

Conference Paper

Phytochemicals, Antioxidant and Antifungal Properties of *Acorus calamus*, *Curcuma mangga*, and *Allium sativum*

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Abstract

The purpose of this study to determine the content of phytochemicals, antioxidant and antifungal properties of the combination of *Acorus calamus*, *Curcuma mangga*, and *Allium sativum*. This research was descriptive qualitative, extractions were done by maceration method with ethanol with 3 different combinations (C₁, C₂ and C₃). Phytochemical test reagent included 4 kinds of test, namely: alkaloids, flavonoids, triterpenoids, saponins and tannins. As for the antioxidant test, the method used was DPPH. The concentration used at 25, 50, 100, 200, and 400 ppm. As for the antifungal test conducted on *Candida albicans* with Kirby-Bauer disc methods with a concentration of 100%, followed by the MIC and MBC test with a concentration of 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78% and 0.39%. Phytochemical test results indicated the presence of the alkaloids, flavonoids and triterpenoids compounds in 3 different combinations (C₁, C₂ and C₃). The highest antioxidant levels founded in C₁ (61.75) followed by C₃ (47.94) and the lowest levels founded in C₂ (42.76). The antifungal test showed the inhibitory zone against *C. albicans*. The highest inhibitory zone was found in C₁ at 5.44 ± 1.78 mm (medium category), followed by C₂ at 4.08 ± 0.86 mm (medium category), and C₃ at 3.05 ± 0.23 mm (medium category). As for the minimum inhibitory concentration (MIC) value got on the concentration of 0:39% and minimum fungisidal concentration (MFC) values were at a concentration of 0.78%.

Keywords: *Acorus calamu*, *Allium sativum*, *Curcuma manga*, *Antioxidant*, *antifungal*, *Candida albicans*.

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1. Introduction

Medicinal plants have been used to tackle many health problems in Indonesia [1]. One of Traditional Medicine to improve fertility is Madura Traditional Herbal, namely

“Subur Kandungan” registered at POM RI and Dep. Kes RI (Ministry of Health) TR 813 256 171. The main content of that herbs are rhizome of *Curcuma mangga*, *Acorus calamus*, and *Allium sativum* [2]. *C. mangga* can be used as a tonic and as an antidote to the poison. In India, *C. mangga* used for cold medicine, reinforcing the stomach, improved digestion, and treated skin diseases [3, 4]. Phytochemical analysis of ethanol extract of *C. mangga* contained flavonoids, triterpenoids, steroids and saponins [5, 6]. *C. mangga* was also contains antioxidant compounds, including kalkons, flavonoids and flavanones. Curcuma extract widely used as an antimicrobial for the content of the active compound is able to prevent the growth of microbes [4, 7].

A. calamus had potential compound act as neuroprotective [9]. Phytochemical analysis of the methanol extract of *A. calamus* shown to contain glycosides, flavonoids, saponins, tannins, polyphenolic, essential oils, and terpenes [10–12]. Ethanol extract of *A. calamus* rhizome inhibited the growth of *C. albicans* in the minimum inhibitory concentration of 2.5 mg/ml. *A. sativum* had many benefits, such as an antibacterial, antiviral, antifungal and antiprotozoal [15–18]. *A. sativum* was able to inhibit the growth of *C. albicans* because it contained volatile fatty acid [14]. One of the cause of infertility was due to the infection of reproductive tract by microorganism. *C. albicans* was a fungus that triggers various reproductive disorders on the female such as candidiosis vaginalis (vaginal discharge). Candidiosis happened if allowed to continue could lead to infertility (sterility). This study aimed to determine the antifungal and antioxidant activity of ethanol extract combination of *A. calamus*, *C. mangga* and *A. Sativum* based on the “Subur Kandungan” herbs in bosting fertility. This was an attempt to provide scientific evidence related to the potential of Madura Traditional herbal medicine to increase fertility in vitro.

