World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Antibacterial potential of butterfly Pea (*Clitoria ternatea*) towards *Aggregatibacter actinomycetemcomitans in vitro*

Rahul Pratama Subianto [1,*], Christian Khoswanto [2] and Indah Listiana Kriswandini [2]

[1] *Undergraduate Student of Dental Medicine Education Program, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[2] *Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.*

World Journal of Advanced Research and Reviews, 2023, 17(01), 868–876

Publication history: Received on 07 December 2022; revised on 19 January 2023; accepted on 22 January 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.17.1.0106

Abstract

Background: Periodontal disease in Indonesia have prevalence of 96.58%, One of the examples of periodontal disease is aggressive periodontitis caused by *Aggregatibacter actinomycetemcomitans* (Aa). Many researches have been done to study compounds that has antibacterial potential against Aa bacteria. Butterfly Pea is a plant abundant in Indonesia and has antibacterial potential.

Purpose: To study the antibacterial potential of Butterfly Pea against Aa bacteria.

Methods: Aa bacteria is cultured and the exposed to variations of Butterfly Pea extracts with various concentration such as 50%, 25%, 12.5%, 6.25%, and 3.125%. Positive control group is exposed to chlorhexidine and negative control group is exposed to DMSO. The results of diffusion test and dilution test are observed.

Results: In the dilution test Aa bacteria grows 100% on negative control group, 73,32% on the extracts with 3.125% concentration, 29.32% on 6.25% concentration, 7.62% on 12.5% concentration, and 0% on 25% & 50% concentration and on positive control group. In the Aa bacterial diffusion test there was an inhibition in the positive group of 24.28 mm, the extract group 12.5%, 25%, and 50% could inhibit 10.45 mm, 13.42 mm, 15.61 mm and the extract group 6.25%, 3.125%, and negative control had no inhibition on bacteria.

Conclusion: Potential Butterfly Pea flower extract as antibacteral compound against Aa with minimum inhibitory concentration of 12.5% and minimum bactericidal concentration of 25%. Butterfly pea flower extract (*Clitoria ternatea*) can inhibit *Aggregatibacter actinomycetemcomitans* bacteria with an average minimum inhibition zone diameter of 10.45 mm.

Keywords: Butterfly Pea; *Aggregatibacter actinomycetemcomitans*; Antibacterial activity; Minimum inhibitory concentration; Minimum bactericidal concentration

1. Introduction

Periodontal disease is a chronic inflammatory condition that destroys the tissues around the teeth and can also be considered a global public health problem, as it is increasing in every region among all socioeconomic classes. Severe periodontal disease is the 11th most common condition worldwide. The prevalence of periodontal disease is reported to range from 20% to 50% worldwide [1]. In general, periodontal disease is caused by plaque bacteria on the tooth surface, where plaque is in the form of a thin layer of biofilm which contains a collection of pathogenic microorganisms

* Corresponding author: Rahul Pratama Subianto

such as *Aggregatibacter actinomycetemcomitans*, *Actinobacillus actinomycetemcomitans, Prevotela intermedia, Tannerella forsythia and Fusobacterium nucleatum* which are soft deposits. Periodontitis initially begins as gingivitis which progresses to periodontitis. Aggressive periodontitis is a rapidly progressing inflammatory disease of the supporting tissues of the teeth, characterized by loss of connective tissue attachment and rapid breakdown of alveolar bone in more than one permanent tooth [2].

Aggregatibacter actinomycetemcomitans is a Gram-negative bacterium has leukotoxins and endotoxins that can damage tissues and the dominant pathogenic bacteria in patients with aggressive periodontitis 3, 4]. The main therapy for aggressive periodontitis can be carried out with mechanical therapy, one of which is by scaling and root planing (SRP) as well as supporting therapy in the form of chemical therapy (antibiotics and mouthwashes) to remove remaining bacteria. The antibiotics used to treat aggressive periodontitis are chlorhexidine, metronidazole and amoxicillin. The repeated and inappropriate use of antibiotics is the main reason for the increase in the number of drug-resistant bacteria. Therefore an alternative that can be done is to use plants that contain antibacterial as a substitute for drugs [5, 6]. Natural ingredients can be used as an alternative treatment for periodontal disease. The popularity of natural ingredients has increased due to their ready to use availability, low cost and minimal side effects. Butterfly pea flowers are classified as plants that can be used as medicine and are quite popular as an alternative to traditional medicine. Butterfly pea flowers have many benefits such as anti-diabetic, antiasthma, anti-inflammatory, antimicrobial and antioxidant. The content of active compounds contained in it include flavonoids, alkaloids, anthocyanins, tannins, saponins [7, 8].

