low oxygen and the interactions among bacteria are the main determinants of the ecology in the root canal.[6]Antibacterial agent including chlorhexidine,is frequently used to inhibit the growth of *E. faecalis*.[7]Chlorhexidine has broad-spectrum antibacterial activity, is low in toxicity, and is soluble in water.As an irrigant and intracanal medicament, chlorhexidine is fairly effective in reducing or eradicating*E. faecalis* in root canals and dentinal tubules.[8] Irrigating the canals by using chlorhexidine for a few minutes was able to remove these bacteria in the dentinal tubules up to 100 µm.[7, 8]It is absorbed to the surface of bacterial cells, destroying the integrity of the cell membrane so that it precipitatesthe cytoplasmic fluid. The mechanism underlying its antibacterial propertiesis through the attraction of cation molecules from chlorhexidine with anion molecules from the bacterial cell membrane.[7]

Chemically produced antibacterial agents have some undesirable effects when applied for a long time causingresistance of bacteria in the oral cavity.Moreover, chlorhexidine is not able to dissolve the remains of necrotic tissue.[9] It has a potential allergic reaction such as anaphylaxis or acute allergic reactions including Quincke edema and urticarial, if exposed to skin.[10, 11]This condition takes the need to look for other alternative treatments such as herbs or traditional ingredients that have the potential to developasantibacterial agents and have relatively low side effects. Okra is a plant that has antibacterial properties. Okra contains active chemical compounds such as flavonoids, saponins, steroids, alkaloids, tannins. These compounds could inhibit the growth of bacteria by damaging the permeability ofthe cell membrane so thatbacterial cell growth isblocked.[12]

Kalarani et al. (2017) found thatthe extractof Okra fruit has the potentialantibacterial effect against *Streptococcus mutans*.[13]Luthfi(2021)showed that okra extract at a concentration of 3.125% influenced the growth of *Aggregatibacter actinomycetemcomitans*and could kill the bacteria at 6.25%.[14]Yuliati et al (2020) demonstrated that okra extract has a MIC value of 3.125% and an MBC value of 6.25% against *Porphyromonas gingivalis*.[15]In addition, Septianingrum NMA et al. (2018) found that the extract at a concentration of 45% could inhibit the growth of*Escherichia coli*.[16]However, the research of the antibacterial effect of okra against*E. faecalis* is not reported. Therefore, this study aimed to investigate the effect of okra extract against *E. faecalis* as an alternative and potential antibacterial agent in root canal treatment.

II. Material And Methods

This study is experimental laboratory research with posttest only control group design. The study was approved by KomisiEtikPenelitianKesehatan(KEPK), Faculty of Medicine, Universitas SumateraUtara. Okra plants were obtained from the fields placed on Jalan Kutilang Medan Sunggal. The extract was prepared at the laboratory of research & development of medicinal plants complex located in TASBI 2 Blok VI no. 57 Medan. The culture of *E. faecalis*ATCC®29212™ was obtained from the Microbiology Laboratory of USU Hospital. An antibacterial test was carried out in the Microbiology Laboratory of the USU Hospital. The sample size was calculated according to Federer'sformula :(t-1) (r-1) ≥ 15, (t = number of treatment, r = number of replication).

Preparation of the extract

Okra fruits as many as 3 kilograms were washed with running water, dried, and stored in a tightly closed container.The extract was prepared by using the maceration method. Simplicia of okra was soaked in 1.5 liters of ethanol 70%. After 18 hours, the solution was filtered, collected, and subsequently drained using a rotary evaporator at a temperature of 40º until the viscous extract was obtained, and stored in the incubator.

