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Screening for Endophytic Fungi from Turmeric Plant (*Curcuma longa* L.) of Sukabumi and Cibinong with Potency as Antioxidant Compounds Producer

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ABSTRACT
Potency of medicinal plant is related to microorganisms lived in the plant tissue. Those microorganisms are known as endophytic microbes that live and form colonies in the plant tissue without harming its host. Each plant may contains several endophytic microbes that produce biological compounds or secondary metabolites due to co-evolution or genetic transfer from the host plant to endophytic microbes. Endophytic fungi research done for turmeric plant (*Curcuma longa* L.) gave 44 isolated fungi as results. Those 44 fungi isolated were fermented in Potato Dextrose Broth (PDB) media, filtered, extracted with ethylacetate and then were analyzed by Thin Layer Chromatography (TLC) method and tested for their antioxidant activity by radical scavenging method. The antioxidant activity of the ethylacetate filtrate extracts either from Sukabumi or Cibinong were higher than the biomass extracts. There were 6 fungi that showed antioxidant activities over 65%, i.e., with code name K.Cl.Sb.R9 (93.58%), K.Cl.Sb.A11 (81.49%), KCl.Sb.B1 (78.81%), KCl.Sb.R11 (71.67%) and K.Cl.Sb.A12 (67.76%) from Sukabumi and K.Cl.Cb.U1 (69.27%) from Cibinong. These results showed that bioproduction by endophytic microbes can gave potential antioxidant compounds.

Key words: Turmeric, *Curcuma longa* L., endophytic fungi, antioxidant, DPPH

INTRODUCTION

Development of biotechnology sciences allow researchers to begin utilizing microbes to produce active compounds. One of them that oftenly studied is endophytic microbes. Endophytic microbes are microbes that live in the plant tissues without harming or endangering their host (Strobel and Daisy, 2003). Endophytic microbes including endophytic fungi can be isolated from plant tissues and grown in fermentation medium and produce identical bioactive compounds with them (Lin *et al*., 2007). This phenomenon started wide studies for the possibility of genetic transfer between plant host and its endophytic fungi that enable to get bioactive compounds produced by plant host from its endophytic fungi. This can lead to enhance production process to be easier, simpler and cheaper without damaging the environment. Development and exploitation of endophytic microbes in industrial process will be a valuable capital for development and advance progress of sciences and technologies in Indonesia (Syarmalina *et al*., 2007).

Turmeric (*Curcuma longa* L.) is one of the specie and medicinal plants widely grown in tropic area, including Indonesia. It has been used in herbal or modern medicines for medication to some ailment such as digestion disorder, antitumor, antioxidant and ant-cholesterol.

Previous research for isolation and purification of endophytic fungi associated with turmeric plant (*Curcuma longa* L.) from Sukabumi and Cibinong obtained 44 endophytic fungi. The aims of this study were to screen and select endophytic fungi that have ability to produce antioxidant compound from those 44 isolates using free radical scavenging method (Yan and Chen, 1995).

MATERIALS AND METHODS

Collection of specimen: Endophytic fungi used in this study were obtained from the culture collection that isolated from several parts of turmeric plant from Sukabumi (39 isolates) and Cibinong (5 isolates) i.e., roots (A), Stems (B), Rhizomes (R), Tubers (U) and Young Tubers (BU).

Instruments used including Laminar Air Flow (LAF) chamber, shaker incubator, vacuum rotavapor, waterbath, UV-Vis Spectrophotometer.

Process involved in screening

Fermentation: Each of 44 fungi were fermented in flasks contained 500 mL Potato Dextrose Broth (PDB) media on shaker incubator at 150 rpm for 3 days.

Filtration and extraction: The broth cultures were filtered by a filter under vacuum condition to separate filtrates and biomass. The filtrates were extracted with ethylacetate. The biomass were dried in oven at 50°C to dry optimally and the dried biomass then extracted with ethylacetate. Each extract were dried using vacuum rotavapor.

Thin Layer Chromatography (TLC) analysis: The mixture of chloroform and methanol (9:1) and n-hexane and ethylacetate (5:1) as mobile phase and silica gel 60 F_{254} TLC plate as stationary phase. Cerium sulphate was used as reagent to get visualized spots.

