



Automatic formed cotton buds delivery agent of antiparasitic drugs from *Annona muricata* leaves as a preventive and curative therapy towards *Otodectes cynotis* infection in pets: An in vivo proof of concept study

Lyvia Angeline Ciputra^a, Muhammad Afiq S. Mappiara^a, Tiara Minarfa S^c, Yuki Nurindar Rizal^b, Abdul Wahid Jamaluddin^{a*}

^aVeterinary medicine, medical faculty, Hasanudddin University, Makassar, 90245, Indonesia

^bMedical faculty, Hasanudddin University, Makassar, 90245, Indonesia

^cFaculty of Pharmacy, Hasanudddin University, Makassar, 90245, Indonesia

*corresponding author: abdulwahidjamaluddin@unhas.ac.id

Abstract

The practice of keeping pet animals have gained popularity in society, with the owning number of pets that reach 67%, particularly 37% for cats and 16% for dogs. Since animals have been considered family members nowadays, keeping them means that the owner is willing to contribute to maintaining their health and hygiene, which is crucial to prevent the spread of diseases that can affect both the pet and their owner. One common issue in animal health is the infection caused by the ear mite called *Otodectes cynotis*, which can lead to external ear canal inflammation. Current treatment for *Otodectes cynotis* infection is utilizing the topical application of synthetic drugs that contain antiparasitic synthetical drugs inside such as ivermectin and doramectin, due to the potential side effects and the threat caused by the tight therapy dose index in antiparasitic drugs, the alternative treatments including modified cotton buds and the use of herbal remedies have been developed to upgrade, to solve and to invent the novelty of science. This research aims to prove the hypothesis of the effectiveness of soursop leaf extract in eradicating *Otodectes cynotis* in animals, prescribing the required concentration to generate effective results yet to investigate the potential of modified cotton buds as a product of equipment. The outcomes of this study are expected to facilitate the development of knowledge in parasitic drugs and herbal medicine substitutes yet to innovate the more practical way to perform drug therapy for external otitis in pet animals caused by *Otodectes cynotis*.

Keywords: pets, Otodectes cynotis, cotton buds, soursop leaf extract

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Introduction

One of the well-known parasites that often infect pet animals is *Otodectes cynotis* mite which has approximate infection prevalences number around 33.3% in dogs and 52.6% in cats (Silva et al., 2020a). *Otodectes cynotis* is an ectoparasite that have predilection site in the external ear canal of cats, dogs, and other carnivores (Waljannah and Siagian, 2021).

Otodectes cynotis infestation in the ear can cause infection resulting in otitis externa or inflammation of the external ear canal (Aritonang et al., 2020). Treatment and prevention of infection due to *Otodectes cynotis* have been fully relying on the use of synthetic chemical drugs through topical administration using hands. The topical treatment uses components such as antibiotics, anti-inflammatories, and anti-parasites including neomycin sulfate, triamcinolone acetonide, permethrin, while the ear drops commonly contain pyrethrins 500 µg/mL in it (Maslim and Batan, 2021; Praing et al., 2021).

On the other hand, the direct application of drugs and drops as a treatment of *Otodectes cynotis* infections has plenty of disadvantages such as limited reach, the potential for the drops to cause a hearing problem, and the threat of microbial transmission through intermediate reservoirs. In addition, the use of synthetic chemical drugs tends to have side effects, and it's relatively expensive compared to the herbal composition. The impact of synthetic drugs is divided into direct accumulated side effects and indirect accumulated side effects which are caused by their inorganic and complex substance component (Weka, 2019). Therefore, the development of natural ingredients for the treatment of *Otodectes cynotis* infection to prevent the additional side effects caused by *Otodectes cynotis* is highly preferable.

