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EFFECTIVENESS OF AVOCADO SEED EXTRACT (*Persea americana* Mill.) ON INHIBIT GROWTH OF *Candida albicans* AND *Trichopyton* sp.

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ABSTRACT

In the world of medicine, avocados (*Persea americane* Mill.) are mostly used as traditional medicine in treating various diseases, but in the avocado seed it turns out that so far, it has only been left and thrown away, even though it contains secondary metabolite compounds that can be used as medicinal ingredients, including alkaloids, flavonoids, tannins, saponins, phenolics, terpenoids, and steroids. This study aimed to determine the effectiveness of an avocado seed extract (*Persea americana* Mill.) concentrations of 5% 10% and 15% b/v against the growth of *Candida albicans* and *Trichopyton* sp. and determine at what concentration is most effective. This research is experimental which is a laboratory study using the method of extraction by maceration and disc diffusion (Kirby-Bauer Test) in testffectiveness of avo (*Persea americana* Mill.) against the growth of *Candida albicans* and *Trichopyton* spto testlts obtained from the avocadoment of the diameter of the inhibition were analyzed by one-way analysis of variance (ANOVA) and correlation test using the IBM SPSS Statistics version 26 program. The results showed that avocado seed extract (*Persea americana* Mill) was effective in inhibiting the growth of *Candida albicans* and *Trichopyton* sp. The most effective concentration was at a concentration of 15% b/v, respectively, where the inhibition of *Candida albicans* was 11.84 ± 0.238 mm while the inhibition of *Trichopyton* sp. was 12.58 ± 0.005 mm (oneway Anova, $\alpha = 0.05$)

Keywords: Effectiveness, *Persea americana* Mill, *Candida albicans, Trichopyton* sp., Kirby- Bauer

INTRODUCTION

Indonesia is one of the countries with the largest biodiversity in the world and extraordinary uniqueness, with no less than 30,000 species of higher plants. There are already 7,000 types of plants known for their benefits, but less than 300 plants can be used as raw materials for the pharmaceutical industry. About 1000 plant species have been identified from the systematic aspects of plant botany. Furthermore, the Indonesian Institute of Sciences (2021) states that Indonesia has approximately 15,000 plants with potential medicinal properties, but only around 7,000 species are used as medicinal raw materials. The World Health Organization (WHO) in 2008 noted that 68% of the world's population still relies on traditional medicine systems that mostly involve plants to cure diseases and more than 80% of the world's population uses herbal medicines to support their health (Maryam et al., 2020; Saifudin et al., 2011; Setiawan, 2022).

One of the plants that can be used in traditional medicine is avocado (*Persea americana* Mill). This is believed to have originated in Central America. Avocado (*Persea americane* Mill.) is a fruit that is loved by the people of Indonesia and generally has a meaty, thick yellowish-green fruit with a brownish seed in the middle. Avocados are mostly used in

traditional medicine for the treatment of various diseases (Marlinda et al., 2012). However, the line of attention from avocados is the seed inside which so far some people only throw it away so that it can cause environmental pollution. For example, when a pile of avocado seeds that are not handled immediately will cause waste and reduce aesthetic value, a pile of avocado seeds when rotting will invite disease vector animals (rats, flies, cockroaches, and worms); seeds that are thrown away will cause blockages in waterways, especially during the rainy season. On the other hand, the avocado seed itself can be used as a medicinal material, and based on the results of phytochemical screening of avocado seeds, it is known to contain secondary metabolite compounds, namely alkaloids, triterpenoids, tannins, flavonoids, and saponins, which are thought to act as antibacterial agents (Anggrella et al., 2014). In addition, other studies have suggested that avocado seeds contain phytosterol compounds, fatty acids, abscisic acid, furanoic acid, dimer flavonoids, and proanthocyanidins, which have been shown to have potential as antifungal agents (Yachya & Sulistyowati, 2016).

Empirically, the ancients used avocado seeds in various remedies, including toothache, hypertension, and diabetes mellitus (Hartati et al., 2022; Sadwiyanti et al., 2009). Hariana's research (2004) also suggested that avocado seeds can be used to reduce blood sugar levels (Marlinda et al., 2012), and people in Nigeria use avocado seeds to treat people with high blood pressure (Ozolua et al., 2009). Avocado seeds also have potential antifungal and larvicidal effects (Leite et al., 2009).