Antioxidant activity test using DPPH (Yan and Chen, 1995): Samples of fungi at 100 ppm concentration were reacted with 0.4 mM DPPH (1,1-diphenyl-1-picrylhydrazyl) solution in tubes. The mixture were incubated in waterbath at 37°C for 30 min. The absorbances were measured at 515 nm using UV-Vis Spectrophotometer.

RESULTS AND DISCUSSION

Extraction: Weight of ethylacetate extracts from filtrate and biomass obtained from extraction process are shown in Table 1.

The results showed that only 2 out of 12 filtrate extracts of endophytic fungi isolated from turmeric stem from Sukabumi have more weight from biomass extracts. This results also seen in endophytic fungi isolated from turmeric root that have only 2 out of 16 filtrate extracts that have more weight from biomass extracts. Different results seen in endophytic fungi isolated from turmeric rhizome that 6 out of 11 filtrate extracts have more weight than biomass extracts. On the other hand, endophytic fungi isolated from Cibinong showed that filtrate extracts have more weight than biomass extracts. Nevertheless, biomass extracts of all endophytic fungi isolated from turmeric plant from Sukabumi and Cibinong have more weight than filtrate extracts generally. This result in line with Dompeipen *et al.* (2011), who report that biomass extracts of endophytic fungi isolated from *Swietenia mahagoni*, *Orthoshipon spicatus* and *Piper* sp. have more weight than filtrate extracts. This may cause the ethylacetate biomass extract contain fatty acids which have relatively great molecular weight in biomass that extracted by ethylacetate. Ethylacetate as non-polar solvent dissolves many hydrophobic compounds such as fatty acids (Harborne, 1984).

TLC analysis: Based on the TLC analysis, the extracellular bioproduction (filtrate extracts) gave richer chemical compounds than intracellular bioproduction (biomass extracts). Biomass extracts mostly contain fatty acids as major compounds. Filtrate extracts contain more secondary metabolites as secretion result of microbes metabolism.

Antioxidant activity: Both filtrate and biomass ethylacetate extracts of endophytic fungi from turmeric plant from Sukabumi and Cibinong have antioxidant activity (Table 2). Endophytic fungi isolated from *Zingiber zerumbet* as

Table 1: Weight of ethylacetate extracts from filtrate and biomass obtained from extraction process

		Extract weight (g)				Extract weight (g)	
No.	Sample code	Biomass	Filtrate	No.	Sample code	Biomass	Filtrate
1	K.Cl.Sb.B1	0.4386	0.0303	23	K.Cl.Sb.R11	0.1568	0.0989
2	K.Cl.Sb.B2	0.3873	0.0802	24	K.Cl.Sb.A1	0.1185	0.0401
3	K.Cl.Sb.B3	0.5537	0.0697	25	K.Cl.Sb.A2	0.1911	0.0629
4	K.Cl.Sb.B4	0.0442	0.0352	26	K.Cl.Sb.A3	0.2390	0.0818
5	K.Cl.Sb.B5	0.4854	0.0414	27	K.Cl.Sb.A4	0.2061	0.0740
6	K.Cl.Sb.B6	0.2403	0.0390	28	K.Cl.Sb.A5	0.1005	0.0567
7	K.Cl.Sb.B7	0.0335	0.0357	29	K.Cl.Sb.A6	0.1774	0.0435
8	K.Cl.Sb.B8	0.3972	0.0581	30	K.Cl.Sb.A7	0.1261	0.0363
9	K.Cl.Sb.B9	0.6547	0.0685	31	K.Cl.Sb.A8	0.1766	0.0796
10	K.Cl.Sb.B10	0.1104	0.0391	32	K.Cl.Sb.A9	0.2368	0.0606
11	K.Cl.Sb.B11	0.0066	0.0419	33	K.Cl.Sb.A10	0.1054	0.0684
12	K.Cl.Sb.B12	0.2263	0.0505	34	K.Cl.Sb.A11	0.0555	0.0855
13	K.Cl.Sb.R1	0.0064	0.0227	35	K.Cl.Sb.A12	0.0242	0.0296
14	K.Cl.Sb.R2	0.0160	0.0378	36	K.Cl.Sb.A13	0.1465	0.0765
15	K.Cl.Sb.R3	0.0136	0.0654	37	K.Cl.Sb.A14	0.2143	0.0498
16	K.Cl.Sb.R4	0.0338	0.0353	38	K.Cl.Sb.A15	0.0198	0.0514
17	K.Cl.Sb.R5	0.0348	0.0232	39	K.Cl.Sb.A16	0.0239	0.0529
18	K.Cl.Sb.R6	0.0757	0.0125	40	K.Cl.Cb.U1	0.0178	0.1067
19	K.Cl.Sb.R7	0.1551	0.0166	41	K.Cl.Cb.U2	0.0327	0.0362
20	K.Cl.Sb.R8	0.0858	0.0207	42	K.Cl.Cb.Bu1	0.2006	0.0246
21	K.Cl.Sb.R9	0.0124	0.0198	43	K.Cl.Cb.Bu2	0.0335	0.0226
22	K.Cl.Sb.R10	0.0126	0.0481	44	K.Cl.Cb.B1	0.0119	0.0635