Previous research on the antibacterial activity of butterfly pea extract showed that the extract had an antibacterial effect on the gram-negative bacteria *Proteus mirabilis* [9]. This study showed that the extract of the butterfly pea flower with a concentration of 15.6% was the minimum inhibitory concentration for the gram positive bacteria *Staphylococcus aureus* [10]. Other studies have also shown that the extract of the butterfly pea flower has a minimum inhibitory concentration of 6.25% and a minimum killing concentration of 25% against *Pseudomonas aeruginosa* bacteria [11]. This prompted researchers to conduct research to prove whether there is antibacterial potential of butterfly pea flower extract against one of the bacteria that causes aggressive periodontitis *Aggregatibacter actinomycetemcomitans*.

2. Material and methods

2.1. Materials and tools

The tools used in the research are a bunsen burner, petri dish, sterile cotton swab, cotton, test tube, ose, incubator, paper disc, micropipette, and caliper. The materials used are disinfectant, spiritus, *Aggregatibacter actinomycetemcomitans*, BHIB, MHA, alcohol, butterfly pea extract, DMSO, and chlorhexidine. The butterfly pea flower which was then determined and extracted by maceration technique with 70% ethanol solvent at the UPT Laboratorium Herbal Materia Medica Batu.

2.2. Preparation of butterfly pea extract

The butterfly pea flower samples obtained were then washed with running water then drained and then dried in the hot sun where the flowers were covered using a black cloth for 6 days and blended to obtain butterfly pea powder. The powder was weighed as much as 100 g and then put into a container with a lid, then added 1 liter of 70% ethanol and stirred for the first 6 hours. Leave it for 18 hours, stirring occasionally. Then strain using cotton and filter paper, collect the filtrate. Evaporate the macerate using a Rotavapor at a temperature of 40 °C or with a water bath at a temperature of 90 ºC while stirring to obtain a thick extract. Butterfly pea flower extract solution with concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%. Weigh 20 grams of butterfly pea extract and dilute with DMSO to obtain a volume of 10 ml while stirring with a stirring rod. Then put in vials and labeled [12, 13, 14].

2.3. Preparation of *Aggregatibacter actinomycetemcomitans*

Aggregatibacter actinomycetemcomitans isolated by the Research Center of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya was collected using an ose needle and then cultured on BHIB media in a test tube. *Aggregatibacter actinomycetemcomitans* in BHIB media were incubated at 37 ºC for 24 hours. After incubated, the bacteria were equalized to 0.5 McFarland or equivalent to 1.5 x 10^8 CFU/ml.

2.4. Antibacterial activity test

In the diffusion test *Aggregatibacter actinomycetemcomitans* were then grown in a petri dish containing MHA. The sample was then divided into the positive control group, treatment group, and negative control group. In the positive

control group, *Aggregatibacter actinomycetemcomitans* in MHA were given a paper disc containing 10 µl of chlorhexidine. For the treatment group, *Aggregatibacter actinomycetemcomitans* in MHA were given a paper disc which each contained 10 µl of butterfly pea extract with a concentration of 50%, 25%, 12,5%, 6,25%, 3,125%. In the negative control group, *Aggregatibacter actinomycetemcomitans* in MHA were given a paper disc containing 10 µl of DMSO. MHA was then incubated at 37 ºC for 24 hours after the paper disc was placed on top of it.

