



FORMULATION AND ACTIVITY TESTS OF ANTI DANDRUFF SHAMPOO ETHANOL EXTRACT OF *Peperomia pellucida* (L.) Kunth LEAVES ON THE GROWTH OF *Pityrosporum ovale* Fungus

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ABSTRACT

*Shampoo is a cosmetic preparation used to clean hair. The leaves of *Peperomia pellucida* (L.) Kunth are one of the plants that can be used to get rid of dandruff. The plants contain alkaloids, saponins, tannins, hydroxylated sesquiterpenes, especially carotol and peppermint which have antifungal activity. Purpose; formulating anti-dandruff shampoo preparations containing suruh leaves, as well as evaluating shampoo preparations which include homogeneity, pH, organoleptic tests, foam height, viscosity, and irritation. Method; The research method used was an experimental method, namely *Peperomia pellucida* (L.) Kunth leaf extract obtained by maceration method, then formulated as an anti-dandruff shampoo preparation starting with making a base consisting of sodium lauryl sulfate, cocamidopropyl betaine, polyquaternium 7, methyl paraben, sodium chloride, citric acid, menthol, rose perfume, and distilled water. Then added the extract of the leaves of the order each with a concentration of 1%, 3%, and 5%. The results obtained were then tested statistically with one way type ANOVA. Results;. The ethanol extract of suruh leaves can be formulated as an anti-dandruff shampoo preparation, and the ethanol extract of suruh leaf anti-dandruff shampoo preparations with a concentration of 5% has provided an effective inhibition against the growth of the fungus *Pityrosporum ovale*. Conclusion; The concentration of the ethanol extract of suruh leaves anti-dandruff shampoo which has an effective inhibitory activity against the growth of the fungus *Pityrosporum ovale* is in formula III with an inhibition zone of 14.2 mm.*

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1. INTRODUCTION

Hair that adorns the human head is an aesthetic need, so that humans spend a lot of time caring for and repairing their hair. Scalp disorders such as sensitivity, oily and dandruff which interfere with normal hair growth often occur. The problem that is still the cause of a person's low self-confidence in carrying out activities is dandruff (1).

Caring for hair has been important for every woman and man since ancient times. In the Middle Ages, more advanced hair products were produced by combining soap with soda, which is the ingredient in the soap itself, late 18th century salons in England. offers a massage service to clean hair to its customers called "shampoo". shampoo and massage originated in India where it was originally known as head massage, usually using alkaline/alkaline natural oils and fragrances (1). It was informed that more than 60% of the world's population has hair problems with dandruff, the prevalence of the Indonesian population suffering from dandruff according to data from the International Date Base, US Census Bureau in 2004 was 43,833,262 out of 238,452,925 and ranked fourth after China, India and US, dandruff can occur anywhere in all races, sex and age (2).

Dandruff is an anomaly of the scalp which is characterized by excessive exfoliation of the horny layer of the scalp forming fine scales. The cause of dandruff can be in the form of excessive secretion of sweat glands or the role of microorganisms on the scalp which produce metabolites that can induce the formation of dandruff on the scalp, with general symptoms of itching on the scalp and redness around the scalp caused by an oily scalp. hormones and fungi (3).

Indonesia is a tropical country which has high humidity which allows various microorganisms to grow well, one of the microorganisms that can grow well in Indonesia is mushrooms. Dandruff mostly occurs in people in the pubertal age group, dandruff or pityriasis sika (dandruff) affects many people in Indonesia, which has a tropical climate, high temperatures and humid air. Men compared to women dandruff is a mild inflammatory disease of oily skin (seborrheic dermatitis) but is more often a problem for sufferers because it reduces one's attractiveness due to dirty hair (4).

Washing too often or too infrequently causes excess oil in the hair and dry dandruff occurs. If you rarely wash, oil will accumulate on your head and can cause wet dandruff (5).

According to BPOM RI head regulation No.HK.00.05.42.1018 concerning cosmetic ingredients states that cosmetics are any ingredients or preparations intended for use on all external parts of the human body (epidermis, hair, nails, lips and external genital organs) or teeth and mucous membranes around the mouth especially for cleaning, deodorizing, changing appearance and/or improving body odor and protecting or maintaining the body in good condition (6).

