

Ethanol Extract (*Colocasia esculenta* (L.) Schott.) Hideung Cultivar as Anti-scabies through In-Vitro

I. Gusti Ayu Manik Widhyastini¹, Febi Nurilmala¹ and Misja¹

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Nusa Bangsa, Bogor 16166, Indonesia

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Abstract: This study aims to determine the potential of taro extracts as anti-scabies. Efforts have been made towards the swarming number of scabies caused by the mites *Sarcoptes scabiei*, which attacks the skins of cattle. Treatments relied more on synthetic chemical drugs that are more effective but are expensive. Taro plants (*Colocasia esculenta* (L.) Schott) is mainly found in farmlands. Talas Bogor is a potential larvacide, able to kill mosquito larvae, predicted to have anti-parasite properties against mites. This research includes phytochemical tests (flavonoid, tannin, saponin, alkaloid, calcium oxalate) of the identified plant, effectiveness test of the taro extract towards the mites through in vitro. The results of the effectiveness test of the wild taro extract (*Colocasia esculenta* (L.) Schott) cultivar Hideung used in in-vitro with probit analysis obtained an LC50 value of 50.11% for 4 hours treatment time, an LC50 value of 24.54% for 6 hours' time, able to exterminate 100% of the mites tested, using Neguvon as a positive control, and 10.96% LC50 value for 3 hours treatment time. Treatment of wild taro extracts (*Colocasia esculenta* (L.) Schott) Cultivar Hideung with 96% ethanol solvent is a potential alternative for scabies treatment.

1 INTRODUCTION

Scabies is generally caused by *Sarcoptes scabiei* variety hominids mites, which are parasites from the Arthropoda class with the order Acarina. Cases of scabies infection occur in both sexes, at all ages, groups, and all socioeconomic levels. Every year, it is measurable that around 300 million prevalence worldwide, this estimate might be too high. Only female mites burrow under the host's skin, causing intense allergic itching (Chosidow O, 2006); (Anderson and Strowd, 2017). Many of these ectoparasite species have their breeding sites very close to their hosts so that they are practically always present.

Throughout the year the presence and number of ectoparasites vary, the speed of this development depends on the temperature, according to the season, (especially in milder and temperate climates (Saad and Desoky, 2016). Then stated that such activities of prophylaxis or control need knowledge of the developmental of the life cycle and on the periods of occurrence of the ectoparasites as well as on their breeding sites (Saad and Desoky, 2016). It is necessary to develop an alternative approach in handling ectoparasites (Fang *et al.*, 2016) In the rabbit; it is normally treated by injecting Ivermectin.

However, this method is expensive and is not always effective (Vu Thi Thu Hang, Chu Duc Tuy, 2012).

Trichlorfon (Neguvon[®], Bayer AG, Leverkusen) was evaluated for its effect on naturally occurring infestations of *Sarcoptes scabiei* in 16 dogs. A 0.1% concentration of trichlorfon was applied as a wash once a week for four weeks. Skin lesions improved gradually and disappeared completely two weeks after the onset of treatment. By week 4, there were no clinical signs of scabies in any of the 16 dogs, and no *S. scabiei* mites or other stages of mite were found on skin scrapings (Sarchahi, 2005). However, the use of chemicals could result in resistance in target species, toxicity, and environmental hazards. (Luo *et al.*, 2015). Based on the increase in cases of scabies on ruminants occurring in Bogor mainly in Babakan Madang (Misja dan Widhyastini, 2016), actions are needed to control the increase in scabies disease on ruminants as well as in humans. One of the solutions to resolve this issue is by searching for natural products that are selective, safe and inexpensive.

Wild taro (*Colocasia esculenta* (L.) Schott.) If consumed can cause itching. It is necessary to use wild taro which is commonly found around farms such as in fields, swamps, on the edge of nets, and home yards. According to Fang *et al.*, (2016) that, natural medicines from plants have advantages such

as having fewer side effects, better patient tolerance, relatively cheaper and acceptable because of the long history of use.

According to M Alcantara,(2013), the chemical constituents of taro plants based on the results of phytochemical screening have shown that taro leaves ethanol extracts consists of compounds including, antioxidant, phenol, flavonoids, tannins, and saponins; and according to Krishnapriya and Suganthi, (2017); (Kumawat *et al.*, 2010), preliminary phytochemical analysis of (*Colocasia esculenta* (L.) Schott.) dried tubers were determined and revealed the presence of alkaloids, glycosides, flavonoids, terpenoids, saponins, and phenols.