Fungi are organisms that are included in the kingdom of fungi and do not contain chlorophyll; therefore, they are heterotrophic, and fungi are saprophytic microorganisms that exist widely on the surface of the body or on the mucosa. The pathophysiology of fungal infections in humans is relatively low compared that with of other pathogenic infections, such as bacteria or parasites. Fungal infections in humans are still more difficult to treat than bacterial infections because humans and fungi are generally eukaryotic organisms that have similar mechanisms of protein formation. Fungal infections are invasive and cause opportunistic infections in immunocompromised humans (patients) (Ahsani, 2014; Mendrofa et al., 2019).

The problem of reproductive tract infections in Asia, who experience vaginal discharge as much as 76% in Indonesia, about 90% of adolescent girls have the potential to experience vaginal discharge because it has a tropical climate so that fungi, bacteria, and viruses are very easy to grow, and vaginal discharge in Indonesia is increasing, as evidenced by the results of research where 52% of women experienced vaginal discharge in 2010 and increased in 2011, which is known to be around 60% of women experiencing vaginal discharge. Women in Indonesia have experienced vaginal discharge, reaching 70% in 2012, while in January to August 2013, almost 55% of women in Indonesia have experienced vaginal discharge. The most common cause of pathological vaginal discharge is the emergence of cases of infection, one of which is *Vulvovaginal Candidasis* caused by fungi, 80%–90% caused by *Candida albicans*, and *trichomoniasis* (TM) is caused by *trichomoniasis vaginalis* with an incidence of approximately 5-20% (Darma et al., 2017).

Generally, opportunistic fungi have lower virulence, but can cause infection when the immune system is not functioning properly. Opportunistic fungal infections are often caused by *dermatophytes* and *candida* fungi. Most opportunistic infections are caused by superficial infections or fungal infections of the skin caused by fungi belonging to skin fungi (*dermatophytes*) such as *Trichopyton* sp, *Epidermopyton* sp. and *Microsporum* sp. Other infections are caused by *Candida, Malassezia furfur, Exophiala, Werneckill, Piedraiahortae,* and *Trichosporon cutaneum* (Baharuddin 2021).

Recently, the discovery of antifungal drugs has developed rapidly, both in topical and systemic forms, but currently there are many antifungal preparations recommended for the treatment of otomycosis, but not enough to guarantee complete healing. Researchers (clinicians) are trying to identify and develop drugs that are most effective in treating a disease (Mendrofa et al., 2019). The use of natural ingredients as inhibitors is a step toward reverting to nature by utilizing the chemical components of these natural ingredients. One of the others is the natural ingredients obtained from avocado seed extract (*Persea americana*

Mill.) Several chemical components, such as alkaloids, tannins, flavonoids, and saponins, are thought to be effective antifungal agents (Kusumo & Nae, 2018). Flavanoid compounds have properties that can damage the fungal cell membrane, causing changes in the permeability of the cell; if the flavonoid compound enters the fungal cell, it is likely that the compound will cause the process of inhibit growth in fungal cells (Diana, 2016; Ferdiansyah et al., 2020).

Various studies on avocado seeds as a reference in the process of developing medicinal materials, such as antibacterials, including the antibacterial effectiveness of ethanol extract of avocado seeds (*Persea americana* Mill.) as a root canal irrigation material against the growth of *Enterococcus faecalis* bacteria (Damayanti et al., 2014), and inhibition test of avocado fruit seed extract (*Persea americana* Mill.) against the growth of *Streptococcus mutans* (Bujung et al., 2017) and the difference in the inhibition of ethanol extract of avocado seeds (*Persea americana* Mill.) against the growth of *Escherichia coli* bacteria with *Staphylococcus aureus* (Anggrella et al., 2014), and as an antifungal, namely the effect of avocado seed decoction water (*Persea Americana* Mill) on the growth of *Candida albicans* fungi In Vitro (Amir et al., 2021), antifungal analysis of avocado seed ethanol extract against the growth of *Colletotrichum* sp. fungus on Raw Chili (*Capsicum frutescens*) (Ferdiansyah et al., 2020).

