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ABSTRACT

Background: The denture usage should be accompanied by good denture cleansing to prevent denture damage and oral disease due to bacterial on the denture. Research on purple leaves (*Graptophyllum pictum*) showed that purple leaves have anti-bacterial and antimicrobial activity. So it is essential to study the effectiveness of purple leaves as denture cleanser towards the growth of S. mutans on flexible denture plates.
Objective: The objective of the study was to compare the effectiveness of purple leaf extract at different concentrations: 1.25%; 2.5%; 10% and 40%, fittident® and aquadest towards S. mutans growth on flexible denture plates.
Methods: This study was in vitro study that used S. mutans from local isolates of the microbiological laboratory faculty of medicine, Universitas Udayana. The isolates were incubated for 24 hours with temperature 37ºC on 18 plates (10x10x2mm). The concentration of purple leaf extract was 1.25%; 2.5%; 10% and 40%. The effectiveness of purple leaf extract, fittident® and aquadest towards the growth of S. mutans can be evaluated from the number of S. mutans colonies on flexible denture base plate (CFU/ml). Data were analyzed using the One Way Anova test with 95% confidence level (α = 0,05%). LSD (Least Significant Different) was tested to determine the significance of differences in the treatment group.
Result: The lowest mean of colonies' growth was found in 10% concentration (21.0000). There was significant difference in colony count between the extract concentration groups (p=0.000). LSD-multi comparisons test on the Post Hoc analysis showed a significant difference between the extract concentrations of 1.25% to 10% (p=0.037), and the 2.5% extract to 10% (p=0.012) and 40% (p=0.027). *Graptophylum pictum* extract showed with highest flavonoid content (4340,30 mg/100 QE wb).
Conclusion: All extract concentrations are effective in suppressing bacterial growth. The optimum concentration to the growth of S. mutans was the extract with 10% concentration but did not differ significantly with the 40% concentration extract.

Keywords: *Graptophyllum Pictum*, S. mutans, flexible denture

Introduction

Tooth loss is a common thing in society due to accidents, illnesses, or natural aging processes. If not treated immediately will be able to produce anatomical, physiological, and functional changes that can even lead to psychological trauma. The use of dentures can help restore phonetic or speech functions, mastication, aesthetic or beauty functions, and preserve oral tissues. The polyamide thermoplastic resin (nylon) is a flexible denture base with distinctive physical and aesthetic properties. This denture has very good flexibility and stability and can be made thinner with certain thicknesses, so it is flexible, lightweight, and not easily broken.[1]

It is essential to prevent denture damage and to minimize the formation of bacterial colonies or candida on the surface of the denture. They can cause dental and oral problems such as halitosis, caries, stomatitis, periodontal disease, inflammation of the palatal mucosa, and denture stomatitis. The causes of denture stomatitis include C. albicans, bacterial infections, allergies, psychological factors, lack of denture hygiene, salivary flow, and nutrients.[3] Colonization of bacteria and fungi causes salivary pH of patients to

be more acidic and a trigger factor of denture stomatitis. The acidic condition is due to carbohydrate fermentation by C. albicans and S. mutans.[4,5]

Denture cleansing can be done mechanically with a soft toothbrush, or by chemical means using a disinfectant. Denture cleansers should preferably be bactericidal and fungicidal, easy to use, and compatible with all denture materials. Ideal denture cleansers should have the following characteristics: nontoxic, easily removed, leaving no irritant, irritating, or dissolving organic and inorganic materials contained in the denture, does not damage denture materials, is stable in storage, and is preferable of a bactericide and fungicide.[6] The use of plant materials as an alternative ingredient of denture cleanser is expected to provide benefits. The material is easy to obtain, cheap, and relatively safer than the chemical-based.[7]

Purple leaf or *Graptophylum pictum* is one of the plants that are often used as traditional medicine, such as hemorrhoid drug.[8] Chemical content of purple leaves consists of alkaloids, flavonoids, tannins, alkaloids, steroids, and saponins. Flavonoids are the largest group of phenol compounds, simple monocyclic phenols, phenylpropanoids, and phenolic quinones.[9] The general properties of phenol can increase cell permeability and precipitate proteins.[10] Flavonoids can inhibit microorganisms because of their ability to form complex compounds with proteins and are antiviral.[9] Research in medicine shows that purple leaves are useful for healing hemorrhoids, anti-bacterial, analgesic, and anti-inflammatory. Research conducted in dentistry shows that 40% purple leaf extract has the highest anti-fungal power to the growth of C. albicans on denture acrylic resin plate.[3] So, it is essential to do research which aims to compare the effectiveness of purple leaf extract as denture cleanser at concentration: 1.25%; 2.5%; 10%, and 40%, towards S. mutans growth on flexible denture plates.

Materials and Methods

Methods

This study was using laboratory experimental research design in-vitro with posttest-only control group design, which was approved by local ethic commission. This study compares the effectiveness of purple leaf extract at different concentrations: 1.25%; 2.5%; 10% and 40%, fittident® and aquadest as denture cleanser towards the growth of S. mutans on flexible denture plates, all group repeated three times.