In taro that is not itchy and edible that (*Colocasia esculenta* (L.) Schott.), Contains a large number of phytochemicals, so taro is recommended for the pharmaceutical industry. Taro traditionally is used as a medicine, the stem of the leaves is believed to be used as the treatment for itchiness and digestive issues in throat and asthma. The stem can be used to heal wounds. Taro has the potential to be an anti-scabies. According to Febi Nurilmala, *et al.*,(2017), In Indonesia, the potential for taro commodities (*Colocasia esculenta* (L.) Schott, which is not itchy has not been supported by proper data compared to other crops such as cassava, sweet potatoes, and potatoes. Diversification of food as a source of choice carbohydrates. Taro are foods that all parts of the plant, such as leaves, stems, and tubers can be consumed. In research Adejumo, (2013), the use of taro (*Colocasia esculenta*) as an alternative energy source to feed animals. *Colocasia esculenta* corm aqueous extract was assessed for its in vitro antioxidant capacity and free radical scavenging potential,(Watal, Unit and Division, 2017)

2 MATERIALS AND METHODS

The materials used in this research include: wild taro root and leaf (*Colocasia esculenta* (L.) Schott. *Kultivar Hideung* result of the determination of Biological Research Center LIPI Bogor, mites *S.scabiei*, ethanol 96%, filter paper, distilled water, *Neguvon*. The object of the study used were rabbits infected with scabies.

2.1 Preparation of Plant Extract

Wild taro leaf stalks were sorted and then washed, then drained and weighed. Afterward, the

ingredients are chopped into smaller pieces, dried by air for 2x24 hours. After drying, it was then put into the oven for three days at 60 ° C so that the sample is arid and then weighed dry and blended until it becomes smooth to form a powder.

The extract of the wild taro extract was carried out by maceration method, which is the material that has become powder and then taken as much as 200 grams of powder, then macerated with 96% ethanol as much as 1000 ml for 72 hours. Followed by filtration to produce a filtrate. The filtrate is evaporated using a rotary evaporator, then evaporated above the water bath until the volume becomes 10% of the initial volume and until a thick extract is obtained.

2.2 Phytochemical Screening

The phytochemical screening method based on (Dhanraj *et al.*, 2013) are as follows:

- **Detection of Alkaloids**
The extract is dissolved individually in a hydrochloric acid solution and then filtered. The filtrate is treated with Hager reagent (saturated picric acid solution). The formation of yellow deposits indicates the presence of alkaloid compounds;
- **Detection of Tannin**
The 4 ml extract was treated with 4 ml *FeCl*, after which appears a green color, concluding as positive for tannin;
- **Detection of Flavonoids**
Alkaline reagent test; the extract is dripped with a few drops of 10% sodium hydroxide solution. Intense yellow formation becomes colorless on the addition of dilute acid, indicating the presence of flavonoids ;
- **Detection of Saponin**
The extract was diluted with distilled water up to 20 ml, and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer foam indicates the presence of saponin. The 0,5 g of extract was shaken with 2 ml of water. If foam persists to produce for ten minutes, it indicates the presence of saponins;
- **Measurement oxalate levels**
Taro powder sample weighed approximately 0.5 grams in Erlenmeyer grindstones and dissolved in water as much as 38 ml and 1 ml of 6N hydrochloric acid. The solution was then heated at 100°C, cooled and filtered. The filtrate was divided into two parts; each section is titrated with ammonium hydroxide using methyl red indicator until the red color changed

to yellow. The results of titration were heated at 90°C with constant stirring, then add 2 ml of 5% calcium chloride and cooled by maintaining a constant stirring, allow the cool solution to stand overnight. The next stage is the separation of the precipitate, and the supernatant is done using centrifuges with a speed of 2.500 rpm for 5 minutes. The precipitate is dissolved in sulfuric acid 20%. All solutions are combined and made into 40 ml. 25 ml of solution titrated with potassium permanganate solution 0,05N with an end point indicated by the formation of a pink color that persists for 30 seconds.