With the mechanism of active substances contained in avocado seeds and the lack of research on avocado seeds as antifungals and not many antifungal products that function as fungicides, it is necessary to conduct further research on the activity of avocado seed extract (*Persea americana* Mill.) as an antifungal, with the aim of determining the effectiveness of avocado seed extract (*Persea americana* Mill.) against the growth of *Candida albicans* and *Trichopyton* sp., and determined the most effective concentration.

RESEARCH METHODS

Research Design

This research is an experimental study which is a laboratory study using the extraction and disc diffusion method (Kirby-Bauer Test) in testing the effectiveness of Avocado seed extract (Persea americana Mill.) against the growth of Candida albicans and Trichopyton sp. Sampling was conducted in Makassar city. Extracts were processed at the Pharmacognosy-Phytochemistry Laboratory of East Indonesia University Pharmacy Makassar. Characterization testing and identification of the chemical content of the samples were performed at the Pharmaceutical Chemistry Laboratory of the University of East Indonesia Makassar. The process of identifying test materials (fungi) and testing the effectiveness of antifungals was performed at the Microbiology Laboratory of Pharmacy, East Indonesia University of Makassar.

Tools and Materials

The tools used consisted of maceration equipment, rotary evaporator (Ika®), autoclave (GEA YX 24 LDJ), stirring rod (IWAKI Pyrex), porcelain cup, Petri dish (IWAKI Pyrex), glass funnel (IWAKI Pyrex), erlenmeyer (IWAKI Pyrex), glass goblet (IWAKI Pyrex), measuring cup (IWAKI Pyrex), scissors, incubator (Mammert T. 75), hallway (Mitotuyo 0-150), Ose needle (round), volumetric flask (IWAKI Pyrex), Laminar Air Flow (Esco), spritz lamp, micropipette (Dragon Med), oven (Memmert UN 55 53 L), tweezers (GOOI TS-11), knife cutter, test tube (IWAKI Pyrex), analytical balance (AND GR-300), analog balance (PGB), Waterbath (WH-6).

The materials used consist of Aquabides (WaterOne), aluminum foil (Total Wrap), pure culture of Candida albicans, pure culture of Trichopyton sp., Avocado fruit seeds (Persea americana Mill), 96% absolute ethanol (Merck), sterile cotton swabs, filter paper, Metronidazole, Medium PDA (Potato Dextrose Agar), Medium SDA (Sabouraud Dextrose Agar), Medium SDB (Saboraud Dextrose Agar), Sodium Clorida (Merck), Na.CMC 1%, Paper Disk (Oxoid), Sterile Cotton Swab (onemed).

Research Procedure

1. Preparation of Research Materials.

   The test material used in this study was avocado ~~fruit~~ seeds (Persea americana Mill) obtained from Makassar City.

   a. Test Material Management

      The test materials that were collected were then wet sorted by washing using running water until clean, cut into small pieces, and dried (dry sorting) by aerating for ± 1 week. After drying, samples were placed in an appropriate container.

   b. Extract Preparation and sample evaporation

      Sample extraction (test material) refers to the research of Indrawati et al. (2022) by using the maceration method, namely the extraction process is carried out by weighing as much as ± 250 g avocado seed simplisia powder, macerated with 96% ethanol as much as 2000 mL until the sample is completely submerged in the maceration vessel or the ratio of the sample to the solvent is 1:10, then the maceration vessel is then closed and allowed to stand in a dark place for 3 × 24 hours with occasional stirring. After three days, the macerated sample was filtered using filter paper to produce the filtrate and residue (first). The residue (first) was re-macerated with 500 mL 96% ethanol, covered, and allowed to stand in the dark for 2 × 24 hours with occasional stirring. After two days, the sample was filtered using a filter paper to produce the filtrate and residue (second). The first and second filtrates were combined and concentrated using a rotary vacuum evaporator at 50^0C with a rotating speed of 50 rpm to remove the solvent. Furthermore, the extract was evaporated again in a water bath at a temperature of $+40^0$C to remove the remaining solvent to obtain a thick extract. The thick ethanol extract was freed from ethanol by adding 5 drops of aquabides to the extract and then reheating it in a water bath until it evaporated until the avocado seed extract was obtained. The extract was then weighed to determine the yield and stored in a closed container before being used for further testing (Indrawati, Isnaeni, et al., 2022).

