

# The Inhibitor Activity Test of Green Okra Fruit Fraction (*Abelmoschus Esculentus*) Against *Candida Albicans*

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## ABSTRACT

This study was done to analyze the inhibitory activity of green Okra Fruit fraction against *Candida albicans*. The green Okra fruit is originated from Cepiring, Kendal. Green Okra fruit is a vegetable that contains compounds including flavonoids, alkaloids, tannins, steroid, triterpenoids stapling that are able to provide antifungal activity. The green Okra fruit was remacerated with ethanol 80 % followed fractination with n-hexane, diethyl ether, and ethyl acetate . The n-hexane, diethyl eter and ethyl acetate Okra fruit can inhibitory activity against *Candida albicans*. The test method for inhibitory activity was the disc method using DMSO as a negative blank and Nystatin as a positive blank. The results obtained Okra Fruit fraction can be used as an antifungi *Candida albicans* and diameter zona of fraction n-hexane, diethyl eter and ethyl acetate were 15,09; 17.11, and 20,28 mm The conclusion of this study is the concentration of the fraction were directly proportional to its inhibitory power, and there weredifferences in and there were differences in antifungal activity. The active campounds contained in the diethyl eter and ethyl acetate fractions of okra fruit was positive for flavonoids, alkaloids, saponins, terpenoids and tannnis, while the n-hexane fraction contained saponins, terpenoids and tannins.

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## 1. Introduction

Skin disease caused by fungus is a very common for Indonesia people because Indonesia is in high humidity area that supports the fungi growth. Drugs that are generally used to treat infections are antibiotics but the use of these antibiotics often has unwanted side effects. Therefore, alternative medicinal agents can be sought from natural ingredients that have fewer side effects. This can be done by utilizing plants that grow well in Indonesia, one of which is okra (*Abelmoschus esculentus* L.).

Okra is a plant from the Malvaceae family with soft stems, with long and pointed fruit shapes and some are green or red.(Sunarjono, 2014). Okra or ladies finger fruit contains active compounds including flavonoids, alkaloids, tannins, steroids/triterpenoids (Kumar et al., 2013; Neldawati and Gusnedi, 2013; Ayushi et al., 2016). The active compound in okra fruit has its own activity as an antimicrobial. Microbial infections, both bacterial and fungal most often occur on the skin (Tranggono, 2007). Research on okra fruit that has been carried out, among others :ethanol extract of okra fruit can inhibit the growth of *Eschericia coli*, *Staphylococcus aureus* and *Streptococcus mutans bacteria*.(Sirait, 2018), but in this study the test was done on fungi.



**Figure 1.** Okra Plants (*Abelmoschus esculentus*L.) (Personal documents)

Fungi are microsized organisms, eukaryotic, not plant categories and are capable of causing infections in humans (Sutanto et al., 2008). One type of fungus that often causes health problems is *Candida* spp, namely dimorphic, opportunistic fungi which are normal flora but if the population increases that can cause health problems including cancer sores, skin lesions, candidauria, candidiasis, female genital infections (Mutschler, 1991; Kurniawan, 2009; Septiadi, Pringgienies and Radjasa, 2013).

The extraction solvent used is ethanol because based on research (Siswarni, Putri and Rinda, 2017) that solvent is effective for attracting the active compound because it contains the hydroxyl group that gives it polar properties, while the alkyl group is a nonpolar group. The ethanol extract was fractionated with n-hexane, diethyl ether and ethyl acetate as solvents to separate the active compounds based on their polarity. The n-hexane, diethyl ether and ethyl acetate fractions were tested for activity against the fungus *Candida albicans* and phytochemical screening was carried out to ensure the content of the active compounds present in each okra fruit fraction by chemical reaction and TLC analysis.

## 2. Method

Equipment used consisted autoclave, stir bars, hot plates, incubators (Mettler), laminar air flow cabinets, micropipettes, tweezers, eyelet needles, vortexes, and other glassware (beakers, petri dishes, reaction tubes, Erlenmeyer flasks, etc.), blender, maceration vessel, porcelain dish, refrigerator, loop needle, micro pipette and caliper, cylinder cup, blue tip

The materials used were nutrient agar medium (Oxoid), nutrient broth (Oxoid), water, Green okra, 80% ethanol, n-hexane, diethyl ether and ethyl acetate, NaOH,  $\text{CHCl}_3$ , HCl, Dragendrof's reagent, Mayer's reagent, Mg powder, amyl alcohol,  $\text{FeCl}_3$ , gelatin, NaCl,  $\text{NaNO}_2$ , NaOH, sulfuric acid, acetic acid and *Candida albicans* cultures.

