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Original Article

Potential of Kenikir (*Cosmos Caudatus* Kunth) Leaves Essential Oil Against *Candida Albicans* ATCC 10231 in Vitro

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Abstract

Background : Kenikir is a medicinal plant whose leaves are often consumed as vegetables. Kenikir leaves contain active compounds such as flavonoids, polyphenols, saponins, tannins, alkaloids and essential oils. These compounds are thought to be able to inhibit the growth of Mycobacterium tuberculosa and *Candida albicans* ATCC 10231 strains. The purpose of this study was to describe the effect of kenikir leaf essential oil on the growth of the *Candida albicans* ATCC 10231 strains. with 4 concentrations and 6 repetitions in *Candida albicans* and the concentration of kenikir leaf essential oil concentration of 0.5%, 1%, 1.5%, 2%.

Methods : This type of research is a pure experimental research by providing treatment in the form of giving essential oil of kenikir leaves with various concentrations on the growth of the *Candida albicans* ATCC 10231 strains. The inhibition test of kenikir leaf essential oil on the growth of the *Candida albicans* ATCC 10231 strains was carried out using the Kirby Bauer disk diffusion method. The research design used is Post Test Only with Control Group Design. Data obtained in the form of inhibition zone diameter, were analyzed using Variant Analysis (Anova), P ≤0.05 and continued with Post Hoc test.

Results : The results showed that 0.5% essential oil concentration had a inhibition zone of 9.67 mm (moderate criteria); essential oil concentration of 1% with a inhibition zone of 9.72 mm with criteria moderate and 1.5% concentration with inhibition zone 11.86% with strong criteria and concentration 2% with inhibition zone 12.67 mm with strong criteria all concentrations affect the growth of Candida albicans with the most optimal concentration of 2% for the *Candida albicans* ATCC 10231 strains.

Conclusion : The higher the concentration of kenikir leaf extract, the inhibitory effect on the growth of *Candida albicans* ATCC 10231 strains was also higher.

Keywords : *Cosmos caudatus* Kunth, *Candida albicans* ATCC 10231 strains, diameter of the inhibition zone

INTRODUCTION

Oral candidiasis is the initial clinical manifestation and most commonly experienced by patients, especially in TB-HIV coinfected patients who have not received antiretroviral (ARV) drugs. The incidence of candidiasis is currently starting to increase globally along with the increase in the immunocompromised population such as people with HIV (Human Immunodeficiency Virus), diabetes mellitus, antibiotic consumption, and pregnant women. Various studies show that the incidence of candidiasis is increasing in this population group. A study reported that the incidence of candidiasis increased in pregnant women by 51.5% and in patients with urogenital tract infections by 10.3%.[1]

This disease is mostly found in people with HIV with a number of cases of 2 million/year (Moyes and Naglik, 2011). In addition, users of broad-spectrum antimicrobials (such as tetracyclines), smokers, users of dental prostheses, diabetic and malnourished patients also suffer.

Candidiasis treatment is generally done by giving antifungal drugs from the azole group. This has led to certain clinical consequences, namely the discovery of azole-resistant isolates as a result of the widespread use of azoles. The development of resistance to the *Candida albicans* pathogen has occurred since 1990 as a result of the effect of fluconazole treatment. Furthermore, as many as 40% of candidiasis patients show resistance by *Candida albicans* to antifungal drugs.[2] Treatment due to fungal resistance has not been developed too much while the number of cases of this infection is increasing. So natural ingredients are needed as an effort to overcome drug resistance.[3]

Kenikir (*Cosmos caudatus* Kunth) is a plant that belongs to the Asteraceae family. This plant is commonly found in Asia, including in Indonesia. Screening of active components in kenikir leaf extract showed the presence of groups of active compounds in the form of terpenoids, flavonoids, phenolics, alkaloids, tannins, steroids and saponins. The polar and non-polar extracts of kenikir leaves showed significant levels of inhibition against five human pathogenic microorganisms, including 2 Gram positive bacteria (*Staphylococcus aureus*, and *Bacillus subtilis*), 2 Gram negative bacteria (*Pseudomonas aeruginosa*, and *Escherichia coli*), and 1 fungus *(Candida albicans)*.

Based on the above background, a research was conducted on the potential of kenikir leaf (*Cosmos caudatus* Kunth) essential oil anti-fungal in vitro.

