

Available online on 15.04.2023 at <http://ajprd.com>

Asian Journal of Pharmaceutical Research and Development

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Research Article

Clinical Study and Toxicity Tests of Disclosing Agent Aloe Vera Gel

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ABSTRACT

Disclosing agents function to visualize and identify dental plaque on the tooth surface, making it very useful for viewing transparent bacterial plaque for the purposes of oral hygiene instruction, evaluation and research. Aloe vera (Aloe vera) has a unique texture because it is in the form of a gel which has anti-inflammatory, wound healing, antibacterial and anticancer properties. Aloe vera can be developed as a disclosing agent. Clinical trials and toxicity tests can be the basis for the clinical use of aloe vera gel. The purpose of this study was to determine the ability of aloe vera gel as a disclosing agent based on clinical trials and toxicity tests. The research method is by carrying out sample preparation, extraction, gel preparation, clinical trials on human test subjects, and toxicity tests with the BSLT method. Data were analyzed descriptively, linear regression. The test results showed that the average plaque score in formula I (thick gel) was 3.62, while the average plaque score in formula II (less viscous gel) was 2.58. This shows that the more viscous aloe vera gel formula is better than the less viscous aloe vera gel. Based on the test, it is known that the average LC50 value is 2596.88 µg/ml. According to the literature, if the LC50 value is above 1000 µg/ml then the sample does not have a toxic effect.

Keywords: Disclosing agent, plaque identification, toxicity**ARTICLE INFO:** Received 16 February 2023; Review Complete 27 March 2023; Accepted 07 April 2023; Available online 15 April. 2023**Cite this article as:**Said F, Rahmawati I, Ningsih NS, Clinical Study and Toxicity Tests of Disclosing Agent Aloe Vera Gel, Asian Journal of Pharmaceutical Research and Development. 2023; 11(2):15-19. DOI: <http://dx.doi.org/10.22270/ajprd.v11i2.12423>

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INTRODUCTION

Disclosing agents function to visualize and identify dental plaque on the tooth surface, so it is very useful to see transparent bacterial plaque for the purposes of oral hygiene instructions, evaluation and research. Disclosing agents that are widely available are generally made from chemicals which have the disadvantage of having an unpleasant taste that makes them less desirable. Another disadvantage is that it can color the mucosa for several hours, so that it can cause embarrassment for patients who will move immediately after using it, and dyes that have the potential to be carcinogens.¹⁻³

Alternatives to using disclosing solutions made from nature need to be considered based on the many disadvantages of chemical based ones. The Ministry of Health suggests developing natural materials for medicinal or medical purposes. Many people's lifestyles lead to the use of natural materials, so the development of natural materials is very promising. This is the basis for developing a disclosing agent made from natural ingredients that can be used by the community as a plaque coloring agent. Natural materials

have the advantage that they are available in abundant quantities and have a high level of safety. Natural materials that can be developed into one of them is Aloe Vera (Aloe vera).^{4,5}

Aloe vera (Aloe vera) is a plant that is often found because it is easy to plant and cultivate. Aloe vera contains minerals such as calcium, iron, zinc, vitamin C and vitamin E. Aloe vera also contains secondary metabolites such as phenols, flavonoids and anthraquinones which are responsible for the properties. Aloe vera can also be used as an anti-inflammatory, wound healer, antibacterial and anticancer. Aloe vera has a unique texture because it is in the form of a gel and is proven safe to use in the mouth because there are aloe vera preparations on the market for mouth sores or sores. Aloe vera plant can be developed as a natural disclosing agent, but the optimal formulation must be researched so that it can be used safely and comfortably.⁶⁻⁹

Research related to the use of aloe vera as a disclosing agent will go through several stages (multi years). In the first year, there will be screening and formulation of aloe vera gel as a disclosing agent. This research is a second year research

proposal covering Clinical Studies and Toxicity Tests of Disclosing Agent Aloe Vera Gel (Aloe vera).

METHODS AND MATERIALS

This research is an experimental study that tested the ability of aloe vera gel as a disclosing agent on human test subjects and as a toxicity test on shrimp larvae using the BSLT method. The research will be conducted in March 2022. Aloe vera samples were taken from cultivated aloe vera in the Kalimantan region. Sample preparation, extract preparation, and formulation of gel dosage forms were carried out at the Banjarmasin PucukSirih Jamu Factory Laboratory which already has a certificate of Good Traditional Medicine Manufacturing Practice (CPOTB) from the Food and Drug Supervisory Agency (BPOM)

The stages of preparation and preparation of the extract were carried out starting with the aloe vera leaf being washed in running water and weighed. The aloe vera leaf and mundar fruit skin were then split into two parts using a knife and the inner flesh was taken to obtain the gel which was then weighed and dried in a drying cabinet at 50°C for 72 hours. The dried aloe vera and mundar fruit peels were weighed and powdered. The powder was then weighed and then mixed with pro-analytical ethanol solvent at 70°C for 6 hours. The solution is filtered using filter paper. The filtered dregs were extracted again until a clear solution was obtained. The filtrate was collected and the solvent was evaporated using a rotary evaporator and dried over a water bath.

