INTERNATIONAL JOURNAL OF SCIENTIFIC DEVELOPMENT AND RESEARCH

International Peer Reviewed & Refereed Journals, Open Access Journal

ISSN Approved Journal No: 2455-2631 | Impact factor: 8.15 | ESTD Year: 2016

Scholarly open access journals, Peer-reviewed, and Refereed Journals, Impact factor 8.15 (Calculate by google scholar and Semantic Scholar | AI-Powered Research Tool) , Multidisciplinary, Monthly, Indexing in all major database & Metadata, Citation Generator, Digital Object Identifier(DOI)

Submit Paper Online | Login to Author Home

Call For Paper

Issue: March 2023
Volume 8 | Issue 3
Impact factor: 8.15
Click Here For more Info

Imp Links for Author

- Publication Guidelines
- Publication Charges
- Pay Publication Charges
- Track Paper
- Hardcopy and DOI
- Sample Paper Format
- Copyright Form
- Undertaking Form
- Past Issue
- Research Area

Imp Links for Reviewer

- Editorial Board
- Join As A Reviewer
- Advantages of Reviewer

Research Area

- Engineering
- Science & Technology
- Pharmacy

Welcome To IJSDR

Call for Paper
Volume 8 | Issue 3 | March 2023
Click Here For Submit Manuscript online

Submit Paper | Author Home | Call for Paper

IJSDR is Scholarly open access journals, Peer-reviewed, and Refereed Journals, High Impact factor 8.15 (Calculate by google scholar and Semantic Scholar | AI-Powered Research Tool), Multidisciplinary, Monthly, Indexing in all major database & Metadata, Citation Generator, Digital Object Identifier(DOI) with Open-Access Publications.

The International Journal of Scientific Development and Research (IJSDR) aims to explore advances in research pertaining to applied, theoretical and experimental Technological studies. The goal is to promote scientific information interchange between researchers, developers, engineers, students, and practitioners working in and around the world. IJSDR will provide an opportunity for practitioners and educators of engineering field to exchange research evidence, models of best practice and innovative ideas.

Indexing In Google Scholar, SSRN, ResearcherID-Publons, Semantic Scholar | AI-Powered Research Tool, Microsoft Academic, Academia.edu, arXiv.org, Research Gate, CiteSeerX, ResearcherID Thomson Reuters, Mendeley : reference manager, DocStoc, ISSUU, Scribd, and many more

IJSDR Important for Current issue

Paper Submission Open For	March 2023
Current Issue	Volume 8 | Issue 3
Last date for paper submission	31-Mar-2023
Notification of Review Result	Within 1-2 Days after submitting paper.
Publication of Paper	Within 1-2 Days after Submititng documents.
ISSN (International Standard Serial	2455-2631