Determination of treatment groups

The study was performed in two stages, with seven groups in both stages. The first stage consisted of: group I (extract of okra10%). group II (extract of ocra 15%), group III (extract of ocra 20%). group IV (extract of ocra 25%), group V (positive control I, Chlorhexidine 2%), group VI (positive control II, Vancomycin), and group VII (negative control, DMSO).The number of replication of this study was six times in each concentration of extract, hence the total amount of samples was 24 for treated groups, 6 for the positive control (Chlorhexidine), 6 for the positive control (Vancomycin), and 6 for negative control.The second stage comprised of: group I (extract of okra30%). group II (extract of ocra 40%), group III (extract of ocra 50%). group IV (extract of ocra 60%), group V (positive control I, Chlorhexidine 2%), group VI (positive control II, Vancomycin), and group VII (negative control, DMSO) with the total number of samples equal to the first stage which was previously mentioned.

Evaluation of antibacterial activity

All 7 tubeswere prepared and added with 1 ml of Mueller Hinton Broth MHB media.The culture of*E. faecalis* ATCC®29212™ was added and suspended in 0.85% NaCl solution until the same turbidity was

obtained with the 0.5 McFarland standard. Drop every 1 ml of okra extract with various concentrations of the extract (stage I and II) with a micropipette into each tube and also 1 ml of positive control solution (1) and negative control. The tubes were incubated at 37º for 24 hours. To examine the MIC and MBC values, all samples were subcultured on Petri dishes containing Mueller Hinton Agar (MHA). The colony of *E. faecalis* grown on the media was counted as a colony-forming unit (CFU).

To measure the inhibition zone, a diffusion test was selected in the study. Sterile disk papers were immersed in the extract solution. Chlorhexidine 2% and vancomycin were positive control, whereas DMSO wasa negative control. One to two loops of *E. faecalis* ATCC®29212™ culture were added onto the media and then scratched tightly on the surface of the medium. The treated disks were placed on MHA with tweezers and then incubated at 37º for 24 hours. The zone of inhibition indicated by the colony-free diameter (clear zone) was measured using a digital caliper with an accuracy of 0.01 mm.

Data analysis

Data were the values of MIC and MBC measurement and analyzed using SPSS v.17.0 software. Data were presented in the mean and standard deviation of six replications of samples. To evaluate the effect of Okra extract against *E. faecalis*in each group, one way ANOVA was selected to analyze the data.

III. Results

Observation of dilution tubes containing the extract of Okra fruits

According to the observation of dilution tubes, the extract at concentrations of 10%, 15%, 20%, and 25% was not seen differences, as a result of opacity from the extracted color. Table 1 shows the dilution tubes in all treatment groups with turbidity.

Table 1. Observation of dilution tubes of the Okra extract at various concentration of 10%, 15%, 20%, 25%

Concentration	Observation					
	I	II	III	IV	V	VI
10%	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
15%	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
20%	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
25%	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid

According to the observed dilution tubes at stage 2, the extracts at concentrations of 30%, 40%, 50%, and 60% were indistinguishable (Figure 1). The cloudy suspension was seen in all treated groups (Table 2).

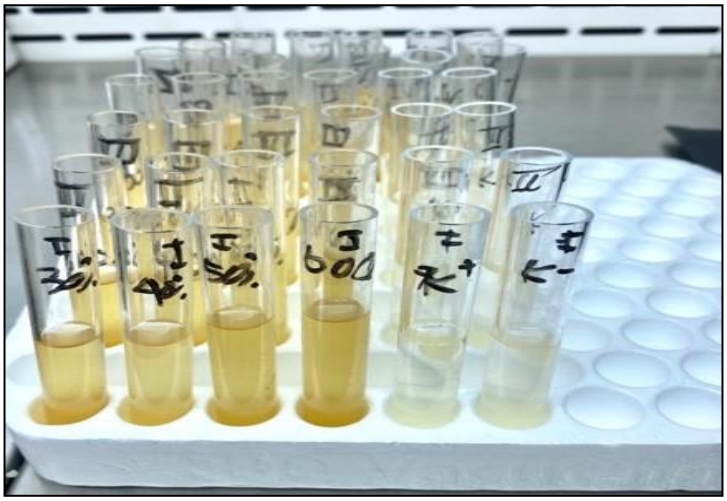

Figure 1. Turbidity in dilution tubes of the okra extract-treated*E. faecalis*. Both positive and negative control showed clear solutionsindicating no bacteria grown in the medium.