2. Extract Characterization Testing (Extract Parameters)

   Extract characterization tests included yield, shape, smell, taste, moisture, and ash content.

3. Chemical Content Examination of Avocado Seed Extract (*Persea americana* Mill)

   The examination of the chemical content of avocado seed extract refers to the research of Indrawati et al. (2023), modified by using phytochemical screening testing with a qualitative approach including:

   a. flavonoid test using magnesium (Mg) powder reagent, concentrated hydrochloric acid (HCl), acetone reagent, boric acid powder, and oxalate powder;
   b. Triterpenoid test using Liebermann- Burchard reagent
   c. Alkaloid test using the Mayer reagent and Dragendorff reagent
   d. Tannin test (phenolic) with 1% FeCl.
   e. saponin test using heated aquabides and vigorous shaking.
   f. glycoside test with Aquabidest and Molish reagent and sulfuric acid (H_2SO_4);
   g. Steroid test with specific perekasi (Liebermann-Burchard)
   h. Phenol test using 5% FeCl reagent and then shaken vigorously (Indrawati et al., 2023)

4. Effectiveness Testing of Avocado Seed Extract (*Persea Americana* Mill).

   a. Preparation and testing of *Minimum Inhibitory Concentration* (MIC) of Avocado seed extract (*Persea Americana* Mill)

      Making and testing the Minmum Inhibitory Concentration of avocado seed extract refers to the research of Indrawati, et.al (2023), namely with the initial concentration of avocado seed extract as the main concentration (stock) of 5% b/v by weighing 5 grams of avocado seed extract then dissolved using 1% Na.CMC and homogenized. The smallest concentrations were made, namely 0.1%, 0.3%, 0.5%, 0.7%, 0.9% v/v by pipetting 0.1, 0.3, 0.5, 0.7 and 0.9 mL each from a concentration of 5% b/v (stock) and then increasing the volume with Na.CMC 1% to 100 mL in a certain container. The next process, pipetted each of the smallest concentrations that

have been made as much as 0.5 mL and then put into a test tube that has been filled with 3 mL each of Saboraud Dextrose Agar (SDB) and 0.1 mL of fungal suspension. For control media (KM), 1 mL of Saboraud Dextrose Agar (SDB) was put into the tube and germ control (KK) 0.9 mL of Saboraud Dextrose Agar (SDB) and 0.1 mL of test fungal suspension was put into the germ tube. All tubes (test tubes containing 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%, control media tubes, and germ control tubes) were vortexed until homogeneous and incubated at room temperature for 24–72 hours, then observed for Turbidity occurred in the test tubes with comparison parameters in the control tubes. The lowest concentration of the test solution that could inhibit fungal growth was characterized by the start of visual clarity. This is the Minimum Inhibitory Concentration (Indrawati et al., 2023).

b. Antifungal effectiveness against *Candida albicans* and *Trichopyton* sp.

The concentrations of avocado seed extract used to test antifungal effectiveness were 5, 10%, and 15%. The 5% concentration was prepared by weighing 5 grams of avocado seed extract and then dissolving it slightly with 1% Na.CMC until the extract dissolved and then putting it into a measuring flask, and the volume was sufficient to the limit mark. The same method was used for the manufacture of concentrations of 10% and 15%, namely by weighing 10 and 15 grams of avocado seed extract, respectively, and then dissolving little by little with 1% Na.CMC until the extract dissolved and then putting into a 100 mL volumetric flask and sufficient volume to the limit mark.

Antifungal effectiveness was determined using the Kirby-Bauer method with paper discs. This method was used with a Petri dish containing 20 mL of Sabouraud Dextrose Agar (SDA) and allowed to solidify. The fungal inoculum was then added by smearing the surface of the media with a cotton bud until evenly distributed. After that, paper discs were placed using tweezers that had previously been soaked for ± 15 minutes in each test sample solution with extract concentrations of 5%, 10%, 15% b/v and negative control (Na.CMC 1% b/v), as well as positive control (Metronidazole 30 ppm). Each Petri dish was incubated at room temperature for 3 x 24 hours. The inhibition area was measured using a slide ruler and the average and standard deviation were calculated (Baharuddin, 2021).

5. Observation and Measurement of Inhibition Diameter.

Observation and measurement of the diameter of the growth inhibition zone of Candida albicans and Trichopyton sp were carried out after a room temperature period of 3 x 24 hours using a sliding bar.

