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Influence of adsorbent-arak ratio and distillation period in bioethanol purification process using Balinese liquor as a raw material

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DOI: https://doi.org/10.37855/jah.2021.v23i01.08
Key words: Arak, adsorbent ratio, distillation period, bioethanol
Show Abstract

Metabolite profile of ethanol extract of Curcuma domestica Val. variety Turina-1

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DOI: https://doi.org/10.37855/jah.2021.v23i01.02
Key words: LC-MS, profile metabolite, ethanolic extracts, Curcuma domestica Val, Turina-1
Show Abstract

Determination of key parameters for grading dehusked coconut using principal component analysis

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DOI: https://doi.org/10.37855/jah.2021.v23i01.03
Key words: Coconut, principal component analysis, score plot, grading
Show Abstract
Metabolite profile of ethanol extract of *Curcuma domestica* Val. variety Turina-1

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Abstract

It is important to know the metabolite compounds profile of *Curcuma domestica* Val. variety 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. variety 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), 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-Tumerone (19.623 %), Curcumin (18.550 %), Bisdemethoxycurcumin-3 (9.064 %), Curcumin-1 (1.706 %), and other compounds with amount less than 1 % (Kaempferol 3-O-glucosyl-rhamnosyl-galactoside, Demethoxycurcumin, ar-Tumerone 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. variety Turina-1 that has the potential as antioxidants is the curcuminoids.

Key words: LC-MS, profile metabolite, ethanolic extracts, *Curcuma domestica* Val, Turina-1

Introduction

Turmeric (*Curcuma domestica* Val) is the second of four priorities for the development of medicinal plants in Indonesia. This development priority is due to the increasing demand for medicinal plants including turmeric. (Nugroho and Ningsih, 2017). 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). The development of turmeric varieties with high curcumin content continues. Research Institute for Medicinal and Aromatic Plants (Balitro) in Bogor, West Java has produced 3 (three) superior varieties of turmeric named Turina-1, Turina-2, and Turina-3 with a curcumin content between 7.46-10.86 %. Based on the results of a quality analysis of the three superior varieties of turmeric, the Turina-1 variety had the lowest curcumin content of 7.46-9.86 % (Bursatrianynyo et al., 2014).

Based on the Revealed Comparative Advantage (RCA) index and Export Product Dynamics (EPD) from 2003 - 2012 Indonesia has good competitiveness in turmeric commodity compared to competing countries namely India and Ethiopia (Kanaya and Firdaus, 2015). Ethiopian Turmeric has advantages compared to Indian saffron in terms of curcumin content. Ethiopian turmeric has curcumin content of 4 %, India is 2 %, while Indonesia is higher at 6 % (Saputri, 2017). The increase in Indonesian turmeric exports is constrained by limited supply. Domestically, turmeric is used for household consumption, industrial raw materials and herbal medicine traders, with an amount that continues to experience an upward trend of around 10-25 % per year (Amiruddin, 2016). To overcome the problem of limited supply, preparing the product in the form of extract will facilitate supply so that the demand for turmeric can be fulfilled.

Turmeric extract (*Curcuma domestica* Val) consists of three diarylheptanoids namely curcumin (CURC), demethoxycurcumin (DMC), and bisdemethoxycurcumin (bisDMC). As an antioxidant, curcumin is a strong metal chelating agent and an efficient free radical scavenger (Nardo et al., 2011). The methoxy phenolic substituent is not directly involved in either metal chelation or in radical scavenging. CURC is proven to be more effective than DMC, which is more effective than bisDMC. DMC is proven to be less effective than CURC, and bisDMC is almost inactive, if it is involved with biologically relevant activities (Cai, et al., 2006). Curcumin is used as a measure of research excellence, based on in vitro or in vivo research shows curcumin has antioxidant, anti-inflammatory properties (Zhou, et al., 2011). Turmeric on average has a comparable proportion of DMC and bisDMC contributions of up to nearly 40 %. In trading bisDMC content in turmeric is even proved to be its main constituent (Amiruddin, 2016).

