The Effect of Basil Leaves Extract (*Ocimum Sanctum* L.) on Mycelial Growth of Postharvest Fungi

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Abstract: Basil (*Ocimum sanctum* L.) contain secondary metabolites which inhibit fungal grow. This research aims to investigate methanolic extract of basil leaves against postharvest fungi. Dried basil leaves were extracted and macerated using methanol. The extract was stored for 3×24 hours at room temperature and homogenized for 24 hours. The homogenise was filtered using whatman filter paper and evaporated at 50 ºC. The thick extract was made a serial concentration 100, 80, 60, 40 and 20 % by dimethylsulfoxide 10%. The postharvest fungi used were *Aspergillus oryzae*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus tamarii*, *Aspergillus niger*, *Aspergillus candidus*, and *Penicillium* sp. The minimum inhibitory concentration was determined by liquid dilution. Ketoconazole 2% and dimethyl sulfoxide were used as positive and negative control respectively. Results showed that basil leaves with concentration 100, 80, 60, 40 and 20% inhibit fungal growth, the highest inhibition occurred on *Aspergillus tamarii* with inhibition 8.7; 8.0; 7.2; 6.0; 5.0 mm respectively. The lowest inhibition occurred on *Penicillium* sp. with inhibition 2.5; 2.0, 1.8; 0.0 mm respectively.

1 INTRODUCTION

Basil (*Ocimum sanctum* L.) is one of the traditional herbs that can be used as antifungi. Solikhah (2015) reported that the antifungi as secondary metabolites were accumulated on leave. Sopianti and Sary (2018) stated that secondary metabolites on basil were alkaloid, saponin, tannin, flavonoid, steroid and eteris oil. Previous study by Omay et al. (2017) reported the potential of basil extract to inhibit *Candida albicans*. Whereas, Berlian et al. (2016) used the leave extract to inhibit *Fusarium oxysporum*. Basil leave extract in inhibiting postharvest fungi such as *Aspergillus niger*, *Fusarium solani*, *Penicillium funicolusum*, *Trichoderma reesei* was reported by Dharmagadda et al. (2005) and Bansod and Rai (2008). Postharvest fungi commonly infect crops and dried stored spices. The used of chemical compounds such as fungicide or fumigants to control fungal infection on agricultural products was expensive and the chemical residues were harmful for human health. The aim of the recent study was to determine the potential of leave basil extract to inhibit mycelial growth of postharvest fungi.

2 MATERIALS AND METHOD

2.1 Preparation of Fungal Isolates

Postharvest fungi used in this experiment were culture collection of Microbiology and Biotechnology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. All of the storage fungi were isolated from dried-stored spices. The fungal isolates were subcultured in potato dextrose agar (PDA) and incubated at 29ºC for 5 days.

2.2 Extraction of Basil Leaves

Ten kilogram of fresh basil leaves were air dried for 4 days and groud using RT 04 Mill Powder Tech. Co LTD Taiwan at 25.000 rpm for 30 second, and 756 g of the simplicia were extracted in 6 L methanol for 3×24 hours. The homogenate was filtered using Whatman filter paper no.1, the filtrate then was evaporated by rotavapor 100 rpm at 50ºC. The extract concentrations used were 100, 80, 60, 40 and 20%, dimethyl sulfoxide 10% used as a solvent.

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2.3 Determination of Basil Leaves Extract in Inhibiting Fungal Growth

Paper disc (5 mm in diameter) containing 10 µl of extract in petri dish (9 cm in diameter) containing 10 mL potato dextrose plate agar. Each fungal isolate was tested by inoculating agar plug containing fungal mycelia. 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.

2.4 Determination of Minimum Inhibitory Concentration of Basil Leaves Extract

Concentration extract used for minimum inhibitory experiment was 20, 40, 60, 80, 85, 90, 95 and 100%. Ketoconazole 2% and dimethylsulfoxide 10% were used as positive and negative control respectively., One mililiter of fungal spore suspension (10^8/ml) then was mixed with 1 ml of each extract concentration, the mixture thes was.homogenized and dilute by a serial dilution until the concentration 10^4 cfu/ml. Each 1 ml thes was pour plate in petri dish containing. PDA. Each treatment was replicate 3 times All plates were incubated at 27°C for 5 days.