Data Analysis

The data obtained from the measurement of the diameter of the obstacles were tabulated, averaged, and then analyzed using the analysis of variance (one-way ANOVA) method and correlation test using the IBM SPSS Statistics version 26 program.

RESULTS AND DISCUSSION

Determination, Extraction and Characterization

Plant determination (plant identification) is a process that specifically determines the name or type of a plant. The purpose of determination is to obtain a specific and targeted plant species, because plants are used in various ways, such as research and medicinal raw materials; therefore, it is necessary to use the right plants so that the results obtained are as objective as possible (Nurhayati et al., 2022). Plant determination carried out at the Botanical Characterization Laboratory, Directorate of Scientific Collection Management BRIN Cibinong, found that the plants used in this study were the Avocado (*Persea americana* Mill) *Lauraceae* tribe with Decree number: B-3753/II.6.2/IR.01.02/10/2024.

Extraction is a method used for the removal of chemical content that can be dissolved in a solvent so that it can be separated from insoluble materials using a certain solvent. Extraction is often used in the process of separating the bioactive content from

plants in terms of wanting to know the yield and characterization that will be produced, one of which is cold extraction (Indrawati, Baharuddin, et al., 2022). Characterization of avocado seed extract (*Persea americana* Mill.) obtained results in the form of yields ranging from 14.08% in the form of concentrated extracts, blackish brown in color, and a distinctive smell with a bitter (astringent) taste. The water content obtained from the avocado seed extract was 4.34%, total ash content was 2.19%, and acid-insoluble ash content was 0.053%.

The water content of avocado seed extract (*Persea americana* Mill.) were in accordance with the established quality standards. According to Voight (1995) and Maryam et al. (2020), the standard (range) water content depends on the type of extract; for dry extracts, the water content is <10%. The moisture content of the extract is most likely greatly influenced by the amount and area of the dried simplisia (samples) well as the length of drying before extraction. Water content is largely determined by the stability of an extract, and a high water content (more than 10%) is very risky, such as causing damage and can usually be overgrown by microorganisms (Baharuddin & Isnaeni, 2020; Maryam et al., 2020).

Ash content was obtained from avocado seed extract (*Persea americana* Mill.) met the predetermined standards, namely, for good quality extracts with a total ash content <10% or ideally ranging from 4.40-4.84%, where in this study, the total ash content was approximately 2.19%. The acid-insoluble ash content of avocado seed extract was approximately 0.053%. This is reinforced in the research of Maryam et al. in 2020, where in their research they obtained a total ash content of less than 10% and acid-insoluble ash content of <2% (Maryam et al., 2020). The results of the extract characterization testing (extract parameters) of avocado fruit seeds (*Persea americana* Mill) are presented in Table 1.

Table I. Extract Characterization Results (Extract Parameters)

No	Specifications	Description	Reference
1.	Yield (%)	14,08	>10%
2.	Shape	Concentrated Extract	Concentrated extract (thick)
3.	Color	Blackish brown	Red-orange or brown-black
4.	Odor	Typical	Distinctive (like rose oil)
5.	Taste	Bitter	Bitter
6.	Moisture Content (%)	4,34	moisture content <10%
7.	Total ash content (%)	2,19	total ash content < 10% or between 4.40-4.84%
	Acid Insoluble Ash Content (%)	0,053	acid insoluble ash content <2%

Chemical Content Identification

Chemical content or phytochemical screening was carried out to determine the class of compounds contained in a plant. In addition, phytochemical screening can help identify compounds that are more dominant in plant extracts. Based on the observations in Table 2, the examination of the chemical content (phytochemical screening) of the avocado seed extract (*Persea americana* Mill) yielded positive results (detected) according to the literature on secondary metabolite compounds in the form of flavonoids, alkaloids, tannins, saponins, phenols (phenolic), whereas terpenoid compounds, glycosides, and steroids with qualitative testing using coloration perekasi did not reveal the content of these compounds (not detected).

