LC-MS Based Metabolite Profiling of Ethanolic Extracts from *Curcuma domestica* Val. varieties Turina-1

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Abstract

It is important to know the metabolite compounds profile of Curcuma domestica Val. varieties Turina-1, as one of the superior varieties of turmeric, so that it can be utilized better. Therefore, the purpose of this research is to study the metabolite profile of ethanol extract of Curcuma domestica Val. Varieties Turina-1. The samples for this research were obtained from BPPT Bogor-Indonesia. These samples were extracted using ethanol (96 %) and then analyzed using UPLC-QToF-MS / MS System (Waters), mass spectrometry: XEVO-G2QTOF (Waters), in ESI positive resolution mode, using gradient method with mobile phase: water, formic acid and acetonitrile. The study found 13 metabolite compounds: Demethoxycurcumin-2 (48.23%), α-Turmerone (19.623%), Curcumin (18.550%), Bisdemethoxycurcumin-3 (9.064%), Curcumin-1, (1.706%), and compounds others with less than 1% (Kaempferol 3-O-glucosyl-rhamnosyl-galactoside, Demethoxycurcumin, ar-Turmerone Bisdemethoxycurcumin, a-Terpinolene, L-Tyrosine and L-Alanine, L-serine). Based on this research, the main metabolite compound contained in the ethanol extract of Curcuma domestica Val. varieties Turina-1 that has the potential as antioxidants is the curcuminoids.

Keywords: *Curcuma domestica, LC-MS, ethanolic extracts, profile metabolite*

1. INTRODUCTION

Turmeric (*Curcuma domestica* Val) is the second of the four priorities for developing medicinal plants in Indonesia, besides ginger, galangal and galingale. This development priority is due to the increasing demand for medicinal plants including turmeric (Sal sim and Munadi, 2006). The value of turmeric exports is also the second largest after ginger, in the period 2011-2015, Indonesia's turmeric exports to the world experienced an average growth of 27.7% per year. Turmeric exports in 2015 increased sharply by 132.5% to USD 10.5 million (Amiruddin, 2016).

Indonesia continues to develop turmeric varieties with high curcumin content. The Indonesian Institute of Medicinal and Aromatic Crops (Balitro) in Bogor, West Java has produced 3 (three) superior turmeric varieties named Turina-1, Turina-2, and Turina-3 with curcumin content between 7.46-10.86 percent. Based on the results of the quality analysis, the three Turmeric-1 varieties with the highest curcumin content were 7.46-9.86 percent (Bursatriannyo et al; 2016). Based on the Revealed Comparative Advance (RCA) index, Ethiopia and India have the highest competitiveness of turmeric for 2011-2015 (Kanaya and Firdaus, 2012). Ethiopian turmeric has advantages compared to Indian turmeric in terms of curcumin content. Ethiopian turmeric curcumin content is 4%, India is 2%, while Indonesia is higher than Ethiopian and Indian turmeric by 6% (International Trade Center, 2010). The increase turmeric exports in Indonesian is constrained by limited supply. Domestically turmeric is used for household consumption, industrial raw materials and herbal medicine traders, with the number continuing to experience an increasing trend of around 10-25% per year (Amiruddin, 2016). To overcome

the problem of limited supply, then preparing the product in the form of extracts will facilitate supply so that the demand for turmeric can be fulfilled both in quantity and in its superiority.

Turmeric extract (Curcuma domestica Val) consists of three diarylheptanoids namely curcumin (CURC), demethoxy-curcumin (DMC), and bisdemethoxy-curcumin (bisDMC). Demethoxy and bisdemethoxy compounds can amount to nearly 40%. In some turmeric extracts bisdemethoxycurcumin has even proven to be the main constituent (Tønnesen et al). Curcumin is used as a measure of product excellence because it has been shown to display effects as an anti-inflammatory compound (Mukhopadhyay et al, 1982; Srimal and Dhawan, 1982, Rao et al., 1982) and strong antioxidants (Srimal and Dhawan, 1982, Rao et al., 1982), chemopreventive agents (Khafif et al, 1998; Leu and Maa, 2002) and chemotherapy (Woo et al., 2003; Moos et al., 2004). and is considered a substance model for the treatment of HIV infection (Moos et al., 2004; Aggrawal et al., 2007; Mazumder et al., 1997). CURC proved to be significantly more effective than DMC (Mazumder et al., 1997; Sui et al., 1993), which in turn is more effective than bisDMC, as an antioxidant (Nardo et al., 2011).