One of the alternative ways to overcome the problem that's been mentioned earlier is by using cotton buds as a drug application instrument. Cotton buds itself is a small plastic bar with edges covered by cotton and can be used to remove cerumen and water, and also to get rid of the itchy sensation inside the ear (Alrajhi et al., 2019a). With the growth of civilization, the cotton buds are now being modified to invent broader functions such as the iodine cotton buds and alcohol cotton buds. Both alcohol cotton swabs and iodine cotton swabs are generally used to collect diagnostic samples and chemical material applications. This new method of drug application is considered to be more non-invasive, easy to use, more efficient, cheaper, and accessible (Jung dan Kim, 2022; Martin et al., 2020). The modification of cotton buds can be done by substituting the liquid form drugs into the middle part of the cotton buds stick that later will be absorbed by the cotton automatically by the diffusion process after one of the cotton bud edge parts is detached. These modified cotton buds have been tested and proven to deliver the drugs with a more specific reach area, precise dosage, and minimum potential of contamination. An alternative to the problems related to medicinal ingredients is the use of organic or herbal-based ingredients. The use of herbal medicines in the veterinary field is applicable for therapeutic, prophylactic, and diagnostic purposes to maintain animal health. The use of herbal medicines is considered to have fewer side effects, and the availability of raw materials that have been used to make them are considered widely available and at more affordable prices. (Herdiana et al., 2021).

One of the herbal medicinal ingredients that is widely grown and easily found is soursop leaves. Based on the preceding research, the soursop leaves have been proven to have anti-cancer, anti-parasite, insecticide, anti-worm, anti-bacterial and antiviral effects. There are many beneficial active substances contained in soursop leaves, including acetogenin, annocatalin, annohexocin, annonacin, anomuricin, and much more (Febrianto, 2018a). The research done by (Lesmana, 2017) shows that soursop leaf extract can cause death to ticks that infected cows. Based on the background mentioned previously, soursop leaf extract might effectively eradicate *Otodectes cynotis* which has a smaller size and softer body structure than ticks. Therefore, for the first time, held research to perform the right treatment method and formulation to kill *Otodectes cynotis* mites using soursop leaf extract. In this research specifically, soursop leaf extract will be injected into the cylindrical

space in the middle of disposable cotton buds. So that when topical treatment is being performed for otitis externa caused by *Otodectes cynosis* there won't be repeated contact done between the instrument and the person who performed it. The formulation and treatment method are expected to be the preventative and curative solution for treating infections caused by *Otodectes cynotis*, simplifying the treatment process, minimizing the potential for transmission of *Otodectes cynotis*, striving welfare of affected animals, and facilitating the development of the knowledge in parasitic drugs herbal medicine substitute.

Materials and Methods

Mites Collection

Mites sample from four mature cats was taken at the Laboratory of the Veterinary Teaching Hospital at Hasanuddin University, Hope Veterinary Clinic, and Jumnih Veterinary Clinic. The cats that were used as experimental animals were mature cats with body weights of about 4 kg.

Orientation and Preparation of the Soursop leaf extract

The equipment used in the making of the extract was a glass jar, 1000 mL Erlenmeyer (Pyrex®), glass funnel (Pyrex®), stirring rod, vacuum rotary evaporator, digital scale, porcelain cup, 100 mL measuring cup (Pyrex®), 500 mL and 250 mL beaker. mL (Pyrex®), dropper pipette, petri dish (Pyrex®), tweezers (Renz®), filter paper, microscope, object glass, cover glass, brown bottle, 100 mL porcelain dish, glass jar, 1cc syringe, And stopwatch. The materials used are soursop leaf *Simplicia* powder, Na CMC, parchment paper, water one, plastic wrap, and ethanol 70%

Soursop leaf *simplicia* powder is then macerated by placing it in a dark glass container and then adding 70% ethanol solvent until the *simplicia* powder is submerged, the solution is then left for 5 days, stirring occasionally. Then the macerate was separated and the dregs were macerated twice with 70% ethanol solvent in the same way for 2 days, then the macerate was separated. All the macerate obtained is combined and then evaporated using a rotary evaporator at a temperature of ± 40 degrees Celsius to obtain a thick extract (Surbakti and Nadiya, 2018). In this research, a suspension solution with a volume of 15 mL was made using a diluent in the form of Na CMC which is capable of dissolving both polar and nonpolar compounds (Octaviani et al., 2021). Dilution aims to obtain different extract concentrations, including 15% and 30%.

