Aloha International Journal of Health Advancement (AIJHA)
ISSN 2621-8224
Volume 2 Number 7, July 2019
http://journal.aloha.academy/index.php/aijha
RESEARCH
DOI: http://dx.doi.org/10.33846/aijha20701

Comparison of Tooth Paste Gel Antibacterial Activity of Ethanol Extract and Ethyl Acetate *Mimosa pudicafolium* Against Bacteria *Streptococcus Mutans* Caries Dental Disease

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Submitted: May 19, 2019 -Revised: June 8, 2019 -Accepted: June 26, 2019 -Published: July 31, 2019

ABSTRACT

Dental caries is a disease of the oral cavity caused by bacteria *Streptococcus mutans*. These diseases cause cavities thus damaging tissues of the teeth. One of the plants that have antibacterial activity is *Mimosa pudicaFolium*, which is known as one type of weed that spreads life. Active substance, alkaloids, flavonoids, saponins, triterpenes and glycosides. Prevention and appropriate treatment can affect the rate of healing of dental caries. One of the prevention is to maintain the cleanliness of your teeth and gums using a toothpaste. The aim of this study to create a gel toothpaste from ethyl aseatat extracts and fractions of ethanol extract of the leaves as well as comparing the *Mimosa pudica* antibacterial activity against *Streptococcus mutans*. This research method made three formulations gel toothpaste with respective weight ratio of extracts and fractions are 5%, 10% and 15%. Gel toothpastes made tested antibacterial activity against *Streptococcus mutans* bacteria in vitro with the negative control is the solvent DMSO and a positive control ciprofloxacin 25 mg/disk. The medium used was media Muller Hinton Agar (MHA). The identification results showed that ethanol extract phytochemicals *Mimosa pudica Folium* contain flavonoids and tannins. While only a fraction of ethyl acetate contained flavonoids. The test results showed that the antibacterial activity of the negative control DMSO not provide a response to the bacteria *Streptococcus mutans*, where as the positive control ciprofloxacin give a strong response at a concentration of 25 µg / disk with a diameter of 25 mm inhibition zone. The test sample gel toothpaste from ethyl acetate fraction of ethanol extract putrimalu leaves the greatest response at 15% concentration of 150 mg/ml of 15.55 mm compared with a concentration of 5%, 10% and ethanol extract on all series of concentration.

Keywords: antibacterials; *Streptococcus mutants*; gel toothpaste; *Mimosa pudica Folium*

INTRODUCTION

Dental caries is a disease of the oral cavity where the mouth is one of the growing microorganisms such as *Streptococcus mutans*, *Streptococcus sanguinis*, and *Candida albicans*. Dental caries cause cavities that can damage the hard tissues of the tooth. Factors causing dental caries is the neglect of oral hygiene, the high humidity in the oral cavity and the presence of leftovers cause plaque buildup. Plaque contains bacteria, one of which is *Streptococcus mutans* which is a gram-positive bacterium, is nonmotil and facultative anaerobes that can metabolize carbohydrates [(1)]. *Streptococcus mutans* is the major bacterial cause of caries[(2)].

Plants *Mimosa pudica* (*Mimosa pudica Linn*) is known as one type of weed species that live creeper, its leaves will be shut when touched or shaken, and will reopen in a few Today, the plant *Mimosa pudica* researched properties, especially as a remedy based on natural materials. Several studies on the efficacy of plant *Mimosa pudica* is treating diarrheal diseases, asthma, inflammatory problems, and urinary tract infection [(3)], anti helmintik, anti-fungal, and antibacterial, antipyretic, antispasmodic and antimicrobial[(4)]. The content of plant secondary metabolites Mimosa pudicaare alkaloids, flavonoids, saponins, triterpenes, glycosides[(5)]. The aim of this study to compare extracts and fractions of ethanol extract of the leaves of Mimosa pudica made gel toothpaste for dental caries to prevent and treat dental caries disease caused by the bacterium *Streptococcus mutans*

METHODS

Tools and Materials

The tools used are glass beaker, flask, measuring cups, aluminum foil, analytical balance, oven, rotary evaporator, mortar stamfer, stir bar, flask, napkins, gloves, masks, separating funnel, stative and clamps.

Materials used are botanicals *Mimosa pudicaFolium*, ethanol 70%, ethyl acetate, nutrient agar medium, nutrient broth, MHA, DMSO, ciprofloxacin discs and blank discs.