The stages of making aloe vera gel used the same concentration of aloe vera extract in all formulations, namely 5%, while the mangosteen fruit extract used was 5, 10, 20% w/w. HPMC dissolved in 50 mL of distilled water in a beaker at 80-90°C while stirring slowly to form a gel mass. Dissolved methyl paraben and propyl paraben, then added the extract while stirring and heating on a hot plate. The extract mixture was put into the stored gel mass and stirred, then added cold aquadest up to 100gr while stirring until homogeneous.

The Plaque Staining Test Phase was carried out by measuring the plaque index of 20 people who did not brush their teeth for 24 hours for two periods, and were willing to eat the same food in each period. Respondents consisted of 20 students from the Health Analyst Department, Pontianak Health Polytechnic with a concentration of 20% and 40%, and the consistency of the plaque index examination in the first period used aloe vera gel which had been dyed with mango steenpeel extract concentrations of 20% and 40%. Data were analyzed by Paired T-Test. Results The average plaque index score with aloe vera gel extract 20% and 40% the results of the two averages were compared to see a significant difference.

The Toxicity Test phase is carried out in several stages:

1. Collection of Artemia salina eggs. Leach
2. Collection of Artemia salina shrimp eggs. Leach was taken from the Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lambung Mangkurat.
3. Preparation of Artemia salina Larvae. Leach

4. Shrimpeggs were hatched 2 days before the test. Prepared vessels for hatchings shrimp eggs. Egg hatching was carried out by immersing 2.5 mg of eggs in a container containing 250 ml of sea water under 18 lamps of 25 watts and equipped with an aerator. The eggs will hatch and become larvae after 24 hours. Artemia salina larvae. Leach that is good to use for the BSLT test is one that is ± 48 hours old and ready to be used as a target for toxicity tests. Because if it lasts more than 48 hours, it is feared that Artemia salina will die. Leach is not caused by the toxicity of the extract but by the limited food supply. The shrimp larvae are separated from the eggs by pipetting into a beaker/vial containing sea water
5. Implementation of Toxicity Test
6. Toxicity test on each sample extract. Prepared containers for testing, for each sample extract concentration requires 4 containers and 1 container as a control. Then, at each concentration of the solution, 10 Artemia salina larvae were added. Leach. Observations were made for 24 hours on the death of the larvae when each concentration was repeated three times and compared to the control. The standard criterion for assessing the mortality of shrimp larvae is when they show no movement during observation. After being observed for 24 hours, the level of toxicity was determined by counting the number of dead larvae. The LC50 value was determined by Linear Regression Analysis (Widiyatni, 2010).

RESULTS

This research is the 2nd year research continuing previous research. The results of previous studies showing high levels of secondary metabolites and minerals in aloe vera originating from West Kalimantan. This underlies the use of aloe vera originating from West Kalimantan in this study. The stages of this research included simplicia production, extract preparation, gel formulation preparation, toxicity testing, and clinical trials. The initial process to the toxicity test was carried out at the PucukSirih Jamu Company in Banjarmasin which already has a certificate of Good Manufacturing Practice for Traditional Medicines (CPOTB) from the Food and Drug Supervisory Agency (BPOM).



Figure 1: Aloe Vera from West Kalimantan

Aloe vera leaves are taken from a place in West Kalimantan. The aloe vera leaf and mangosteen rind are then split into two parts using a knife and the inner flesh is taken to get the gel as shown in picture 2.



Figure 2: Aloe Vera Flesh

Aloe vera meat originating from West Kalimantan has unique characteristics because it is semi-solid and has a soft texture. Aloe vera flesh that comes from West Kalimantan has a thick texture. Processed aloe vera meat has the same characteristics as those used in the previous year because it was taken from the same plantation in West Kalimantan. It is intended that the results obtained are in line with previous research. Then it was weighed and dried in a drying cabinet at 50°C for 72 hours. The dried aloe vera and mangosteen rind were weighed and powdered.