Table 2: Inhibition percentage of filtrate and biomass ethylacetate extracts

No.	Fungi code	Inhibition (%)	No.	Fungi code	Inhibition (%)
Filtrate					
1	K.Cl.Sb.R1	26.03	23	K.Cl.Sb.A12	67.76
2	K.Cl.Sb.R2	62.11	24	K.Cl.Sb.A13	50.38
3	K.Cl.Sb.R3	60.77	25	K.Cl.Sb.A14	48.74
4	K.Cl.Sb.R4	63.20	26	K.Cl.Sb.A15	57.68
5	K.Cl.Sb.R5	56.17	27	K.Cl.Sb.A16	42.82
6	K.Cl.Sb.R6	55.33	28	K.Cl.Sb.B1	78.81
7	K.Cl.Sb.R7	38.26	29	K.Cl.Sb.B2	24.21
8	K.Cl.Sb.R8	52.78	30	K.Cl.Sb.B3	33.41
9	K.Cl.Sb.R9	93.58	31	K.Cl.Sb.B4	34.32
10	K.Cl.Sb.R10	57.99	32	K.Cl.Sb.B5	54.24
11	K.Cl.Sb.R11	71.67	33	K.Cl.Sb.B6	42.49
12	K.Cl.Sb.A1	62.22	34	K.Cl.Sb.B7	40.07
13	K.Cl.Sb.A2	46.22	35	K.Cl.Sb.B8	33.29
14	K.Cl.Sb.A3	54.03	36	K.Cl.Sb.B9	22.52
15	K.Cl.Sb.A4	25.57	37	K.Cl.Sb.B10	30.23
16	K.Cl.Sb.A5	47.86	38	K.Cl.Sb.B11	42.57
17	K.Cl.Sb.A6	58.69	39	K.Cl.Sb.B12	48.87
18	K.Cl.Sb.A7	48.99	40	K.Cl.Cb.U1	69.27
19	K.Cl.Sb.A8	63.85	41	K.Cl.Cb.U2	29.02
20	K.Cl.Sb.A9	47.48	42	K.Cl.Cb.Bu1	42.86
21	K.Cl.Sb.A10	51.01	43	K.Cl.Cb.Bu2	31.45
22	K.Cl.Sb.A11	81.49	44	K.Cl.Cb.B1	54.18
Biomass					
1	K.Cl.Sb.R1	7.94	23	K.Cl.Sb.A12	4.65
2	K.Cl.Sb.R2	30.98	24	K.Cl.Sb.A13	1.89
3	K.Cl.Sb.R3	12.64	25	K.Cl.Sb.A14	4.66
4	K.Cl.Sb.R4	15.10	26	K.Cl.Sb.A15	17.00
5	K.Cl.Sb.R5	3.24	27	K.Cl.Sb.A16	14.61
6	K.Cl.Sb.R6	4.36	28	K.Cl.Sb.B1	38.37
7	K.Cl.Sb.R7	5.15	29	K.Cl.Sb.B2	4.81
8	K.Cl.Sb.R8	0.00	30	K.Cl.Sb.B3	8.84
9	K.Cl.Sb.R9	10.18	31	K.Cl.Sb.B4	21.56
10	K.Cl.Sb.R10	25.17	32	K.Cl.Sb.B5	0.89
11	K.Cl.Sb.R11	36.69	33	K.Cl.Sb.B6	0.89
12	K.Cl.Sb.A1	5.56	34	K.Cl.Sb.B7	12.98
13	K.Cl.Sb.A2	9.95	35	K.Cl.Sb.B8	8.05
14	K.Cl.Sb.A3	2.71	36	K.Cl.Sb.B9	7.94
15	K.Cl.Sb.A4	6.17	37	K.Cl.Sb.B10	1.16
16	K.Cl.Sb.A5	3.10	38	K.Cl.Sb.B11	3.10
17	K.Cl.Sb.A6	1.42	39	K.Cl.Sb.B12	7.11
18	K.Cl.Sb.A7	2.90	40	K.Cl.Cb.U1	23.99
19	K.Cl.Sb.A8	4.26	41	K.Cl.Cb.U2	31.27
20	K.Cl.Sb.A9	3.53	42	K.Cl.Cb.Bu1	23.54
21	K.Cl.Sb.A10	2.27	43	K.Cl.Cb.Bu2	20.75
22	K.Cl.Sb.A11	35.40	44	K.Cl.Cb.B1	22.82