As a new variety developed, it is necessary to know the proportion of the diarylheptanoids of Turina-1 turmeric so that its superiority is known. This study aims to determine the metabolite profile of Turina-1 turmeric varieties ethanol extract. With the known proportions of curcumin (CURC), demethoxycurcumin (DMC), and bisdemethoxycurcumin (bisDMC), the variety of turmeric becomes more competitive.
Materials and method

**Material and Equipment:** Turmeric (*C. domestica* Val.) variety Turina-1 seed was obtained from Badan Pengkajian dan Penerapan Teknologi /BPPT (Agency for the Assessment and Application of Technology) Bogor, Indonesia. Turmeric was planted in experimental gardens in Antap Village, Candi Kuning, Tabanan, Bali, Indonesia. Turmeric was harvested at age of nine months. The chemicals used were ethanol (BrathacoChemical), acetonitrile (Merck), formic acid (Merck), and aquadest.

**Preparation of turmeric extract:** First, the turmeric was washed, drained and then sliced ± 1 mm and dried in an 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 12 h. 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. The endpoint for evaporation process is characterized by ethanol, which is no longer dripping.

**Research and Analysis:** Identification of turmeric extract used LC-MS with specifications of UPLC-QToF-MS / MS System

**Identification of ethanol extract components of turmeric variety Turina-1**

<table>
<thead>
<tr>
<th>Peak number</th>
<th>Compound</th>
<th>Elemental composition</th>
<th>Measured mass</th>
<th>Calculated mass</th>
<th>Retention Time</th>
<th>Relative area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L-serine</td>
<td>C12H31NO3Si3</td>
<td>321.287</td>
<td>321</td>
<td>0.46</td>
<td>0.006</td>
</tr>
<tr>
<td>2</td>
<td>L-Alanine</td>
<td>C9H23NO29H23NO2Si2</td>
<td>233</td>
<td>233</td>
<td>2.32</td>
<td>0.007</td>
</tr>
<tr>
<td>3</td>
<td>α-Terpeneolene</td>
<td>C10H16</td>
<td>136.109</td>
<td>136</td>
<td>2.58</td>
<td>0.124</td>
</tr>
<tr>
<td>4</td>
<td>Demethoxycurcumin</td>
<td>C20H19O5</td>
<td>119.109</td>
<td>119.050</td>
<td>3.17</td>
<td>0.692</td>
</tr>
<tr>
<td>5</td>
<td>Bisdemethoxycurcumin</td>
<td>C13H11O3</td>
<td>215.070</td>
<td>215.070</td>
<td>3.49</td>
<td>9.064</td>
</tr>
<tr>
<td>6</td>
<td>Curcumin</td>
<td>C21H21O6</td>
<td>285.112</td>
<td>285.081</td>
<td>4.07</td>
<td>18.550</td>
</tr>
<tr>
<td>7</td>
<td>Demethoxycurcumin</td>
<td>C19H22O</td>
<td>218.166</td>
<td>218.167</td>
<td>4.72</td>
<td>48.223</td>
</tr>
<tr>
<td>8</td>
<td>α-Turmerone</td>
<td>C18H17O4</td>
<td>285.112</td>
<td>285.081</td>
<td>4.07</td>
<td>19.623</td>
</tr>
<tr>
<td>9</td>
<td>Curcumin 1</td>
<td>C17H17O4</td>
<td>216.151</td>
<td>216.151</td>
<td>5.47</td>
<td>1.706</td>
</tr>
<tr>
<td>10</td>
<td>ar-Turmerone</td>
<td>C17H20O</td>
<td>309.112</td>
<td>309.112</td>
<td>6.03</td>
<td>0.516</td>
</tr>
<tr>
<td>11</td>
<td>Bisdemethoxycurcumin</td>
<td>C15H27NO3Si2</td>
<td>325.192</td>
<td>325</td>
<td>6.38</td>
<td>0.098</td>
</tr>
<tr>
<td>12</td>
<td>L-Phosphobistephenol</td>
<td>C8H7O</td>
<td>756.549</td>
<td>756.113</td>
<td>7.88</td>
<td>0.895</td>
</tr>
</tbody>
</table>