Table 2. Observation of dilution tubes of the Okra extract at various concentration of 30%, 40%, 50%, 60%

Concentration	Observation					
	I	II	III	IV	V	VI
30%	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
40%	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
50%	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
60%	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid

Otherwise, the dilution tubes in positive control group I (chlorhexidine) were allclear, but not negative control (DMSO) (Table 3).

Table 3. Observation of dilution tubes of the Okra extract at positive and negative control groups

Control	PengamatanTabungDilusi					
	I	II	III	IV	V	VI
Positive	Clear	Clear	Clear	Clear	Clear	Clear
Negative	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Okra Extract against *E. faecalis* ATCC®29212™

MIC and MBC were determined by dilution test on *Mueller Hinton Agar* media and then the formed colonies on petri dish were counted as colony-formingunits (CFU). It shows that the MIC and MBC values of the extract at a concentration of 10%, 15%, 20%, 25% were not acquired as the number of colonies was more than 300 CFU/ml (Table 4).

Table 4. The amount of *E. faecalis* colonies grown on the petri dish following treatment of okra extract at the concentration of 10%, 15%, 20%, 25%

Concentration	Colony Forming Unit (CFU/ml)					
	I	II	III	IV	V	VI
10%	>300	>300	>300	>300	>300	>300
15%	>300	>300	>300	>300	>300	>300
20%	>300	>300	>300	>300	>300	>300
25%	>300	>300	>300	>300	>300	>300

In the first stage of this study, okra fruit extracts with concentrations of 10%, 15%, 20%, 25% were not found to have the MIC and MBC valuesagainst *E. faecalis* ATCC®29212™. Furthermore, the concentrations of extract were increased to 30%, 40%, 50%, and 60%. As shown in Table 5, the extract of okra could inhibit the growth of *E. faecalis*at a concentration of 50% (MIC), while eradicated the bacteria at a concentration of 60% (MBC).

Table 5. The values of MIC and MBC on the extract of okra fruits against *E. faecalis* ATCC®29212™

Concentration	Colony Forming Unit (CFU/ml)					
	I	II	III	IV	V	VI
30%	>300	>300	>300	>300	>300	>300
40%	>300	>300	>300	>300	>300	>300
50%	287	274	288	292	279	263
60%	0	0	0	0	0	0

Positive control I (chlorhexidine) completely blocked the growth of*E. faecalis* showing the value of 0 CFU/ml (Table 6). Otherwise, the colonies of *E. faecalis* were highly increased as many as more than 300 CFU/ml in the negative control group (Table 6).

Table 6. The number of *E. faecalis* colonies grown in the positive and negative control

Control	Colony Forming Unit (CFU/ml)

