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Research Article

Screening of Volatile Compounds of Brotowali (*Tinospora Crispa*) and Antifungal Activity Against *Candida albicans*

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ABSTRACT

Brotowali (*Tinospra crispa*) has antifungal potential of *Candida albicans* against. This plant contains a compounds was berberine, Columbine, alkaloids, saponins, tannins, and flavonoids as antipyretic, analgesic, antiparasitic, antiseptic, antifungal, antidiabetic and antitumor. The research was conducted by means of extraction and fractionation of stem brotowali with solvent n - hexane, chloroform, ethyl acetate with increasing polarity. Each fractions in Thin layer chromatography (TLC) to see the profile of the chromatogram and the same profile combined into one fraction then subsequently tested of the fungus *C. albicans* activity by diffusion method and the combined active fractions were identified by UV - Vis and GC-MS. Based on the results obtained TLC 5 fractions F1 - F5 was combined, then the antifungal activity test results showed that the fraction of one (F1) has antifungal activity *C. albicans* against with inhibitory potential of 27.73 ± 0.16 %. Identification results based on UV - Vis spectra of F1 containing the OH groups, aliphatic CH, carbonyl group (C = O), methylene (- CH2) and the CO group. GC -MS identification showed the phenolic compounds, fatty acids, and terpenoids. Compounds that are antifungal is Hexadecanoid and 9.12 Octadecadienoic acid.

Keywords: *Tinospora crispa*, antifungal, fractionation, identification, *Candida albicans*

INTRODUCTION

Infectious diseases can be caused of death even of14 million people annually die (Schlein, 2009). *Candida albicans* (*C. Albicans*) are fungus causing the infection, both locally as oral and vaginal infection. Treatment of infections was caused by *C. albicans* usually using antifungal ketoconazole for example, the use of ketokonasol provide a satisfactory treatment outcome but side effects such as fever, vomiting, muscle spasms, and hypotension. Given the problems so many are turning to alternative medicine use traditional medicine (Brooks *et al*., 2004). One of the plants is brotowali (*Tinospra crispa*) containing of resin, starch, glycosides, pikroretosid, bitter substances pikroretin, Harsha, berberine alkaloids and palmatin so that functions as antipyretic, analgesic, antiparasytik, antiseptic, anti-tumor and antidiabetik (Dalimartha , 2008). The extract of stem brotowali as bacteriostatic effect to *Escherichia coli* and Staphylococcus aureus against (Widowati *et al*., 1994).

The extract stems , leaves and roots brotowali have been potentialy as antimicrobial (Gram positive and Gram negative) and anti- fungal (*C. albicans*) (Zakaria & Matjais (2006), Shahriar *et al*., (2011) and Mohammed *et al*., (2012), some one of plant extracts such as *Tinospora crispa*, *Anacardium occidentale*, leaves of *Hibiscus cannabinus* and *Garcinia atroviridis* also reported to inhibit the growth of *S. aureus* and *E. coli* (Zakaria *et al*., (2011)

By knowing the benefits brotowali plants as anti-bacterial and antifungal , it is necessary to conduct further research to clarify the compounds contained in the trunk brotowali that have antifungal activity on Candida albicans .

MATERIALS AND METHODS

Materials

Materials are using in this study were stem of brotowali (tinospora crispa), ethanol 70%, chloroform, *n*-hexane, ethyl acetate, distilled water, *C. albicans isolates*, media Sabouraud dextrose agar, and media Sabouraud dextrose broth.

Procedure

Pulverizing stem of brotowali: Brotowali rods was taken from Purwokerto in June of 2013 to August of 2013, then a small cut. Drying is done in an oven at 40oC. Simplicia which has been dried to maximum moisture content of 10%. So that pulverizing with a grinder.