From the medical aspect, dandruff is still getting less attention, but from the cosmetic aspect, dandruff is a very significant problem, so there are many cosmeceutical anti-dandruff in the form of shampoos, creams, gels and lotions available in the market. In general, dandruff sufferers seek self-medication by buying anti-dandruff shampoos, but in reality anti-dandruff drugs are only able to control dandruff but cannot cure it (7).

Dandruff is a scalp disease caused by the fungus *Pityrosporum ovale* which is a fungus of the genus *Malassezia* Sp. Dandruff can be in the form of fine to coarse scales of gray or white color found on the head which occurs due to the detachment of the stratum corneum on the scalp, factors that trigger dandruff are the growth of the fungus *Pityrosporum ovale*, sebaceous gland activity and individual susceptibility, moisture is one of the causes of dandruff. an important factor in the occurrence of dandruff because it can trigger the growth of the fungus *Pityrosporum ovale* (8).

The fungus of the genus *Malassezia* Sp is a unique species among the kingdom fungi because it is the only species that forms part of the normal human cutaneous commensal flora, besides

that the *Pityrosporum ovale* species can cause several cutaneous diseases, systemic diseases in humans with the appropriate predisposing factors and dermatitis in many types of animals and their positions are closely related between commensals and pathogens (8).

Synthetic antifungal drugs (antifungal) are commercially known and relied upon in disease management. Among the synthetic antifungal drugs are amphotericin, griseofulvin, nystatin and ketoconazole. However, synthetic drugs cause serious side effects and require doctor supervision. Apart from being expensive, people are starting to try to use traditional medicines from herbal plants which are a source of domestic natural resources and are worth exploring (9).

Peperomia pellucida (L.) Kunth is one of the plants that is often used as a traditional medicinal ingredient by the community, this plant is a wild plant that lives and grows in groups, this plant is widespread in areas that have a humid and shady climate. such as mountainous areas, traditionally use plants in treating several diseases such as abscesses, acne, boils, inflammation of the skin, kidney disease and stomach ache. The people in Gorontalo use the herbs of the medicinal plant to treat pain in the bones by washing the herbs and then boiling them so that the result of the decoction is consumed. Apart from that, the leaves of *peperomia pellucida* (L.) Kunth are also used by the people of Gorontalo to get rid of body odor. processed by mashed leaves, then mashed and then rubbed on the armpits (10).

The part of the plant that is often used by the community is the whole plant or it is called a herb and even in the Philippines this plant is known by the local community as pansit-pansitan which can be used as medicine, among other things, to reduce uric acid levels, as well as to treat kidney problems, in Kalimantan by the local population It is widely used by boiling and drinking the boiled water to treat rheumatic pain due to high uric acid. In addition, it is used as a medicine to treat kidney disease, diarrhea, colds, anti-oxidants and hypertension, while in South America the people use boiled leaves and stems for the treatment of gout. and arthritis (11)

In a previous study, Mulyani et al (2018) examined "The formulation and anti-oxidant activity of *Peperomia pellucida* (L.) Kunth leaf extract lotion from her research showed that *Peperomia pellucida* (L.) Kunth leaf extract lotion with a concentration of 0.550 mg/ml (formula III) meets the requirements of the physical properties test and has anti-oxidant activity with an average % inhibition activity of $93.29\% \pm 5.487$ (12).

Tasya's research, C et al (2017) "Formulation of anti-dandruff shampoo preparations of henna leaf extract (*Impatiens Balsamina* L.) and its activity test against the fungus *Candida Albicans* ATC 10231 in vitro" proved that anti-fungal activity testing showed that the shampoo was prepared with a concentration of 5% having the best antifungal activity followed by shampoo with a concentration of 10% and shampoo with a concentration of 15%, all three of them fall into the category of strong microbial growth inhibition response, henna leaf extract can be formulated as an anti-dandruff shampoo (12).

Elisabeth Arundhina et al (2016) research showed that the best results were on *candida albicans* compared to *pityrosporum ovale*, the results of the inhibition zone were continued with testing to find out MIC using the tube dilution method and KBM using the number of colony calculation methods with concentrations of 2, 1.75, 1.50 1, 25 and 1% (w/v) for *candida albicans*, while concentrations of 10,9,8,7,6 and 5% (w/v) for *pityrosporum ovale* are 9% (w/v) MBC for *candida albicans* is 1, 75% (w/v), while for *pityrosporum* it is 10% (w/v) (13).