Curcumin is a strong metal chelating agent and efficient free radical scavenger. Methoxy phenolic substituents are not directly involved in either metal chelation or radical scavenging. DMC is proven to be less effective than CURC, and bisDMC is almost inactive, if it is associated with both biologically relevant activities (Dairam et al., 2007). This study focused on finding out the metabolite profile ethanol extract Turina-1 varieties of turmeric. With known proportions (CURC), demethoxy-curcumin (DMC), and bisdemethoxy-curcumin (bisDMC). then the effectiveness of Turina-1 extract. as in active ingredients that are biologically useful it can be known.

2. MATERIALS AND METHODS

2.1 Material and Equipment

Turmeric seed variety Turina 1 was obtained from BPPT Bogor, planted in experimental gardens in Antap Village, Candi Kuning Bedugul, Tabanan Bali, turmeric was harvested at age of nine months. The chemicals used are ethanol (Brathaco Chemical), LC– MS grade acetonitrile (purity 99.98%), methanol (purity 99.99%), ammonium formate (purity 99.95%) and formic acid (purity 99.98) were purchased from Fluka analytical, Sigma-Aldrich Corporation (St. Louis, MO USA). Water used in the entire analysis was of LC–MS grade. Other chemicals used were of analytical grade obtained from commercial sources.

2.2 Preparation of Turmeric Extract

First, the turmeric was washed, drained and then sliced ± 1 mm, and dried-oven at 55 ± 2 ° C until it reached the water content of a maximum 10%. The dried turmeric were turned into powder and sieved with 80 mesh, then macerated/soaked in ethanol 96% with a ratio of 1:6 for the powder and its solvent. The maceration process was conducted in 2 phases with each phase lasting for 24 hours. During each phase, the mixture was stirred twice. The filtrate was then separated using a rotary evaporator at 40°C and a pressure of 100 m Bar.

2.3 Research and Analysis

Identification of turmeric extract using LC-MS with specifications of UPLC-QToF-MS / MS System (Waters), processing data using Mass Lynk version software. Experimental conditions: LC Acquity UPLC BEH C.18 1.7µm, 2.1x50 mm. Setting the tool temperature to 40ºC, flow rate 0.3 mL / min, sample injection: 5 uL. The mobile phase is, water, formic acid and acetonitrile, with the gradient method as listed in Table 1. Mass spectroscopic conditions are as follows: XEVO - G2QTOF (Waters), the separation model is (ESI model) with the following conditions: 3 kV capillary voltage, sample voltage 38 V, desolvation temperature 300ºC, carrier temperature 110ºC, gas velocity separation of 500 L/hour and gas cone speed of 16 L/hour. Identification is done by comparing the molecular weight of the compound with the data in the system bank using phenol explorer and some literature in the journals.

Table 1. The Gradient Method Used in Separating Turmeric Extract

The gradient method	Mobile phase	
time (min)	%A	%B
0	95	5
1	95	5

6	0	100
7	0	100
7.5	95	5
9	95	5

Note : A : H_2O +0.1% formic acid B : acetonitrile + 0.1% formic acid

3. RESULTS

Based on LC-MS (Liquid Chromatography Mass Spectroscopy) test, turmeric extract contains 13 constituent components as stated in the chromatogram. Figure 1. Thirteen components are then identified to determine the components contained therein. identification results are presented in Table 2. Components identified were sourced from turmeric extract, namely: Demethoxycurcumin-2 (48.23%). α-Turmerone (19,623%). Curcumin (18,550%). Bisdemethoxycurcumin-3 (9,064%). Curcumin-1. (1,706%). others with less than 1% compounds (Kaempferol 3-O-glucosyl-rhamnosyl-galactoside. Demethoxycurcumin. ar-Turmerone Bisdemethoxycurcumin. a-Terpenole. L-Tyrosine and L-Alanine. L-serine). The results of component identification in this study are in accordance with the research of Herebian et al (2009).