3 RESULTS AND DISCUSSION

3.1 Inhibition Zone of Basil Leaves Methanolic Extract on Mycelial Growth of Postharvest Fungi

Leave extract of basil leaves potential to inhibit mycelial growth of postharvest fungi (Table 1). As shown in Table 1 all fungal species were affected by the presence of extract, however, each of the species has different inhibition zone. The higher extract concentration is followed by the increasing of inhibition. Among the postharvest fungi, *Aspergillus tamarii* was the most affected by basil extract (6.98 mm) followed by *A. candidus* (6.48 mm). Whereas, *Penicillium* sp. was the less affected (1.26) followed by *A. oryzae* and unknown yeast (2.58 mm).

Fardiaz (1989) stated that the ability of antifungal compounds in inhibiting mycelial growth was determined by concentration of the compound and fungal species. The range of inhibition zone indicate that basil leave extract has mild to low potential in inhibiting storage fungi. We assumed that the use of crude extract lowering antifungal compounds. Davis and Stout (1971) reported that the effect of antifungi on mycelial growth as indicated by inhibition zone with mild (5 and 10 mm in diameter) and low inhibition (0 to 4 mm in diameter). The presence of chemical compounds in the basil extract might inhibit mycelial growth. Previously studied by Kadian and Parle (2012) reported that eteris oil in basil leaves contain methyl chavicol and linalool that has antifungal activity, the compounds affect on ergosterol in fungal cell membrane.

Table 1: Inhibition zone (mm) of basil leaves methanolic extract (%) againts postharvest fungi

Fungal isolates	Extract concentration (%) / inhibition zone (mm)							
	K-	K+	20	40	60	80	100	Average
Aspergillus oryzae	0	5.2	1.8	2.1	2.6	3.0	3,4	2.58
A. flavus	0	7.0	4.0	5.0	5.3	5.7	6,6	5.32
A. niger	0	5.5	2.9	3.0	3.7	4.0	4,2	3.56
A. candidus	0	8.0	5.5	5.8	6.6	7.0	7,5	6.48
A. fumigatus	0	6.0	2.8	3.0	3.2	3.6	4,0	3.32
A. tamarii	0	9.5	5.0	6.0	7.2	8.0	8,7	6.98
Penicillium sp.	0	3.0	0.0	0.0	1.8	2.0	2,5	1.26

Table 2: Minimum inhibitory concentration methanolic extract of basil leave on the growth of postharvest fungi

Extract (%)	Fungal species/mycelial growth						
	A. oryzae	*A. flavus*	*A. niger*	*A. candidus*	*A fumigatus*	*A. tamarii*	*Penicillium* sp.
K-	+	+	+	+	+	+	+
K+	-	-	-	-	-	-	-
100	+	-	+	-	+	-	+
95	+	-	+	-	+	-	+
90	+	-	+	-	+	-	+
85	+	+	+	-	+	-	+
80	+	+	+	+	+	+	+
60	+	+	+	+	+	+	+
40	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+

Ruijter et al. (2004) reported that xerophilic fungi accumulate polyol such as glycerol, eryhritol, and arabitol to maintain their grow in low water activity. Whereas, the effect of tannin in basil leaves was reported by Ajizah (2004), the compound reduce membrane permeability.

3.2 Determination of Minimum Inhibitory Concentration of Basil Leaves Extract

Minimum inhibitory concentration of the extract to all fungi was shown in Table 2. Basil leave with concentration lower than 85 % has no effect on all fungi tested. The minimum inhibitory of the extract begin to inhibit fungal mycelia at concentration 85% particularly on *Aspergillus candidus* and *Aspergillus tamarii*. Some species of fungi such as *A. oryzae*, *A. niger*, *A. fumigatus* and *Penicillium* have no effect, even at the highest concentration (100%).

4 CONCLUSION

Basil leaves extract reduce mycelial growth of postharvest fungi, however, the potential of the extract in inhibiting the fungal growth was different for each species.

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