Zingiberacean plant which one family with turmeric reported have antioxidant activity (Nongalleima *et al*., 2013). Antioxidant activity of filtrate ethylacetate extracts of endophytic fungi isolated from turmeric rhizome from Sukabumi showed the highest with 93.58% inhibition (Kcl.Sb.R9) and endophytic fungi isolated from turmeric stem showed the lowest value with only 22.52% inhibition (KCl.Sb.B9). Average inhibition of filtrate ethylacetate extracts of endophytic fungi from all parts of turmeric plant from Sukabumi showed that rhizome organ was the highest value (57.99%), followed by root and stem with 53.42 and 40.42% respectively. Antioxidant activity of biomass ethylacetate extracts of endophytic fungi isolated from turmeric stem from Sukabumi showed the highest value with 38.37% inhibition (KCl.Sb.B1) and endophytic fungi isolated from turmeric rhizome showed the lowest value with no inhibition (KCl.Sb.R8). Average inhibition of biomass ethylacetate extracts of endophytic fungi from all parts of turmeric plant from Sukabumi showed that rhizome organ was the highest (13.77%), followed by stem and root with 9.64 and 7.51%, respectively.

Based on antioxidant activity both filtrate and biomass ethylacetate extracts, the isolate from Sukabumi have the highest than isolate from Cibinong. This may caused by the influences of environmental differences and other ecological conditions where those fungi live. This conditions gave effects to chemical compounds they produced. For example, curcuminoid content in turmeric plant, as host of endophytic fungi in this study, is diverse depend on their varieties, location and growth condition (Li *et al*., 2011).

Based on the results, extracellular bioproduction (filtrate extracts) gave higher antioxidant activity than intracellular bioproduction (biomass extracts), either for endophytic fungi from Sukabumi or Cibinong. As supported by TLC analysis data, biomass extracts mostly contain fatty acids that less active as antioxidant. Filtrate extracts contain many secondary metabolites that may act as protection for the endophytic fungi of host plant (Theantana *et al*., 2007). There were 6 fungi that showed antioxidant activity over 65% i.e., KCl.Sb.R9 (93.58%), KCl.Sb.A11 (81.49%), KCl.Sb.B1 (78.81%), KCl.Sb.R11 (71.67%) and KCl.Sb.A12 (67.76%) from Sukabumi and Kcl.Cb.U1 (69.27%) from Cibinong (Table 2).

CONCLUSION

Based on the test results, six isolated endophytic fungi found that have potential antioxidant activity with inhibition percentage more than 65%.

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