	I	II	III	IV	V	VI
Positive	0	0	0	0	0	0
Negative	>300	>300	>300	>300	>300	>300

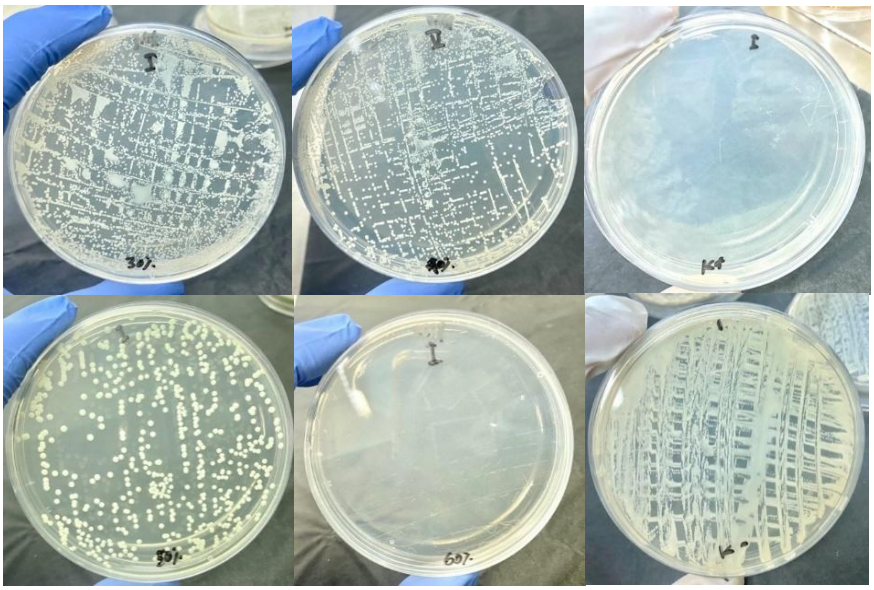

Figure 2. Growth of *E. faecalis* colonies observed in the negative control, the extract of okra fruits treated bacteria at a concentration of 30%. 40%, and 50%. While there were no bacteria grown on positive control and the okra extract at the concentration of 60%.

Diameter of inhibition zone of the extract of okra fruits against *E. faecalis* ATCC®29212™

The inhibition zone was determined by diffusion test and the diameter of the clear area surrounding disk paper was measured using a caliper. Results showed that the extract of okra fruits 40%, 50%, 60% inhibited *E. faecalis*colonies with the mean value of inhibition zone were 9,5±0,3; 13,0±0,7; 15,4±0,3 respectively. Otherwise, there was no inhibition zone found following the treatment of the extract at a concentration of 30% (Table 7).

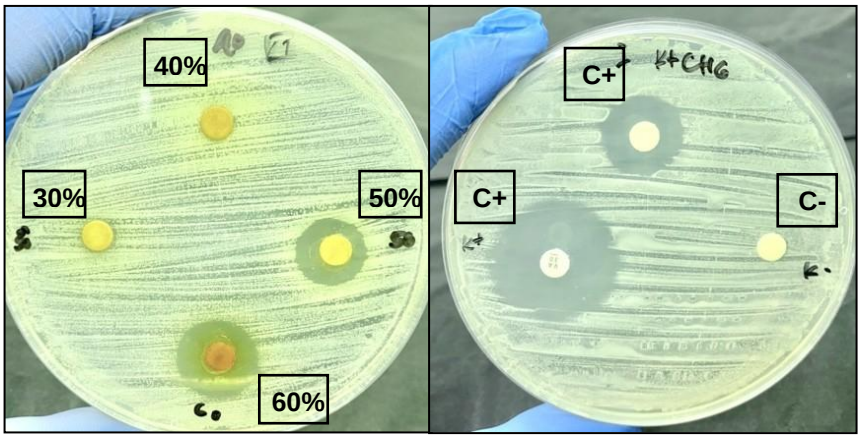

Figure 3. The formation of inhibition zone on disk paper in control positive and the okra extract-treated medium containing *E. faecalis*.

Table 7. The diameter of inhibition zone after-treatment of the various concentration of the okra fruits extract against*E. faecalis* ATCC®29212™

Concentration	Inhibition zone (mm)						
	I	II	III	IV	V	VI	Mean ±SD

30%	0	0	0	0	0	0	0±0
40%	9,5	9,5	10	10	9	9,5	9,5±0,3
50%	14	12,5	13	13	12	13,5	13,0±0,7
60%	16	15	15,5	15	15,5	15,5	15,4±0,3
Positive Control I	19	20	18	20	20	19	19,3±0,8
Positive Control II	28	28	27	29	27	28	27,8±0,7
Negative Control	0	0	0	0	0	0	0±0

IV. DISCUSSION

E. faecalis is a bacterium with high resistance which often affects the failure of root canal treatment. This study using okra fruit extract as an antibacterial agent against *E. faecalis* was successful in determining the MIC, MBC, and zone of inhibition by increasing the concentration. Our study showed that the extract of okra fruits at lower concentrations of 10%, 15%, 20%, and 25%did not affect the inhibition of *E. faecalis*growth. However, several previous studies demonstrated the effectiveness of the okra extract against some other bacteria at very low concentrations compared with the current study.[13-16]It is presumably caused by various factors, such as bacterial resistance and virulence, the ability to compete with other microorganisms (invasion of these bacteria into the dentinal tubules), and the ability to survive in low nutritional conditions.[2, 3]