Preparation of extracts: Brotowali stem powder weighed 250 grams, the maceration for 3 x 24 hours with ethanol 70%. The extract was filtered, the filtrate evaporated to the Rotary evaporator until thick, and weighed. Methanol extracts tested antifungal activity to the *C. albicans*

Fractionation of ethanolic extract using column chromatography: Fractionation performed using column chromatography . The stationary phase used 254GF silica gel, mobile phase used was solvent n-hexane, chloroform, ethyl acetate and used based on the level of polarity. Then each eluent was collected every 5 ml was followed by thin layer chromatography (TLC), the fraction that

produces the same profile are merged then tested activities, and identification of the most active followed by Infra red and GC-MS.

Table 1. Results of grouping fraction

No	Fractoin	Vial Eluat	Weigh of Fraction (mg)	Colour
1	F_1	10-12	1000	green
2	F_2	12-15	1000	dark green
3	F_3	16	500	yellowish green
4	F_4	17-18	2900	reddish brown
5	F_5	19-20	3000	dark brown

Antifungal test

Sabouraud Dextrose Agar (SDA): SDA medium was prepared by dissolving 40 g of dextrose , 10 g Mycological peptone , 15 g agar, 500 ml of distilled water was added , and then diluted with distilled water to a volume of 1000 ml . The solution was homogenized in the above magnetic stirrer . Then the media is inserted into a petri dish , and then sterilized by autoclaving at 121 ° C for 15 min .

Table .2. The results of the activity of the ethanol extract on *C.albicans*

Extract/ Control	Conc. (ppm)	% potential inhibition	Potential inhibition ± SD
Extract	750	39.3	
	750	37.2	36.967 ± 2.4583
	750	34.4	
	500	29.8	
	500	32.6	31.733 ± 1.6773
	500	32.8	
	250	20.6	
	250	21.4	20.967 ± 0.4041
	250	20.9	
Ketokonazol	25	100	100±0

*0Sabouraud Dextrose Broth (SDB):*Media SBD dibuatdengan Mycological by mixing 10 g peptone , 40 g dextrose , 1 liter of distilled water, after which the solution is homogenized in atasmagnetic stirrer. Then the media is inserted into a petri dish, and then sterilized using an autoclave at temperature of 121oC for 15 minutes .

Activity against Candida albicans test: Activity assay was performed using the disc diffusion method . Paper discs dipped first into the test solution for 5-10 minutes and then the paper has been placed on medium containing bacterial culture in a petri dish (Jawetz et al . , 2005) . Bark extract concentration used was 3 concentration is 750 ppm , 500ppm and 250ppm . Each of these fractions obtained created the 3 concentrations. Then there are 2 additional group that is one positive control group with ketoconazole at a concentration of 2 % . 1 negative control using C.albicans in SDB medium.

Analysis of data

Percentage inhibition (Mariawati, 2008)

Observations were made by calculating the percent of potential barriers:

$$I = \frac{d2-d1}{d1} \times 100\%$$

Description:

I = Potential barriers

d1 = diameter of the disk

d2 = average diameter of inhibition zone

Identification of compounds: Identification of the active fraction of stem brotowali performed by FTIR spectrophotometry and GCMS

Table 3: The results of antifungal activity assay of *T.crispa* fraction on *Candida albicans*

Fraction	% potential inhibition 250ppm	250ppm	250ppm	% Potential inhibition ± SD
F1	27,6	26.7	28,6	27.733 ± 0.9609
F2	-	-	-	-
F3	-	-	-	-
F4	-	-	-	-
F5	-	-	-	-
Control (+)	97,3	94.6	99,2	97.033 ± 2.3116
Control (-)	-	-	-	-