2. Material and Method

The study design was descriptive qualitative. Independent variables consisted of three variations of a combination of *A. calamus*, *C mangga* and *A.sativum* were employed for 3 combination. Combination 1 (C1) consist of of 28%: 36%: 36%, Combination 2 (C2) that is 30%: 30%: 40%, and Combination 3 (C3) that is 25%: 40%: 35% containing *A. calamus*, *C mangga* and *A.sativum* respectively. The herb concoction were extracted and examined the phytochemical using reagen test, antioxidant activity using the DPPH and antifungal test using disc diffusion method to evaluate the inhibitory zone, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC).

2.1. Phytochemical screening

Phytochemical screening of *A. calamus*, *C. mangga* and *A. sativum* ethanol extracts included alkaloid and saponin tests referring to Halimah and Hayati [13], flavonoids, triterpenoids, steroids, saponins, and tannins tests referring to Indrayani [19].

2.2. Antioxidant Activity test

The extract was dissolved in ethanol and made various concentrations of 25, 50, 100, 200, and 400 ppm. The extracts (4.5 mL) were added 1.5 mL of 0.1 mM DPPH and incubated at 37 °C for 65 minutes, then measured at a wavelength of 514.9 nm. As the blanks were used ethanol and 0.1 mM DPPH, while the control used were ascorbic acid (Vitamin C). The scavenging activity percentage was determined by specific formulation [13]. The IC₅₀ values were calculated by obtaining a regression equation from extracts concentration (x) with percentage scavenging activity of free radicals (y) using the program "prisma5 GraphPad software, Regression for analyzing dose-response data".

2.3. Antifungal Activity Test

Antifungal activity test used paper disc (Ø 6 mm). *C. albicans* as much as 0.1 mL were inserted into sterile petridishes, then inserted ± 5 ml of liquid water medium and allowed to solidify. Sterilized sterile discs with 100% C₁, C₂ and C₃ extracts were placed on Sabouraud Dextrose Agar (SDA) medium for 30 minutes, then incubated at 37°C for 18-24 hours [20]. The positive control (C +) is nystatin 1%. The clear zone indicated antifungal activity. Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC), were undertaken with streak plate of the test results in a dilution antifungal solid. All media that provided inhibitory zone were took to MIC and MFC test. First tube contained control of extract combination, 2nd tube contained the control of culture, 3-10 tube contained the test solution made serial dilution of 50%, 25%, 12.5%, 6.25%, 3.125%, 0.78% and 0.39%. Then observed on the tubes to how visible turbidity. MIC was characterized by clear tube. Samples were taken from all tubes, then inoculated on solid media (SDA) and incubated for 18-24 hours at 37°C for the determination of MFC. Fungus colonies grown were calculated using colony counter according to the standard plate count were 30-300 colonies per plate.

TABLE 1: Phytochemical screening result.

Group of compound	Reagens tests	A.calamus	C.mangga	A.sativum	Combination		
					C1	C2	C3
Alkaloid	Dragendorff	+	-	+	+	+	+
	Mayer	+	-	-	-	-	-
Flavonoid	Wilstater	-	+	-	+	+	+
Triterpenoid	Lieberman-Burchard	+	+	+	+	+	+
Steroid	Lieberman-Burchard	-	-	-	-	-	-
Saponin	Forth	-	-	-	-	-	-
Tannin	FeCl ₃	-	-	-	-	-	-

3. Results

3.1. Phytochemical Screening

The results of phytochemical observations are presented in Table 1.

3.2. Antioxidant Activity Test

The highest percentage of scavenging at concentration 200 ppm produced by C₂, C₃ and C₁, while vitamin C, percentage of scavenging at concentration of 50 ppm. In general, any concentration increase, percentage of scavenging also increase (Fig 1. and Table 2). These results which stated that the scavenging activity percentage would also increased with increasing concentration of the extract [25]. The percentage of scavenging of all combination (C₁, C₂, C₃, C₊) decreased at concentration of 400 ppm. Antioxidant ability started weakening in large concentrations as they related to the rate of oxidation. The influence of the amount of concentration on the rate of oxidation depended on the structure of the antioxidant, conditions and samples to be tested [26]. That's different with Vitamin C (positive control/C₊) as a comparison, in low concentrations had been able to ward off free radicals.