### 2.1 Preparation of Green Okra Fruit Ethanol Extract

Clean the selected intact okra fruit then dried and blended, to smooth, after that sifted the blended Okra fruit. . Remaceration used 80% ethanol solvent. 200 gr green Okra fruit powder is remaceration in 1L ethanol 80% and every 24 hours replaced with new solvent for 5 days.

### 2.2 Preparation of Extract Fractination

Fractionation was carried out using 80% ethanol extract with 50 ml of distilled water and then fractionated with n-hexane, diethyl ether and ethyl acetate using a separating funnel. The resulting collectionation vaporized to be thick fraction.

### 2.3 Phytochemical Screening

#### a. Identification of Flavonoids

The n-hexane fraction, diethyl ether fraction and ethyl acetate fraction of Okra fruit were added with the magnesium metal and 2% HCl in a test tube with addition amyl alcohol. Red, yellow/orange color on the amyl alcohol layer if it positive contains flavonoids.

#### b. Identification of Saponins

The n-hexane fraction, the diethyl ether fraction and the ethyl acetate fraction of Okra fruit were shaken vertically for 10 seconds in a test tube, the result was foam, plus one drop of 1% HCl to form a stable foam if it contained positive saponins.

**c. Identification of Alkaloids**

The n-hexane fraction, diethyl ether fraction and ethyl acetate fraction of okra fruit were added with 10 ml of HCl solution (1:10) divided by each solution into 3 test tubes, then added 5 ml of the precipitating reagent.

**d. Identification of Tannins**

The n-hexane fraction, diethyl ether fraction and ethyl acetate fraction of okra fruit were added with 1% FeCl<sub>3</sub> solution. The formation the dark blue or green-black color shows the presence of a phenol group compound, then in the other tube the sample is added to the solution of NaCl and gelatin with the formation of precipitate that indicates the positive tannin.

**e. Terpenoid Identification**

The n-hexane fraction, diethyl ether fraction and ethyl acetate fraction of okra were added with Liebermann-Burchard reagent then concentrated H<sub>2</sub>SO<sub>4</sub> was added, shaken and observed. A positive terpenoid test results in a red or violet color.

**f. TLC Test**

Three chambers were prepared and each chamber was added with eluent for identification of alkaloids, flavonoids, tannins and saponins and then saturated with the system used as follows:

**Table 1.**

Identification of Active Compound of KRA Fruit Fraction

Sample	Stationary phase	Mobile phase	Detection
Flavonoids	Silica gel GF254	Butanol:acid acetate:water(4:1:5) (Harbone, 1987)	Ammonia vapor
Alkaloids	Silica gel GF254	Ethyl acetate:methanol:water (100:13,5:10) (Harbone, 1987)	Dragendorf
Saponins	Silica gel GF254	Chloroform:methanol:water (64:50:10) (Hanani, 2015)	Anisaldehyde – H <sub>2</sub> SO <sub>4</sub> (p)
Tannins	Silica gel GF 254	Methanol : water (6:4) (Hanani, 2015)	FeCl <sub>3</sub> 5%
Terpenoids	Silica gel GF254	n-hexane : ethyl acetate (5;5) (Hanani, 2015)	Liebermann-Burchard

**2.4 The Inhibitor Activity Test****a. Preparation of Test Solution**

A total of 5 grams of the n-hexane fraction, the diethyl ether fraction and the thick ethyl acetate fraction were weighed and dissolved in DMSO (Dimethyl sulfoxide) to obtain the extract concentration of 500/ml and diluted to obtain a concentration of 300 and 100 mg/ml.

**b. Sterilization equipment**

The equipment such as erlenmeyer, test tubes, cotton swabs, and others for antifungal testing were sterilized in an autoclave at 121°C for 15 minutes.

**c. Preparation of Sabouraud Dextrose Agar 4% media**

The preparation used 9.75 grams of Sabouraud Dextrose Agar 4% dissolved in 150 ml of aqua distillate then sterilized for 20 minutes at 121°C in an autoclave.

**d. Making Stock of *Candida albicans***

Before being used to test the antifungal activity, *Candida albicans* must be rejuvenated. The first step was to make a slanted agar culture, one ose of the fungi was scratched onto a new SDA (Sabouraud Dextrose Agar) medium, then incubated at 25°C for 3 x 24 hours.(Desmara, 2016).

**e. Preparation of *Candida albicans* suspension**

With a sterile round loop needle, *Candida albicans* cultures were taken from the mushroom stock, then inoculated and incubated at 25°C for 3 x 24 hours. The suspension was added with NB media or fungal colonies to obtain an absorption of 0.080 to 0.100 at a wavelength of 625 nm with a UV spectrophotometer instrument according to the Mc standard. Farland(Desmara, 2016).

