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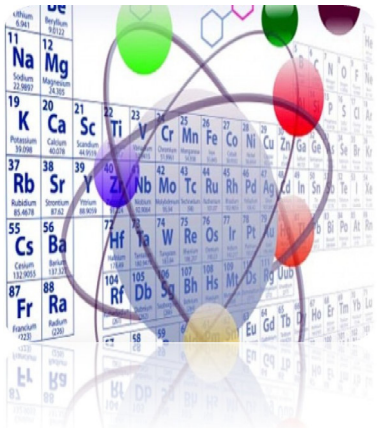

Study of Phytochemicals, Toxicity, Antibacterial Activity of Ethyl Acetate Leaf Extract Extract (Paperomiapellucida L)

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ABSTRACT

Suruhan Leaf (Paperomiapellucida L) is a weedy plant that is used by rural communities as herbal medicine. In this study, phytochemical tests have identified compounds contained in the ethyl acetate extract, then the toxicity test was carried out by the Brint Shrimp LethallytyTocycity (BSLT) method using Artemia Salina Leach shrimp larvae, an antibacterial activity test for bacteria: Bacillus cereus (ATCC) 11778), S. aureus (ATCC 25923), E. coli (ATCC 25922), S. saprophytic (ATCC 49907), Catsobacterfrendi (ATCC 18090), P.acne (ATCC 27853), Epidermis (ATCC 12228) with diffusion methods, various of concentration 1; 5; 10; 25% as control used chloramphenicol 30 mcg, the highest inhibitory zone at a concentration of 25% showed a strong antibacterial succession of 20.7; 11.1; 29.7; 15; 13.5; 10; 17.3 mm. The results of the identification of compounds in ethyl acetate extract are alkaloids, flavonoids, steroids, saponins, and tannins. The toxicity of LC50 is 71.28 ppm (toxic).

Keywords: Paperomiapellucida L; Phytochemicals; Toxicity; Antibacterial.

I. Introduction

Water leaves (PaperomiaPellucida L) are wild weed plants found in South America and Asia that are classified as a family of piperaceae, a place to grow around in damp shady places and enough water. Various researchers from different places find the secondary metabolite activity of these plants as anti-inflammatory, antioxidant, antiamoebic, antibacterial, cholesterol, antimalarial[1,2,3]. Natural product compounds (secondary metabolites) from plants are grouped compounds: Alkaloids, Terpenoids, Flavonoids, Phenolics, Saponins, Kumarins, Quinone dyes, and Carotenoids, these are things that need to be researched and developed because they have multi-potential activities[4].

Figure 1. Suruhan leaves (Paperomia pellucida L)

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Image of leaf Suruhan (Paperomia pellucida L)

Secondary metabolites which identifiedof plants has been done by the phytochemical method [5,6,7,8]. Toxicity tests on plants that have potential as medicinal plants can be carried out by the BSLT method (Brine shrimp lethality test [9,10,11]. Lots of evidence can be shown that in plants that contain compounds phenolic has antibacterial activity [12,13]. Various types of solvents used for extraction from nonpolar to polar solvents produce various secondary metabolites and its activity [14], and even though the plant is the same species, but where it grows differently the environment can affect the types of secondary metabolites and their activities as well[15].

II. Experimental Method

Samples were obtained from around the UNIMED campus. Which was collected in March 2019

2.1. Instruments and Materials

The instruments used in this study are Erlenmeyers, measuring cups, beaker glass, stirring rods, watch glass, test tubes, pipettes, measuring flasks, tube racks, glass funnels, analytic balance sheets, hot plates, autoclaves, petri dishes, evendorf tube, vortex, paper disc, rotary evaporator, blender, micropipette/stirring fan, incubator, autoclave, blender, fan, maceration equipment, maceration equipment, analytical balance, glassware, refrigerator, volume pipette.

2.2. Materials

The chemicals used in the study were obtained from chemical stores: hexane, chloroform, acetone, ethyl acetate, ethanol Citric acid, MHA (oxsoid, CM0337), MHB (HiMedia: GM 391), aquades, test bacteria obtained from USU pharmaceutical chemistry: Bacillus cereus (ATCC 11778), S. aureus (ATCC 25923), E. coli (ATCC 25922), S. saprophytic (ATCC 49907), Catsobacterfrendi (ATCC 18090), P.acne (ATCC 27853), Epidermis (ATCC 12228) . DMSO (SIGMA), blank disc paper (oxoid), standard chloramphenicol (Oxoid), NaCl, Chloramphenicol powder (sigma), ammonia (Merck), petroleum ether (Merck), chloroform (Merck), HCl (Merck), Dragendroff reagents (sigma), ammonia (Merck), petroleum ether (Merck), chloroform (Merck), HCl (Merck), Dragendroff reagents (sigma), ammonia (Merck), petroleum ether (Merck), chloroform (Merck), HCl (Merck), Dragendroff reagents (sigma) Merck), Mayer reagents, CH_3COOH anhydrous (Merck), H_2SO_4 (Merck) Lieberman-Buchard reagents (Sigma), magnesium plates (Merck), amyl alcohol (Merck), $FeCl_3$ (Merck), Stiassny reagents (sigma), sodium acetate and Sigma NaOH (Merck), NB (Oxoid CM0067). vitamin C that functions as a positive control, DPPH, methanol (p.a) that is as a solvent and negative control, aquades, lead acetate 1%.