**Note:** A: H2O +0.1 % formic acid   B: acetonitrile + 0.1 % formic acid  (Waters). Processing data used Mass Lynk version software. Experimental conditions: LC Acuity UPLC BEH C.18 1.7µm. 2.1 x 50 mm. Setting the tool temperature to 40 °C, flow rate of 0.3 mL/min. sample injection: 5 µL. The mobile phase were water, formic acid and acetonitrile with the gradient method as listed in Table 1. Mass spectroscopic conditions were as follows: XEVO - G2QTOF (Waters), the separation model is (ESI) model with the following conditions: 3 kV capillary voltage, 38 V sample voltage, desolation temperature of 300 °C, carrier temperature of 110°C, 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.
Metabolite profile of ethanol extract of \textit{Curcuma domestica} Val. variety Turina-1

**Results**

**Metabolite profile in turmeric (C. domestica Val) extract of Turina-1:** Based on LC-MS (Liquid Chromatography Mass Spectroscopy) test, turmeric extract contains 13 constituent components as listed in the chromatogram Figure 1. Thirteen components are then identified to determine the components inside. The identification results are presented in Table 2. The identified components are sourced from ethanol extract of turmeric, namely: Demethoxycurcumin-2 (48.23 %), \( \alpha \)-Turmerone (19.623 %), Curcumin (18.550 %), Bisdemethoxycurcumin-3 (9.064 %), Curcumin-1 (1.706 %) and other compounds with less than 1 % amount (Kaempferol 3-O-glucosyl-rhamnosyl-galactoside, desmethoxycurcumin, ar-Turmerone Bisdemethoxycurcumin, a-Terpinolene, L-Tyrosine and L-Alanine, L-serine). The results of the identification of components in this study are consistent with Herebian’s research. (Herebian, et al., 2009).

**Discussion**

It is known that there are about 235 components in turmeric, especially phenolic compounds and terpenoids. The compounds that have been identified consist of 22 diarylheptanoids and diarylheptanoids classes, 8 phenylpropene and other phenolic groups, 68 monoterpenes, 109 groups of sesquiterpenes, 5 groups of diterpene, 3 groups of triterpenoids, 4 groups of sterols, 2 alkaloids and the other 14 groups combined (Herebian, et al., 2009). Curcuminoids are a group of diarylheptanoids that are the main bioactive of turmeric. The most common curcuminoid in turmeric is curcumin and has long been used for medicinal purposes.

Curcumin has very low water solubility, thus limiting its use as an oral drug. Curcumin in the form of turmeric extract has an antiangiogenic effect five times higher than pure curcumin. This is due to the presence of other curcumin derivative components and other components contained in turmeric extract. Therefore, turmeric extract is stated to be more pharmacologically potential than pure curcumin (Liu, et al., 2008). The low bioavailability of curcumin has been proven in clinical studies and animal experiments (Yue, et al., 2012), the presence of lipophilic components (such as turmerone) in turmeric extract can affect the absorption of curcumin. Turmerone significantly increases curcumin transport into intestine cells so that absorption of curcumin increases significantly. Thus, giving turmeric extract containing turmerone is more effective in treating diseases than just curcumin.