**f. Preparation of media containing a suspension of *Candida albicans***

Media Sabouraud Dextrose Agar 4% heated with free fire then cooled to a temperature of 45 - 50°C. Added 5 l of *Candida albicans* suspension (1.0 x 10<sup>8</sup> CFU/ml) and homogenized, after that 20 ml was poured into a petri dish and allowed to solidify.

**g. Positive Control**

Nystatin with a concentration of 500,000 IU was crushed and diluted with sterile distilled water to volume of 25.0 ml (concentration 20,000 IU/ml) was used as a positive control.

**h. Green Okra Fruit Antifungal Activity Test**

Sabouraud Dextrose Agar (SDA) media was poured into 10 ml sterile petri dishes. 5 l of *Candida albicans* suspension was inoculated into 20 ml of SDA media, then the fungal culture suspension and media were homogenized. Aseptically SDA media containing mushroom culture was poured into a petri dish that had been filled with the first layer and a cylinder cup to form a well. When the upper media solidified, the cylinder cup was taken and each well was filled with n-hexane fraction, diethyl ether fraction and 50µL okra ethyl acetate fraction, Nystatin positive control solution and DMSO negative control solution. Each group was replicated five times. The petri dishes were incubated at 250°C for 3x24 hours. The diameter of the inhibition zone around the well indicates the presence of antifungal activity and measurements were done with a caliper.

**3. Result and Discussion**

**3.1 Results**

The next process was phytochemical screening to determine the content of chemical compounds in the n-hexane, diether ether and ethyl acetate fractions of okra fruit.

**Table 2.**  
Okra Fruit Screening Result (*Abelmoschus Esculentus* L)

Compounds	Reaction of Screening	n-hexane fraction	Diethyl ether fraction	Ethyl acetate fraction
<b>Alkaloids</b>	HCl 2N + heated cooled, filtered + dragendroff (Depkes RI, 1995)	(+)	(+)	(+)
	HCl 2N + heated cooled, filtered + meyer (Depkes RI, 1995)	(-)	(+)	(+)
	HCl 2N + heated cooled, filtered + bouchardat (Depkes RI, 1995)	(-)	(+)	(+)
<b>Flavonoids</b>	Mg powder + HCL(p) + amyl alcohol (Indrayani, Soetjipto and Sihasale, 2006)	(-)	(+)	(+)
<b>Saponins</b>	Aquadest then shaken + HCL 2N (Depkes RI, 1995)	(-)	(+)	(+)
<b>Terpenoids</b>	Liebermann-Burchard reagent (Depkes RI, 1995)	(+)	(+)	(+)
<b>Tannins</b>	Hot water then filtered + 1% FeCl3 (Widowati et al., 2006)	(+)	(+)	(+)

Description: (+) = contains the compound being tested  
(-) = does not contain the compound tested

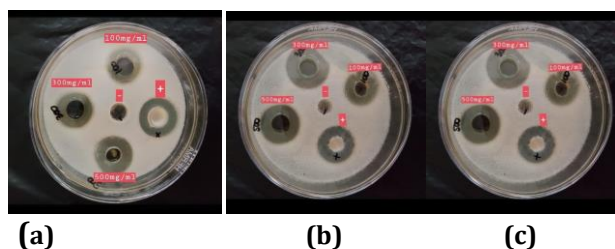
**Table 3.**  
Thin Layer Chromatography Test Result of Okra Fruit Fraction

Compounds	n-hexane fraction	Diethyl ether fraction	Ethyl acetate fraction
<b>Alkaloids</b>	-	(+)Orange Stain Rf 0.75	(+)Orange Stain Rf 0.75

Compounds	n-hexane fraction	Diethyl ether fraction	Ethyl acetate fraction
<b>Flavonoids</b>	-	(+)Yellow stain Rf 0.60	(+)Yellow stain Rf 0.83
<b>Saponins</b>	(+)purple stain Rf 0.90	(+) purple stain Rf 0.85	(+)Bluish green stain Rf 0.41
<b>Terpenoids</b>	(+)Brown stains Rf 0.50	(+) Brown stains Rf 0.70	(+)Brown stains Rf 0.70
<b>Tannins</b>	(+)Dark green stain Rf 0.70	(+) Dark green stain Rf 0.75	(+)Dark green stain Rf 0.31