RESEARCH METHODS

This type of research is a pure experimental research by providing treatment in the form of giving essential oil of kenikir leaves with various concentrations on the growth of the *Candida albicans* ATCC 10231 strains. The research design used is Post Test Only with Control Group Design. There are two groups, namely the group that was given treatment. The first group was the experimental group which was treated and then measured and the experimental group. The second group was the comparison or control group that did not receive treatment, but only measured.

Preparation of sample (Fungi)

In this study, the sample used *Candida albicans* ATCC 10231 obtained from the collection of Balai Laboratorium Kesehatan dan Kalibrasi Yogyakarta. *Candida albicans* ATCC 10231 was rejuvenated in solid Sabouroud Dextrose Agar (SDA) medium containing peptone, glucose and agar. Furthermore, fungal inoculation was carried out in liquid Sabouroud Dextrose Agar (SDA) media at a temperature of 37° C for 48 hours according to the incubation period in an incubator shaker.

Essential Oil Isolation

Before the isolation process is carried out, the kenikir leaves are rinsed with clean running water to remove dirt and dust attached. The isolation process in this study uses the water vapor distillation method where the volatile oil content of kenikir leaves is separated based on the principle of the difference in partial pressure of the volatile compound content with the water vapor phase and the boiler continuously until complete and ends with condensation of the mixed vapor phase into water distillate along with the compounds contained in the water. completely or partially separated. The solvent used is distilled water.

Essential Oil Characterization

Characterization of kenikir leaf essential oil using gas chromatography-mass spectrophotometry (GC-MS) to determine the compound content of kenikir leaf essential oil.

Antifungal Effectiveness Test

The effectiveness test carried out in this study used the disc diffusion method with variations in concentrations of 0.5%, 1%, 1.5%, 2% against *Candida albicans*. The positive control used was ketoconazole 1% and CMC 1% solvent as a negative control.

The inhibition test of kenikir leaf essential oil

The concentrations used in the experimental group were the concentration of kenikir leaf essential oil on the fungus *Candida albicans* ATCC 10231 strains 0.5%; 1.0%; 1.5%; 2.0%, the control group consisted of positive control and negative control. Positive control used 1% ketoconazole while negative control used 1% Carboxy methyl cellulose (CMC). The number of repetitions used was six repetitions in each group.

The inhibition test of kenikir leaf essential oil on the growth of the fungus *Candida albicans* ATCC 10231 strains was carried out using the Kirby Bauer disk diffusion method:

a. Sabouraud Dextrose Agar (SDA) media as much as 20 ml was poured into a disposable petri dish.
b. The suspension of *Candida albicans* fungus that has been made according to the standard Mc Farland 0.5, is poured as much as 1 ml into a petri dish that already contains SDA media, homogenized and then waited for it to solidify.
c. Kenikir leaf essential oil is diluted into various concentrations of 0.5%; 1.0%; 1.5%; 2.0% using 1% CMC solvent.
d. Make a positive control of 1% ketoconazole and 1% negative control of CMC.
e. The paper discs were immersed for 5 minutes into each concentration of kenikir leaf essential oil and into the control. The number of discs inserted into each concentration was 8 and in each control.
f. Paper discs were affixed to the media that had been inoculated with *Candida albicans* ATCC 10231 strains fungal suspension.
g. Wrapped in paper and plastic then stored in a box container at room temperature for 24 hours.
h. The zone of inhibition of the growth of *Candida albicans* ATCC 10231 strains was measured using a caliper.

RESULTS

Analysis Results Oil Chemical Compound essential Leaf Kenikir with GC-MS

Analysis result with GC-MS will obtained two data are chromatogram originating _ from results analysis gas chromatography (GC) and spectra mass from results analysis spectroscopy mass (MS). Chromatogram from analysis with gas chromatography showed 39 peaks compound with 15 peaks compound identified main. Gas chromatography here working as tool separator various component mixture in sample, while spectrometer mass working for detect each molecule components that have been separated on the system gas chromatography . From the GC-MS chromatogram it will be obtained information amount detected compounds and from _ GC-MS spectra will be obtained information structure detected compounds .

Content chemical oil essential leaf mistress of results GC-MS analysis, oil essential from leaf mischievous contains 39 compounds chemistry, and 15 compounds among them have content chemistry above 1 % (Table 1.).

Based on Figure 21, 39 peaks were detected with 15 peaks component dominant which is composer oil essential leaf mischievous. From the components composer oil essential leaf mischievous These compounds , 1,3,6-Octatriene, 3,7-dimethyl-, (E)- (CAS) .BETA is component main and include in group monoterpene compounds .