After MHA was incubated, an inhibition zone formed. The inhibition zone formed appears as a clear zone around the paper disc. The diameter of the inhibition zone was measured using a caliper in millimeters. The test was repeated four times for accuracy. In the dilusion test Prepare a test tube containing 7 ml of BHIB media according to the repetition and treatment. Put 1 ml of the bacterial suspension into the tube containing BHIB, then add 1 ml of the extract according to each concentration, then vortex and incubate for 24 hours at 37 ºC in an anaerobic atmosphere. After an incubation period of 24 hours, then observe MIC and KBM by observing each tube, if: Clear tube: Bacteria killed = extract can inhibit bacteria. Cloudy tube: Bacteria not killed = extract cannot inhibit. The MIC and MBC values are affected by concentrated extract concentrations, so it is necessary to subculture all dilution tubes at each concentration into solid media, to determine whether the turbidity is caused by the presence of bacterial growth or not, and at the same time to determine MIC and KBM, the entire tube The dilution was continued to the subculture stage on solid media, with full spread and incubated for 24 hours at 37 ºC under anaerobic conditions. If the media is visible results: The lowest concentration still contains bacterial colonies indicating the presence of bacteriostatic = MIC value, the lowest concentration where there is no growth of bacterial colonies indicates a bactericidal effect = MBC value. The entire procedure was repeated four times for each extract to avoid refraction.

2.5. Data Analysis

Data were analyzed using IBM SPSS Statistics 26.0 for windows. Normality of data distribution was assessed using the Shapiro-Wilk test because the number of samples was less than 50 (n<50). Levene's test is used to assess whether each group has homogeneous data. because the data were not normally distributed and were not homogeneous, a comparative test was used using kruskal wallis to compare the results between each group and to find out whether there were differences in the inhibition of *Aggregatibacter actinomycetemcomitans* growth.

3. Results

The result of this research is the calculation of the average inhibition zone diameter of *Aggregatibacter actinomycetemcomitans* (diffusion method) and the minimum inhibitory concentration & minimum bacterisidal concentration (dilution method).