RESULTS AND DISCUSSION

Powdered crude drug of stem of brotowali using ethanol 70% and produce yield to the weight of the powder was 11.10% w/w. Results grouping fraction (Table 1) was obtained compound with large amounts of polar compounds. The chromatograms (Figure 1B and 1C), indicating that each faction has a different chromatogram profile and a description of the differences in the content of compounds according to the polarity of the solvent. Chromatogram (Fig 1D) showed a weak blue spots on the observation of visible light in ethylacetate fraction,. Weak blue color is indicative of terpenoid compounds in ethanolic extract, chloroform fraction and ethyl acetate fraction (Jork *et al.*, 1989). TLC results in Figure 1D and 1E show that the ethanolic extract and fractions I looked into purple spots after spraying anisaldehyde sulfuric acid than the initial spot. Stahl (1985) suggests the use of anisaldehyde sulfuric acid reagent to determine the presence of terpenoid compounds and patches (initially colorless) after being sprayed will be colored purple, blue, red, gray or green. Anisaldehid can extend the system chromophore compounds and the ethanolic extract fractions also showed the presence of a compound class I terpenoid. After TLC fractions were then tested antifungal activity against *C.albicans*. Antifungal activity against *C.albicans* extracts are presented in Table 2. Test results against *C.albicans* ethanol extract showed that the extract has the ability to inhibit the growth of *C.albicans*, at a concentration of 250 ppm could inhibition on 20 967 ± 0.4041 and the higher the concentration the greater the potential. Testing continued activity of the fractions were