Track Paper

Track Submitted Paper

Important Links

Call For Paper

Join As Reviewer

Conference Proposal

CLICK HERE
For Conference Prposal

ISSN

INTERNATIONAL STANDARD SERIAL NUMBER
ISSN 2455-2631

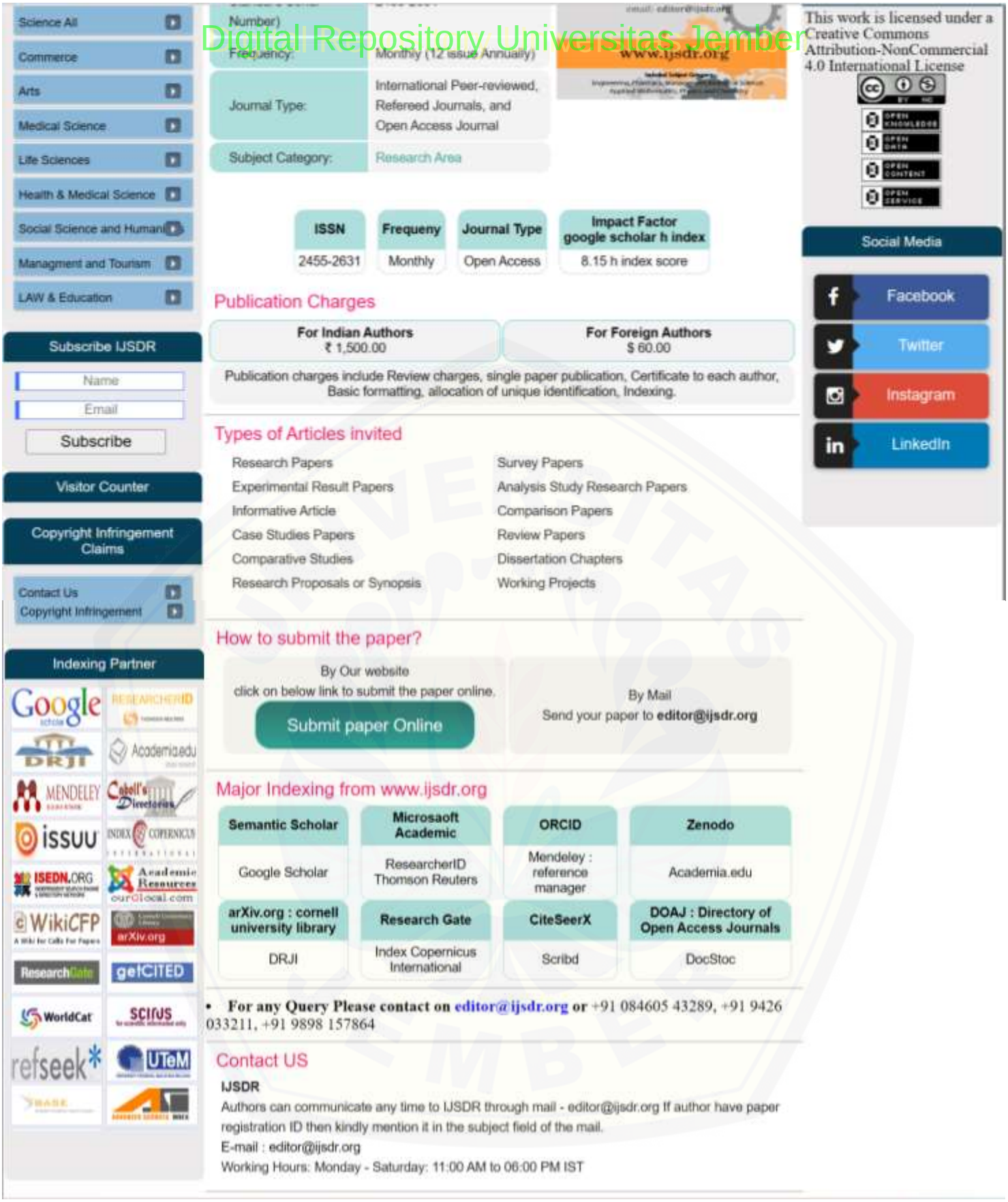
9 772455 263002 >

DOI (A digital object identifier)

figshare

Providing A digital object identifier by DOI
How to GET DOI and Hard Copy Related

Open Access License Policy



Science All
Commerce
Arts
Medical Science
Life Sciences
Health & Medical Science
Social Science and Humani
Managment and Tourism
LAW & Education

Subscribe IJSDR
Name
Email
Subscribe

Visitor Counter

Copyright Infringement Claims

Contact Us
Copyright Infringement

Indexing Partner

Number)
Frequency: Monthly (12 issue Annually)
Journal Type: International Peer-reviewed, Refereed Journals, and Open Access Journal
Subject Category: Research Area

www.ijsdr.org

ISSN	Frequeny	Journal Type	Impact Factor google scholar h index
2455-2631	Monthly	Open Access	8.15 h index score

Publication Charges

For Indian Authors	For Foreign Authors
₹ 1,500.00	$ 60.00

Publication charges include Review charges, single paper publication, Certificate to each author, Basic formatting, allocation of unique identification, Indexing.

Types of Articles invited

- Research Papers
- Experimental Result Papers
- Informative Article
- Case Studies Papers
- Comparative Studies
- Research Proposals or Synopsis
- Survey Papers
- Analysis Study Research Papers
- Comparison Papers
- Review Papers
- Dissertation Chapters
- Working Projects

How to submit the paper?

By Our website
click on below link to submit the paper online.
Submit paper Online

By Mail
Send your paper to **editor@ijsdr.org**

Major Indexing from www.ijsdr.org

Semantic Scholar	Microsaoft Academic	ORCID	Zenodo
Google Scholar	ResearcherID Thomson Reuters	Mendeley : reference manager	Academia.edu
arXiv.org : cornell university library	**Research Gate**	**CiteSeerX**	**DOAJ : Directory of Open Access Journals**
DRJI	Index Copernicus International	Scribd	DocStoc

- **For any Query Please contact on editor@ijsdr.org or** +91 084605 43289, +91 9426 033211, +91 9898 157864

Contact US

IJSDR
Authors can communicate any time to IJSDR through mail - editor@ijsdr.org If author have paper registration ID then kindly mention it in the subject field of the mail.
E-mail : editor@ijsdr.org
Working Hours: Monday - Saturday: 11:00 AM to 06:00 PM IST

Social Media
Facebook
Twitter
Instagram
LinkedIn

https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwi146-bvPL9AhUGS2wGHXIUCZIQFnoECAsQAQ&url=https%3A%2F%2Fwww.ijsdr.org%2Fpapers%2FIJSDR2206077.pdf&usg=AOvVaw2Ud7rdYnn-gvNpHdjFLlxq