**Table 4.**  
Comparison of Inhibition Diameters of Okra Fruit Fraction

Concentration	n-hexane fraction	Diethyl ether fraction	Ethyl acetate fraction
100mg/ml	7.06	10.85	15, 03
	7.05	10.98	14, 98
	7.03	10.95	15, 22
	7.00	10.90	15,11
	7.04	10.93	15.01
Average SD	7.04±0.0230	10.92±0.0497	15.0±0.045
300mg/ml	7.80	11.63	16.87
	7.95	11.75	17.02
	7.85	11.70	17.11
	7.90	11.65	17.29
	7.99	11.60	17.27
Average SD	7.90±0.0760	11.67±0.0594	17, 11±0.872
500mg/ml	11.45	12.04	20.22
	11.50	12.03	20.34
	11.40	12.05	19.79
	11.55	12.01	20.18
	11.30	12.00	20.88
Average SD	11.44±0.0962	12.03±0.0207	20.28±0.869



**Figure 2.** Comparison of Inhibition Diameter of Okra Fruit n-hexane fraction (a), diethyl ether fraction (b) and ethyl acetate fraction (c)

### 3.2 Discussion

The sample used was fresh okra (*Abelmoschus esculentus* L.) from green okra plantation in Cepiring village RT.07 RW.01 Cepiring Getas kulon, Kendal as much as 20 kg as much 800 g of dried green okra fruit was mashed in a blender and sieved with a mesh sieve no 30/40 to reduce the size of the okra simplicia so as to facilitate the extraction process (DepKes RI, 1986). The extraction process was carried

out by remaceration with 80% ethanol as solvent then weighed 200 g of green okra fruit powder repressed for 5 days with 1000 ml of 80% ethanol. The yield obtained in this study was 31.42%.

The next process was the fractionated the viscous extract to separate the compounds, 10 grams of green okra fruit extract was added and 50 ml of distilled water was added and 150 ml of n-hexane solvent was put into a separating funnel and then fractionated. The solvents used were n-hexane, diethyl ether and ethyl acetate. The yield percentage obtained was in the n-hexane fraction of 11.98%, the diethyl ether fraction of 6.54%, and the ethyl acetate fraction of 5.36%.

The next process was phytochemical screening to determine the content of chemical compounds in the n-hexane, diethyl ether and ethyl acetate fractions of okra fruit. Phytochemical screening of alkaloids showed that the n-hexane, diethyl ether and ethyl acetate fractions were positive for alkaloids because they were able to form minimal precipitates with two precipitating reagents from different groups. Nitrogen from alkaloid compounds can form covalent bond then forms the orange or brick red precipitate (Svehla, 1990). While alkaloids with Mayer have the same reaction to form coordinate covalent bonds with metal ions to make white precipitate appears (McMurry and Fay, 2004). The metal ion  $K^+$  will form a coordinate covalent bond with nitrogen in the alkaloid to form a potassium-alkaloid complex which forms a dark brown precipitate in the precipitation reaction with Bouchardat reagent.

A positive flavonoid result is indicated by the formation of a yellow, orange or red color in the tested solution (Indrayani, Soetjipto and Sihasale, 2006). The results of the diethyl ether and ethyl acetate fractions were positive for flavonoids, while the n-hexane fraction was negative because the amyl alcohol layer was green. The formation of red, yellow or orange color is because the Mg and HCl metals in this test reduce the benzopyron core contained in the flavonoid structure (Prashant et al., 2011).

The results of the reaction test for the saponin compound of the diethyl ether fraction and the ethyl acetate fraction were positive containing saponins with terstable froth, while the n-hexane fraction showed negative results for saponins because there was no stable foam formed. Foam is a relatively stable structure composed of an air pocket surrounded by a thin layer of liquid, with the gas-in-liquid dispersion stabilized by a surface tension-lowering chemical (DepKes RI, 1995).

The results of the reaction test for tannin compounds proved that the n-hexane fraction, diethyl ether fraction and ethyl acetate fraction positively contained tannins characterized by the formation of a blue-black color with 1%  $FeCl_3$  due to the presence of  $Fe^{3+}$  ions as the central atom and tannins have O atoms have lone pairs of electrons. It can coordinate to the central atom as the ligand (Sa'adah, 2010). Tannin compounds are also capable of forming complexes with protein compounds. In this study, gelatin compounds were used, the formation of complexes was indicated by the presence of a white precipitate.

The results of phytochemical screening of okra fruit powder in research (Nurfatwa, 2018) as well as positive for flavonoids, alkaloids and tannins but for saponins of the research was negative. Tandil et al., 2020, showed the results of phytochemical screening of okra fruit extract were also positive for flavonoids, alkaloids, saponins and tannins.