2.3. Procedures

15 kg of leaf plants (PaperomiaPellucida L) separated roots and washed with water. Blanching by boiling at a temperature of 1000C for 5 minutes with 0.05% citric acid solution media drained, dried in the room while fanming occasionally and reversed. The drying process is carried out in a shady place, protected from sunlight, during the drying process using a fan to avoid mold and caterpillars. Drying is done in a shady place intended to avoid damaging the metabolite content of the sample due to direct contact with sunlight. With such drying conditions, it is expected that the content of secondary metabolites contained in the sample will not be damaged, after drying the leaves are smoothed using a blender to expand the surface so that the leaf powder can be extracted maximally.

2.4. Phytochemical Screening of Secondary Metabolites

1. Flavonoids

Test for the presence of flavonoid compounds was carried out by taking 0.5gram extracts, adding two drops of 5% FeCl3 solution to the drip plate sample. The color change to greenish or black and blue indicates the presence of flavonoids.

2. Alkaloids

Test for the presence of alkaloid compounds was carried out by adding 0.5 gram of sample with 1 mL of HCl 2N and supplemented with 9 mL of distilled water and then added with 5 drops of Dragendorff reagent, the presence of brick red precipitate indicated the presence of alkaloids.

3. Saponins

Test for the presence of saponin compounds is done by adding 1 mL of sample with 10 mL of distilled water until 10 minutes and leaving the foam and then waiting for up to 10 minutes if the foam does not disappear add 2N HCl. If the foam

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does not disappear indicating the presence of compound saponins.

4. Steroids and Terpenoids

Test the presence of steroid and terpenoids compounds is done by adding 0.5 grams of extract with 10 drops of acetic anhydrous plus 2 drops of concentrated sulfuric acid shaken and left for a few minutes, the emergence of red and purple color shows positive triterpenoids, the emergence of green and blue color shows positive steroids .

5. Tanins

Test for the presence of tannin compounds was carried out by adding 0.5 gram extract with 10 mL aquadest and added with 3 drops of FeCl3 1%, the appearance of a blackish green color showed positive containing tannin.

2.4. Antibacterial

Equipment and Media Sterilization

All tools made of glass are sterilized in an autoclave at a temperature of 1210C 2 atm pressure for 15 minutes.

2.5. Media Making and Sterilization

Mueller-Hinton (MHA)

Bacterial growth media are made in heat-resistant capped bottles using Mueller-Hinton (MHA) media. The MHA flour was weighed using a 38-gram weighing bottle and mixed with 1 Liter of distilled water into a heat-resistant bottle than in a corner until homogeneous, put into a 1210C temperature autoclave pressure of 2 atm for 15 minutes. Furthermore, in a hot state, the media is poured into a petri dish and left until the media solidifies, the process of making media is carried out in laminar-flow.

Media Nutrient Broth (NB)Bacterial growth media are made in heat-resistant capped bottles using Nutrient Broth (NB) media. NB flour is weighed using a 13-gram weighing bottle and mixed with 1 Liter of distilled water into a heat-resistant bottle than in a corner until homogeneous, put into an autoclave at 1210C at 2 atm pressure for 15 minutes. Furthermore, in a hot state, the media is poured into a petri dish and left until the media solidifies, the process of making media is carried out in laminar-flow.

Media Nutrient Broth (NB)

Bacterial growth media are made in heat-resistant capped bottles using Nutrient Broth (NB) media. NB flour is weighed using a 13-gram weighing bottle and mixed with 1 Liter of distilled water into a heat-resistant bottle than in a corner until homogeneous, put into an autoclave at 1210C at 2 atm pressure for 15 minutes. Furthermore, in a hot state, the media is poured into a petri dish and left until the media solidifies, the process of making media is carried out in laminar-flow.

Renewal of Pure Culture

Each test bacterium: Escherichia coli, Staphylococcus epidermis, Lactobacillus, Propionibacterium acnes rejuvenated before use. Bacteria bred on sterilized MHA agar, then incubated for 24 hours at 37 0C (incubator (Memmert))

Bacterial suspensions preparation

Cultured bacteria are taken one strike using a cotton bud then the bacteria are suspended in 0.9% NaCl as much as 2 mL in an impulse tube. Suspending the bacteria into 0.9% NaCl, vortex, compared to the turbidity level with McFarland Standard 0.5 (0.05 ml of Barium Chloride in 9.95 mL of Sulfuric Acid, 1.5 x 10-8 / mL).