To further find the effective concentration of the okra extract against *E. faecalis*, the investigation was continued by increasing the concentration to 30%, 40%, 50%, and 60%.The extract of okra fruits has MIC and MBC values at a concentration of 50% and 60%, respectively. Moreover, the results showed that the extract with concentrations of 40%, 50%, 60% showed the diameter of inhibition zone on disk paper against *E. faecalis* ATCC®29212™. The inhibitory zone of the okra fruit extract at concentrations of 60% and 50% had a strong inhibitory level against *E. faecalis* ATCC®29212™, while at a concentration of 40% it had a moderate inhibitory level.There was a significant difference in the diameter of the inhibition zone in all treatment groups in inhibiting the growth of *E. faecalis* ATCC®29212™. This value indicates that the extract at concentrations of 30%, 40%, 50%, 60% was able to inhibit the growth of *E.faecalis*. The results of this study are in line with a study by Kalarani et al. (2017) which stated that the okra fruit extract with different diameters of the inhibition zone in all treatment groups inhibited the growth of *Streptococcus mutans*.[13]

Plant extracts with active compounds have a significant effect on antibacterial activity. The components of active substances contained in the okra fruit include flavonoids, alkaloids, tannins, steroids, and saponins.[17] These secondary metabolites are likely involved in the antibacterial action in blocking the growth of *E. faecalis*. Flavonoids are active compounds that play a role in forming complexes between hydrogen and walls and can directly inhibit the growth and function of microorganisms in the microbial cell cycle.[18] Alkaloids have the ability toan antibacterial by interfering with the peptidoglycan constituent components in bacterial cells, so that the cell wall layer is not formed completely and causes the death of the cell.[19] Steroids can inhibit growth by interfering with the process of membrane and cell wall formation. Saponins cause damage to bacterial membrane permeability so that the materials needed for life are lost and can cause cell death.[20]

V. CONCLUSION

Our study concludes that the extract of Okra fruits has an antibacterial effect against *E. faecalis* with the MIC values at a concentration of 50% and the MBC values at a concentration of 60%. Therefore, it is required to further study the effect of the okra extract against *E. faecalis* in an animal model to investigate the efficacy of the extract in dental tissues, so that it may be used as a potential alternative antibacterial agent.

ACKNOWLEDGEMENT

This article is an output from research that has been sponsored by Talenta fund from Lembaga Penelitian University Sumatera Utara, No123/UN5.2.3.1/PPM/SPP-TALENTA USU/2021

REFERENCES

[1]. COGULU D, UZEL A, ONCAG O, AKSOY SC, ERONAT C. Detection of Enterococcus faecalis in Necrotic Teeth Root Canals by Culture and Polymerase Chain Reaction Methods. European Journal of Dentistry. 2007;1:216-21.
[2]. Zoletti GO, Pereira EM, Schuenck RP, Teixeira LM, Siqueira JF, Jr., dos Santos KR. Characterization of virulence factors and clonal diversity of Enterococcus faecalis isolates from treated dental root canals. Res Microbiol. 2011;162(2):151-8.
[3]. Sakko M, Tjäderhane L, Rautemaa-Richardson R. Microbiology of root canal infections. Primary Dental Journal. 2016;5:84-9.
[4]. Baugh D, Wallace J. The Role of Apical Instrumentation in Root Canal Treatment: A Review of the Literature. Journal of Endodontics. 2005;31(5):333-40.
[5]. Tomson PL, Simon SR. Contemporary Cleaning and Shaping of the Root Canal System. Prim Dent J. 2016;5(2):46-53.
[6]. Dioguardi M, Di Gioia G, Illuzzi G, Arena C, Caponio VCA, Caloro GA, et al. Inspection of the Microbiota in Endodontic Lesions. Dent J (Basel). 2019;7(2).