The Effects of Robusta Coffee Bean *(Coffee canephora)* Polyphenols Extract on Growth and Morphological Alteration *Streptococcus mutans*

Didin Erma Indahyani[a], Izzata Barid[a], Niken Probosari[b], Dzanuar Rahmawan[c]

[a]Oral Biology Departement, Dental Medicine University Of Jember, Jl. Kalimantan 32 Jember. 68121. Indonesia
[b]Pediatric Departement, Dental Medicine University of Jember, Jl. Kalimatan 32 Jember. 68121. Indonesia
[c]Departement of Biomedic Bhakti Wiyata Institute of Health Sciences, Kediri. Indonesia

Abstract

Background. Coffee polyphenols are antibacterial because they can inhibit growth and damage the cell wall. *S mutans* is a bacterium that plays a role in the caries process.
Purpose. This study aimed to analyze the effect of polyphenols extract of robusta coffee beans on the growth and morphological changes of *S mutans* bacteria.
Method. This research was conducted with two stages of observation. The first step is to observe the growth of *S. mutans* in treatment with polyphenolic extract of robusta coffee beans using the minimum inhibitory concentration (MIC) growth test and minimum bactericidal concentration (MBC). The second stage is to observe the morphology of *S. mutans* bacteria by analyzing changes in the shape and size of the cell membrane of *S. mutans* using Scanning Electron Microscope (SEM). Data were observed by a non-parametric test.
Result. The results showed that in the MIC test, 0.2 ml concentration of robusta coffee polyphenol extract significantly ($p < 0.05$) caused inhibition of growth of *S. mutans* while in the MBC test at the same concentration detected *S. mutans* existence. This indicates that the coffee bean polyphenol extract is not bactericidal. Analysis of SEM showed 12.5% concentration of polyphenol extract resulted in larger cell size and cell morphology changes with blebs on the cell wall and ghost cell formation. **Conclusion.** It was concluded that polyphenolic extract of robusta coffee beans caused inhibition of bacterial growth of *S. mutans* by influencing morphological changes of the bacterial cell wall. However, the extract did not show bactericidal properties at the same concentration.

Keywords: **robusta coffee, *S. mutans*, polyphenols, bacterial growth, bacterial morphology ;**

Introduction

Robusta coffee is an excellent product in Indonesia. Robusta/canephora coffee has a bitter and more acidic taste than arabica coffee. The taste is associated with a high content of polyphenols and caffeine.[1] Polyphenol are organic materials with one or more hydroxyl groups attached to the phenyl ring and synthesized from alanine or tyrosine phenyls. Polyphenols are not involved in plant average growth and development but act as a mechanism for plant growth against viruses, bacteria, fungi etc.[2] The structure of polyphenol is generally divided into flavonoids and non-flavonoids. What flavonoids include flavonols, flavanones, flavones, anthocyanins, proanthocyanidins (tannins), hydroxystilbenes, aurones, etc. Polyphenols are toxic to bacteria because they can inhibit hydrolytic enzymes (proteases and carbohydrates) or interact to activate microbial adhesin, cell envelope transport proteins, non-specific interactions with carbohydrates, etc.[3] In addition, polyphenols have antibacterial activity and can suppress bacterial virulence factors by inhibiting biofilm formation, reducing ligant host adhesion, neutralizing bacterial toxins and showing synergism with antibiotics.[4] Proanthocyanidins (Tannins) can inhibit bacterial growth by destroying cariogenic bacterial membranes[5], Proanthocyanidins (Tannins) can inhibit cariogenic bacterial growth by destroying cell membranes. The bacteria produce acid and form biofilms5. Coffee proanthocyanidins reduce the growth of S Mutans by inhibiting the glucosyltransferase and F-ATPase. That is cause their acid production is less. The oligomeric structure of type A epicatechin, flavonols, and phenolic acids from proanthocyanidins increase the permeability of cell membranes..[6] Phenolic acids (and hydroxycinnamic acids in particular) increased the cell membrane permeability of the LAB strains.[7]

Usually, bacteria use cell membranes as barrier diffusion. Therefore, the integrity of the cell membrane is needed to maintain chemotactic balance. It has a vital role in the membrane-associated energetic metabolism of LAB.[8] Increasing permeability cause reduced or lost resistance extracellular layer resulting in high reversibility, and the drug can easily pass through**.** High cell membrane permeability results in easy passage to cells, often associated with increased drug activity and toxicity. Therefore, evaluating membrane permeability has an important role.[9]
The interaction between the cell wall and other surfaces (e.g. the host) controls the exchange of the bacteria with the surrounding environment. That shows the importance of the structure and chemical composition of the cell wall because it can determine the nature of the interactions between cell membranes and host and abiotic surfaces.[10] The cell wall is essential in determining the shape and preventing cell lysis due to osmotic pressure. Therefore, cell integrity becomes the target of antibiotics by interfering with their metabolism. These disorders weaken the cell wall, which results in easy rupture. Thus, a weak cell wall may no longer be able to

withstand turgor pressure, resulting in loss of membrane integrity and leakage of cytoplasm. The large hole produced as a result of this event causes cell death.