In Vitro Test

1. The mite's life was first assessed using a microscope with an objective lens using 40x magnification, the indication of living mites is by them actively doing movement. The percentage of mites motility before given treatment was nearly 100%
2. The cerument taken from ear canal which contains *Otodectes cynotis* mites then divided into 4 groups of observation. The first group was given treatment with 30% concentration of soursop leaves extract, the second group was given 15% concentration of soursop leaves extract, the third group was given the ivermectin with a concentration of ---%, and the last group was given no treatment and being left into the open air (negative control)

***Otodectes cynotis* Mite Death Confirmation Test**

This test is used as a parameter for the effectiveness of soursop leaves on the mortality of *Otodectes cynotis* mites. The benchmark for the death of *Otodectes cynotis* mites is the absence of movement of the *otodectes cynotis* within five minutes continuously even after the treatment that's been given such as tipping using a needle pin.

Manufacturing and Testing Activity of Customized Cotton Buds Sticks with Active Substance Reservoir

The extract is injected into a reservoir of customized cotton buds with a predetermined dose and formula and then functionally tested to see the power and speed of cotton absorption of the extract after one end of the cotton bud is broken. Through a designed automatic system, hygiene will be maintained when applying soursop leaf extract topically. Apart from that, the test was continued by applying cotton buds directly to infected animals and then observing the cerumen through a microscope. The effectiveness of the extract via cotton buds will be seen through a comparison of the results of cerumen examination in infected animals given the drug by drops.

Research Variables

1. The independent variables are the amount of extract produced and the concentration of soursop leaf extract
2. Control variables are Na CMC solvent, ivermectin, *Otodectes cynotis* mites, and customized cotton buds
3. The dependent variables are the number of deaths of *Otodectes cynotis* mites and the time of death of *Otodectes cynotis*.

Results and Discussion

The spotted deer captive area spans approximately 5,261.4 m². The main enclosure contains two smaller enclosures and a shelter. The deer are reared semi-intensively, with food provided by both the management and visitors to the breeding facility. At the time of this study, the deer population consisted of 34 individuals, comprising 8 adult males, 19 adult females, and 7 fawns.

Table 1. The mortality time results after the treatment

Groups	15%	30%	ivermectine
1	1.28.00	10.57	2.17.02
2	1.33.21	16.27	2.01.27
3	1.32.06	14.30	1.57.06
4	1.50.20	15.26	2.11.34

Table 2. Anova One-Way Test

	Sum of Squares	df	Mean Square	F	Sig.
Between Gropus	26951.167	2	13475.583	215.993	.000
Within Gropus	561.500	9	62.389		
Total	27512.667	11			

Table 3. Post Hoc Test (Duncan Method)

PER LAK UAN	N	Subset for alpha = 0.05		
		1	2	3
P2	4	14.0000		
P1	4		95.7500	
P3	4			1.2625E2
Sig.		1.000	1.000	1.000

The posthoc test used the Duncan method because the variables were all given different treatment

Conclusion

The soursop leaves (*Annona muricata*) potential to kill *Otodectes cynotis* mites has been confirmed and proven to effectively kill the mite within the average time of 1 hour 35 minutes for an extract with 15% concentration and 13 minutes for an extract with a 30% concentration. Meanwhile, the mite-killing drug containing ivermectin on the market that's been used in most cases shows the performance of killing mites in an average time of almost 2 hours. Considering the narrow therapeutic dose, the potential toxicity that can be produced by ivermectin, as well as the index of action time which tends to be slower than the soursop extract itself, soursop leaves can then be considered to be a substitute for ivermectin in the treatment of *Otodectes cynotis* mite infections in pets.

Conflict of Interest

We certify that there is no conflict of interest with any financial, personal, or other relationships with people or organizations related to the material discussed in this manuscript.

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