[7]. Mohammadi Z, Jafarzadeh H, Shalavi S. Antimicrobial efficacy of chlorhexidine as a root canal irrigant: a literature review. J Oral Sci. 2014;56(2):99-103.
[8]. Ruksakiet K, Hanak L, Farkas N, Hegyi P, Sadaeng W, Czumbel LM, et al. Antimicrobial Efficacy of Chlorhexidine and Sodium Hypochlorite in Root Canal Disinfection: A Systematic Review and Meta-analysis of Randomized Controlled Trials. J Endod. 2020;46(8):1032-41 e7.
[9]. Zehnder M. Root canal irrigants. J Endod. 2006;32(5):389-98.
[10]. Torricelli R, Wüthrich B. Life-threatening anaphylactic shock due to skin application of chlorhexidine. Clin Exp Allergy. 1996;26(1):112.
[11]. Snellman E, Rantanen T. Severe anaphylaxis after a chlorhexidine bath. J Am Acad Dermatol. 1999;40(5 Pt 1):771-2.
[12]. de Carvalho CCCR, Cruz PA, da Fonseca MMR, Xavier-Filho L. Antibacterial properties of the extract of Abelmoschus esculentus. Biotechnology and Bioprocess Engineering. 2011;16(5):971-7.
[13]. G K, Govindarajan M, A L, Prabhu A, Krishnakumar R, Dhurikaliyamorthy S. Antibacterial Effect of Abelmoschus Esculentus (Okra) Extracts on Dental Caries Derived Streptococcus Mutans. IOSR Journal of Dental and Medical Sciences. 2017;16(04):63-6.
[14]. Luthfi M, Yuliati, Oki A, Sosiawan A, Cida B. Effectiveness of okra fruit (Abelmoschus esculentus) extract against Aggregatibacter actinomycetemcomitans (Aa) as a bacterium that causes aggressive periodontitis. Journal of International Oral Health. 2020;12(6).
[15]. Yuliati, Luthfi M, Rachmadi P, Cida BP, Wijayanti EH. The potency of Okra Fruit Extract (Abelmoschus esculentus) Against Porphyromonas gingivalis as the Cause of Chronic Periodontitis. Journal of International Dental and Medical Research. 2020;13:519-24.
[16]. Septianingrum NMAN, Hapsari WS, Syariffudin A. IDENTIFIKASI KANDUNGAN FITOKIMIA EKSTRAK OKRA MERAH (Abelmoschus Esculentus) DAN UJI AKTIVITAS ANTIBIOTIK TERHADAP BAKTERI Escherichia coli. Jurnal Insan Farmasi Indonesia. 2018;1:170-7.
[17]. Bello HS, Mustapha A, Isa MA, Rahila TC. THE INVITRO ANTIBACTERIAL ACTIVITY OF OKRA (Abelmoschus esculentus) AGAINST SOME SELECTED BACTERIA FROM MAIDUGURI, NORTHEASTERN NIGERIA. International Journal of Biosciences and Nanosciences. 2015;2:84-44.
[18]. Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26(5):343-56.
[19]. Cushnie TP, Cushnie B, Lamb AJ. Alkaloids: an overview of their antibacterial, antibiotic-enhancing, and antivirulence activities. Int J Antimicrob Agents. 2014;44(5):377-86.
[20]. Arabski M, Węgierek-Ciuk A, Czerwonka G, Lankoff A, Kaca W. Effects of Saponins against Clinical <i>E. coli</I> Strains and Eukaryotic Cell Line. Journal of Biomedicine and Biotechnology. 2012;2012:286216.

Filia Dana Tyasingsih, et. al. "POTENCY OF OKRA FRUITS (Abelmoschus esculentus) AS ANTIBACTERIAL AGENT AGAINST Enterococcusfaecalis ATCC®29212™ (In Vitro)." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 21(09), 2022, pp. 25-31.