The bacteria that causes caries is S mutans. Under certain conditions, these bacteria can reveal their pathogenic properties, which determine their virulence. S mutans colonize the oral cavity through adhesion mechanisms on solid surfaces and form a biofilm. Another characteristic that causes colonization in the oral cavity is its ability to survive in an acidic environment and its interactions with other specific bacteria.[11] *S.mutans* cells have a diameter of 0.5-0.75 µm, in pairs or short chains, and lengths without capsules. In acidic conditions, both broth and solid cocci medium can form a short stem with a length of 1.5-3.0 µm in length.[12] The purpose of this study was to analyze the effect of polyphenols extract of robusta coffee beans on the growth and morphological changes of *S mutans* bacteria.

MATERIALS AND METHODS

1. Bacterial strains and growth

Streptococcus mutans strain JC2 was obtained from the microbiology dental faculty-Unair Surabaya laboratory, which has been tested for purity, grown in Brain Heart Infusion Broth (BHI-B) culture medium. Brain hearth infusion broth (BHI-B) was prepared by mixing 3.7 grams of BHI-B powder with 100ml of sterile aquadest in Erlenmeyer, stirring until homogeneously heated to 121°C, then sterilized by inserting it in an autoclave at 12°C for 15 min. The bacterial suspension was prepared by one ose *S mutans* inserted in 2 ml of BHI-B in the reaction tube, then incubated at 37°C for 24 hr. Suspension of *S mutans* taken from the incubator, shaken until homogeneous and measured turbidity with spectrophotometer up to a level of 0.5 McFarland is 1.5 x 108 CFU / ml.

2. Polyphenol extract of robusta coffee beans

This extract is done in the laboratory of CIA (Chemistry Identification and Application) Chemistry Department FMIPA University of Jember is as follows Coffee beans that have been washed and dried, mashed into powder by using a hammer mill to size 30 mesh (500µm). The powder is mixed with ethanol solvent and is contacted for 30 min to 5 hr. Purification is done by mixing it with petroleum ether to remove lipids and pigments for 30 min to 5 hr. Next, chloroform is used to remove caffeine and wax for 30 min to 5 hr. Then the extract is distilled using a rotary evaporator at 50°C under 40 millibars pressure to remove the existing solvent, resulting in polyphenol extract.

3. MIC and MBC test

The MIC test was performed by diluting the polyphenolic extract of robusta coffee beans obtained at a concentration of 2 ml. Taking 3.1 ml of extract (P1), diluted gradually ie 50% P2, 25% P3, 12.5% P4, 6.25% P5, 3.12% P6, 1.56% P7. The eighth tube (P8) is a positive control, and tube 9 (P9) is a negative control. Tubes that have been filled with polyphenols extract added BHI-B 1 ml and suspension of *S mutans* with a turbidity level of 0.5 Mc Farland of 0.1 ml. For tube eight, the antibiotic added ampicillin 250 mg, and for tube nine, added again BHI-B. All tubes were incubated for 24 hr at 37°C. The absence of bacterial growth is characterized by the clarity of the tube.

The MBC test was performed using a blood agar medium (blood agar). After the MIC test, the test preparation was taken 1 microliter, then inoculated with the dispersion method and incubated at 37°C for 24 hr. Growth of *S. mutans* with green discolouration on the agar medium.

4. Test structure and morphology of *S mutans*

This test is performed using SEM. After the MIC test, the *S. mutans* cell suspension was taken from the tube of th[3]. MIC test, inserted into Eppendorf using a Pasteur pipette, then mixed with 2% glutaraldehyde fixation solution for 2-3 hr at 4°C, then centrifuged at 1000 rpm for 15 s. Washed with phosphate buffer solution pH 7.4 3 times, each for 5 s at 4°C, then centrifuged at 1000 rpm for 15 s. Furthermore, it was fixed with 1% solution of osmic acid for 1-2 hr at 4°C, then centrifuged at 1000 rpm for 15 s. Washed with phosphate buffer solution pH 7.4 3 times, each 5 s at 4°C, then centrifuged at 1000 rpm for 15 s. Dehydration with ethanol stratified: 30%, 50%, 70%, each for 15 - 20 minutes with a temperature of 4°C, then centrifuged at 1000 rpm for 15 s. Continued dehydration with 80% ethanol and 90% twice each for 15-20 minutes at room temperature, then centrifuged at 1000 rpm for 15 s. The resulting sediment is mixed with absolute acetic acid (as a preservative until waiting for drying time). Then the sediment *S. Mutans* pipette used a paste pipette and dripped on the glass object that was cut with an area of 16 mm2 and was cleaned with alcohol, then *S. mutans* dried. The drying of *S. mutans* is done by using the Critical Point Drying (CPD) tool to prevent bacterial cell structure. A coating process follows this by attaching to a holder using special glue. The coating is applied using pure gold or carbon using Vacum Evaporator; then, the sample is ready to be observed and photographed on SEM (Jeoltype JSM-T100, Germany) with selected magnification.