Table II. Chemical Content Inspection Results

Content Test	Reagents	Testing Results	Reference	Description
Flavonoids	0.1 g mg	red or yellow or		Detected

	powder + and 1 mL HCl P + 2 mL amyl alcohol (shaken)	intense yellow color formed	orange color on the amyl alcohol layer	(+)
	Acetone P + As. Borate + Oxalate Powder	light yellow fluoroscent solution	intensive yellow fluorescence (in UV light 366 nm)	Detected (+)
Terpenoid	Liebermann-Burchard	no discoloration	red, dark red, or purple color is formed	No Detected (-)
Alkaloids	- Mayer	formed (↓) yellowish white color	(↓) agglomerate white or yellow in color.	Detected (+)
	- Dragendorff	formed (↓) light brown (reddish)	(↓) brown or orange-brown in color	Detected (+)
Tannins	$FeCl_3$ 1%	blue-black color formed	blue or blackish green color	Detected (+)
Saponins	H_2O (hot) and then shaken vigorously	1.9 cm high foam formed	formed a stable foam of not less than 10 minutes 1 to 10 cm high and + 1 drop of HCl 2N froth does not disappear	Detected (+)
Glycosides	2 ml of water + 5 drops of Molish's recombinant then +2 ml H2SO4 P through the tube wall	no purple ring formed	formation of a purple ring at the boundary of the two liquids, indicating the presence of sugar (glycone) or glycoside bonds	No Detected (-)
Steroids	Liebermann-Burchard	no blue-green color	blue or blue-green color is formed	No Detected (-)
Phenols (Phenolic)	5 drops of FeCl3 5% (shaken vigorously)	blue-black color formed	blue-black color formed	Detected (+)

In general, flavanoids, tannins, saponins, alkaloids, and phenols (phenolics) are antimicrobial agents (antibiotics, antiseptics and antifungal agents are included), where flavanoids have a mechanism of action by forming complex compounds against extracellular proteins that disrupt the integrity of cell membranes while tannins have a mechanism of action by inhibiting reverse transcriptase and DNA topoisomerase enzymes so that microbial cells cannot form, because they have the ability to activate microbial cell adhesins, activate enzymes, and disrupt protein transport in the inner layer of cells. In another case, saponins, which act as antimicrobials by damaging the cell wall permeability, can cause cell death. Alkaloids (pyridine alkaloids) also act as antibacterial and antifungal agents, and their mechanism of action is thought to interfere with the constituent components of peptidoglycan in microbial cells, so that the cell wall layer is not formed intact and can cause cell death. Phenol compounds (phenolics) inactivate important enzyme systems and inhibit microbial growth by denaturing proteins and damaging membranes. In addition, it can

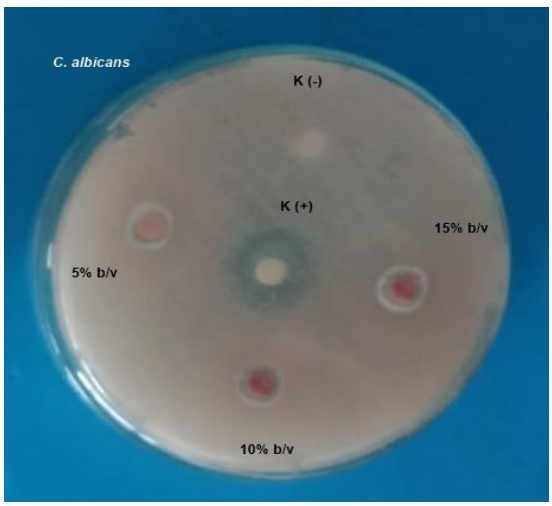
change the structure and morphology of cells, causing imbalances in microbial metabolism, reducing cell wall permeability, inhibiting virulence factors such as enzymes and toxins, and suppressing the formation of bacterial biofilms (Indrawati et al., 2024).