The results of the measurement inhibition zone diameter on Candida albicans ATCC 10231 strains. Growth

The results of the measurement of the diameter of the resulting inhibition zone were then analyzed descriptively, analytically and statistically. The results of the descriptive analysis are presented in table 2.

From the graph the average diameter of the Candida albicans ATCC 10231 strains growth inhibition zone, then analyzed analytically using the antifungal strength criteria according to Davis and Stout, as happened in table 2.

After being analyzed descriptively and analytically, the data were then analyzed statistically using the SPSS 16.0 for Windows program. The results of the normality test of Shapiro Wilk's data show that the significance value of the data for the variable diameter of the inhibition zone is entirely sig >0.05, which means that all data distributions are normally distributed.

The results of the Kruskall Wallis test showed a significance result of 0.000. The value of sig < 0.05 so that H0 is rejected and Ha is accepted which indicates that

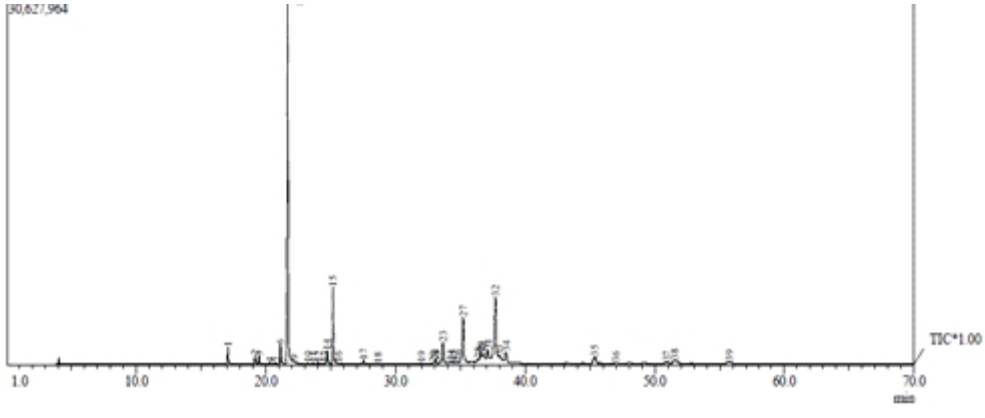

Figure 1. Chromatogram of kenikir leaf essential oil

TABLE 1
Composition compounds found in oil _ essential leaf mischievous with GC-MS (abundance >1%)

NO	Peak	Retention Time (minutes)	Abundance (%)	Possible compounds	Compound Group
1	1	17.063	1.73	Bicyclo 3.1.0 hexane, 4-methyl-1-(1-methylethyl	monoterpene
2	6	21.103	1.91	1,3,6-Octatriene, 3,7-dimethyl-, (Z)- (CAS) cis-3,7	monoterpene
3	8	21.670	40.28	1,3,6-Octatriene, 3,7-dimethyl-, (E)- (CAS).BETA	monoterpene
4	14	24.724	1.36	p-Mentha-1,5,8-triene	monoterpene
5	15	25.160	7.84	p-Mentha-1,5,8-triene	monoterpene
6	23	33.659	3.19	2,4-DIISOPROPENYL-1-METHYL-1-VINYL-	sesquiterpene
7	27	35.201	7.75	beta.- CARYOPHYLLENE	sesquiterpene
8	29	36.566	1.89	beta.- Selinene	sesquiterpene
9	30	36.759	1.94	.alpha .- amorphene	
10	31	37.102	2.88	.alpha .- Farnesene	sesquiterpene
11	32	37.687	14.77	germacrene d	sesquiterpene
12	33	38.019	1.86	delta.- Guaiene	sesquiterpene
13	34	38,519	2.03	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	sesquiterpene
14	35	45.360	1.79	(-)-Caryophyllene oxide	sesquiterpene
15	38	51.518	1.50	.alpha .- Santalol	sesquiterpene

Source: primary data processed

there is an effect of various concentrations of kenikir leaf essential oil on the growth of the fungus *Candida albicans*.