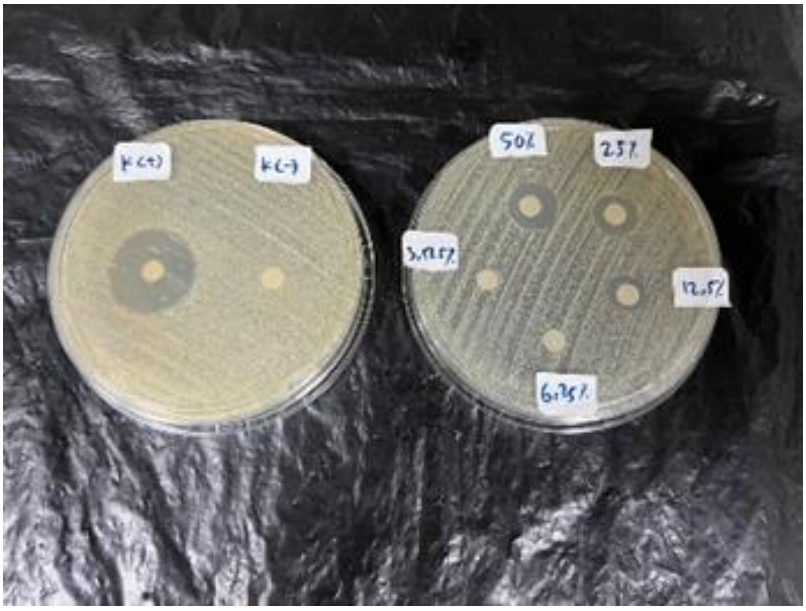

Figure 1 Inhibition Zone Results from the First Repetition

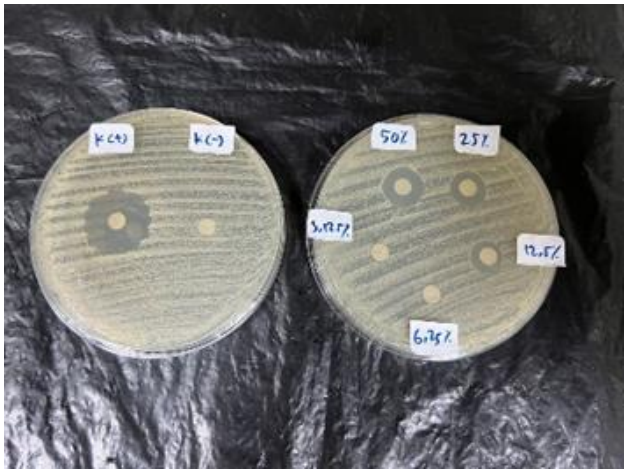

Figure 2 Inhibition Zone Results from the Second Repetition

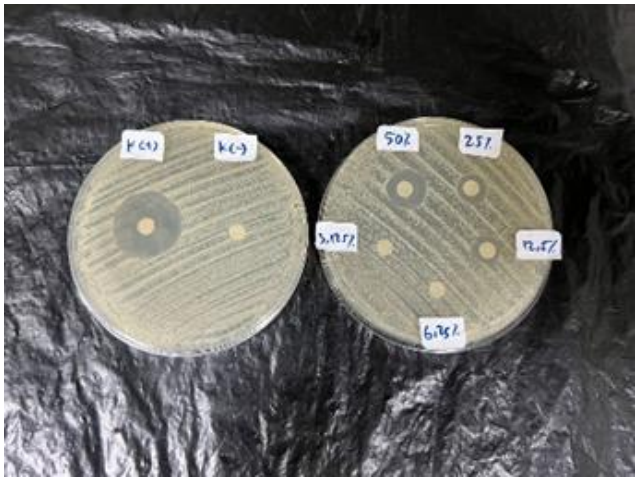

Figure 3 Inhibition Zone Results from the Third Repetition

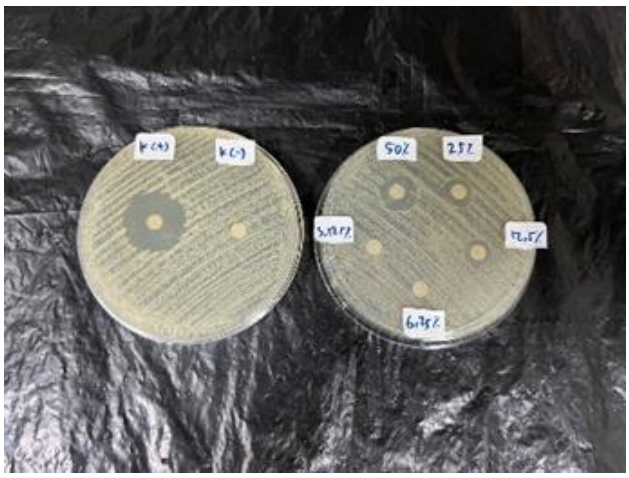

Figure 4 Inhibition Zone Results from the Fourth Repetition

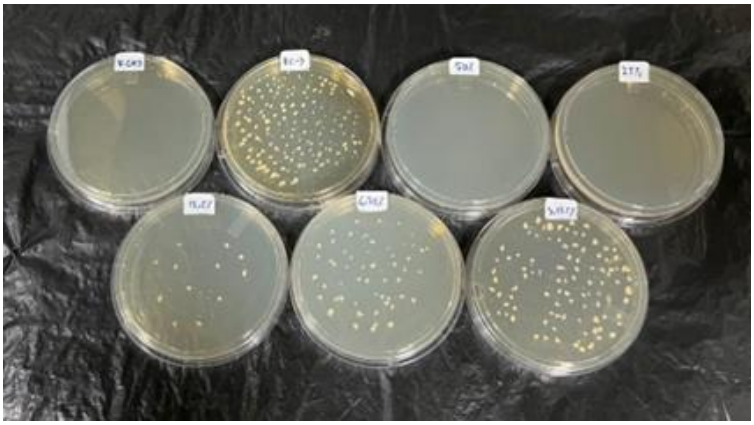

Figure 5 Colony count in each control from the first repetition

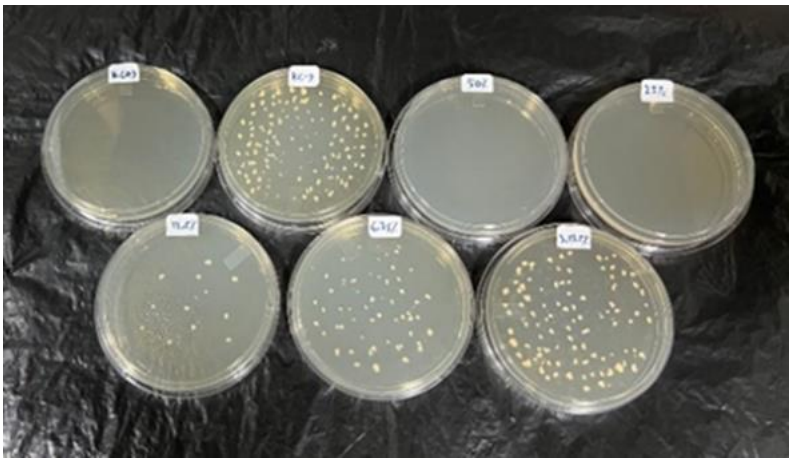

Figure 6 Colony count in each control the second repetition

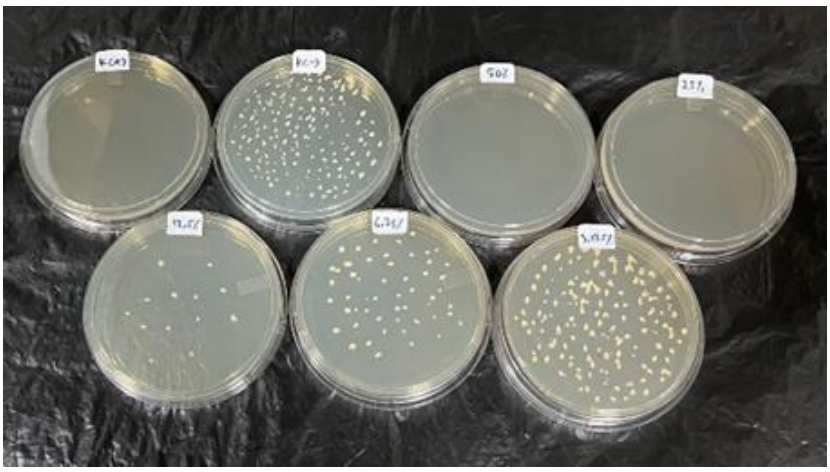

Figure 7 Colony count in each control the third repetition

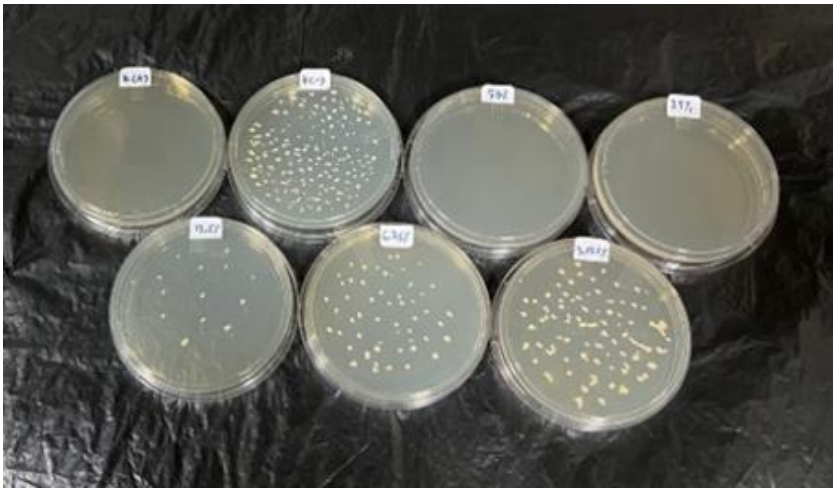

Figure 8 Colony count in each control the fourth repetition

Table 1 Inhibition zone diameter of *Aggregatibacter actinomycetemcomitans* (diffusion method)

Treatment Group	Diffusion Test (mm)				
	Replication 1	Replication 2	Replication 3	Replication 4	Average inhibition zone
50%	16.05	15.24	15.35	15.81	15.61 mm
25%	13.35	13.02	13.77	13.54	13.42 mm
12.5%	10.10	10.28	10.82	10.60	10.45 mm
6.25%	0.00	0.00	0.00	0.00	0.00
3.125%	0.00	0.00	0.00	0.00	0.00
K(-)	0.00	0.00	0.00	0.00	0.00
K(+)	24.63	25.05	23.47	23.98	24.28 mm
Min	0.00				
Max	25.05				
Mean	9.11				
SD	8.96				

Based on the research data (table 1), there was no inhibition zone formed from 6,25%, 3,125% butterfly pea extract and DMSO as a negative control group. Butterfly pea extract can inhibit the growth of *Aggregatibacter actinomycetemcomitans* starting at a concentration of 12,5% with an average inhibition zone diameter of 10.45 mm. The largest inhibition zone diameter was from 50% butterfly pea extract with an average inhibition zone diameter of 15.61 mm, although it was still lower than chlorhexidine as a positive control group with an average inhibition zone diameter of 24.28 mm.