Preparation of Extract

Mimosa pudicaFolium are separated from the stems, sorted dry and wet, dried using an oven at 50^0C until a moisture content below 10%, and a blender to obtain a powder.

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Powder obtained by maceration extracted using 70% ethanol for 3 days, filtered, concentrated juice extracts obtained using rotaryevaporator until thick.

Identification of Phytochemicals

The identification of phytichemicals were:

1) Steroids / triterpen. Condensed extract samples are added 20 drops of acetic acid anhydride and 1 drop of concentrated sulfuric acid (Liebermann Burchard reagent) to form a blue or blue-green color indicates steroid, while red, pink or purple indicates triterpenoids[6].
2) Flavonoids. Samples coupled with a little water in a test tube, add a bit of metal magnesium and 5 drops of 2 N HCl, heated for 5-10 minutes, hot filtered and allowed to cool, the filtrate plus amyl alcohol, strong shaking. A positive reaction to the formation of a layer of red in amyl alcohol [7]
3) Alkaloids. Extracts basified with ammonia, chloroform added. Chloroform liquid is filtered, the filtrate is placed in a test tube and then added 2 N HCl, shaken, until separation occurs. The filtrate plus reagent Dragendorff show sediment or turbidity colorless to brown, and other filtrate added reagent Mayer showed white precipitate or turbidity.
4) Saponin. 1g of extract added with warm water, shaken vertically for 10 seconds, allowed to stand for 10 seconds. 1-10 cm tall foam forming stable for not less than 10 minutes, indicating the presence of saponins. In addition 1 drop of HCl 2 N, the foam does not disappear [8]
5) Tanin, 200 mg of extract diluted in 20 ml of hot water and then shaken until homogeneous. after cold added $FeCl_3$ 3% showed a positive result if the solution formed a blue-black or brownish green.

Fractionation

Weighed ethanol extract of the leaves as much as 20 grams *Mimosa pudica*, added 100 ml of distilled water, shaken ad dissolves enter into a separating funnel. Furthermore, the partition with 100 ml of solvent n-hexane to obtain the clear fraction of n-hexane. Fraction acquired distilled water was added with 100 ml of ethyl acetate, is repeated until it produces ethyl acetate fraction with a constant color. Further ethyl acetate fraction was concentrated using a rotary evaporator. Calculated yield of ethyl acetate fraction.

Formulation

Table 1. Formulation ethanol extract

Material	F1	F2	F3
The ethanol extract	5%	10%	15%
Sorbitol	10%	10%	10%
Glycerin	10%	10%	10%
Carbopol 934	0.5%	0.5%	0.5%
TEA	0.75%	0.75%	0.75%
Nipagin	0.1%	0.1%	0.1%
Aqua ad	100	100	100

Table 2. Formulation ethyl acetate fraction

Material	F1	F2	F3
Ethyl acetate fraction	1%	3%	5%
Sorbitol	10%	10%	10%
Glycerin	10%	10%	10%
Carbopol 934	0.5%	0.5%	0.5%
TEA	0.75%	0.75%	0.75%
Nipagin	0.1%	0.1%	0.1%
Aqua ad	100	100	100

Make gel toothpaste: Weighed all the ingredients, then develop carbopol 934 in distilled water. Development is carried out for 24 hours. Dissolve nipagin in sorbitol. Then mix all ingredients with carbopol, mixing using a mixer with rpm 558. Add triethanolamine to reach a neutral pH.

Antibacterial Activity Test

1) Preparation of the test solution
   Created a test solution of each formulation, namely to gel toothpaste extract 5%, 10% and 15%. As for toothpaste gel fraction of 5%, 10% and 15%. The test solution is diluted with DMSO.
2) Antibacterial activity test

