The Potential Methanolic Extract of Coffee Leaves (*Coffea Canephora* L.) in Inhibiting Storage Fungi and Yeast

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Abstract: Robusta coffee (*Coffea canephora* L.) leaves contain alkaloids, flavonoids, and phenolics that antifungal activity. This study aims to determine the ability of robusta coffee leaf extract in inhibiting six spesies of postharvest fungi and yeast (*Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus tamarii*, *Penicillium* sp., *Candida albicans* and yeast). The leaves were obtained from farmers at Berastagi, Karo Regency, North Sumatra. The extract was obtained by maceration using methanol. Antifungal activity was determined using disc diffusion method at concentrations 20, 40, 60, 80 and 100%. The minimum inhibitory concentrations were determined by dilution method at concentrations 20, 40, 60, 80, 85, 90, and 95%.The positive control used was ketoconazole 2% and negative control dimethyl sulfoxide 10%. Results showed coffee leaves extract has anti-fungal activity againts storage fungi and yeast which were characterized by an inhibition zone from 9.27 to 0.62 mm. The most effective extract at concentration 100% with the highest inhibition 9.27 mm againts *A. oryzae*. Whereas, the minimum inhibitory concentration was at 85% againts *A. oryzae* and *C. albicans.*

1 INTRODUCTION

Storage fungi that contaminate agricultural products become a serious problem particularly on tropical countries. The ability of the fungi to grow at low moisture content might increase their population during storage and it cause spoilage and mycotoxin contamination (Pitt and Hocking 2009; Bala 2017). *Aspergillus* and *Penicillium* were storage fungi that commonly found contaminate food and foodstuffs during storage (Pitt and Hocking 2009).

The used of chemical substances such as phosphine and ethylene oxyde gas (EtO) as fumigants were effective to inhibit fungal growth, however, the substances are carcinogen when inhaled and it leaves harmful chemical residues. Coffee leaves contain caffeine, alkaloids, saponin, flavonoid, phenolic, terpenoid and tannin (Hasanah 2017).

Nartowicz (1979) reported that caffeine was the most predominant compound in coffee. This compound inhibit *A. flavus*, *A. parasiticus, Penicillium citrinum* and *P. urticeae*. The other components such as phenol, trigonelline and chlorogenic acid were reported has antifungal activity (Fardiaz, 1995; Karou et al. 2005)). The potential of coffee leaves extract (*Coffea canephora*) in inhibiting *C. albicans* was reported by Erisha (2017) and Putri (2017). The purpose of the recent study was to investigate robusta coffee leaves extract (*Coffea canephora*) in inhibiting storage fungi and yeast.

2 MATERIALS AND METHOD

2.1 Preparation of Fungal Isolates

The experiment was conducted from April to October 2019 at Microbiology and Biotechnology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. All fungi isolates used in this experiment were culture collection of Microbiology and Biotechnology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. All fungal isolates were previously isolated from dried-stored spices that sold by retailers at traditional markets in Medan, North Sumatera. The fungal isolates were were sub-cultured in potato dextrose agar (PDA) and incubated at 29^{o}C for 5 days.

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2.2 Extraction of Coffee Leaves

As much as ten kilogram of fresh coffee leaves (*Coffea canephora*) obtained from subsistence farming at Berastagi, North Sumatera. All of the fresh leaves were air dried for 4 days and powdered using RT 04 Mill Powder Tech. Co LTD Taiwan at 25.000 rpm for 30 second and 2000 g simplicia obtained were extracted in methanol 1:2 (w/v,). Homogenization was conducted every 1×24 hour for 3×24 hours. The suspension was filtered using Whatman filter paper no.1, the residu obtained was add by fresh methanol several times until clear filtrate was obtained. Filtrate then was air-dried by rotavapor 100 rpm at 50ºC. The extract concentrations made were 100, 80, 60, 40 and 20%, dimethyl sulfoxide 10% used as a solvent.

2.3 Determination of Fungal Spore Density

All isolates of fungi subculturing on potato dextrose agar plates (5 days at 28°C) in petri dish (diameter 9 cm). Each petri was added by 10 ml distilled water containing 0.05% Tween 80. The spores then were harvested using small brush. The spore suspension used was made 10^6/ml.

2.4 Determination of Inhibition of Coffee Leaves Extract on Storage Fungi and Yeast

Well diffusion method was used in this experiment. Paper disc (5 mm in diameter) containing 10 µl extract concentration was placed in petri dish (9 cm in diameter) containing 15 mL potato dextrose agar plate. Each fungal isolate was tested by inoculating agar plug. Ketoconazole 2% and dimethyl sulfoxide 10% were used as positive and negative control respectively. All plates were incubated at 27°C for 5 days. Three replicates were used for each concentration.