Antimicrobial Activity

The paper disk diffusion test was carried out to determine the antibacterial power of the sample against Escherichia coli, Staphylococcus epidermis, Lactobacillus, Propionibacterium acnes bacteria. This test is quantitative and repeated three times. Before the paper disc diffusion test is carried out, first, a sample extract solution made by weighing the extract concentration of 1.25% as much as 100 mg then dissolved in 1 mL DMSO. Concentrations of 100, 50, 25 and 12.5 mg / mL were prepared by serially diluting the extract with DMSO[16]. As a positive control, 0.02% Chloramphenicol (as much as 0.002 grams of Chloramphenicol dissolved in 10 mL distilled water (0.025 w / v). Selective media, so that which was made then given a 100 µl bacterial suspension was flattened using a spider. Paper discs inserted into media that had been containing as many as 5 bacteria, 1-4 paper disks were saturated with 20 µl test extract, as a positive control 1 Chloramphenicol added to the media, and the solvent (DMSO) saturated into the 5th paper disc as a negative control, then incubated at 37oC for 24 hours The clear area around the disc shows

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no bacterial growth which is then measured using a digital callipers.

Toxicity (BSLT Method)

Preparation of larvae Artemiasalina Leach

Preparation of larvae done by taking eggs Artemiasalina Leach as much as 1 g. The hatching is done by immersing the egg in artificial seawater as much as 2 L and given lighting with 40-60 watt incandescent lamps and aerated for 48 hours. Artificial seawater made by dissolving 40 g of salt in 2 L of water then filtered.

Stock Solution Preparation

Test solution made with a concentration of 2000 ppm. From the 2000 ppm test solution, further solutions made with concentrations of 100, 50, 25 and 12.5 ppm by dilution. For control (0 ppm) done without the addition of extracts.

Toxicity Test

Test solutions with concentrations of 100, 50, 25 and 12.5 ppm, each pipette as much as 6 mL put into a test tube and ten tail larvae added two days old. Each concentration was carried out twice and compared with controls. An observation I carried out for 6 hours with an interval of 1 hour. Furthermore, observation II was carried out at 12, 18 and 24 hours. The number of dead shrimp larvae counted every 6, 12, 18 and 24 hours.

III. Results and Discussion

Identification of the compounds obtained in the ethyl acetate extract of leaves paperomiapellucida L can be seen in the table 1.

Table1. Phytochemical of ethyl acetate extract of leaves (Peperomiapellucida L)

Flavonoid	Alkaloid	Saponin	Tanin	Steroid
++	+	+	++	++++

Toxicity tests performed with the BSLT method can be seen in the table 2. LC50 values can be determined using the probit method. It is necessary to look for a straight line equation from the research data. The x-axis is the log of concentration, and the y-axis is a probit which obtained by looking at the probit table in each value of % death.

Table 2. : BSLT (Brine shrimp lethality test) of ethyl acetate extracts Peperomiapellucida leaf

Treatment	Death of Artemia Salina Leach Concentration (ppm)				Control Negative
	50	250	500	1000	
1	5	6	8	10	0
2	5	6	8	9	0
3	4	6	7	10	0
4	4	6	7	7	0
Total	18	24	30	36	0
Average	4,5	6	7,5	9	0
Death	45%	60%	75%	90%	0

Table 3. Death of larvae onEthyl Acetate Extract leaves (Peperomiapellucida l)

Concentration of Extract	Log Concentration of extract	% Death of Larvae on the extract	Probit value (LC_{50})
1000	3	90%	6,28
500	2,698	75%	5,67
250	2,397	60%	5,25
50	1,698	45%	5,00

From the table data, we get the straight-line equation Y = 0.923x + 3.289, as shown in the following figure 2.