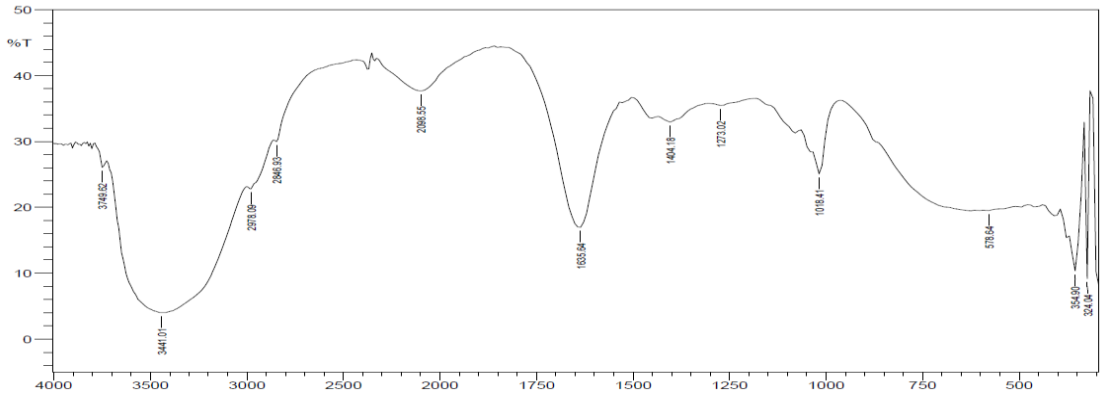

Figure 1: Spectra Infra red spectrophotometry of fraction I

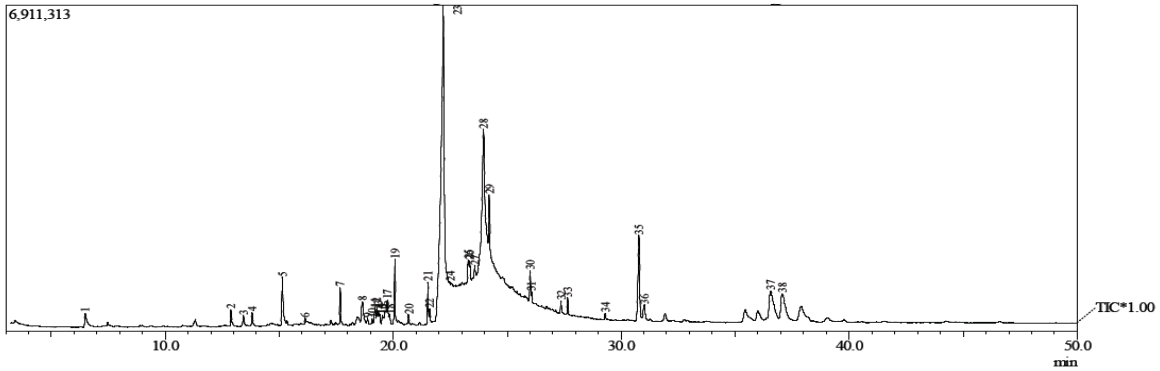

Figure 2. Spectra Gas chromatography of fraction I

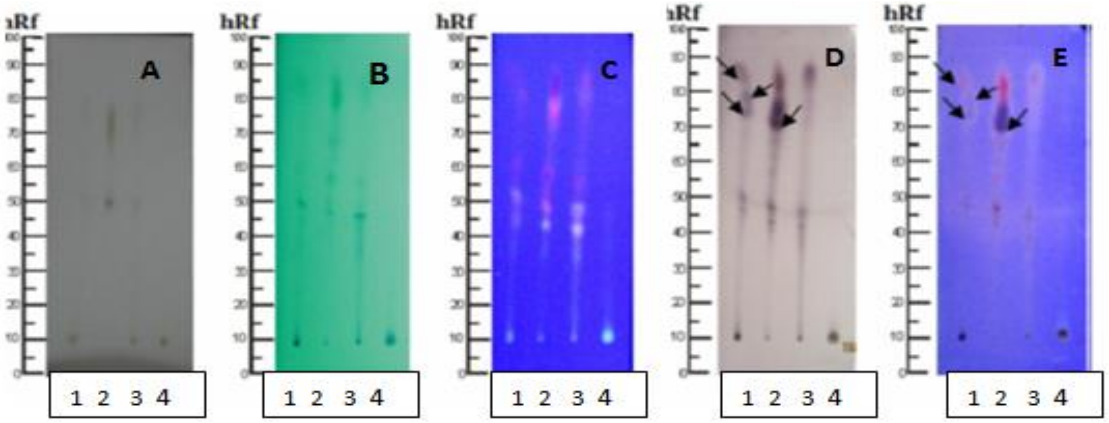

Figure 3: Chromatograms of ethanolic extract and its fractions brotowali rod before and after spraying anisaldehyde sulfuric acid. A visible light, UV 254 B, C UV 366m (A, B and C seen before sprayed), D and E of visible light after being sprayed UV 366, 1. Extracts ethanol, 2. Fraction of chloroform, 3. Fraction Nheksan and 4 fraki ethylacetate. presence of terpenoid compounds indicated by arrows

obtained (Table 3). The results of activity on *C.albicans* of brotowali fraction showed that only fraction I, which is able to inhibit the growth of *C.albicans*, while fraction 2-5 not potentially inhibit the growth of *C.albicans*. there is likely because fraction I containing compounds such as terpenoids shown by identidication by TLC (Fig 1 and 2) Identification by IR spectrometer (Figure 1) showed the main absorption bands at wave numbers (Vmax) 3441.01; 2978.09; 2098.55; 1635.64; 1404.18; 1273.02; and 1018.41 cm-1. IR spectrometer fraction I gave absorption bands at 3441.01 cm-1 with strong density and width indicate that the-OH group hydrogen bonding experience. Uptake in the number 2978.09 cm-1 and 2098.55 cm-1 indicate the presence of aliphatic CH. Ribbon at 1635.64 cm-1 wave is karakteritis to the carbonyl group (C = O). Wave number 1404.18 cm-1 shows the methyl group of-CH (-CH3) and the wave of 1273.02 cm-1 indicate the presence of methylene (-CH2). While the number gelompang 1018.41 cm-1 indicate the presence of CO group. Peaks detected in the GCMS not all have potential as antifungal of 27 compounds, for more details are presented in Table 4. Table 4 shows that the fraction I *T.crispa* bark contains several types of classes of compounds are compounds phenols, fatty acids and