The IC₅₀ was a measure of how effective a drug was. It indicated how much of a particular drug or other substance was needed to inhibit a given biological process. The Lowest IC₅₀ founded in C₊ (vitamin C) at 27.59 (very strong), followed by C₂ at 42.76 (very strong), C₃ at 47.94 (very strong) and C₁ at 61.75 (active). If the IC₅₀ < 50

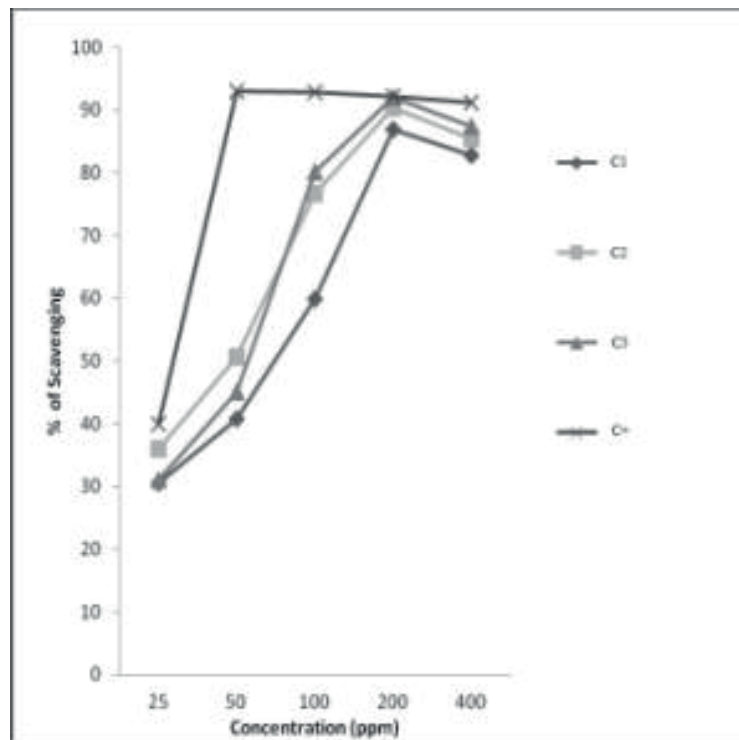


Figure 1: Determination of a DPPH radical scavenging activity.

TABLE 2: The Antioxidant level of ethanol extract of the combination of *A. calamus*, *C. mangga* dan *A. sat*

No	Treatment	Percentage of scavenging					R(squere)	IC ₅₀	Level
		25ppm	50ppm	100ppm	200ppm	400ppm			
1.	C1	30.56	40.74	59.87	86.95	82.79	0.9435	61.75	Active
2.	C2	35.89	50.58	76.63	90.35	85.48	0,9505	42.76	Very Strong
3.	C3	30.96	44.98	80.14	92.00	87.34	0,9456	47.94	Very Strong
4	C+ (Vitamin C)	39.85	93.02	92.91	92.18	91.20	0.9172	27.59	Very Strong

ppm, it is a very strong antioxidant power, IC₅₀ 50-100 ppm active antioxidant power, IC₅₀ 101-250 ppm moderate antioxidant power, IC₅₀ 250-500 ppm weak antioxidant power and IC₅₀ > 500 ppm antioxidant power is off [26].

3.3. Antifungal Activity Test

Based on the results of the study, the ethanol extract of the combination of 1, 2, and 3 showed antifungal activity of *C. albicans*, they were characterized by the formation of a inhibitory zone of fungal growth test. The result showed that the highest inhibitory zone of the treatments was C1 5.44±1.78 mm (medium), followed by C2 4.08±0.86 mm (medium) and C3 4.08±0.8 6mm (medium), nystatin as a positive control showed

TABLE 3: The Inhibitory zone of ethanol extract of the combination of *A. calamus*, *C. mangga* dan *A. sativum*.