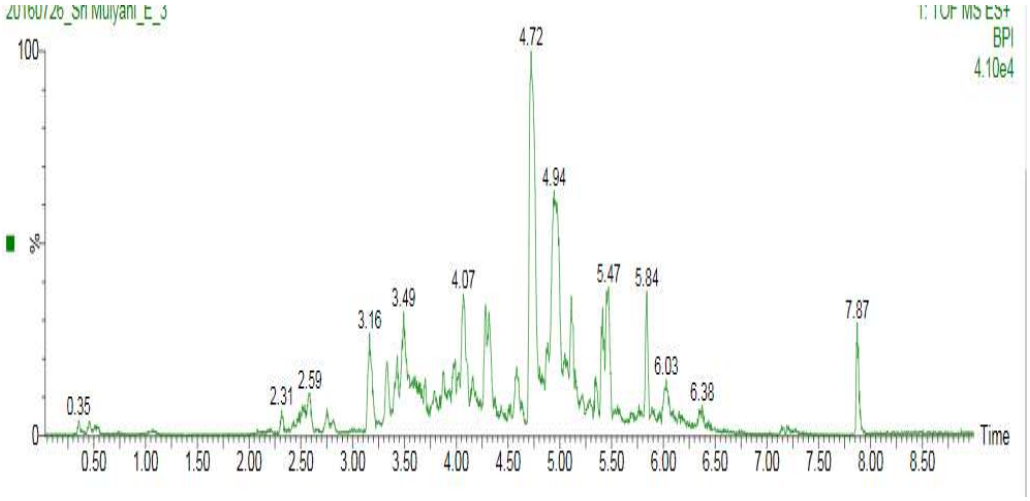

Figure 1. LC-MS chromatogram of extract turmeric *Curcuma domestica* Val. varieties Turina-1

Table 2. Identify the components of turmeric extract (*Curcuma domestica* Val) varieties Turina-1 by LC-MS

Peak number	Compound	Elemental composition	Measured mass	Calculated mass	Retention Time	Relative area (%)
1	L-serine	C12H31NO3Si3 C9H23NO29H2	321.287	321	0.46	0.006
2	L-Alanine	3NO2Si2	233	233	2.32	0.007
3	a-Terpenolene	C10H16	136.1097	136	2.58	0.124
4	Demethoxycurcumin	C20H19O5	119.10911	119.05024	3.17	0.692
5	Bisdemethoxycurcumin 3	C13H11O3	215.06998	215.06998	3.49	9.064
6	Curcumin	C21H21O6	285.11246	285.08095	4.07	18.550
7	Demethoxycurcumin 2	C8H7O	119.10911	119.05024	4.72	48.223
8	α-Turmerone	C15H22O	218.16652	218.16712	4.94	19.623
9	Curcumin 1	C17H17O4	285.11246	285.08095	5.47	1.706
10	ar-Turmerone	C15H20O	216.15087	216.15104	5.84	0.516
11	Bisdemethoxycurcumin	C19H17O4	309.11213	309.11198	6.03	0.497
12	L-Tyrosine	C15H27NO3Si2	325.192	325	6.38	0.098
13	Kaempferol 3-O-glucosyl-rhamnosyl-galactoside	$C_{33}H_{40}O_{20}$	756.5495	756.1129	7.88	0.895
	Total					100

4. DISCUSSION

Has been identified about 235 compounds of turmeric, especially phenolic and terpenoid compounds. Identified compounds consist of 22 diarylheptanoids and diarylpentanoid, 8 phenylpropene and other phenolic groups. 68 groups of monoterpenes. 109 class of sesquiterpenes. 5 groups of diterpenes. 3 triterpenoid groups. 4 groups of sterols. 2 groups of alkaloids. and 14 other compounds (Herebian et al., 2009) Curcuminoids are a group of diarylheptanoids which are the main bioactive ingredients of turmeric. The most common curcuminoid in turmeric is curcumin and has been consumed for hundreds of years for medicinal purposes. In 2016, the growth of the herbal cosmetics industry reached 30% (Salim and Munadi, 2006), this also had an impact on the world's need for turmeric, because the active ingredient of curcumin was needed as a cosmetic active ingredient.