Morphological changes were observed by analyzing the size changes and the formation of bacterial cell protrusions with criteria 0.46µm - 0.54µm was 0; 0.55µm - 0.62µm is 1; 0.63µm - 0.70µm is 2 and 0.71µm - 0.78µm is 3. The formation of the bulge is as follows. No bulge is A, and there is a bulge is B. Ghost cell formation with criteria no ghost cell is X, and their ghost cell is Y.

RESULTS

1. MIC test

The test results showed that at a concentration of 2 ml (100%), tube P1, looks clear. This result is similar to the eighth tube (negative control) given antibiotic solution. At the same time, tubes P2 through P7 show the same turbidity as the P9 tube (positive control) (see Fig. 1).

Figure 1. MIC test results that show turbidity level on each tube

2. MBC Test

MBC test shows that there is a red colour change to greenish colour. The color change is caused by the growth of *S mutans* in the agar blood agar. Color changes occur in all groups except the negative control group (P8) given antibiotics. A minor discolouration is seen in group 1 (100% concentration) (figure 2).

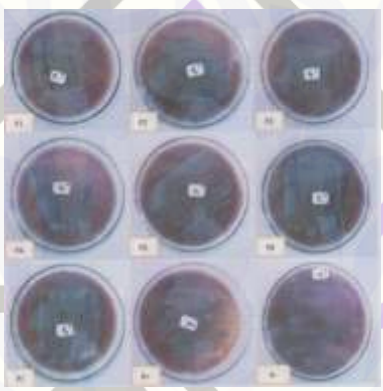


Figure 2. Changes in color of the agar medium from red to green

3. SEM Test

The result of observation by using a Scanning Electron Microscope (SEM) on *S. mutans* cell wall which was not given polyphenol extract of robusta coffee beans (control) and given a polyphenolic extract of robusta coffee beans concentration of 12.5%, 25%, 50%, 100% analyzed by how to see changes in cell wall morphology ie changes in size, the formation of the bulge (blebs) and ghost cell formation. The results can be seen as follows.