Figure 3: Drying Cabinet

Dried aloe vera or known as simplicia is then crushed using a blender to obtain aloe vera powder. The powder obtained was in the form of powder, white in color, had a distinctive plant odor, and tasted slightly bitter. Next, the extract is made. The extract was prepared by maceration method using 96% ethanol solvent. Aloe vera extract has a gel-like texture. There was almost no organoleptic difference between the aloe vera extract obtained in this study and the previous year.

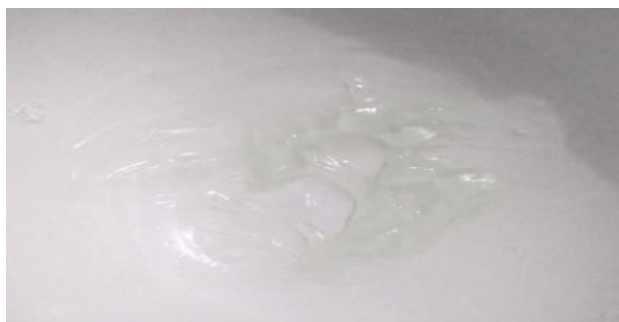


Figure 4: Aloe Vera Extract

The aloe vera extract obtained was then formulated with mangosteen rind extract. The addition of mangosteen peel extract causes a color change in the gel. At first the gel was clear, after adding mangosteen rind extract it turned dark purple. The percentage of adding mangosteen rind based on the results of previous studies is 40%. The results of the formulation of aloe vera gel with mangosteen rind are shown in the figure below.



Figure 5: Aloe Vera Gel With Mangosteen Skin Coloring

Based on the formulation results, it was found that aloe vera gel with mangosteen rind coloring has a dark purple color, soft texture, semisolid dosage form, and a characteristic odor. These results are in accordance with the desired results using the same manufacturing procedure in the previous year's study. In the previous year's study a physical evaluation of the gel preparation was carried out. Based on visual observations, the results obtained are not much different.

The disclosing agent gel formula was then tested for toxicity using the Brine Shrimp Lethality Test (BSLT) method. This test aims to determine the possibility of toxicity that can occur in the preparation. The test results are presented in the table below.

Table 1: BSLT Method Toxicity Test Results on Aloe Vera Gel

Rate (µg/ml)	Percent Mortality		
	Replication 1	Replication 2	Replication 3
100	10	0	0
500	10	20	0
1000	20	20	20
1500	30	30	30
LC50	2923,88 µg/ml	2509,63 µg/ml	2357,14 µg/ml
Everage LC50	2596,88 µg/ml		
Result (> 1000 µg/ml)	NO TOKSIK		

Testing is carried out using 3x replication to ensure the results obtained are valid and consistent. Based on the test, it is known that the average LC50 value is 2596.88 µg/ml. According to the literature, if the LC50 value is above 1000 µg/ml then the sample does not have a toxic effect.

The aloe vera gel formula was then tested on humans. There are two formulations tested on humans with different levels of viscosity (viscosity) between the two. The first formula has a denser gel consistency with a viscosity of 3,843 cps,

while the second formula has a more liquid consistency with a viscosity of 3,273 cps. Viscosity value measurement using Brookfield's viscometer. The results of testing on humans as a disclosing agent are shown in the table below.

Table 2: Test Results for Aloe Vera Gel Disclosing Agent

No.	Respondent Code	Formulation I	Formulation II
1.	AD	3,83	2,60
2	CA	3,33	1,20
3	CC	4,10	1,30
4	CR	4,50	3,00
5	DM	4,40	3,80
6	DW	3,16	1,80
7	FL	3,80	4,00
8	FK	3,16	2,80
9	FA	2,80	2,00
10	FS	4,00	3,10
11	MP	3,50	3,30
12	NA	4,00	1,20
13	NW	3,80	4,00
14	RR	4,00	2,60
15	RI	3,60	3,80
16	RO	3,00	1,60
17	SR	3,16	2,60
18	ST	3,00	2,80
19	SN	4,10	0,50
20	TK	3,16	3,50
Average		3,62	2,58

Before being assigned as research respondents all subjects were asked to fill out informed consent. Plaque clinical examination was carried out on 20 respondents who had agreed to informed consent. Respondents were given two treatments, namely the first treatment by applying formula I gel and the second treatment with formula 2 gel. Respondents were given education not to brush their teeth in the morning, not to fast, and the examination was carried out on two consecutive days in the morning. All respondents were treated with the application of aloe vera gel combined with mangosteen peel extract, then the plaque score was assessed. The test results showed that the average plaque score in formula I (thick gel) was 3.62, while the average plaque score in formula II (less viscous gel) was 2.58. This shows that the more viscous aloe vera gel formula is better than the less viscous aloe vera gel.