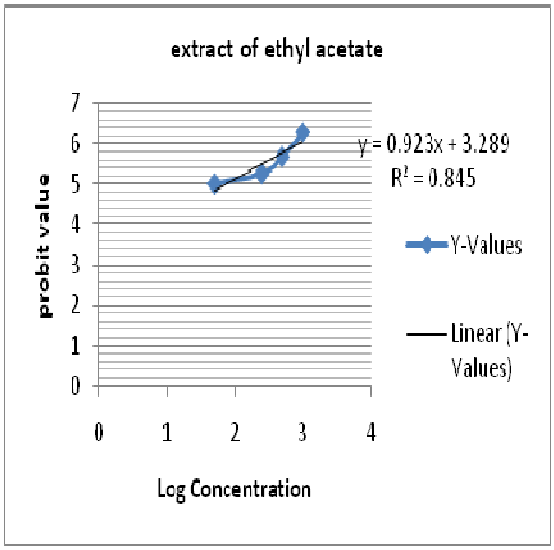

Figure 2. Graph LC_{50} Of ethyl acetate extract of leaves peperomia pellucida L

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The LC50 value with Y is 5. The LC50 value obtained by finding the antilog of x, then the LC50 value of the extract of Suruhan leaves of Ethyl Acetate is obtained at 71.28 ppm. The designation of the toxic effects produced indicates the disruption of the cell formation process. In this case, it is assumed to be a cancer cell[17]. Determination of LC50 can do in several ways, including probit log concentration charts, graph methods, mathematical calculations.

Determination of the probit concentration graph method do by placing the percentage of responses of each group of animals in the ordinate and logarithm of the dose of the drug that gave absently [18].

By looking at the data table and comparing it with the results obtained it showed that the ethyl acetate extract from the leaves of the mess is very toxic

Inhibitory zone of Ethyl acetate leaf extract (Paperomiapellucida L)against on several types of bacteria was shown in the table 5.

Table 4. LC50 Toxicity Value Table

No	LC50 value (µg/ml)	Level of toxicity
1	0-250	Very toxic
2	250-500	Toxic
3	500-750	Moderate toxic
4	750-1000	Non-toxic

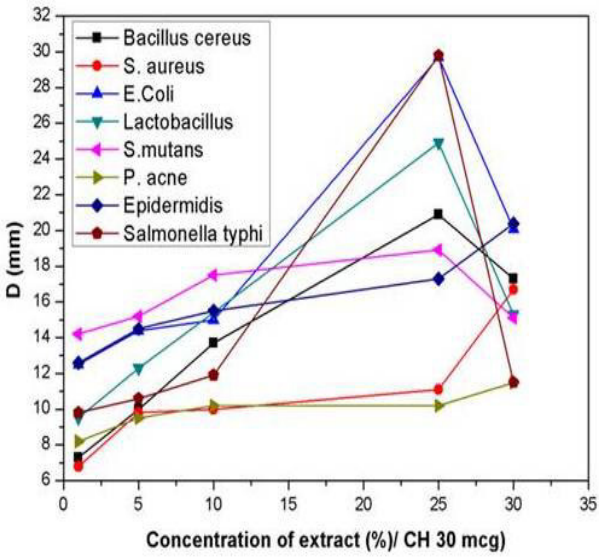

Figure 3. Inhibition Ethyl acetate extract of leaves (Paperomiapellucida L) against several microorganism

Ethyl acetate extract of Suruhan leaves (Paperomiapellucida L) at a concentration of 1% gives inhibition to more than 50% of E. coli bacteria (ATCC 25922, S. saprophticus (ATCC49907), P. acne (ATCC 27853) and Epidermidis (ATCC 12228) as control used chloramphenicol (30 mcg).

Table 5. Inhibition ethyl acetate extract of leaves paperomiapellucida L against Microorganisms

Concentration of extract (%) / (CH 30 mcg)	Micoorganism / D (mm)							
	Bacillus cereus (ATCC1 1778) / D (mm)	S. aureus (ATCC 25923)/ D (mm)	E.Coli (ATCC 25922) / D (mm)	Lactobacillus (ATCC) / D (mm)	S.mutans (ATCC / D (mm)	P. acne (ATCC 27853) / D (mm)	Epidermidis (ATCC 12228) / D (mm)	Salmonella typhi (ATCC
1	7,3	6,8	12,5	9,5	14,2	8,2	12,6	9,8
5	10	9,8	14,4	12,3	15,2	9,5	14,5	10,6
10	13,7	10	15	15,4	17,5	10,2	15,5	11,9
25	20,9	11,1	29,7	24,9	18,9	10,2	17,3	29,8
CH (C 30 mcg)	**17,3**	**16,7**	**20,1**	**15,3**	**15,1**	**11,5**	**20,4**	**11,5**

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From these findings that this plant has the prospect of being used as an herbal medicine as an antibacterial

IV. Conclusion

This plant has extremely toxicity; this illustrates potent bioactivity used as medicinal plants.Ethyl acetate extracts Leaf leaves (Paperomiapellucida L) have secondary metabolite compounds: alkaloids, flavonoids, steroids, saponins and tannins that have the potential to be used as herbal plants as antibacterial.The higher the concentration of the ethyl acetate extract of the leaves of the leaf, the wider the obstacles to the test bacteria were tested using comparative antibiotic chloramphenicol.

Suggestion. In this plant, other activity tests and their chemical structures need to test.As a medicinal plant, it needs to be cultivated to increase the inventory of Indonesian herbal plants

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