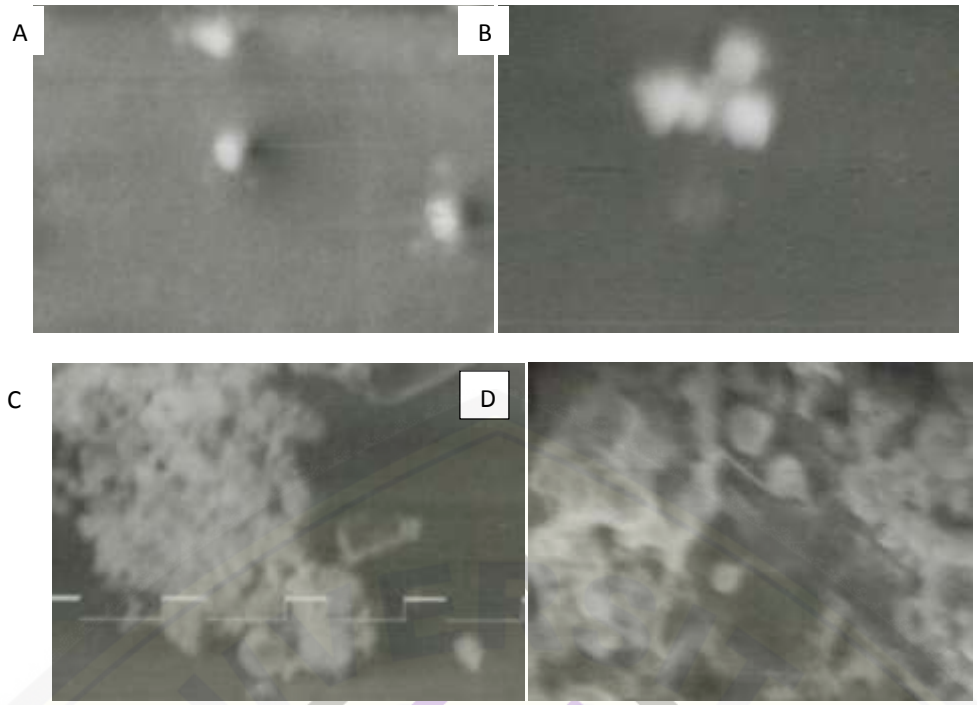


Figure 3 Scanning Electron Microscope (SEM) with morphological changes of size change in S. mutans given a polyphenolic extract of robusta coffee beans 3500 times magnification. (a) *S. mutans* was not given robusta coffee polyphenol extract (control); (b) *S. mutans* given a polyphenolic extract of robusta coffee beans with a concentration of 12.5%; (c) *S. mutans* given a polyphenolic extract of robusta coffee beans with 25% concentration; (d) *S. mutans* given a polyphenolic extract of robusta coffee bean with the concentration of 50%;

The result of observation of *S. mutans* cell wall damage with morphological changes in the form of size change (ghost) and shape (blebs). At concentrations of 12.5% polyphenolic extract the robusta beans have changed the blebs in the cell membrane, whereas the ghost changes appear to begin at 25% concentration. Changes in cell wall morphology in the form of size changes can be seen in the following tables and figures.

Table 1. Changes in cell shape and size

Concentration (%)	Size (ghost)	Shape (blebs)
Control	0	0
12,5	0	3
25	3	3
50	2	3
100	3	3

The criteria of damage to *S. mutans* cell wall in the form of a change in size are marked 0 if the size of bacteria (0.46µm - 0,54µm), 1 (0.55µm - 0.62µm), 2 (0.63µm - 0.70µm) 3 (0.71µm - 0.78µm). the criteria of damage to *S. mutans* cell wall in the form of a bulge (blebs) are marked A (no bulge), B (no bulge).

Table 4.2 cruskal-Wallis test results for damage to *S. mutans* cell wall in the form of size changes

Chi-Square	Df	*Asymp. Sig*
14,000	4	0,007

Chi-Square shows calculated statistics for the median test, df (degree of freedom) shows the number of groups (in our case = 5) minus 1, Asymp. The sign indicates the level of significance of the test.

Mann-Whitney test results showed a significant difference between each treatment group. Significant differences occurred in the treatment group, ie P1 with P2, P1 with K, P2 with P3, P2 with P4, P2 with K, P3 with K, and P4 with K.

DISCUSSION

The results of the MIC and MBC test show the ability of polyphenol coffee extract inhibition on the growth of low *S. mutans*. This can be seen in the minimum inhibitory concentration test of growth inhibition at 100% concentration. Minimum inhibitory concentration (MIC) shows the effect of polyphenols on the inhibition of biofilm-forming enzymes without the occurrence of bacterial killings.[13] Inhibition of growth is thought to be related to inhibition of biofilm formation. Biofilm serves to attach to host

cells and tissues. Such properties are important for bacterial colonization leading to pathogenicity.[11] Polyphenols inhibit the enzyme glucosyl transferase, the enzyme that performs the metabolism of sucrose into monosaccharides and fructose. The metabolism serves to produce water-insoluble glucan/glucan binding protein that promotes bacterial attachment and biofilm maturation. In addition, polyphenols also inhibit quorum-sensing bacteria, the mechanism of regulation of the battery that expresses the specific gene en masse. It is responsible for the physiological behaviour of biofilm formation.[14] Biofilms facilitate the transfer of genes between species of bacteria through transformation or conjugation.[15] This causes the bacteria to replicate DNA and produce new DNA.[16] In addition, the biofilm matrix is able to provide nutrients for the colonies within its environment and allows for metabolic cooperation of other bacteria where its metabolism results are used as other bacterial nutrients.[17] The inhibition of biofilm formation leads to the growth of *S.mutans* also inhibited, because it is unable to communicate with other bacteria to coordinate the growth factor factors and decrease the source of nutrients for growth.