DISCUSSION

In this study, the same steps were carried out with the previous research, namely harvesting materials, wet sorting, cutting, washing, drying, extraction, and making aloe vera gel formulas using dyes from mangosteen rind. The next stage is the toxicity test using the Brine Shrimp Lethality

Test (BSLT) method and clinical trials on test subjects to determine the ability of the gel as a disclosing agent.

Samples were taken at one location with the aim that the amount and composition of the chemical compounds were the same. Aloe vera that is harvested is mature leaves that are dark green and still fresh. The ability to produce secondary metabolites is more in mature plants, so the content of the compounds contained is also more optimal. Samples were washed with running water and weighed.¹⁰⁻¹²

The powder was then weighed and then mixed with pro-analytical ethanol solvent at 70°C for 6 hours. The temperature was chosen to avoid damage to the flavonoid content in plants. This process aims to reduce the water content contained in the sample so as to inhibit the growth of fungi, molds and bacteria. The simplicia that has been dried is then carried out in the process of separating again from impurities and samples that have been damaged by carrying out the dry sorting stage.

The powder form in simplicia is produced due to the smoothing process which is carried out using a blender. The color and smell obtained are based on the characteristics of the plants used. The bitter taste in the sample is thought to be caused by the presence of tannin compounds in plants. Tannin compounds will cause a bitter taste in the oral cavity because they are able to form cross-links to glycoproteins and proteins.¹³⁻¹⁶

The extract was prepared by maceration method using 96% ethanol solvent. This method was chosen because the process is simpler and does not require heating, so it does not damage the heat-resistant compounds. 96% ethanol solvent is used because it is a universal solvent that is widely used to extract polar, semi-polar and non-polar compounds from natural materials. Aloe vera extract has a gel-like texture. There was almost no organoleptic difference between the aloe vera extract obtained in this study and the previous year.^{17,18}

The extract obtained was then formulated in a gel dosage form with the addition of mangosteen rind extract. The results of organoleptic observations showed that aloe vera gel dyed with mangosteen rind extract had a dark purple color, soft texture, semisolid dosage form, and a characteristic odor. The purple color comes from the color of the purple mangosteen rind extract. Color is the main component that is expected to appear from the gel that is made in addition to the ability to stick and the ideal physical properties of a gel preparation.

Toxicity test was carried out using the BSLT method with the help of shrimp larvae. Samples were made into several concentration series, then tested on shrimp larvae with larval mortality parameters. The results in the table show that all samples are not toxic because they are at an LD50 above 1000 µg/mL. The toxicity test of plant compounds requires an appropriate, fast and simple method. One of the methods used is toxicity test using *Artemia salina* shrimp larvae. Leach. This method is known as the Brine Shrimp Lethality Test (BSLT). The BSLT method was chosen because this method is often used for pre-screening active compounds contained in plant extracts because it is simple, fast, cheap, easy, reliable, and the results are representative.¹⁹

Observation time of *Artemia salina* shrimp larvae. Leach is carried out for 24 hours. Symptoms of poisoning appear slowly over a long time after administration of a substance. This means that poisoning does not occur immediately when administering a substance, but the substance is excreted first so that with a long time the effect will be seen. At each increase in concentration there is an increase in the mortality rate of shrimp larvae. This shows that the higher the concentration of a substance given, the greater the number of dead shrimp larvae, so that it correlates with an increase in the toxic effect of a compound.²⁰

Disclosing agents work by giving color to the plaque so that there is a color difference between the teeth and the dental plaque. The advantage of using a disclosing agent is that patients can carry out an independent assessment of dental plaque, increase awareness of the importance of cleaning dental plaque, and patients can identify areas where there is plaque so that cleaning becomes optimal. Disclosing agents can be made with natural ingredients, one of which is the content of plant dyes with a gel base made from aloe vera. The use of a combination of aloe vera gel with mangosteen peel extract will add to the function of the gel. Apart from being disclosing agents, aloe vera and mangosteen rind have antibacterial and antifungal activity, so their use will add added value to the gel.^{21,22}

CONCLUSION

The test results showed that the average plaque score in formula I (thick gel) was 3.62, while the average plaque score in formula II (less viscous gel) was 2.58 which indicated that the aloe vera gel formula was thicker better than aloe vera gel which is less viscous. The results of the toxicity test using the Brine Shrimp Lethality Test (BSLT) method revealed an average LC50 value of 2596.88 µg/ml. According to the literature, if the LC50 value is above 1000 µg/ml then the sample does not have a toxic effect.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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