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a) First, Preparation of media for oblique is weighed nutrient agar (NA) of 2.8 g was added 100 ml of distilled water, using a hot plate heated to boiling. 5 ml NA poured into a sterile test tube, sterilized in an autoclave for 15 minutes at a temperature of 121^0C , put the desired angle and then wait until hardened.
b) Second, planting test bacteria on an agar medium slant. *Streptococcus mutans* bacteria culture taken using a needle ose round, tightly scrawled on the media for oblique zigzag from the bottom up, incubated at room temperature (37^0C) for 24 hours.
c) Third, the manufacture of liquid media Nutrient Broth (NB) that weighed as much as 3.25 gn NB media added 250 ml of distilled water, heated using a hot plate and stirred until boiling and homogenized, poured into the flask 50 ml, 30 ml NB, sterilized by autoclave during 15 minutes the temperature of 121^0 C for 24 hours.
d) Fourth, the planting of test bacteria in a liquid medium. Taking a colony of bacteria that has grown on a slant medium ose using sterile needles, inserted into a liquid medium, and incubated for 24 hours in a shaker at 120 rpm.
e) Fifth media manufacture Muller Hinton Agar (MHA). MHA media weighing as much as 38 grams, then added to 1000 ml of distilled water, stirred and heated using a hot plate, in an autoclave for 15 minutes at a temperature of 121^0 C. Then the media was poured into a sterile petri dish of 15 ml and carried in the LAF.
f) Sixth inhibition test. Blank discs soak for 5 minutes on each sample and the test solution as DMSO as a negative control. Positive control used was chloramphenicol and done 3 times replication. Furthermore incubated for 24 hours at a temperature of 37^0C. Observe the inhibition zone by making measurements using calipers. Diameter of clear zone in millimeters (mm) [(9)].

a. Data analysis
b. Statistical data analysis, test the normal distribution (Kolmogorov-Smirnov) will be used to test whether or not the data is normally distributed. If the data were not normally distributed ($p < 0.05$), then followed by a non-parametric test. If the data were normally distributed ($p > 0.05$), then followed by a parametric test one-way analysis of variance (ANOVA). Test followed by Post Hoc test to see whether there is a difference between each treatment group. As for analisis correlation between the magnitude of inhibition zone against antibacterial activity will be conducted by analysis of correlation test level of 95% [(10)].

RESULTS

Process extraction *Mimosa pudica Folium* used maceration because the method simple, extraction method by immersing powder bulbs with 96% ethanol solvent ratio of 1: 10 for 3x 24 hours. Extraction maceration leaf extract Mimosa pudicagain weight at 377.4 grams with a yield of 59.90% as shown in Table 3.

Table 3. Extraction

No.	Dry weight (grams)	Extract Weight (grams)	The yield (%)
1	630	377.4	59.90

After the extraction process, followed by liquids fractionation using a partition separating funnel. The partition process using different solvent solubility is solvent n-hexane and ethyl acetate.

Table 4. Results fractionation

No.	Solvent	Weight fraction (g)	The yield (%)
1	N-hexane	58.80	14,700
2	Ethyl acetate	70.50	23.5

Based on table 4 can be seen the results of fractionation of the ethanol extract of the leaves were *Mimosa pudica* with a weight fraction of n-hexane fraction of 58.80 grams with a yield of 14.70% and a weight of 70.50 grams of ethyl acetate fraction with a yield of 23.50%. The purpose of the fractionation process was to obtain chemical compounds or secondary metabolites simplified by the polarity of the solvent. N-hexane solvent was a polar solvent that will attract the compounds that were non-polar where as the semi-polar solvent was ethyl acetate will be interesting compounds that were semi-polar. Identification of important phytochemicals carried out to determine the compounds contained in extracts and fractions of ethanol extract of *Mimosa pudica Folium*.

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Table 5. Results of phytochemical screening

test material	The ethanol extract	Ethyl acetate fraction
Alkaloids	-	-
Flavonoid	+	+
Tannin	+	-
Saponin	-	-
Steroids / triterpen	-	-

Information: (+) Contains secondary metabolites, (-) does not contain secondary metabolites

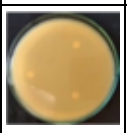
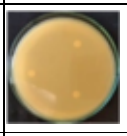
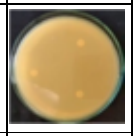
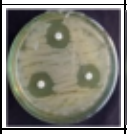
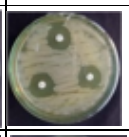
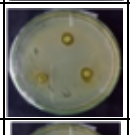
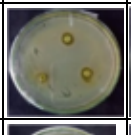
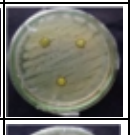
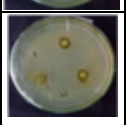
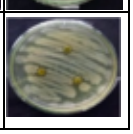
The test results phytochemical extracts and fractions of ethanol extract of *Mimosa pudicaFolium*, showed that positive *Mimosa pudicaFolium* extract contains flavonoids and tannins. While only a fraction of ethyl acetate containing flavonoids. Flavonoids and tannins have a property or as an antioxidant and antibacterial activity. In an effort to increase the use of natural materials for the prevention and treatment of dental caries disease, researchers will formulate extracts and fractions of ethanol extract of *Mimosa pudica Folium*as toothpaste in gel form. Furthermore gel toothpaste will be tested activity against *Streptococcus mutans* bacteria in vitro.