Based on the above background, the authors are interested in raising the title "Formulation and Activity Test of Anti-Dandruff Shampoo Preparation of Ethanol Extract of *Peperomia pellucida* (L.) Kunth Leaves on the growth of *Pityrosporum ovale*".

2. RESEARCH METHODE

This type of research was carried out experimentally. This research includes, sampling, sample preparation, sample processing, extract preparation, preparation of shampoo preparations and evaluation of shampoo preparations.

The sample is part of the population whose characteristics are to be studied. The samples used were 5 kg of fresh suruhan leaves obtained from Tanjung Mas Village, Simpang Kanan District, Aceh Singkil Regency and *Pityrosporum ovale* Mushrooms obtained from the Microbiology Laboratory, Faculty of Pharmacy, University of North Sumatra

3. RESULT AND ANALYSIS

Based on the research that has been carried out, 4 formulas were made with different concentrations to see whether or not there were differences between the formulas and an evaluation of the formulas was carried out which consisted of organoleptic tests, pH, foam height, viscosity and irritation. So it can be seen in the table below

Organoleptic Test Results

Table 1. Organoleptic Test Results

No	Formula	Bau	Warna	Bentuk
1	F0	Khas suruhan	Putih	Cairan Kental
2	F1	Khas suruhan	Hijau Muda	Cairan Kental
3	F2	Khas suruhan	Hijau Tua	Cairan Kental
4	F3	Khas suruhan	Hijau Kehitaman	Cairan Kental

Information:

F0 : Shampoo does not contain 96% ethanol extract of suruh leaf (blank)

F1 : Shampoo containing 96% ethanol extract of 1% suruh leaves

F2 : Shampoo containing 96% ethanol extract of 3% suruh leaves

F3 : Shampoo containing 96% ethanol extract of 5% suruh leaves

Based on organoleptic observations of shampoo preparations with the addition of ethanol extract of suruh leaves at a concentration of 1% it produces a light green color, at a concentration of 3% it produces a dark green color, while at a concentration of 5% it produces a blackish green color. The preparation of suruhan leaf extract shampoo has a distinctive smell of suruhan. F0 shampoo (blank) produces a translucent white color. And all shampoo preparations with different concentrations have a thick liquid texture and a distinctive aroma.

pH Test Results

Table 2. pH test results

Formula	pH I	pH II	pH III	Rata-rata	Keterangan
F0	6,0	6,2	6,3	6,1	Asam
F1	5,4	5,3	5,5	5,4	Asam
F2	5,3	5,2	5,3	5,2	Asam
F3	5,2	5,1	5,2	5,1	Asam

Information:

- F0 : Shampoo does not contain 96% ethanol extract of suruh leaf (blank)
 F1 : Shampoo containing 96% ethanol extract of 1% suruh leaves
 F2 : Shampoo containing 96% ethanol extract of 3% suruh leaves
 F3 : Shampoo containing 96% ethanol extract of 5% suruh leaves

Based on the results of pH observations with concentrations of 0%, 1%, 3%, and 5% it was found that the average pH values were 6.1, 5.4, 5.2 and 5.1.

High Foam

Table 3. Foam Height Test Results

Formula shampoo	PI (cm)	PII (cm)	PIII (cm)	Rata-rata
F0	12,2	12,4	12,1	12,23
F1	13,8	13,5	13,6	13,63
F2	14,3	14,2	14,1	14,2
F3	15,7	15,8	15,6	15,7

Information:

- F0 : Shampoo does not contain 96% ethanol extract of suruh leaf (blank)
 F1 : Shampoo containing 96% ethanol extract of 1% suruh leaves
 F2 : Shampoo containing 96% ethanol extract of 3% suruh leaves
 F3 : Shampoo containing 96% ethanol extract of 5% suruh leaves

Based on the results of the foam height test carried out at each shampoo preparation concentration of 0%, 1%, 3%, and 5%, it was found that the average foam height was 12.23 cm, 13.63 cm, 14.2 cm and 15.7 cm.