2.3 Mite Sample Preparation

The mite samples used in this study were *Sarcoptes scabiei* mites obtained from rabbit skin scrapings infested with *S. scabiei* naturally (Luo et al., 2015); (Sashidharan, Basavaraj, and Bates, 2016); (Ari Puspita Dewi .Haryuningtyas, 2008). The infested skin was scraped using a scalpel or cutter knife until it bled, then the scrapings were collected into a petri dish (Sashidharan, Basavaraj, and Bates, 2016); (Ari Puspita Dewi .Haryuningtyas, 2008) which was filled with physiological NaCl. Each petri dish was filled with ten mite samples (Ari Puspita Dewi .Haryuningtyas, 2008).

2.4 Test the Effectiveness of Wild Taro Extract

The method of test the effectiveness of wild taro extract, are as follow:

- Preliminary test
The preliminary test begins with the concentration of the concentrated extracts into the following concentrations of 0%, 20%, 40%, 60%, and 80%. Each treatment consisted of 10 *Sarcoptes scabiei* with three replications, to determine the concentration of which treatment is causing death by 50% of the population of *Sarcoptes*. Criteria for the death of *Sarcoptes* is that they do not move or do not react when touched in the treatment. A positive control using *Neguvon* and for negative control using distilled water. The treatment stood for 6 hours; then observation was done every hour under a microscope with 100x magnification, the data of dead mite was recorded and analyzed;
- Effectiveness Test
In the effectiveness test, the concentration used was the concentration obtained in the

preliminary test which caused 50% death of the *Sarcoptes* population, which became the reference for determining the range of concentration intervals on the actual toxicity test, i.e., by raising and lowering the concentration in the preliminary test, by a close-range interval. The test concentration used were: 0, 25%, 30%, 35%, 40%, 45% and 50%. Treatment of *Neguvon* used were 10%, 20%, and 25%. Calculation of dead mites is carried out every hour; observations carried out for 6 hours. The data obtained were then analyzed using *probit* analysis to determine the LC50 value in each treatment hour;

▪ Data Analysis

The data obtained were then analyzed using a *Probit* analysis to determine the LC50 value in each hour of treatment.

This analysis is used in biological testing to determine the response of the subjects studied by the presence of stimuli, in this case of wild taro extract by knowing the response in the form of mortality of mites.

3 RESULTS AND DISCUSSION

Based on the "*Herbarium Bogoriense*," LIPI Bogor Botanical Field of Biological Research Center, wild taro is used in taro type of *Colocasia esculenta* (L) Schott. *Hideung Cultivar*.

3.1 Secondary Metabolism

The assessment of a plant can be considered a food source, the main thing to note is the nutritional value of a plant. However, the presence of other toxic compounds in a plant can also affect plant characteristics and nutritional content. These toxic factors act as anti-nutrients and affect organisms. Antinutrients are chemicals which have been evolved by plants for their defense, among other biological functions. Anti-nutrients reduce the maximum utilization of nutrients (especially proteins, vitamins and minerals), thus preventing optimal exploitation of the nutrients present in food and decreasing the nutritive value (Mcewan, 2008). Anti-nutrients vary in chemical structures, ranging from amino acids to proteins; from simple amines to alkaloids, glycosides and many phenolic compounds. The biological effects of all these

chemicals are diverse and complex. Analysis of secondary metabolites was carried out using extracts from Bogor taro leaves and stems (*Colocasia esculenta* (L) Schott) Hideung Cultivar identifies compounds such as alkaloid, flavonoid, tannin, saponin, and calcium oxalate compounds, (Table 1). The anti-nutrient levels were generally low and thus may not pose an immediate effect on the health of consumers. Reduction of the antinutrients through processing (cooking, frying, roasting) was observed to enhance the nutritional value of these tubers. (Mcewan, 2008). The results of the phytochemical test showed that there were flavonoids, tannins, saponins, and calcium oxalate. According to Temesgen and Ratta,(2015),anti-nutrients which found in taro root have negative implications for taro as food, yet they also have positive implications for taro as a crop that can be grown with minimal use of fungicides and pesticides. The main antinutrients that exist in taro are mucilage, oxalic acid, tannins, cyanide, lectins, alpha-amylase inhibitors, protease (trypsin and chymotrypsin) and inhibitors)(Temesgen and Ratta, 2015). Flavonoids function as anti-bacterial by forming complex compounds against extracellular proteins that interfere with the integrity of bacterial cell membranes (Kumar and Pandey, 2013). In addition to flavonoids which have the ability as an anti-bacterial, alkaloids can also be an anti-bacterial. (Kumar and Pandey, 2013); (Cushnie, Cushnie, and Lamb, 2014) The suggested mechanism is by disrupting the constituent components of peptidoglycan in the cell so that the cell wall layer is not formed completely and causes the cell death (Cushnie, Cushnie, and Lamb, 2014). In the test results of leaf extracts and stems of wild taro plants, showed the presence of tannin compounds. According to Ashok and Upadhyaya, (2012) medical tannins can be used, among others, to heal burns, stop bleeding, stop the infection, heal wounds internally antidiarrheal, hemostatic, and ant hemorrhoids. Tanin can form a protective layer over the open tissue to make the wound uninfected even more.