Table 6. Zone resistor

Materials testing	Concentration (%)	Zone of Inhibition (mm)		
		I	II	III
DMSO	-	-	-	-
Ciprofloxacin	25 ug / disk	25.20	25.00	25.30
Gel toothpaste Extract	5	7.10	7,20	7.10
	10	8.0	8.20	8.20
	15	10.12	10,11	10.12
Gel toothpaste Faction	5	8.40	8.40	8.42
	10	11.33	11.33	11.30
	15	15.55	15.54	15.55

Based on the above table, the effectiveness of antibacterial activity can be seen from the zone of inhibition is formed. The zone of inhibition explains that the classification of bacterial growth inhibition responses were seen based on the diameter of clear zone consists of 4 groups: a weak response (diameter ≤5 mm), medium (5-10 mm diameter), strong (10-20 mm diameter) and very strong (diameter ≥20 mm).

Table 7. Test material

No.	Test material	Concentration		
		5%	10%	15%
1	DMSO			
2	Ciplrofloxacin			
3	Gel toothpaste ethanol extract			
4	Gel toothpaste ethyl acetate fraction			

DISCUSSION

Use of the gel formulation is intended for local effect, which in this study gel toothpaste of extracts and fractions of extracts *Mimosa pudica folium*has antibacterial activity against *Streptococcus mutans* bacteria causing dental caries. The advantage is when the gel formulation to dry and form a thin layer with high adhesion, does not clog pores and is easily washed with water [(11)]. Additionally gel formulation has a high water content, thereby reducing the risk of inflammation. The aim of this study was to compare the antibacterial activity of gel toothpaste extracts and fractions of ethanol extract of *Mimosa pudicafolium* against *Streptococcus mutans*, determine the effect of variations in the concentration of extracts and fractions of ethanol extract of *Mimosa pudicafolium* who is able to inhibit the bacteria *Streptococcus mutans.*

Table 6.Based on the description above showed that the solvent DMSO did not provide a response to the bacteria *Streptococcus mutans*, whereas the positive control ciprofloxacin give a strong response at a concentration of 25ug / disk with a diameter of 25 mm inhibition zone. The test sample ethanol extract of the leaves respond well Mimosa pudica at a concentration of 15%, ie 150 mg / ml with inhibition zone diameter of 10.17 mm, giving a strong enough response, while at a concentration of 10%, ie 100 mg / ml provides 8.13 inhibitory zone mm and a 5% concentration of 50 mg / ml provides 7.13 mm inhibition zone. antibacterial activity of ethanol extract fraction obtained *Mimosa pudica* leaves the biggest inhibition zone is at a concentration of 15% that is 150 mg / ml of 15.55 mm, while the 10% concentration of 100 mg / ml provides inhibitory zone 11.32 and a concentration of 5% ie 50 mg / ml provides 8.41 mm inhibition zone.

Statistical analysis of the activity of extracts and fractions of ethanol extract of *Mimosa pudicafolium* is normality Kolmogorov-Smirnov ie, p= 0.200 and Shapiro-Wilkie, p = 0.472 for the control (+), p = 0.629 for the extract and p = 0.834 for ethyl acetate fraction is > 0.05 meaning that the value of all the inhibition zone test group are normally distributed. Test homogenity of variance significance value (Sig) 0.718> 0.05 meaning variants of the test groups are the same or homogeneous. Annova test significance value of 0.000< 0,005 that has meaning the average value of inhibition zone test group is significantly different from that of control (+), ethanol extract and ethyl acetate fraction has a different antibacterial activity.

CONCLUSION

Based on the above research shows that the gel toothpaste from a fraction of ethyl acetate at a concentration of 15%, ie 150 mg / ml had inhibitory zone greatest value is 15.55 mm compared to the gel toothpaste from putrimalu leaves ethanol extract concentration of 5%, 10% and gel toothpaste from the ethanol extract at all concentrations series.

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