Viscosity Test

Table 4. Viscosity Test

Formulasi	Daya Lekat (Detik)			Rata-rata
	P I	P II	P III	
F0	8,9	8,26	8,32	8,4
F1	11,1	11,3	11,5	11,3
F2	4,2	4,21	4,63	4,3
F3	3,2	3,5	3,14	3,2
F4	7,18	7,20	7,22	7,2

Information:

- F0 : Shampoo does not contain 96% ethanol extract of suruh leaf (blank)
 F1 : Shampoo containing 96% ethanol extract of 1% suruh leaves
 F2 : Shampoo containing 96% ethanol extract of 3% suruh leaves
 F3 : Shampoo containing 96% ethanol extract of 5% suruh leaves

Based on the results of the viscosity test for each shampoo concentration of 0%, 1%, 3% and 5%, the average viscosity results were 1,085 cP, 1,261 cP, 1,563 cP, and 1,486 cP.

Results of the Diameter of the Bacterial Inhibition Zone

Table 5. Results of Pityrosporum ovale Inhibition Zone Diameter

Formula	Diameter Zona Hambatan Berbagai konsentrasi
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	P1 (mm)	P2 (mm)	P3 (mm)	Rata-rata (mm)
F0	12,25	12,65	11,75	12,21
F1	12,7	12,45	13,55	12,9
F2	12,85	13,3	12,75	12,9
F3	12,2	13,75	16,65	14,2
Kontrol (+)	19,9	17,8	18,4	18,7

Information:

F0 : Shampoo does not contain 96% ethanol extract of suruh leaf (blank)

F1 : Shampoo containing 96% ethanol extract of 1% suruh leaves

F2 : Shampoo containing 96% ethanol extract of 3% suruh leaves

F3 : Shampoo containing 96% ethanol extract of 5% suruh leaves

Based on the test results in table 5, it shows that the anti-dandruff shampoo of the ethanol extract of *Peperomia pellucida* (L.) Kunth leaves has anti-fungal activity against the fungus *Pityrosporum ovale*.

Data Analysis

Table 6. One Way ANOVA Statistical Test Results

Konsentrasi	Uji Normalitas Shapiro-Wilk (sig.)	Data Uji Homogenitas (sig.)	ANOVA (sig.)
Kontrol (-)	0,878		
F1	0,417	0,051	0,000
F2	0,328		
F3	0,669		
Kontrol (+)	0,537		

Information:

F0 : Shampoo does not contain 96% ethanol extract of suruh leaf (blank)

F1 : Shampoo containing 96% ethanol extract of 1% suruh leaves

F2 : Shampoo containing 96% ethanol extract of 3% suruh leaves

F3 : Shampoo containing 96% ethanol extract of 5% suruh leaves

DISCUSSION

Organoleptic Test

This organoleptic test was carried out by observing changes in the shape, smell and color of the shampoo preparation. The results of the organoleptic examination of the shampoo preparations, the preparations made have the form of a viscous liquid, having a variety of colors ranging from F0 which is slightly clear white, F1 is light green, FII is dark green while FIII is blackish green and has a distinctive odor of suruh leaves. The higher the concentration of the extract in the shampoo, the stronger the odor of the leaves. The difference in color for each formula with a concentration of 0%, 1%, 3% and 5% was due to the addition of suruh leaf extract. At a concentration of 3% it produces a dark green color due to the addition of small amounts of suruhan leaf extract, at a concentration of 1% it produces a light green color in this case due to the addition of too little extract, in this case this is evidenced by research conducted by Nielma Aulia et al. ., (2020) on kaffir lime peel extract with concentrations of 5%, 10%, and 15%. Where at a concentration of 15% it has a stronger distinctive odor and a more concentrated color (14).

Test the pH

Measurement of the pH of anti-dandruff shampoo preparations was carried out using a pH meter, while the pH of shampoo according to SNI No.06-2692-1992 ranged from 5.0 to 9.0, where this figure is the normal pH of the skin so that the shampoo made does not irritate the scalp. The pH of a shampoo that is too acidic or too alkaline can irritate the scalp (15).

The way to measure pH is by using a digital pH meter where the pH meter is first calibrated with a standard buffer solution (pH 7.00) and an acidic pH buffer solution (4.00) until the tool shows the pH number. The sample is made in a concentration of 1%, namely weighed in 1 gram of the preparation and then dissolved in 100 ml of distilled water. Then the pH is dissolved into the solution, let the pH show the pH number until it is constant and the number shown is the pH of the preparation being tested (15).