Table 4. Compounds of the result of GC

No	Peak	Name compounds	R. Time	Area %	Rumus Molekul	
1	5	Vanilin	15.135	2,60	C8H8O3	Fenol
2	6	2,5-cydohexadiene	16.133	0,14	C14H20O2	Asam lemak
4	9	Patcouli alcohol	18.872	0,21	C15H26O	Terpenoid: Sesquiterpen
5	10	Nonyl phenol	19.073	0,22	C15H24O	Fenol
6	11	Nonyl phenol	19.192	0,79	C15H24O	Fenol
7	12	Nonyl phenol	19.277	0,70	C15H24O	Fenol
8	13	Nonyl phenol	19.350	0,55	C15H24O	Fenol
9	14	Nonyl phenol	19.417	0,36	C15H24O	Fenol
10	15	Nonyl phenol	19.525	0,58	C15H24O	Fenol
11	16	Nonyl phenol	19.642	0,79	C15H24O	Fenol
12	17	Phenol	19.731	1,30	C14H22O	Fenol
13	18	Nonyl phenol	19.850	0,45	C15H24O	Fenol
14	19	1-nonadecene	20.074	1,99	C19H38	Fenol
15	20	1-acetoxy-2-(dexyloxy)ethene	20.677	0,37	C16H32O3	Asam lemak
16	21	Hexadecanoic acid	21.523	1,42	C17H34O2	Asam lemak
17	22	2,5 cyclohexadiene-1,4-dione	21.605	0,60	C14H20O2	Asam lemak
18	23	Hexadecanoic acid	22.201	37,20	C16H32O2	Asam lemak
19	24	Hexadecanoic acid	22.492	0,75	C16H32O2	Asam lemak
20	25	9,12-octadecadienoic acid	23.296	0,82	C19H34O2	Asam lemak
21	26	9-octadecadienoic acid	23.347	0,75	C19H34O2	Asam lemak
22	27	9,12-octadecadienoic acid	23.575	0,70	C18H32O2	Asam lemak
23	28	9,12-octadecadienoic acid	23.958	19,92	C18H32O2	Asam lemak
24	31	Hexanedioic acid	26.072	0,36	C22H42O4	Asam lemak
25	32	1,2-benzene dicaroxylic acid	27.368	0,43	C24H38O4	Asam lemak
26	33	Cyclooctacosane	27.656	0,57	C28H56	Asam lemak
27	34	Cyclotetracosane	29.302	0,24	C24H48	Asam lemak
28	38	Stygmast-5-en-ol	37.067	3,75	C29H50O	Terpenoid: Sesquiterpen

terpenoids group. Vanillin, phenol and phenol Nonyl included in the class seyawa phenol. While alcohol and stygmast patcouli-5-en-ol compounds belong to the class of terpenoids sesquiterpenes. Sesquiterpenes are terpenoid compounds built by 3 isoprene units. This class of compounds also has a sizeable bioactivity as antimicrobial, antifungal and antibiotic (Ali *et al*., 2008; Lenny, 2006; Guo *et al*., 2008). 2,5-cydohexadiene addition, 1-Acetoxy-2- (dexyloxy) ethene, Hexadecanoic acid, 2,5-cyclohexadiene-1,4-dione, 9,12-octadecadienoic acid, 9-octadecadienoic acid, 1,2 - benzene dicaroxylic acid, and Cyclotetracosane included in the sam-fat compounds that can inhibit the growth of microbes (Noviyanti, 2010).

CONCLUSIONS

Based on these results, it can be concluded that; The ethanol extract of the stem bark *T.crispa* has antifungal activity against *C.albicans* (20.59%) The results obtained fractionation 5 fractions with greatest antifungal activity in fraction I (27.73%) Identification using IR spectrophotometry and GCMS performed on fractions I, and obtained three classes of antifungal compounds are potentially as phenols (vanillin, Nonyl phenol and phenol), fatty acid group (2,5-cydohexadiene, 1-Acetoxy-2-(dexyloxy) ethene, hexadecanoic acid, 2,5-cyclohexadiene-1,4-dione, 9,12-octadecadienoic acid, 9-octadecadienoic acid, 1,2-benzene dicaroxylic acid, and Cyclotetracosane) and terpenoids (Patcouli alcohol, Stygmast -5-en-ol).

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