After the phytochemical screening test, it was continued with the confirmation of the target compound, namely flavonoids using the Thin Layer Chromatography (TLC) method. Based on the results of the identification of compounds by TLC, it supports the content of compounds contained in each fraction and such as the results of chemical reactions that the diethyl ether and ethyl acetate fractions of okra fruit were positive for flavonoids, alkaloids, saponins, terpenoids and tannins, while the n-hexane fraction was positive for saponins, terpenoids and tannins. The results of the identification of Thin Layer Chromatography are presented in Table 3.

After the phytochemical screening test and TLC it was continued with the antifungal activity test against the fungus *Candida albicans*. The concentration of the fungal suspension in the antifungal test was equalized with the absorbance of McFarland solution. The turbidity of the mushroom suspension will affect the number of mushrooms planted it is due to the heterogeneity of the fungus then the number of mushrooms planted is not homogenous so that it may affect the results of observations. The same incubation time in each replication will result the homogenous fungal growth because the fungal metabolism process has been running perfectly. The n-hexane, diethyl ether and ethyl acetate fractions of okra were made with concentrations of 100mg/ml, 300mg/ml, and 500mg/ml, respectively. Nystatin was used as a positive control because nystatin only be bound by sensitive fungi. The antifungal effect depends on the presence of ergosterol binding to the fungal cell membrane. Nystatin can be fungistatic and fungicidal against the fungus *Candida albicans*. As a result the formation bonds between sterols and antibiotics had change in membrane permeability so that fungal cells will lose various

compounds (Setiabudy and Bahry, 2007). The negative control used was DMSO (Dimethyl Sulphoxide) because DMSO did not have antibacterial and antifungal activity (Martindale, 1982). The media used for observation and maintenance of mushrooms was Sabouraud Dextrose Agar (SDA) (Jawetz, Melnick and Adelberg, 2005). SDA media has a pH of 5.6 which contains peptone, agar, and dextrose. The low pH of SDA media does not support bacterial growth (Lay and Hastowo, 1992).

Through the well method, the antifungal activity was tested by diffusing antifungal compounds into the media. The advantage of this well method is the sample volume used is more than other methods, the resulting clear zone has a regular shape, the antifungal compound will diffuse in all directions because the sample in the well can be in direct contact with the fungus and is able to diffuse evenly, both vertical and horizontal (Jawetz, Melnick and Adelberg, 2005). The antifungal activity test of the n-hexane fraction, diethyl ether fraction and ethyl acetate fraction were replicated 5 times, then the inoculation results were incubated for 3x24 hours at 25°C. The results obtained were clear zones that were calculated using a caliper. The antifungal activity test results from the n-hexane and ether fractions are presented in table 4

The ethyl acetate fraction has greater potential as an antifungal compound, it is indicated by the average inhibition zone of the n-hexane fraction, diethyl ether fraction and ethyl acetate fraction. One of the differences in the diameter of the inhibition zone of the antifungal activity is due to the high and low levels of the active compound or substance contained in the fraction. The size of the clear zone diameter results is influenced by the test concentration used, the higher the test concentration used, the larger the clear zone diameter results, it can be observed in Figure 2.

The diameter of the inhibition zone of the n-hexane fraction, diethyl ether fraction and ethyl acetate fraction were tested using SPSS (statistical product and service solution). The two-way ANOVA test, showed the differences or effects on the n-hexane fraction, diethyl ether fraction and ethyl acetate fraction of okra fruit with different concentrations of the inhibition zone because the significant value was less than 0.05. Based on the results of the post-ANOVA test, there was a significant difference in the clear zone between the n-hexane fraction, diethyl ether fraction and ethyl acetate fraction at concentrations of 100mg/ml, 300mg/ml, and 500mg/ml because the significant value showed less than 0.05. The differences Significant can be interpreted that there was a real influence.

#### 4. Conclusion

Based on the research that has been done, the following conclusions are obtained:

- a. The content of compounds contained in each fraction and such as the results of chemical reactions that the diethyl ether and ethyl acetate fractions of okra fruit were positive for flavonoids, alkaloids, saponins, terpenoids and tannins, while the n-hexane fraction was positive for saponins, terpenoids and tannins.
- b. The n-hexane fraction, diethyl ether fraction and ethyl acetate fractions of okra fruit had antifungal activity against the growth of *Candida albicans*
- c. There were differences in antifungal activity between the n-hexane fraction, diethyl ether fraction and ethyl acetate fractions of okra fruit with concentrations of 100mg/ml, 300mg/ml, and 500mg/ml on the growth of *Candida albicans*

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