Bacteria and reagents

This study used S. mutans ATCC 35668 from the microbiology laboratory, Faculty of Medicine, Universitas Udayana. The S. mutans has incubated for 24 hours at 37^0C with suspension according to 0.5 Mc. Farland standard ($3x10^6$ CFU/ml), on the surface of the thermoplastic nylon plate size 10x10x2 mm. Mueller Hinton agar was used for the disc diffusion method.

Preparation of purple leaves extract

Graptophyllum Pictum or purple leaves is a traditional medicine act as anti-bacterial, analgesic, and anti-inflammatory for healing hemorrhoids. The extracting process consists of extracting and evaporating processes. Purple leaves as much as 100 grams are dried and ground and then extracted using 70% ethanol for 3 hours. The liquid extract was concentrated with a Vacuum Rotary Evaporator to obtain 100% purple leaf extract. The extract is then weighed with an analytical balance. The dilution method was used to obtain a solution of purple leaf extract concentrations of 1.25%: 1.25 grams of purple leaf extract plus distilled water up to 100 ml. Making a solution of purple leaf extract of 2.5%, 10% and 40% was carried out the same method with the weight of purple leaf extract 2.5 g, 10 g, and 40 g.

Preparation of Denture Cleansers

The flexible denture plates were sterilized using an autoclave with temperature 121°C for 15 minutes. Then, it immersed in sterile saliva for 1 hour, then rinsed with PBS and inserted into test tube containing TSB (trypticase soy broth) media containing S. mutans suspension, then incubated for 24 hours at 37°C. After that, the plates were inserted into a closed test tube, each containing a purple leaf extract of 1.25%,

2.5%, 10%, and 40% concentrations, and sterile aquades (control). The immersion period used is 5 minutes. Furthermore, the plate was rinsed with PBS and then inserted into test tube containing TSB media. All test tubes were vibrated on vortex for 1 minute to release S. mutans attached to plate, then S. mutants were grown on Nutrient Broth - MHB agar, then incubated for 24 hours at 37°C, then counting bacterial colonies using colony counter.

Statistical Analysis

Data were analyzed using One-Way Anova test with 95% confidence level (α = 0,05%). LSD (Least Significant Different) was then tested to determine the significance of differences in the treatment group.

Result

The mean of S. mutans colonies on flexible denture base plate in each *Graptophylum pictum* extract concentration group can be seen in **table 1**. It was showed that 2,5% *Graptophyllum pictum* extract had the highest mean of colonies growth (43.3333), while the lowest mean of colonies growth was in 10% concentration (21.0000).

The result of One-Way ANOVA test (**Table 2)** showed that there was a significant difference ($p < 0.05$) of bacterial colony count between some extract concentration groups. In **table 3,** the results of the LSD-multi comparisons test on the Post Hoc analysis showed significant difference between the extract concentrations of 1.25% to 10%, and the 2.5% extract to 10% and 40%. All extract concentrations have significant differences in suppressing bacterial growth when compared to the control group. Extracts with 10% concentration were considered to have the most optimum effectiveness. The extracts at these concentrations were significantly different with 1.25% and 2.5% concentration, but did not differ significantly with the extracts with concentrations of 40%.

The qualitative phytochemical analysis (**Table 4**) showed that the *Graptophylum pictum* plant extract contains a mixture of phytochemicals as Flavonoid, Tannin and Saponin. In vitro antioxidant activity of *Graptophylum pictum* extract were determined using spectrophotometric methods. In **table 5** the *Graptophylum pictum* extract showed flavonoid content (4340,30 mg/100 QE wb), tannin content (1104,62 mg/100g TAE wb) and total phenolic content (1082,25 mg/100g GAE wb). However, the *Graptophylum pictum* exhibited very weak antioxidant capacities (3038,41 mg/L GAEAC) with the IC 50% value is 212,77 mg/ml.

Table 1. The mean of S. mutans colonies on flexible denture base plate (CFU/ml)

Group	Number of Samples	Mean
1,25% extract concentration	3	38.6667
2,5% extract concentration	3	43.3333
5% extract concentration	3	25.0000
10% extract concentration	3	21.0000
20% extract concentration	3	40.0000
40% extract concentration	3	24.3333
Fittydent®	3	0.3333
Aquades	3	26.6667
Bacterial suspension	3	298.0000
Total	27	57.4815

Table 2. The result of One-Way ANOVA test

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	179114.667	5	35822.933	420.073	.000
Within Groups	1023.333	12	85.278		
Total	180138.000	17			