Saponins are secondary metabolites with high molecular weight. They present in a wide range of plant species and are distributed throughout the bark, leaves, stems, roots and even flowers (Moghimpour and Handali, 2015). Saponins are bitter in taste Saponin has the ability as an anti-septic which functions to kill germs (Nimenibo-uadia, Ugwu, and Erameh, 2017), hepatoprotective, anti-ulcer, anti-tumor, antimicrobial, adjuvant and anti-

inflammatory activities (Moghimpour and Handali, 2015).

Oxalate compound is generally known anti-nutrients found in taro, which creates a feeling sharp stabbing. Oxalate is also in the know can form complexes with metal compounds that reduce nutrient bioavailability of minerals in taro. Compounds are known calcium oxalate is insoluble in water and has a tendency to precipitate (solidified) in the kidneys or the urinary tract, thus forming calcium oxalate crystals acute when the concentration is high enough (M Alcantara, 2013). Reported that oxalate levels were higher in petioles of taro cocoyam than in leaves (du Hang and Preston, 2010). Oxalate salts, such as sodium and potassium, can dissolve, whereas calcium oxalate salts are insoluble. Calcium oxalate is a salt formed from oxalic acid. Calcium oxalate tends to settle in the kidneys or in the urinary tract to form calcium oxalate crystals; these crystals play a role in the formation of kidney stones at high levels, sharp-edge calcium oxalate crystals, (Mcewan, 2008);(Watal, Unit and Division, 2017). *Colocasia esculenta* has been broadly investigated for proximate composition and antinutrient screening, but the data were not comparable because of variations in genotypes, locations, and experimental analysis. Generally, taro plants, have an astringent and itchy taste that can cause swelling of the lips, mouth, and throat if eaten without proper processing, (Mcewan, 2008). Oxalate compounds found in taro plants are chemical compounds responsible for poisoning, oxalate compounds are also found in other plants, (E Yuen, 2001).

Table 1: Qualitative analysis of some metabolites of extract *Colocasia esculenta* (L) Schott. Hideung Cultivar in alcohol extract of leaf and stem

Sample	Flavonoid	Tannin	Saponin	Alkaloid	Calcium Oxalate
Leaf					
1	++	++	+	-	+
2	++	++	+	-	+
3	++	++	+	-	+
Stem					
1	++	++	+	-	+
2	++	++	+	-	+
3	++	++	+	-	+

3.2 The Potential Test of Wild Taro Extract

The potential test of wild taro extract was carried out by a bioassay test method by calculating the *probit* value to determine LC50. The test was performed in two experimental stages: preliminary test and effectiveness test of extract. Collection of scraped mite samples from rabbits infected with scabies is collected in a petri dish. The scraping is done at room temperature at about 22-30 ° C. This is to ensure samples survive during testing and as a requirement in testing the potential of the extract. According to (Chosidow, O. 2006) *Sarcoptes scabies* mites have a temperature of less than 27-30 °C which is approximately four days outside the host and still caton infect and penetrate. Female mites live longer than male mites in the same condition.

3.2.1 Preliminary Test

The preliminary test used variations of extract stalks and leaves of wild taro (*Colocasia esculenta* (L.) Schott *hideung cultivar*). This is the effect of the addition of wild taro extract concentration by determining the LC50 of mortality. The concentrations used were 0%, 20%, 40%, 60%, and 80%. The calculated *probit* value of the initial test of wild taro extract for 6 hours is 28.18%.