DISCUSSION

This study was conducted to determine the potential of kenikir leaf essential oil on the growth of the fungus *Candida albicans* ATCC 10231 strains. In this study, the disc diffusion method was used because this method is good for determining the activity of antifungal agents. The potential of kenikir leaf essential oil can be seen from the diameter of the inhibition zone formed on Saboraud Dextrose Agar (SDA) media which had previously been inoculated with *Candida albicans* suspension and incubated for 24 hours at room temperature. The media used in this research is *Saboraud Dextrose Agar* (SDA) media which is a selective medium for isolating fungi and yeasts.[24]

The essential oil that has been obtained is then made in various concentrations, namely concentrations of 0.5%, 1.0%, 1.5% and 2.0% using 1% Carboxymethyl Cellulose (CMC) solvent. The diameter of the inhibition zone was indicated by the formation of a clear zone around the paper disc that had been soaked for 5 minutes in citronella essential oil measured using a caliper starting from the smallest concentration of 0.5% to the

TABLE 2
Results of Measurement of Inhibitory Zone Diameter on *Candida albicans* ATCC 10231 strains. Fungus Growth

Replication	Inhibitory Zone Diameter (mm)					
	Essential oil of Kenikir				Ketokonazol	CMC
	0.5%	1.0%	1.5%	2.0%		
1	10.2	8.14	10.00	11.20	21.6	21.6
2	10.9	8.90	10.40	11.80	21.6	21.6
3	7.38	9.00	11.60	11.90	21.6	21.6
4	9.10	9.80	11.76	11.70	21.6	21.6
5	11.58	10.46	13.00	14.10	21.6	21.6
6	8.90	12.00	14.40	15.30	21.6	21.6
Amouth	58.06	58.3	71.16	76.00	21.6	21.6
Average	9.67	9.72	11.86	12.67	21.6	21.6

Source: Processed Primary Data, 2019

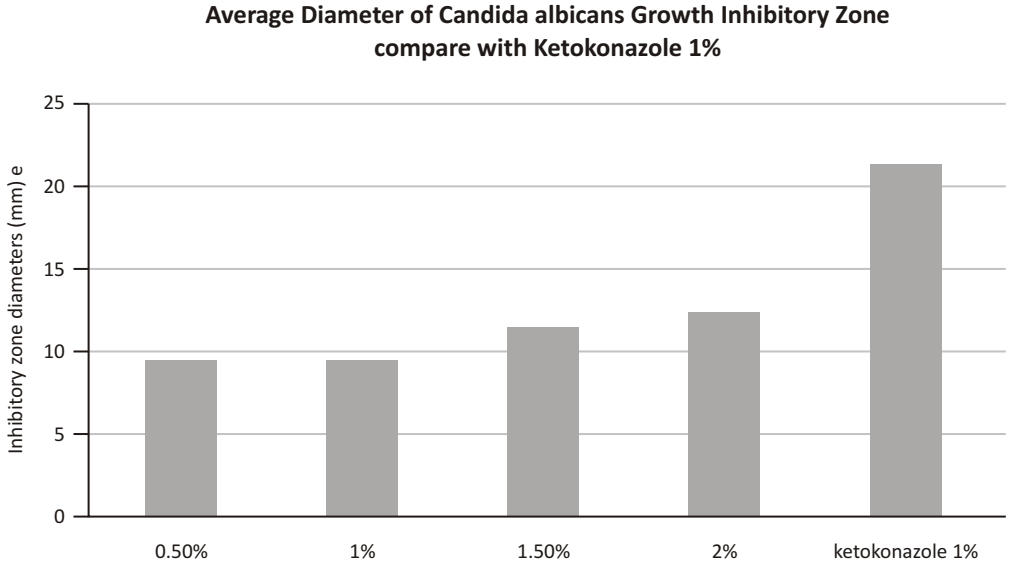

Figure 2. Graph of Average Diameter of *Candida albicans*. Fungus Growth Inhibitory Zone

largest concentration of 2.0% including positive control of 1% ketoconazole. and 1% CMC negative control. CMC was used as an essential oil solvent and 1% ketoconazole solvent in the positive control because it was able to bind water so that water molecules were trapped in the gel structure formed by CMC and were able to dissolve substances that were not soluble in water.[5]

The mechanism of kenikir leaf essential oil compounds as antifungals is to inhibit the synthesis of ergosterol (the main sterol forming fungal cell membranes) so that the membrane protein structure is damaged and membrane permeability increases which will cause the death of *Candida albicans* fungal cells. The inhibition zone is a clear zone around the discs which widens as the concentration of essential oil of kenikir leaves increases.[67] From the research that has been done, the essential oil of kenikir leaves can inhibit the growth of *Candida albicans* at concentrations of 0.5%, 1.0%, 1.5% and 2.0% with the diameter of the inhibition zone respectively 9.67 mm, 9.72 mm, 11.86 mm, and 12.67 mm while the results of the inhibition zone diameter measurement in 1% ketoconazole positive control was 21.6 mm. The results of the measurement of the diameter of the inhibition zone in essential oils at the largest concentration of 2.0% were still low compared to the results of measurements in the positive control of 1%