Treatment	Inhibitory Zone (mm)±SD	Inhibitory Zone Level
C1	5.44±1.78	Medium
C2	4.08±0.86	Medium
C3	3.05±0.23	Medium
Nystatin (C+)	17.68±0.45	Strong

a strong inhibitory zone (17.68±0.45 mm) (Table 3). The following response categories based growth inhibition by inhibitory zone diameter, e.g; Strong (> 6 mm), Medium (3-6 mm) and Weak (0-3 mm) [27].

Nystatin chosen as positive control because it is one of the best antifungal drug. Nystatin is a polyene best class in destructive fungal cell membrane (ergosterol). Mechanism of action of the polyene class was to damage ergosterol irreversibly [28]. Ergosterol was a very important component of fungus cell membrane. The size of the inhibitory zone depended on the amount of the extractable compound. So that the combination extract could have increased or decreased inhibitory zone. The decreasing and increasing of the inhibitory zone caused by the components of the substances contained in the plants can mutually weaken, strengthen, repair or alter at all [29].

3.4. Test results of the MIC and MBC

Test compounds could be say to have antifungal power if the test media had greater clarity than the control of fungal. Based on Table 3 below, can be seen that the concentration of 0.39% have clarity compared to the control of *C. albicans*, so that the minimum inhibitory concentration can be determined at 0.38% concentration.

To confirm the value of MIC and MFC, it was confirmed by the streak plate method at all concentrations produced from the test solution. The result showed that at concentration 0,39% there was still fungi growth, whereas at concentration 50, 25, 12,5, 6,25, 3,13, 1,56 and 0,78% had not seen anymore the growth of fungi (Table 5). Thus, There were concluded that the MIC of the combinations ethanol extracts (C1, C2 and C3) were 0.39% and the MFC from the combinations ethanol extracts were 0.78%.

TABLE 4: The MIC of ethanol extract of the combination of *A. calamus*, *C. mangga* dan *A. sativum*.

Concentration	C1			C2			C3		
	I	II	III	I	II	III	I	II	III
Extract Control (extract of combination 100%)	-	-	-	-	-	-	-	-	-
0,39%	-	-	-	-	-	-	-	-	-
0,78%	+	+	+	+	+	+	+	+	+
1,56%	+	+	+	+	+	+	+	+	+
3,13%	+	+	+	+	+	+	+	+	+
6,25%	+	++	++	++	+	+	++	+	++
12,5%	++	++	++	++	++	++	+++	+++	+++
25%	++	+++	++	++	+++	++	+++	+++	+++
50%	++	++++	+++	+++	++++	+++	++++	++++	++++
Control of Fungal (<i>C. albicans</i> 100%)	++++	++++	++++	++++	++++	++++	++++	++++	++++

Description: Very cloudy: +++++, Snappy: +++, Somewhat cloudy: ++, Clear: -

TABLE 5: The MFC of ethanol extract of the combination of *A. calamus*, *C. mangga* dan *A. sativum*.

Concentration	C1	C2	C3
Control of Fungal	123×10^9	123×10^9	123×10^9
0,39%	78×10^6	$61,2 \times 10^6$	$80,67 \times 10^6$
0,78%	0	0	0
1,56%	0	0	0
3,13%	0	0	0
6,25%	0	0	0
12,5%	0	0	0
25%	0	0	0
50%	0	0	0
Control of extracts	0	0	0

4. Discussion

The phytochemical tests results of all ethanol extract combination (C1, C2 and C3) were alkaloids, flavonoids and triterpenoids. These compounds were able to inhibit the activity of free radicals. The compounds had potent antioxidant properties were flavonoids, tannins, phenols, alkaloids and saponins [30]. Phytochemical test rhizome

extract of *A. calamus* contained secondary metabolites such as alkaloids, flavonoids, polyphenols and essential oils, while the phytochemical test results extracts of *A. cativum* were tannins, alkaloids and saponins [32]. The content of secondary metabolites *C. mangga* extracts were curcumin, flavonoids, polyphenols, and essential oils, which contain antioxidant compounds, including kallon, flavonoids, flavanones [33].