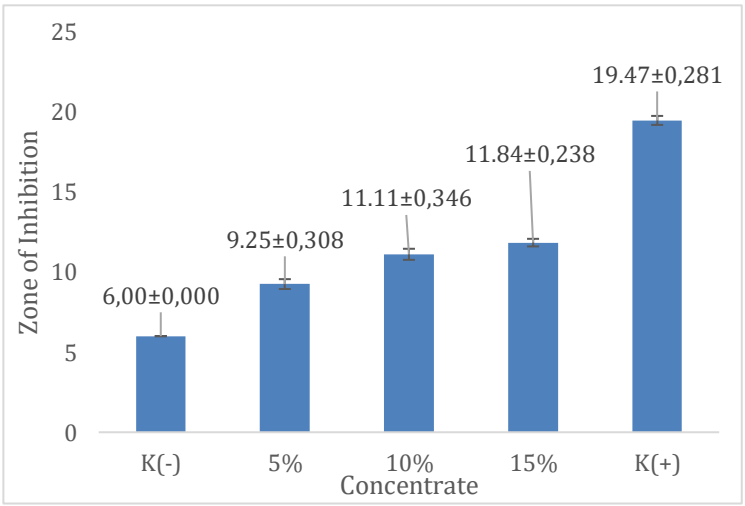
Antifungal Effectiveness

Antifungal effectiveness was tested to measure antifungal activity and susceptibility to antifungals. To measure antifungal activity, the zone of inhibition technique, *minimum inhibitory concentration* (MIC), and percentage of growth inhibition were used. Based on the observations in Table 3 and Figure 1, the greater the concentration of avocado seed extract (*Persea americana* Mill), the greater the inhibition produced against the growth of *Candida albicans*.

Table III. Results of Testing the Effectiveness of Avocado Seed Extract (*Persea americana* Mill) against the growth of *Candida albicans*

Test Fungi	Diameter of Zone of Inhibition (mm) Avocado Seed Extract (*Persea americana* Mill)				
	Control (-)	**5% b/v**	**10% b/v**	**15% b/v**	**Control (+)**
Candida albicans	6,00	9,08	11,09	11,74	19,66
	6,00	9,07	11,09	11,68	19,61
	6,00	9,61	11,15	12,12	19,15
Total	**18,00**	**27,76**	**33,33**	**35,54**	**58,42**
Average	**6,00**	**9,25**	**11,11**	**11,84**	**19,47**
Standard Deviation (SD)	±0,000	±0,308	±0,346	±0,238	±0,281





Note:
K(-) : Na.CMC 1%
5% b/v : Avocado Seed Extract Concentration 5%
10% b/v : Avocado Seed Extract Concentration 10%
15% b/v : Avocado Seed Extract Concentration 15%,
K(+) : Metronidazole 30 ppm

Figure 1. Diameter of Zone of Inhibition of Avocado Seed Extract (*Persea americana* Mill) against *Candida albicans* growth

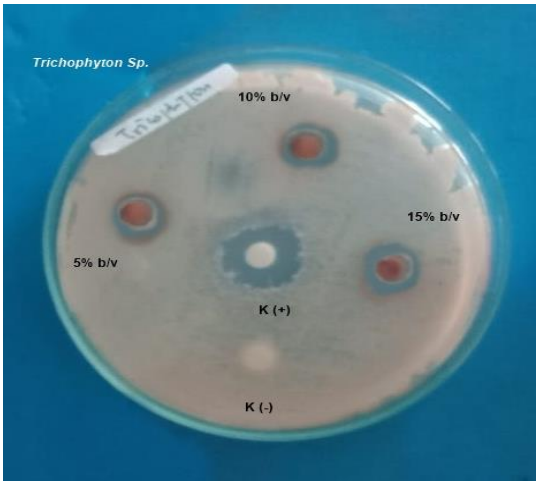
Variations in the concentration of avocado seed extract (*Persea americana* Mill) used to test the effectiveness of this antifungal greatly affected the inhibition. The results of the data analysis used in the analysis of variance (one-way ANOVA) yielded a Sig value. 0.000 (<0.05), where there was a significant difference between the concentration variations of avocado seed extract and the inhibition zone produced by *Candida albicans*.