TABLE 3
Inhibitory Strength of Kenikir leaves (*Cosmos caudatus* Kunth) Essential Oil on Inhibitory Zone Diameter of *Candida albicans* ATCC 10231 strains

Davis and Stout			Results	
Inhibitory Zone Diameter	Criteria	Concentration of essential oil of kenikir leaves	Inhibitory Zone Diameter	Criteria
< 5 mm	Weak	0.5 %	9.67 mm	Medium
5–10 mm	Medium	1.0 %	9.72 mm	Medium
10–20 mm	Strong	1.5 %	11.86 mm	Strong
>20 mm	Very strong	2.0 %	12.67 mm	Strong

Source: Processed Primary Data, 2019

ketoconazole. However, the diameter of the inhibition zone of essential oils on the growth of the fungus *Candida albicans* formed is in the moderate to strong category according to the criteria for the strength of the inhibition zone according to Davis and Stout.[8]

Potency oil essential leaf mischievous seen from the diameter of the inhibition zone formed on the previous *Saboraud Dextrose Agar* (SDA) media has inoculated suspension mold Candida albicans and incubated for 24 hours. Inhibition zone diameter showed with formation of a clear zone around disc paper that has been given oil fragrant lemongrass essentials with concentrations of 0.5%, 1.0%, 1.5% and 2.0% were measured with use period push start from concentration smallest 0.5% to concentration largest 2.0% including control positive ketoconazole 1% and control negative CMC 1%. Media used in research this is *Saboraud Dextrose Agar* (SDA) media which is a selective medium isolation fungi and yeast. SDA media contains casein , peptone and dextrose that play a role as supply nutrition growth mushrooms . The pH of SDA media is sour that is about 5.6 so that allow for growth mushrooms and yeast, other than that so you can hinder growth bacteria.[9]

Research conducted by 12 essential oil Lemongrass can inhibit the growth of *Tricophyton fungus rubrum* at a concentration of 0.5% at 8.96 mm and 10% at 24.9 mm , *Microsporum canis* at a concentration of 0.5% at 7.13 mm and 10% at 22.56 mm, *Epidermophyton floccosum* at a concentration of 0.5% of 6.13 mm and 10% of 19.5 mm. From various study the in accordance with results research obtained by researchers, that _ oil essential fragrant lemongrass could also hinder growth mold *Candida albicans* at concentrations of 0.5%, 1.0%, 1.5% and 2.0% with inhibition zone diameter by in a row namely 9.67 mm, 9.72 mm, 11.86 mm, and 12.67 mm .

Concentration the smallest taken in the study this is 0.5% and increasing by 0.5 on each the concentration . That thing show significant difference _ if seen from difference mean diameter of inhibition zone every concentration . The difference in the mean diameter of the inhibition zone of *Candida albicans* between 0.5% essential oil concentration and 1% concentration is 0.05 mm, between 1% and 1.5% concentration is 2.14 mm, between 1.5% and 2% concentration is 0.81 mm. Inhibition zone diameter oil essential to growth mold *Candida albicans* caused by enter in category currently until strong according to criteria inhibition zone strength according to Davis and Stout. Difference the average diameter of the inhibition zone produced in each group concentration oil essential leaf kenikir and group control show significant results _ so that could concluded that on every concentration oil essential potential as antifungal with different abilities.[12]

Mechanism of oil essential leaf mischievous as antifungal is with formation an inhibition zone i.e. clear zone around increasingly discrete discs widen along increase concentration oil essential lemongrass fragrance given . Concentration oil essential largest used is 2.0% has a diameter of inhibition zone as big as 1 2.67 mm still have difference as big as 12.7mm compared with the diameter of the inhibition zone formed in the control positive ketoconazole 1.0% i.e of 8.93 mm.