The low inhibition of polyphenol extract of robusta coffee is also seen in the results of observations on SEM that do not indicate the presence of bacteria that lisis but found many bacteria that undergo morphological changes that size appears larger than normal and the existence of bulge or blebs on the cell wall Cells are protected by their cell walls from osmotic pressure. Cell walls that have damaged their cell walls. This causes a decrease in the tolerance of cells to their environment, for example, the osmotic pressure is too low or the ionic strength is high. Therefore, the structure of the cell wall, namely the peptidoglycan layer, is the main element when there is an interaction with antibiotics. Thus the cell wall plays a role in maintaining the survival of bacteria.[2] The interaction between peptidoglycan in the functioning cell wall also affects the integrity of the cell, because it will provide inhibitory forces, and the modulositive channel (MS) that modulates turgor. Turgor is produced by selectively accumulating solutes to molar concentrations, especially when cells are immersed in high osmolarity mediums. Interaksi antara pepdidoglikan di dalam dinding sel berfungsi juga mempengaruhi integritas sel, karena akan memberikan kekuatan penghambatan, dan saluran modulositif (MS) yang memodulasi turgor. Turgor diproduksi dengan mengumpulkan zat terlarut secara selektif ke konsentrasi molar, terutama ketika sel direndam dalam media osmolaritas tinggi. Tekanan turgor akan meningkat lebih dari 10 atm dalam hitungan detik apabila terjadi penurunan tekanan osmotik eksternal secara tiba tiba (disebut downshock) oleh karena memicu pergerakan air yang cepat ke dalam sel. Tekanan tekanan yang menyebabkan pelebaran sel akan diakomodasi oleh dinding sel yang bersifat elastis..[18] Different osmotic pressures move the cytoplasmic membrane out through the pore pores and give rise to bulge formation. Bulge is a rather large bulge. The bulge that occurs in a smaller size is called a bleb. This bluff is to maintain the integrity of the cell wall peptidoglycan layer and the cytoplasmic membrane in order to prevent the cytoplasm from escaping. Bleb formation in this study is the positive charge of polyphenol extract. Cushnie, et al[19], states that the positive charge of antibiotics and the hydrophobic fatty acid chain is a feature that allows interaction with phosphate groups with negatively charged and peptidoglycan hydrophobic fatty acids on the outer membrane resulting in an increase in the surface area that causes the membrane to be bound tightly on the peptidoglycan and can not develop, this increased surface area will fold outward resulting in blebs.[19]

Polyphenols interact with proteins or phospholipids from the lipid bilayer. The interaction causes disruption of the lipid bilayer, increasing membrane permeability, affecting membrane fluidity, inhibiting respiration, and altering ion transport processes. The inhibition caused by phenol material is caused by the reduction of iron or hydrogen bonds with essential proteins such as microbial enzymes or other interactions to activated microbial adhesives, cell envelope transport proteins, and non-specific interactions with carbohydrates. The morphological study, apoptosis such as cytoplasmic membrane blebbing, nuclear contraction, nuclear fragmentation and contact inhibition on treatment with extracts.[20] The formation of blebs on the bacterial cell wall of *S. marcescens* treated with antimicrobial tea polyphenols (TP Morphology changes were first observed by transmission electron microscopy after treatment with TP, which indicated that the primary inhibition action of TP was to damage the bacterial cell membranes The permeability of the outer and inner membrane of *S. marcescens* dramatically increased after TP treatment, followed by the release of small cellular molecules.[5] Pattern of cell-shape deformation i.e. 1 bulge or sometimes multiple bulge cells often cause cracking around the bulges.[21]

Conflict of Interest Statement

We have no conflict of interest.

Acknowledgements

Thank you to the Head of the Oral Biology Laboratory, Dental Medicine University of Jember and the staff who have facilitated this research. We also extend our big thanks to the students, Tri Mulyana Agustin, colleagues and parties involved in this research.

CONCLUSIONS

It was concluded that polyphenolic extract of robusta coffee beans caused inhibition of bacterial growth of S. mutans by influencing morphological changes of the bacterial cell wall. However, the extract did not show bactericidal properties at the same concentration.

REFERENCES

1. Hecimovic I, Ana Belščak-Cvitanovic, Dunja H, Draz˘enka K, 2011, Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting, *Food Chemistry*, 129 : 991–1000

2. Papuc, GVG, Corina N.P, Valentin N, and Georgeta S, 2017, Plant Polyphenols as Antioxidant and Antibacterial Agents for Shelf-Life Extension of Meat and Meat Products: Classification, Structures, Sources, and Action Mechanisms Camelia Comprehensive *Reviews in Food Science and Food Safety*, 16

3. Khamenei B., Iranshahy M., Sohail V., Bazzaz S.F., 2019, Revie on plant antimicrobials: a mechanistic viewpoint, *Antimicrobial Resistance and Infection Control*, 8 : 118. https://doi.org/10.1186/s13756-019-0559-6

4. Dahlia M., 2012, Polyphenols As Antimicrobial Agents, *Current Opinion In Biotechnology*, 23 : 174-181

5. Yi S , Wei W, Fengling B, Junli Z, Jianrong L, Xuepeng L, Yongxia X, Tong S, Yutang H, 2014, Antimicrobial effect and membrane-active mechanism of tea polyphenols against *Serratia marcescens*, *World Journal of Microbiology and Biotechnology*, 30(2) : 451–460