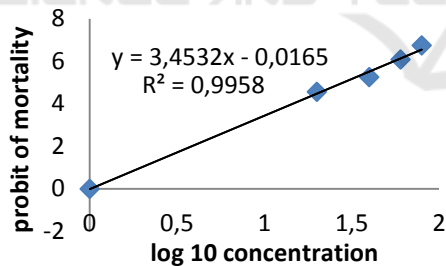


Figure 1: Calculating LC50 using probit analysis on equations for 6 hours in preliminary test

LC50 value of the toxicity of wild taro extract against *Sarcoptes scabies* mites in preliminary tests every hour at initial concentrations can be seen in Table 2.

Table 2: Result of LC50 Ethanol Extract of Wild Taro in the Preliminary Test

Ethanol Extract of Wild Taro	
Time(hour)	LC ₅₀ (%)
1	169,8
2	109,6
3	69,1
4	53,7
5	45,7
6	28,18

The LC50 value of the toxicity of wild taro extract on *Sarcoptes scabies* mites on the effectiveness test every hour at the actual concentration can be seen in Table 2. LC50 values obtained at the preliminary test concentration (0%, 20%, 40%, 60%, and 80%) in every hour. Based on the results, the LC50 value of 28.18% is the smallest concentration of extracts that can kill 50% of test mites for 6 hours. The LC50 value will be used as the concentration range in the actual test.

3.2.2 Test the Effectiveness of Wild Taro Extract

The concentration toxicity test used in the actual stage refers to the concentration of the preliminary test (LC50) which is in the concentration range of 28.18% (table.1), so that the actual test used concentration of 0; 25; 30; 35; 40; 45; and 50%, the test was carried out with three repetitions, and *Neguvon* was tested as a positive control.

The results of the acquisition of mite's mortality percentage data showed that the percentage increase increased in each increase in the concentration of treatment. Acquisition of mortality percentage on the effectiveness test of wild taro extract was able to kill mites up to 100% with a concentration of 50% extract for 6 hours.

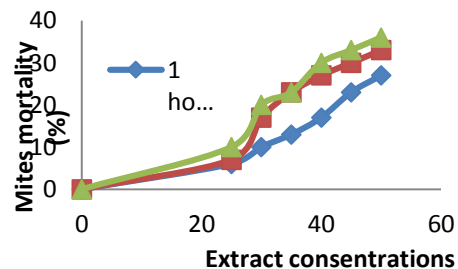


Figure 2: Probit analysis of mites mortality with extract concentrations for 1-3 hours

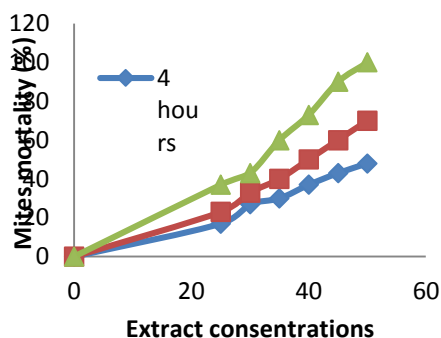


Figure 3: Probit analysis of mites mortality with extract concentrations for 4-6 hours

The data obtained from the percentage of mite mortality showed at the first hour at 25% extract concentration was able to kill 6% of the test mites, 30% extract concentration was able to kill 10% of the test mites, while the extract concentration of 35% was able to kill mites by 13% of the test mites. Then at a concentration of 40% increased to 17%, at the concentration of 45% was able to kill mites 23% and a maximum at 50% extract concentration at the first hour of mite mortality percentage of 27% (Figure 2).

The percentage of mortality increased along with the increase in concentration at every hour, up to the 6th hour at 50% extract concentration capable of killing 100% mites from test mites (Figure 3).

If calculated based on the calculation of probit analysis from observational data every hour of treatment, it requires different extract concentrations to kill 50% of the test mite population — the results of the calculation of the probit value obtained by the linear regression equation with the predicted LC50 value of 24.54% for 6 hours.

Table 3: A Comparison of LC50 for Extracts of Wild Taro *Hideung Cultivar* and *Neguvon*

Time (hour)	Ethanol Extract of Taro LC ₅₀ (%)	Neguvon LC ₅₀ (%)
1	93.3	87.09
2	70.79	42.65
3	64.56	10.96
4	50.11	
5	39.80	
6	24.54	

At 2 hours, it was predicted that the LC50 value for wild taro extract was 70.79%, meaning that at 2

hours effective concentration treatment to kill 50% of test mites required extract concentrations is of 70.79%.