Invasion of candida fungus begins with an adaptive form of the fungus (yeast) that is inhaled or attached to the body. This fungus will become a pathogen if there are conditions that allow for multiplication and produce mycotoxicity.[11]

The cell membrane of *Candida albicans* consists of lipids and proteins that function as a barrier that prevents the movement of water or water-soluble substances from one space to another. Ergosterol is a layer of sterols that functions to help membrane permeability and regulate most of the liquid properties of fungi.[13]

Fluconazole will only bind to sensitive fungi or yeasts. The antifungal activity depends on the presence of sterols in the fungal or yeast cell membranes, especially ergosterol. As a result of the formation of bonds between sterols and antibiotics, there is a change in cell membrane

permeability so that cells will lose various molecules.[14] The hydrophobic molecules that make up essential oils will attack the ergosterol in the fungal cell membrane, causing changes in membrane permeability and membrane damage, which in turn causes the fungal cell molecules to come out, causing cell death. Essential oil molecules can also interfere with the work of enzymes bound to yeast cell membranes, thereby interfering with the formation of cell membranes. In other words, essential oils can kill and inhibit the growth of fungi.[15]

Mechanism of ketoconazole in control positive as antifungal is with hinder the enzyme 14-α-sterol demethylase, which destroys ergosterol biosynthesis for membrane cytoplasm and causes accumulation of 14-α-methylsterol. Methylsterol this could disturb chain result phospholipids , damage function system enzymes on the membrane cell so that hinder growth mushrooms.[17,18]

Analysis result show that compound main contained _ in oil essential oil essential leaf mischievous is 1,3,6-octatriene,3,7-dimethyl-,(E)-(CAS) with rate more than 35%. Oil essential part big arranged on component monoterpenes and sesquiterpenes. Based on Table 2. Compounds included _ group monoterpene there are five, namely Bicyclo 3.1.0 hexane, 4-methyl-1-(1-methylethyl, 1,3,6-Octatriene, 3,7-dimethyl-, (Z)- (CAS) cis-3,7; 1,3,6 -Octatriene, 3,7-dimethyl-, (E)- (CAS). BETA; p-Mentha-1,5,8-triene; p-Mentha-1,5,8-triene. Compounds included group sesquiterpene there is ten , namely beta.-CARYOPHYLLENE; beta.-Selinene; beta.-CARYOPHYLLENE; beta.-Selinene; alpha.- amorphene; alpha.- Farnesene; germacrene d; delta.- Guaiene; Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-; (-)-Caryophyllene oxide; .alpha.-Santalol.

Factors that can influence results research that has been conducted is making suspension mushrooms and abilities different disc absorption, degree acidity (pH) of media, temperature , and humidity.[7] Making suspension mold conducted with compare with Mc Farland standard visually. Suspension mushrooms made by researchers can so have level different turbidity with existing Mc Farland standards. Whereas ability Different disc absorption can also be influence result of the resulting inhibition zone diameter, because researcher no could sort out which disk has good absorption _ or not . _ This thing controlled by researchers with soaking disc disc in same time _ that is about 5 minutes .

From various description on has in accordance with proposed hypothesis _ that is oil essential Lemongrass (*Cymbopogon* nardus _ L. Rendle) has power resistor as antifungal to growth mold *Candida albicans*. The more tall concentration oil given essential , then the more high content _ substance antifungal in oil fragrant lemongrass essentials, so that the diameter of the inhibition zone formed the more big .

Some of the other factors that contribute to the quality of essential oils are the quality of the soil in which the plants are grown, the temperature in the area, the climate/annual amount of rainfall in which the plants are grown, the height of the live plants, the distillation process, the time gap between crop harvesting and distillation, storage. oil after extraction, type of distillation equipment used etc.[3]

Factors that can affect the results of research that have been carried out are the manufacture of fungal suspensions and different disk absorption capabilities, the degree of acidity (pH) of the media, temperature, and humidity.[4] The mushroom suspension was made by visually comparing it with Mc Farland's standard. The mushroom suspension made by the researcher may have a different turbidity level with the existing Mc Farland standard. Meanwhile, the absorption ability of different disks can also affect the results of the resulting inhibition zone diameter, because researchers cannot sort out which disks have good absorption or those that do not.

CONCLUSION

Essential oil of kenikir leaves (*Cosmos caudatus* Kunth) has the potential as antifungal against the growth of *Candida albicans* ATCC 10231 strains.

The diameter of the inhibition zone of C*andida albicans* ATCC 10231 strains formed at various concentrations of essential oil of kenikir leaves (*Cosmos caudatus* Kunth) 0.5%, 1.0%, 1.5%, 2.0% had an inhibition zone diameter of 9.67 mm. , 9.72 mm, 11.86 mm and 12.67 mm.

The optimal concentration of essential oil of kenikir leaves (*Cosmos caudatus* Kunth) which is able to inhibit the growth of *Candida albicans* ATCC 10231 strains is 2.0% which has an inhibition zone diameter of 12.67 mm.

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