2.5 Determination of Minimum Inhibitory Concentration

Various extract concentrations (20, 40, 60, 80, 85, 90 and 95% (w/v) were made. Ketoconazole 2% and dimethylsulfoxide 10% were used as positive and negative contol respectively. One mililiter of each extract was mixed homogenously with 1 ml spore suspension (10^6/ml), Serial dilution made was 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} One mililiter of each dilution was pour plate in petri dish containing PDA medium. All plates were incubated at 27°C for 5 days, Three replicates were made for each concentration. The mycelial growth on each concentration was observed.

3 RESULTS AND DISCUSSION

3.1 Inhibition of Coffee Leaves Methanolic Extract on Mycelial Growth of Storage Fungi and Yeast

Coffee leave extract potential to inhibit mycelial growth of storage fungi and yeast (Table 1). In general, the effect concentration of coffee leave extract on fungal growth showed each fungal species has different response. High extract concentration is followed by the increase of inhibition area. Among storage fungi, *Aspergillus oryzae* was the most inhibited followed by *A. tamarii*, and *A. niger*. Whereas, *A. candidus* and yeasts were the less sensitive (< 5 mm).. In our research found that yeast particularly *C. albicans* begin to inhibit at 20% extract, The highest inhibition (2.93 mm) on yeast occured at 100% extract. We assumed that cell wall of asexual yeast might resist on the extract exposure, as previous study by Jawetz et al. (2005) reported that yeast cells with asexual chlamidospores have resist on antifungi.

3.2 Minimum Inhibitory Concentration of Coffee Leaves Methanolic Extract on Mycelial Growth of Postharvest Fungi

Minimum inhibitory concentration of the extract to all fungal species was shown in Table 2. As shown in Table 2 each fungal species have different minimum inhibitory concentration.

Table 1: Inhibition zone (mm) of coffee leaves methanolic extract (%) againts storage fungi and yeast

Fungal isolates	Extract concentration (%) / inhibition zone (mm)							
	DMSO (K-)	Ketoconazole (K+)	20	40	60	80	100	Average
Aspergillus candidus	0	4.21	0.62	0.84	1.40	1.70	2.57	1.42
A. flavus	0	6.60	1.80	2.90	3.70	3.70	4.90	3.40
A. fumigatus	0	5.70	1.90	3.10	3.40	4.40	5.40	3.64
A. niger	0	9.13	2.40	3.10	5.60	6.60	7.60	5.06
A. oryzae	0	10.8	5.10	6.30	7.10	8.70	9.27	7.29
A. tamarii	0	9.60	4.10	6.20	7.80	6.80	8.10	6.60
Candida albicans	0	3.20	2.00	2.13	2.20	2.40	2.73	2.29
Penicillium sp.	0	8.87	0.80	2.10	3.80	5.50	6.50	3.74
Yeast	0	3.53	2.17	2.36	2.40	2.50	2.93	2.47

K - negative control, K + positive control

Table 2: Minimum inhibitory concentration methanolic extract of coffee leaves on mycelial growth of storage fungi and yeast

Extract (%)	Fungal species/Minimum inhibitory concentration of coffee leave extract								
	A. candidus	*A. flavus*	*A. fumigatus*	*A. niger*	*A. oryzae*	*A. tamarii*	*Candida albicans*.	*Penicillium* sp.	yeast
K-	+	+	+	+	+	+	+	+	+
K+	-	-	-	-	-	-	-	-	-
100	+	-*	-*	+	-	+	-	+	+
95	-*	+	+	+	-	+	-	+	+
90	-	+	+	+	-	+	-	+	+
85	-	+	+	+	-*	+	-*	+	+
80	+	+	+	+	+	+	+	+	+
60	+	+	+	+	+	+	+	+	+
40	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+

K (-) spore suspension as negative control
K (+) ketoconazole as positive control
\+ mycelial growth occurred
\- no mycelial growth
* minimum inhibitory concentration

In compare to ketoconazole that kill all fungal mycelia, the presence of extract at 100% concentration was potential to kill *A. flavus*, *A. fumigatus*, *A. oryzae* and *C. albicans*. The extract has no effect both on *Penicillium* sp. and yeast. The other fungal species require minimum inhibitory concentration to inhibit their grow such as *A. candidus* (95%), *A. oryzae* (85%), *C. albicans* (85%). Previous study by Handayani and Purwoko (2008) showed similar results that the presence of coffee leaf extract inhibit fungal mycelia.

4 CONCLUSION

Methanolic extract of coffee leave inhibit the growth storage fungi and yeast. However, the inhibition and minimum inhibitoory concentration was different depend on the fungal species.

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