The highest antioxidant power founded in C+ (vitamin C) at 27.59 (very strong), followed by C2 at 42.76 (very strong), C3 at 47.94 (very strong) and C1 at 61.75 (active). The active compound combinations of ethanol extract of *A. calamus*, *C. Mangga* C. and *A. sativum* which had potential as an antioxidant and antifungal were flavonoids, alkaloids and triterpenoids. Combination 2 (C2) that is AC 30%: CM 30%: AS 40% might have a best composition that contained optimum antioxidant properties.

One of flavonoid compounds that had been found to be effective as antioxidants was quercetin [34]. The antioxidant activity of quercetin was very strong because of its ability to capture free radicals. This was because quercetin had a phenolic hydroxyl group which was able to form a new radical. Flavonoids have antioxidant activity of reactive oxygen species and free radicals and also to strengthen the mucosal defense system through stimulation of gastric mucus secretion [35].

Alkaloids also had potential as an antioxidant and antifungal. Alkaloid, especially indole, had the ability to stop the chain reaction of free radicals efficiently. Radical compounds derived from these amine compounds had a very long termination stage. Other alkaloids that had antioxidants properties were quinolone, that could act as a buffer hydroxyl radicals and melatonin [36, 37]. Quinolone played an important role on keeping cells from the effects of radiation and toxicity of drugs. Alkaloids which had antimicrobial activities were cryptolepine. Alkaloid compounds could inhibit the synthesis of nucleic acids and ergosterol of *C. albicans* [38-40]. Ergosterols were a component of *C. albican* plasma membrane. Ergosterols played a role in the formation of chitin which were polysaccharide component of the cell wall and had an important role in the germination of *C. albicans*. Triterpenoids compounds had antifungal activity by affecting the permeability of the cell membrane that could lead to cell membrane lysis [41].

Triterpenoids in the ethanol extract of these combination (C1, C2 and C3) could use as an antioxidant and antifungal. Terpenoids was a triterpenoids class and one of potential antimicrobial. In addition these compounds were widely used to cure skin disorders. Triterpenoids had antifungal properties, insecticide, antibacterial, and antiviral [41].

Group of triterpenoids which affected the antimicrobial was triterpena pentacyclic-Amyrin α and β -Amyrin. This is possible as a protective compound microbial attack [42, 43].

The highest inhibitory zone of the treatments was Combination 1 (AC 36%: CM 36%: AS 28%) 5.44 ± 1.78 mm (medium), but have not significant different compared with C2 (AC 30%: CM 30%: AS 40%) 4.08 ± 0.86 mm (medium) and C3 (AC 25%:CM 40%:AS 35%) 3.05 ± 0.23 mm (medium). The composition of C1 referred to the "Subur Kandungan" herb, while C2 and C3 were variation of "Subur Kandungan" herb composition. This facts showed that the composition and phytochemical content of this herbs supported to increase fertility. MIC and MFC can be used as consideration for determining the concentration of ingredients composition and get the best combination to overcome vaginal discharge which can cause infertility due to *C. albicans*

5. Conclusion

The conclusion of this research were (1) The phytochemical tests results of the combination of *A. calamus*, *C. mangga* dan *A. sativum* (C1, C2 and C3) were alkaloids, flavonoids and triterpenoids. (2) All the combination have antioksidant activity. The highest antioxidant power founded in C2 at 42.76 (very strong), followed by C3 at 47.94 (very strong) and C1 at 61.75 (active). (2). The highest inhibitory zone of the treatments was Combination 1 (AC 36%: CM 36%: AS 28%) 5.44 ± 1.78 mm (medium), but have not significant different compared with C2 (AC 30%: CM 30%: AS 40%) 4.08 ± 0.86 mm (medium) and C3 (AC 25%:CM 40%:AS 35%) 3.05 ± 0.23 mm (medium).The minimum inhibitory concentration (MIC) of C1, C2, and C3 against *C. albicans* at a concentration of 0.39%, while the minimum fungicidal concentration (MFC) at a concentration of 0.78%.

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