6. Akhlaghi N., Sadeghi M., Fazeli F., Akhlaghi S., Mehnati M, Sadeghi M., 2019, The antibacterial effects of coffee extract, chlorhexidine, and fluoride against Streptococcus mutans and Lactobacillus plantarum: An in vitro study, *Dent Res J* , 16:346-53. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6749852/

7. Campos FM., JA Couto, AR. Figueiredo, IV. Tóth, A.O.S.S. Rangel, T.A. Hogg, 2009, Cell membrane damage induced by phenolic acids on wine lactic acid bacteria, (accessed on April 8, 2022) https://pdfs.semanticscholar.org/577a/7288c06a935b21bc7334b1d8747388ae35f4.pdf, https://core.ac.uk/reader/70664660

8. Lemos JA.,Palmer SR., Zeng L., Wen ZT., Kajfasz JK., Freire's IA., Abranches J., Brady LJ., 2019, The Biology of Streptococcus mutans, *Microbiol Spectr*,7(1): doi:10.1128/microbiolspec.GPP3-0051-2018.

9. Chitemerere TA and S Mukanganyama, 2014, Evaluation of cell membrane integrity as a potential antimicrobial target for plant products, *BMC Complement Altern Med*, 14: 278.

10. Ramstedt M., R Nakao, Sn Wai, Be Uhlin, J Boily, 2011, Monitoring Surface Chemical Changes In The Bacterial Cell Wall Multivariate Analysis Of Cryo-X-Ray Photo Electron Spectros Copy Data, *The Journal Of Biological Chemistry*, 286 (14) : 12389–12396

11. Krzyściak W, A. Jurczak, D. Kościelniak, B. Bystrowska, and A. Skalniak, 2014, The virulence of *Streptococcus mutans* and the ability to form biofilms, *Eur J Clin Microbiol Infect Dis*. 2014; 33(4): 499–515.

12. Grönroos L, 2000, Quantitative and Qualitative Characterization of Mutans Streptococci in Saliva and in the Dentition, *Dissertation*, Institute of Dentistry University of Helsinki, Finland

13. Sendamangalam V, OK Choi, D Kim and Y Seo, 2011, The anti-biofouling effect of polyphenols against *Streptococcus mutans*, *The Journal of Bioadhesion and Biofilm Research*, 27

14. Kociolek MG, 2009, Quorum Sensing Inhibitors and Biofilms, *J.Ant.Inf. Agents Med. Chem*, 8 (4) :315-326

15. Abe K., Nomura N., Suzuki S., 2020, Biofilms: Hot spots of horizontal gene transfer (HGT) in aquatic environments, with a focus on a new HGT mechanism, *FEMS Microbiol Ecol*, 96(5): fiaa031. 10.1093/femsec/fiaa031

16. Sumei Liao S., Klein MI., Heim KP., Yuwei Fan C., Bitoun JP., San-Joon Ahn. Robert C., Burne A., Hyun Koo C/. Jeannine Brady EL., Zezhang T. Wena, 2014, Streptococcus mutans Extracellular DNA Is Upregulated during Growth in Biofilms, Actively Released via Membrane Vesicles, and Influenced by Components of the Protein Secretion Machinery, *Journal of Bacteriology*, 196(13): 2355–2366, https://doi.org/10.1021/acs.langmuir.1c01410

17. Matsumoto-Nakano M., 2017, Role of Streptococcus mutans surface proteins for biofilm formation, *Japanese Dental Science Review*, 54: 22—29, https://doi.org/10.1016/j.jdsr.2017.08.002

18. Reuter M, NJ. Hayward, SS. Black, S Miller, DTF Dryden, IR. Booth, 2013, Mechanosensitive channels and bacterial cell wall integrity: does life end with a bang or a whimper?, *J. R. Soc. Interface*, 11 : 20130850., http://dx.doi.org/10.1098/rsif.2013.0850

19. Cushnie TPT, NH O'Driscoll, AJ Lamb, 2016, Morphological and ultrastructural changes in bacterial cells as an indicator of antibacterial mechanism of action, *Cell. Mol. Life Sci*., 73:4471–4492, https://www.researchgate.net/publication/305040042

20. Moirangthem DS, NC Talukdar, N Kasoju, U Bora , 2012, Antioxidant, antibacterial, cytotoxic and apoptotic activity of extracts of Cephalotaxus griffithii Hook. f. (stem bark), *BMC Complement Altern Med*., 12: 30. Published online 2012 April 3. doi: 10.1186/1472-6882-12-30, https://www.biomedcentral.com/manuscript/review/attachment/pdf/1813466398654778.pdf

21. Yao Z., Kahne D., Kishony R., 2012, Distinct Single-Cell Morphological Dynamics under Beta-Lactam Antibiotics, *Molecular Cell*, 48 (14) :705–712, https://doi.org/10.1016/j.molcel.2012.09.016