Then the LC50 value at 3 hours obtained 64.56%, at 4 hours obtained the LC50 value of 50.11%, while at 5 hours obtained the LC50 value of 39.80%. The lowest extract concentration obtained at six hours is 24.54% (table3).

The positive results of the LC50 values obtained from wild taro extract with 6 hours of observation were 24.54%, at that concentration wild taro extract was able to kill mites up to 100%. The results of LC50 values on Neguvon as a positive control, at 3 hours of treatment were able to kill mites up to 50% at a concentration of 10.96%, (Table 3).

Recent studies suggested that aqueous and ethanol extracts from plants used in allopathic medicine are potential sources of anti-viral, anti-tumor and anti-microbial agents, etc. (Prajapati et al., 2011). According to (Sarchahi, 2005) Trichlorfon (metrifonate) is an organophosphate used as an insecticide and pesticide for plants and livestock (*Neguvon*) as well as an anthelmintic for animals, principally the horse. It can be used systemically in dogs for its anthelmintic pesticidal properties. Thus, it can be recommended that a 0.1% solution of trichlorfon is an effective and safe treatment for canine sarcoptic mange, but beyond the limitations of the study, the results of research on wild taro extract still have the potential as an acaricide because it can kill 50% mites within 6 hours with a concentration of 24.54%. This shows that the extract of wild taro has the potential as an anti-scabies.

According to Hay et al., (2012), the pathogenesis of scabies involves many complex immunological and inflammatory pathways, some of which we have just begun to understand. Inflammation of the skin, papules, and pruritus results from a hypersensitivity reaction delayed by specific immune-mediated antigens. Beginning 3-4 weeks after the main infestation is usually asymptomatic. In subsequent infestations, however, symptoms reappear much more quickly, in about 1-2 days. Further action aimed at achieving realistic control targets must involve further research topics, such as long-term health consequences of scabies infection or an explanation for cycle events, and purification and trials of appropriate regimens for community-based scabies control using ivermectin and topical agents and options for the treatment of scabies, (Luo et al., 2015). All different attacks of part-time ectoparasites and endoparasitic lice, mites, or insects make it necessary to block their aggressive attacks with

repellents to be placed on animals and to reduce the number of aggressors around the herd with insecticide applications. And acaricides on the skin of farm animals and the ectoparasite breeding sites. Such activities of prophylaxis or control need knowledge of the developmental of the life cycle and on the periods of occurrence of the ectoparasites as well as on their breeding sites (Saad and Desoky, 2016).

However, the use of chemicals could result in resistance in target species, toxicity, and environmental hazards, Natural medicines from these plants are gaining popularity because of several advantages such as often having fewer side effects, better patient tolerance, relatively cheaper and acceptable because of the long history of use (Tabassum and Hamdani, 2014)

4 CONCLUSIONS

A plant will be a food source, especially getting attention is its nutritional content. However, the content of other compounds that are toxic to a plant will affect the nutritional value of the plant.

Analysis of secondary metabolites was carried out using extracts from Bogor taro leaves and stems (*Colocasia esculenta* (L) Schott) Hideung Cultivar identifies compounds such as alkaloid, flavonoid, tannin, saponin, and calcium oxalate compounds.

Colocasia esculenta has been broadly investigated for proximate composition and antinutrient screening, but the data were not comparable because of variations in genotypes, locations, and experimental analysis. Most taro cultivars have an astringent taste and can cause swelling of lips, mouth, and throat if eaten unprocessed. Implications for taro as food, yet they also have positive implications for taro as a crop that can be grown with minimal use of fungicides and pesticides. Collection of scraped mite samples from rabbits infected with scabies is collected in a petri dish. The scraping is done at room temperature at about 22-30 ° C. This is to ensure samples survive during testing and as a requirement in testing the potential of the extract. Wild taro extract (*Colocasia esculenta* (L). Schott) Hideung Cultivar with 96% ethanol solvent has potential as anti-scabies with the LC50 value of 24.54% capable of killing 100% mites within 6 hours. Nuevovon is effective for sarcoptic, but beyond the limitations of the study, the results of research on wild taro extract still have the potential as an acaricide. This shows that the extract of wild taro has the potential as an anti-

scabies. However, the use of chemicals could result in resistance in target species, toxicity, and environmental hazards

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