Table 2 Colony count results (dilution method)

Treatment Group	Colony count (CFU/ml)				
	Replication 1	**Replication 2**	**Replication 3**	**Replication 4**	**Percentage**
50%	0	0	0	0	0.00%
25%	0	0	0	0	0.00%
12.5%	13	14	11	12	7.62%
6.25%	49	47	54	41	29.16%
3.125%	154	112	109	106	73.32%
K(-)	165	171	159	161	100.00%
K(+)	0	0	0	0	0.00%
Min	0				
Max	171				
Mean	49.21				
SD	63.56				

Based on the research data (table 2), it can be observed that at a concentration of 25%, 50% and chlorhexidine as a positive control there is no formation of bacterial colonies at all. At a concentration of 3.125% to 12.5% it can be observed that there are still bacterial colonies that are formed. The 25% concentration group is the minimum bacterisidal concentration where bacterial colony growth is 0%. The 12.5% concentration group is the minimum inhibitory concentration where bacterial growth is only 7.62% when compared to the negative control group where bacterial growth can be said to be 100%. Because only 7.62% of bacterial colonies can grow, it can be said that the inhibition power of 12.5% concentration is 92.38%. This is in accordance with the minimum inhibitory concentration requirements, namely inhibition >90%.

Table 3 Kruskal-Wallis test results

Test Statistics[a,b]		
	Dilution	**Diffusion**
Kruskal-Wallis H	26.679	26.679
df	6	6
Asymp. Sig.	0.000	0.000

a. Kruskal Wallis Test; b. Grouping Variable: Group

The Kruskal-Wallis test results found that P = 0.000 (P<0.05). it can be interpreted that there are groups that have significant differences compared to other groups in the dilution test and the diffusion test.

4. Discussion

This study examined the potential of butterfly pea flower extract (*Clitoria ternatea*) in inhibiting and killing *Aggregatibacter actinomycetemcomitans* bacterial colonies. This study chose butterfly pea extract because it is a plant that has a high population in tropical climates such as Indonesia. This research was conducted experimentally *in vitro* laboratory with the colony counting method and measuring the diameter of the inhibition zone in bacterial cultures exposed to butterfly pea extract with various concentrations ranging from 50%, 25%, 12.5%, 6.25% and 3.125%. In addition, there was also a positive control group that was exposed to chlorhexidine and a negative control group that was only given DMSO [12, 13, 14].

Potential of butterfly pea flower extract in inhibiting or killing *Aggregatibacter actinomycetemcomitans* bacteria can be measured by observing the minimum inhibitory concentration (MIC) and minimum bacterisidal concentration (MBC).

The requirement for a concentration expressed as MIC is that at least a compound with that concentration can inhibit bacteria by more than 90%. The requirement for a concentration expressed as MBC is that at least a compound with that concentration can inhibit bacteria close to 100%. This can be observed in the dilution test.

In the dilution test, the MIC value was obtained at a concentration of 12.5%. This can be observed because at a concentration of 12.5% only 7.62% of bacteria can grow compared to the population of bacteria in the negative control group. This shows that the concentration of 12.5% has an inhibition of 92.38% and is in accordance with the MIC requirements where the inhibition of the compound against bacteria is more than 90%. Then at concentrations of 25% and 50% no colonies of *Aggregatibacter actinomycetemcomitans* bacteria could be found or in other words at these concentrations bacterial growth was 0% when compared to the negative control group. Taking this into account, it can be determined that the MBC is 25%. then the results of the diffusion test confirmed that the inhibition zone was formed starting at a concentration of 12.5% which was the smallest inhibition zone with an average inhibition zone diameter of 10.45 mm and a concentration of 50% was the concentration with the largest inhibition zone with an average inhibition zone diameter of 15.61 mm.