Based on the results of measuring the pH using a pH meter on the anti-dandruff shampoo preparations, it was found that each concentration of the shampoo preparation had an average pH, namely at the F0 concentration of 6.1, at the FII concentration the result was 5.4, while at the FII concentration it was 5.2 cm and at concentration FIII get a pH value of 5.1. (16). These results indicate that the pH of shampoos of various concentrations meets the requirements for scalp pH. From all these pH observations, the pH values varied, this was influenced by the addition of active substances from the leaves of the order. The pH of the solution for most of the cosmetic ingredients is acidic or weakly basic or amphoteric, whose solubility depends on the pH of the solution and the dissociation constants of weak acids and bases and the amphoteric isoelectric point. An increase in pH will increase the solubility of weak acids, while a decrease in pH will increase the solubility of weak bases. An increase in amphoteric solubility occurs at a pH above or below the isoelectric point (17).

Foam Height Test

The ability test of foam height was carried out to determine the ability of shampoo preparations to produce foam against aquadest. Anti-dandruff shampoo is made with a solution of 2 grams of suruh leaves in distilled water, then put into a 100 ml closed measuring cup and shaken for 20 seconds by inverting the measuring cup regularly. The height of the foam formed is observed (18).

In the foam height test, the results showed that each foam height of various concentrations was in accordance with the parameters, namely 1.3-2.2 cm. The foam height test results obtained from all shampoo preparation formulas ranged from 12.23 to 15.7 cm. The highest foam obtained from the four anti-dandruff shampoo formulas of the ethanol extract of suruhan leaves was found in shampoos containing 5% suruhan leaf extract, namely 15.7 cm, while the lowest foam was found in shampoos that did not contain suruh leaf extract (F0), namely 12.23 cm. The test showed that there was an increase in foaming power between the extracted shampoo and the non-extracted shampoo preparation, this was due to the leaves of *Peperomia pellucida* (L.) Kunth's order containing saponins. According to Harbone (1996) saponins are soapy so they can form foam, as also proven by research conducted by Anayanti Arianto et al (2018) on citronella oil with a concentration of 5%, 7.5% and 10% which is at a concentration of 10%. already able to get high foam. Foam is one of the factors that influence consumer choice of a type of shampoo, the amount of foam in shampoo is influenced by the type and concentration of surfactants where surfactants themselves are known to function as foamers. Surfactants are active ingredients in shampoos in the form of synthetic cleaning detergents which are suitable for the condition of the user's hair, for example in soaps, detergents work by lowering the surface tension of liquids because they are amphiphilic, so they can dissolve dirt adhering to the surface of the hair. There is no principle of

using detergents in soaps and shampoos, but selection of surfactants according to the condition of the hair (4). In this case, it was also proven by research conducted by Mardinda Belia et al (2016). On the extract of Alamanda leaves with a concentration of 15% and 30%, it showed that there was an increase in foaming power between the extracted shampoo preparations and the non-extracted shampoo preparations. % has the highest foam (19).

Viscosity Test

Based on the results of the Brookfield Viscometer examination for the preparation of suruh leaf extract shampoo, it was found that the viscosity produced from various concentrations obtained on average were F0 1,085 cP, FI 1,261 cP, FII 1,563 cP, and FIII 1,486 cP. Meets the viscosity requirements of 400-4000 cp. From these observations, the concentration of the anti-dandruff shampoo with suruh leaf extract, the highest viscosity value was in the shampoo containing the suruh leaf extract and had a concentration of 3% with a viscosity value of 1,563 cP, while the lowest viscosity value was found in the shampoo that had a concentration of F0 (which did not contain leaf extract). messenger) with a viscosity of 1.085 cP.

The results of the viscosity measurements show that there is an increase and decrease in the viscosity value at each various concentrations. One of the factors that can influence this is the concentration of *Peperomia pellucida* (L) leaf extract. Kunth used. The viscosity of the shampoo preparation decreased with increasing concentration of suruhan leaf extract. This is because suruhan leaf extract has a water content, evidenced by research conducted by Nina Jusnita and Riska Arguar Syah (2017) on bitter melon leaf extract with concentrations of 1%, 2%, 3%, and 4% that the viscosity of the shampoo preparation decreases with increasing extract bitter melon leaves which at a concentration of 4% has the lowest viscosity (20).