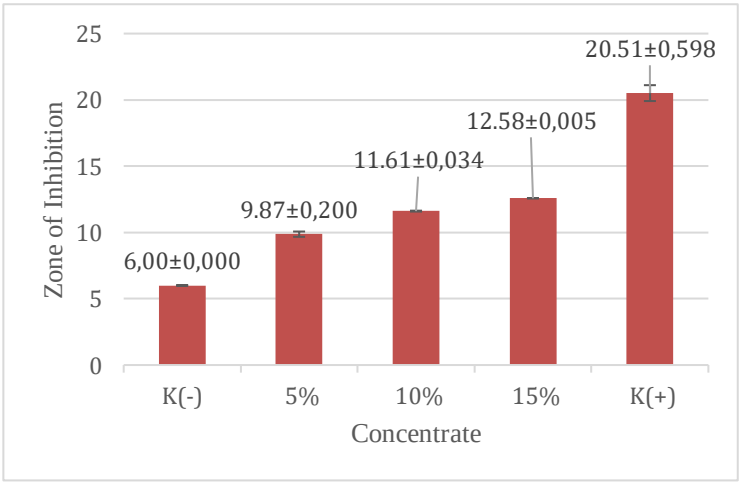
In the process of determining the effectiveness of Avocado (*Persea americana* Mill) seed extract with an incubation period of 2-3 x 24 hours, it was seen that there was an increase in inhibition that occurred but the results obtained were in the moderate category, both at a concentration of 10% b/v and 15% b/v. In other words, avocado seed extract at these concentrations was only effective in inhibiting the growth of *Candida albicans* with an incubation period of 72 hours. This is reinforced by Baharuddin (2021), who suggests that determining the category of fungal growth inhibition response, namely for clear zone diameters < 10 mm, has a weak growth inhibition response, 10–15 mm has a moderate growth inhibition response, 16–20 mm has a strong growth inhibition response, and > 20 mm has a very strong growth inhibition response (Baharuddin, 2021).

In table 4 figure 2 it is also found that the diameter of the inhibition zone of Avocado seed extract (*Persea americana* Mill) against *Trichophyton* Sp. growth activity with an incubation period of 2-3 x 24 hours at room temperature, which shows that the greater the concentration of Avocado seed extract (*Persea americana* Mill), the greater the inhibition produced against *Trichophyton* Sp. growth.

Table IV. Results of Testing The effectiveness of avocado seed extract (*Persea americana* Mill) against the growth of *Trichophyton* sp.

Test Fungi	Diameter of Zone of Inhibition (mm) Avocado Seed Extract (*Persea americana* Mill)				
	Control (-)	**5% b/v**	**10% b/v**	**15% b/v**	**Control (+)**
Trichophyton sp.	6,00	9,85	11,59	12,58	20,19
	6,00	10,08	11,59	12,58	20,14
	6,00	9,68	11,65	12,59	21,20
Total	**18,00**	**29,61**	**34,83**	**37,75**	**61,53**
Average	**6,00**	**9,87**	**11,61**	**12,58**	**20,51**
Standard Deviation (SD)	±0,000	±0,200	±0,034	±0,005	±0,598





Note:
K(-) : Na.CMC 1%
5% b/v : Avocado Seed Extract Concentration 5%
10% b/v : Avocado Seed Extract Concentration 10%
15% b/v : Avocado Seed Extract Concentration 15%,
K(+) : Metronidazole 30 ppm

Figure 2. The diameter of the Zone of Inhibition of Avocado Seed Extract (*Persea americana* Mill) against the growth of *Trichophyton* sp.

Variations in the concentration of avocado seed extract (*Persea americana* Mill) used to test the effectiveness of this antifungal agent significantly affected the inhibition. The results of the data analysis used in the analysis of variance (one-way ANOVA) yielded a Sig value. 0.000 (<0.05) in an incubation period of 2-3 x 24 hours, where there was a significant difference between the concentration variation of avocado seed extract and the inhibition zone produced by *Trichophyton* sp.

In the process of determining the effectiveness of Avocado (*Persea americana* Mill) seed extract with an incubation period of 2-3 x 24 hours, it was seen that there was an increase in inhibition that occurred but the results obtained were in the medium category, both at a concentration of 10% b/v and 15% b/v. In other words, avocado seed extract at these concentrations was only effective in inhibiting the growth of Trichophyton Sp. with an incubation period of 72 hours. Baharuddin reinforces (2021), which in his research suggests that determining the category of fungal growth inhibition response, namely for clear zone diameters < 10 mm has a weak growth inhibition response, 10 - 15 mm has a moderate growth inhibition response, 16 - 20 mm has a strong growth inhibition response and > 20 mm has a very strong growth inhibition response (Baharuddin, 2021).

CONCLUSION

Based on these results, it can be concluded that avocado seed extract (Persea americana Mill) effectively inhibited the growth of Candida albicans and Trichopyton sp. The most effective concentration was 15% b/v (one-way ANOVA, $\alpha = 0.05$).

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