**Curcuminoid on ethanol extract of turmeric (C. domestica Val) variety Turina-1:** Based data on Table 2, turmeric ethanol extract containing curcuminoids consisting of Curcumin (CUR), desmethoxycurcumin (DMC) and Bisdemethoxycurcumin (bis-DMC), as well as essential oils that play an important role in subsequent reactions. Approximately, there are 25 essential oil compounds that have been found in turmeric extract. There are quantitative variations in each of the chemical components of essential oils depending on where the turmeric plant is grown (Jayaprakasha, et al., 2005). The largest portion of Turina-1 turmeric extract is 78.732 % curcuminoid, the second largest is \( \alpha \)-Turmerone. The largest composition of curcuminoid in the extract is desmethoxycurcumin (DMC), and the smallest is Bisdemethoxycurcumin (bis-DMC) (Table 3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin (CUR)</td>
<td>25.73</td>
</tr>
<tr>
<td>Desmethoxycurcumin (DMC)</td>
<td>62.13</td>
</tr>
<tr>
<td>Bisdemethoxycurcumin (bis-DMC)</td>
<td>12.14</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Why curcumin is more reactive than DMC and bis-DMC, sofar the molecular reaction mechanism is not fully understood. This causes the content of curcumin in turmeric extract is important to consider. Curcumin and its analogs are potential inhibitors of low-density lipoprotein oxidation, inhibitory reactions occur due to abstraction of H atoms from phenolic groups and possible involvement of 4-hydroxy-3-methoxyphenyl groups (Chen, et al., 2006). The antioxidant activity of curcumin is associated with the release of electrons from the keto-enol group to the hydroxy-phenol group, apart from that the methoxy phenolic substitution also plays an important role. Curcumin is a strong metal chelating agent and an efficient radical scavenger. The content of curcumin is important given the antioxidant effect of each constituent. Although the molecular mechanism is not fully understood, it is clear that antioxidant activity is associated with electron withdrawal from the keto-enol group to the hydroxy-phenolic group, methoxy phenolic substitution also plays an important role (Chen et al., 2006). Curcumin is a powerful metal chelating agent and an efficient radical scavenger, metal chelating is carried out at the center of the keto-enol group (Somparn et al., 2007). Metabolite profile major (87.986 %) of ethanolic extract \textit{C. domestica} Val, variety Turina-1 is curcuminoid potentially antioxidants. The bis-DMC decay mechanism from steady-state (S1-state) is discussed and compared with curcumin using steady-state absorption and fluorescence techniques. The results show that differences in observed S1-state dynamics between bis-DMC and curcumin can be ascribed to differences in donor H-acceptor acceptors from donor phenolic OH and differences in the strength of intramolecular H bonds in keto-enol groups in two molecules (Nardo, et al., 2011). Compared to other varieties of turmeric that currently exist in Indonesia, Turina-1 turmeric has a higher curcumin content. To produce curcumin levels > 7 % turmeric this variety should be harvested at the minimum age of 9 (nine) months. Research shows that increasing the age of turmeric harvest from 9 months to 11 months does not significantly increase the content of curcumin with a curcumin content ranging from 7.0 to 7.59 % (Dewi, et al., 2016).

A total of 15 metabolites were characterised from ethanolic extracts of \textit{C. domestica} Val, variety Turina-1: Demethoxycurcumin 2 (42.60 %), Curcumin 27.44 %, \( \alpha \)-Turmerone 17.29 %, Bisdemethoxycurcumin 3 (7.99 %), Curcumin 1 (2.28 %), compounds less than 1 %: ar-Turmerone, Demethoxycurcumin, Bisdemethoxycurcumin, Ribonic acid, isodemethoxycurcumin, a-Terpenolene, L-Tyrosine, L-Alanine, Shikimic acid, Uridine,
L-Alanine, L-serine. The composition of curcuminoid extract is Cur 25.758 %, DMC 62.128 % and Bis-DMC 12.144 %.

Acknowledgments

Our deepest gratitude goes to the Ministry of Research, Technology, and Higher Education (RISTEKDIKTI) and the Institute for Research and Community Service (LPPM), Udayana University which has funded this research through Applied Research in 2019.

References