Results of Bacterial Inhibition Zone Diameter

The antifungal test used is the well method. This method is carried out by using hole punchers and wells then adding positive controls, negative controls, FI, FII, and FIII then placing them on solid media that has been inoculated with the fungus to be tested. As for the well procedure, the fungus that has been diluted in 0.9% NaCl is taken 0.1 ml into a petri dish then add 10 ml of PDA media and homogenize. After the media has solidified, punch holes in the media using a punching tool or wells, then insert the test preparation into the agar media and then wrap it in parchment paper and incubate it for 24 hours.

Anti-dandruff shampoo preparations with various concentrations were tested for their activity against the growth of the fungus *Pityrosporum ovale* and using PDA (Potato Dextrose Agar) as a growth medium. The mushrooms used came from the Microbiology Laboratory of the Faculty of Pharmacy (USU). This media was chosen because it can support the growth of the *Pityrosporum ovale* fungus which has the characteristics of being able to grow fast under acidic conditions compared to normal or alkaline pH, PDA (Potato Dextrose Agar) also contains carbohydrates (21).

Pityrosporum ovale fungus testing was carried out at a concentration of 1%, 3%, 5% and as a comparison the negative control, namely shampoo base without suruhan leaf extract and the positive control, namely bidara leaf anti-dandruff shampoo, in each treatment showed that there was an inhibition zone marked in the area clear around the wellbore.

Among the anti-dandruff shampoo formulas, the largest inhibition zone for the *Pityrosporum ovale* fungus was found in shampoo containing a concentration of 5% with an inhibition zone of 14.2 mm and shampoo with a positive control having an inhibition zone of 18.7 mm. While the

lowest inhibition zone was found at a concentration of 1% and 3% with an inhibition zone of 12.9 mm, whereas . In the negative positive control with an inhibition zone of 12.21 mm.

Based on the test results, it can be seen that there is an increase in the zone of inhibition for each concentration. This is due to the presence of chemical compounds present in the suruhan leaf extract, which affect the possibility of the presence of flavonoid compounds against the *Pityrosporum ovale* fungus by denaturing protein bonds in the cell membrane, so that the cell membrane becomes lysed, it is possible that the flavonoids will penetrate into the cell nucleus. By entering the flavonoid into the cell nucleus, it causes the fungus not to develop, the flavonoid group can act as an antifungal because it has phenol which can denature proteins and can damage cell membranes.

Tannin compounds can also react with enzymes if they enter the cytoplasm. Enzymes that react with these compounds lose their ability to work so that the metabolic processes catalyzed by enzymes cannot take place. This causes inhibition of fungal growth. Saponins have anti-bacterial and fungal effects by interfering with monosaccharide groups and their derivatives. Saponin compounds have been known as secondary metabolic compounds in plants that are able to suppress fungal growth.

The mechanism of action of saponins as antifungals is related to the interaction of saponins with cell sterol membranes. Saponins work by lowering surface tension causing disruption of the absorption of substances needed by fungi for growth so that cells will swell and burst.

Data Analysis

Data analysis was carried out to see significant differences between groups using statistical tests. The normality test has a p value > 0.05 , meaning that there is no significant relationship between groups. The normality test results obtained sig. at each concentration > 0.05 which means the data is normally distributed.

The homogeneity test results obtained sig. 0.051 (> 0.05) which means the data has a homogeneous variation. Then the one-way Anova test was then carried out

The results of the ANOVA test show the sig. 0.000 (< 0.05) so that it can be concluded that the inhibition zone values at concentrations of 1%, 3% and 5% showed a significant difference. This means that the concentration has a significant effect on the inhibition zone formed.

4. CONCLUSION

The conclusion in this study is that the ethanol extract of suruh leaves which has an effective inhibitory activity against the growth of the fungus *Pityrosporum ovale* is in formula III with an inhibition zone of 14.2 mm produced by Formula III with a concentration of 5%.

Anemia of 31 respondents pregnant women with hyperemesis as many as 16 respondents (51.6%) and pregnant women with emesis as many as 15 respondents (15%). the probability is 0.001 or < 0.05 .

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