Table 3. The results of LSD-multi comparisons test on the Post-Hoc analysis

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1,25% Extract concentration	2,5% Extract concentration	-4.667	7.540	0.548	-21.09	11.76
	10% Extract concentration	17.667*	7.540	**0.037***	1.24	34.09
	40% Extract concentration	14.333	7.540	0.082	-2.09	30.76
	Bacterial suspension	-258.667*	7.540	**0.000***	-275.09	-242.24
2,5% Extract concentration	1,25% Extract concentration	4.667	7.540	0.548	-11.76	21.09
	10% Extract concentration	22.333*	7.540	**0.012***	5.91	38.76
	40% Extract concentration	19.000*	7.540	**0.027***	2.57	35.43
	Bacterial suspension	-254.000*	7.540	**0.000***	-270.43	-237.57
10% Extract concentration	1,25% Extract concentration	-17.667*	7.540	0.037	-34.09	-1.24
	40% Extract concentration	-3.333	7.540	0.666	-19.76	13.09
	Bacterial suspension	-276.333*	7.540	**0.000***	-292.76	-259.91
40% Extract concentration	1,25% Extract concentration	-14.333	7.540	0.082	-30.76	2.09
	Bacterial suspension	-273.000*	7.540	**0.000***	-289.43	-256.57
Bacterial suspension	1,25% Extract concentration	258.667*	7.540	**0.000***	242.24	275.09
	2,5% Extract concentration	254.000*	7.540	**0.000***	237.57	270.43
	10% Extract concentration	276.333*	7.540	**0.000***	259.91	292.76
	40% Extract concentration	273.000*	7.540	**0.000***	256.57	289.43

Noted: * The mean difference is significant at the 0.05 level.

Table 4. The result of qualitative phytochemical test

No	Parameter	Method	Result
1	Alkaloid	Qualitative	Negative
2	Flavonoid	Qualitative	Positive
3	Tannin	Qualitative	Positive

4	Steroid	Qualitative	Negative
5	Saponin	Qualitative	Positive

Table 5. The result of quantitative phytochemical test

No	Parameter	measure	Measure	Content
1	Flavonoid	Spectrophotometric	mg/100 QE wb	4340,30
2	Antioxidant capacity	Spectrophotometric	mg/L GAEAC	3038,41
3	IC 50%	Spectrophotometric	Mg/L	212,77
4	Phenol total (polyphenol)	Spectrophotometric	mg/100g (GAE) wb	1082,25
5	Tannin	Spectrophotometric	mg/100g TAE wb	1104,62

Abbreviations: TAE (Tannic acid equivalent); QE (Quercetine equivalent); GAE (Garlic acid equivalent); **GAEAC** (Gallic acid equivalent antioxidant capacity); wb (wet basin)

Notes: IC50% Result (>200mg/L = very weak antioxidant), (150mg/L <IC50% <200mg/L = weak antioxidant), (100mg/L <IC50% <150mg/L = moderate antioxidant), (50mg/L<IC50%<100mg/L = strong antioxidant), (IC50%<50mg/L=very strong antioxidant)

Discussion

In the early formation of salivary-pellicles, gram-positive bacteria, Streptococcus sp. became the first bacteria to attach to the base of the denture and form a colony. S. mutans is one of them which extracellular polysaccharide (PSE) and other bacteria do not own it. The substrate becomes the path for bacteria and other fungi attached to the base of the denture. The colonization of bacteria and fungi will proliferate into plaque leading to denture stomatitis.[11] The denture base material also affects the ability of S. mutans bacteria attachment on the denture base. Whereas, based on the results of the research on removable denture base ingredients shows that the number of S. mutans bacteria on the nylon thermoplastics plate are less than the heat-cured acrylic resins. Based on this study was showed that the highest colonies' growth was in 2,5% *Graptophyllum pictum* extract concentration, while the lowest colonies' growth average was in 10% concentration.

This study showed that the Purple leaf or *Graptophylum pictum* plant extract contains a mixture of phytochemicals as Flavonoid, Tannin, and Saponin. Purple leaf extract contains anti-bacterial flavonoids, capable of inhibiting the growth of S. mutans by forming complex compounds against extracellular proteins that interfere with the integrity of bacterial cell membranes.[12] Flavonoids work using protein denaturation, thereby increasing the permeability of cell membranes. Denaturation of proteins causes a disruption in cell formation that alters the composition of protein components. The function of the disrupted cell membrane may cause increased cell permeability, resulting in cellular damage.[3] Denaturation of proteins can damage cells irreversibly and irreparably. Tanin is also thought to have the ability to inhibit growth or kill S. mutans by collapsing and precipitating proteins from the solution by forming an insoluble compound.[13] Based on this, the effectiveness of purple leaf extract is lower in suppressing bacterial growth at concentrations below 10%, possibly due to lower levels of flavonoids and tannins. Still, at concentrations of 40%, their effectiveness has no significant difference with a 10% concentration of the possibility because the levels of flavonoids and tannins have reached optimal ability. However, the *Graptophylum pictum* exhibited very weak antioxidant capacities, with the IC 50% value is 212,77 mg/ml.

Conclusion

All extract concentrations are effective in suppressing the growth of bacteria, but which has the optimum effect is an extract with a concentration of 10%.

Author Contribution

All authors have contributed to all process in this research, including research design, data collection, and its analysis, writing the manuscript for article publication

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Conflict of Interest

The authors declare no conflict of interest regarding the publication of this article

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