The antibacterial effect of butterfly pea flower extract is probably due to the many active compounds it contains. Some of these active compounds include alkaloids, saponins, tannins, and anthocyanins. Alkaloids inhibit bacterial growth by disrupting the constituent components of peptidoglycan which then cause bacterial cells susceptible to lysis. Alkaloids also inhibit bacterial growth through the process of DNA interaction [15]. Saponin inhibits bacterial growth by acting as a 'detergent' agent. It can cause a decrease in the surface tension of bacterial cells which then leads to a decrease in the bacterial cell wall permeability [16]. Tannin inhibits bacterial growth by inhibiting the bacterial absorption of glucose and amino acids [17]. The mechanism underlying the antimicrobial activity of anthocyanins includes cell membrane and intracellular interactions of these compounds. Bacteria exposed to anthocyanins will experience irregularities in the outer membrane and cytoplasmic leakage [18].

From the results of this study it is known that the higher the concentration of butterfly pea flower extract, the greater its ability to inhibit bacteria. This can happen because the higher the concentration of the extract, the higher the phytochemical compounds contained therein.

This research shows that the extract of butterfly pea flower has potential as an antibacterial against *Aggregatibacter actinomycetemcomitans*. This is because the butterfly pea flower extract contains various phytochemical compounds as antibacterial against *Aggregatibacter actinomycetemcomitans*. In future research, butterfly pea flower extract can be developed into an alternative topical drug or mixed ingredient in mouthwash for the treatment of periodontitis and other oral infections.

5. Conclusion

The potency of butterfly pea flower extract (*Clitoria ternatea*) as an antibacterial was obtained for 12.5% MIC and 25% MBC for *Aggregatibacter actinomycetemcomitans*. Butterfly pea flower extract (*Clitoria ternatea*) can inhibit *Aggregatibacter actinomycetemcomitans* bacteria with an average minimum inhibition zone diameter of 10.45 mm.

Compliance with ethical standards

Acknowledgments

We would like to thank the Research Center of the Faculty of Dental Medicine, Universitas Airlangga for allowing and providing a place for research to be carried out.

Disclosure of conflict of interest

There is no conflict of interest between the authors.

Statement of ethical approval

An ethical clearance certificate which is a requirement for conducting this research has been obtained from the Health Research Ethical Clearance Commission of the Faculty of Dental Medicine, Universitas Airlangga (No. 790/HRECC.FODM/X/2022).

References

[1] Vos, T. et al. (2017) 'Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016', The Lancet, 390(10100), pp. 1211–1259. DOI: 10.1016/S0140- 6736(17)32154-2.

[2] Andriani, I. and Chairunnisa, F. A. (2019) 'Periodontitis Kronis dan Penatalaksaan Kasus dengan Kuretase', Insisiva Dental Journal: Majalah Kedokteran Gigi Insisiva, 8(1), pp. 25–30. DOI: 10.18196/di.8103.

[3] Andayani, R., Chismirina, S., Habdani, A.D. (2012). EFEK ANTIBAKTERIAL REBUSAN TEH HIJAU TERHADAP PERTUMBUHAN *AGGREGATIBACTER ACTINOMYCETEMCOMITANS* SEBAGAI PERIODONTOPATOGEN PERIODONTITIS AGRESIF. dentika Dental Journal, Vol 17, No. 2: 172-176.

[4] Mythireyi, D. and Krishnababa, M. (2012) '*Aggregatibacter actinomycetemcomitans*, an Aggressive Oral Bacteria - A Review', International Journal of Health Sciences and Rese, 2(5), pp. 105–117.

[5] Alibasyah, Zulfan & Setya, Diana & Sinda, Maya. (2020). AKTIVITAS ANTIBAKTERI EKSTRAK ETANOL 70% DAUN BIDURI (Calotropis gigantea) TERHADAP *Aggregatibacter actinomycetemcomitans* ATCC 29523. Cakradonya Dental Journal. 12. 56-63. 10.24815/cdj.v12i1.17831.

[6] Afrina, Chismirina, S., Magistra, R.Y. (2016). 'KONSENTRASI HAMBAT DAN BUNUH MINIMUM EKSTRAK DAUN JERUK NIPIS (Citrus aurantifolia) Terhadap *Aggregatibacter actinomycetemcomitans* SECARA *IN VITRO*.' Cakradonya Dent J, 8(1):1-76.