In this study it was proved that Turina-1 turmeric curcumin content was 7%, with the largest components demetoxycurcumin (48.92%) curcumin (20.26%) and bisemethoxycurcumin (9.56%). These results are consistent with several previous studies that show demetoxycurcumin is the largest and bismethoxycurcumin is the smallest component. Compared to other varieties of turmeric, Turina-1 has a higher content of curcumin. To produce curcumin content > 7% turmeric Turina 1 variety should be harvested at least 9 months of age. The results of the study (Dewi and Hartiati, 2016), showed that increasing the age of turmeric harvest from 9 months to 11 months did not significantly increase the content of curcumin with curcumin content ranging from 7.0 to 7.59%.

The development of Turina-1, Turina-2 and Turina-3 turmeric varieties, is intended in addition to meeting domestic needs for export. Turina-1 curcumin content is the smallest, but the curcumin is higher than the average Ethiopian turmeric content of 4% and India 2% (Salim and Munadi, 2006). This high potency of curcumin needs to be developed in view of an increase in Indonesian turmeric exports to the main destination countries of Indonesian turmeric exports, such as to India whose exports rose 225.3% to USD 8.3 million, Vietnam (up 107.6%), and Argentina (up 162.3%) (Salim and Munadi, 2006). India is interested in supplying wet turmeric from Indonesia with a volume of up to 7,000 tons per month, but this is constrained by the limited supply of Indonesian turmeric (Amiruddin, 2016).

The content of curcumin is important considering the antioxidant effects of each constituent. Although the molecular mechanism is not yet fully understood, it is apparent that antioxidant activity is associated with electrons withdrawal from group keto-enol group to hydroxy-phenolic moieties, substitution of phenolic methoxy also plays an important role (Cai et al., 2006; Jayaprakasha et al., 2006; Chen et al., 2006; Somparn et al., 2007). Curcumin is a powerful metal chelating agent and an efficient radical scavenger. Metal seams occur at the centre keto-enol groups and are strongly affected by the enol proton. Mobility and acidity (Schaich et al., 1994). The DMC has been shown to be less effective than CURC, and bisDMC is almost inactive, with respect to these two biologically relevant activities (Dairam et al., 2007).

Differences between bisDMC and CURC could be ascribed to either the difference in H-bond acceptor/donor properties of the phenolic OH, or the difference in strength of the intramolecular H-bond in the keto-enol moiety. BisDMC undergoes slower or faster deactivation from the singlet state compared to CURC depending on the environment. The photodecomposition seems to be slightly more extensive than what is reported for CURC under the same conditions. Consequently, modification of the CURC molecule by removal of the methoxy substituents does not necessarily improve photostability or photosensitizing potential of the sensitizer. This is consistent with our latest results on the antibacterial phototoxic effect of bisDMC (Haukvik et al., 2011)

5. CONCLUSIONS

A total of 15 metabolites were characterised from ethanolic extracts *Curcuma domestica* Val, varieties Turina-1: Demethoxycurcumin 2 (42,60%), Curcumin 27.44%, α-Turmerone 17,29%, Bisdemethoxycurcumin 3 (7,99%), Curcumin 1(2,28%), compounds less than 1%: ar-Turmerone, Demethoxycurcumin, Bisdemethoxycurcumin, Ribonic acid,

Bisdemethoxycurcumin, a-Terpenolene, L-Tyrosine, L-Alanine, Shikimic acid, Uridine, L-Alanine, L-serine. Metabolite profile major (>80%) of ethanolic extract *Curcuma domestica* Val, varieties Turina-1 is curcuminoid potentially antioxidants.

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