[7] Pratap, G. M. J. S., Manoj, K. M. G., Sai, S. A. J., Sujatha, B., and Sreedevi, E. (2012) 'Evaluation of three medicinal plants for anti-microbial activity.', Ayu, 33(3), pp. 423–428. doi: 10.4103/0974- 8520.108859.

[8] Yurisna, V. C., Nabila, F. S., Radhityaningtyas, D. ., Listyaningrum, F., & Aini, N. (2022). Potensi Bunga Telang (*Clitoria ternatea* L.) sebagai Antibakteri pada Produk Pangan. JITIPARI (Jurnal Ilmiah Teknologi Dan Industri Pangan UNISRI), 7(1), 68–77. https://doi.org/10.33061/jitipari.v7i1.5738

[9] Dhanasekaran, S., Rajesh, A., Mathimani, T., Melvin Samuel, S., Shanmuganathan, R. and Brindhadevi, K. (2019) 'Efficacy of crude extracts of *Clitoria ternatea* for antibacterial activity against gram negative bacterium (Proteus mirabilis)', Biocatalysis and Agricultural Biotechnology. Elsevier Ltd, 21(September), p. 101328. DOI: 10.1016/j.bcab.2019.101328.

[10] Indrianingsih, A. W., Wulanjati, M. P., Windarsih, A., Bhattacharjya, D. K., Suzuki, T. and Katayama, T. (2021) '*In vitro* studies of antioxidant, antidiabetic, and antibacterial activities of Theobroma cacao, Anonna muricata and *Clitoria ternatea*, Biocatalysis and Agricultural Biotechnology.

[11] Rashid, S. A., Tong, W. Y., Leong, C. R., Abdul Ghazali, N. M., Taher, M. A., Ahmad, N., Tan, W. N. and Teo, S. H. (2021) 'Anthocyanin Microcapsule from *Clitoria ternatea* : Potential Bio-preservative and Blue Colorant for Baked Food Products', Arabian Journal for Science and Engineering. Springer Berlin Heidelberg, 46(1), pp. 65–72. DOI: 10.1007/s13369-020-04716-y.

[12] Puti, S. F. (2022). Pengaruh Konsentrasi Sari Bunga Telang (*Clitoria ternatea*) Terhadap Karakteristik Fisikokimia dan Organoleptik Permen Jelly (Doctoral dissertation, Universitas Andalas).

[13] Montironi, I. D., Cariddi, L. N., & Reinoso, E. B. (2016). Evaluation of the antimicrobial efficacy of Minthostachys verticillata essential oil and limonene against Streptococcus uberis strains isolated from bovine mastitis. Revista Argentina de microbiologia, 48(3), 210-216.

[14] Baena-Santillán, E. S., Piloni-Martini, J., Santos-López, E. M., Gómez-Aldapa, C. A., Rangel-Vargas, E., & Castro-Rosas, J. (2021). Comparison of the antimicrobial activity of Hibiscus sabdariffa calyx extracts, six commercial types of mouthwashes, and chlorhexidine on oral pathogenic bacteria, and the effect of Hibiscus sabdariffa extracts and chlorhexidine on permeability of the bacterial membrane. Journal of medicinal food, 24(1), 67-76.

[15] Cushnie TPT, Cushnie B, Lamb AJ. Alkaloids: an Overview of Their Antibacterial, Antibiotic-enhancing and Antivirulence Activities. International Journal of Antimicrobial Agents. 2014; 44(5): 377-386.

[16] Weng A, Thakur, Melzig, Fuchs. Chemistry and Pharmacology of Saponins: Special Focus on Cytotoxic Properties. Botanics: Targets and Therapy. 2011; 1: 19.

[17] Kaczmarek B. Tannic Acid with Antiviral and Antibacterial Activity as A Promosing Component of Biomaterials: A Minireview. Materials. 2020; 13(14).

[18] Cisowska A1., D. Wojnicz., AB Hendrich. 2011. Anthocyanins as antimicrobialagents of natural plant origin. Nat Prod Commun. 6(1):149-56