INTRODUCTION: Curcuma rotunda (Zingiberaceae) is widely used as a spice in some Asian countries such as Indonesia, Malaysia, and Thailand. Commonly known as temu kunci in Indonesia, C. rotunda can be used to cure diseases such as stomach pain and discomfort, viral and bacterial infection, and diuretic agent, traditionally. It commonly used by Indonesian women after child birth under the traditional name ‘jamu.’ The rhizomes of this plant are effective against inflammatory diseases such as wounds, swellings, and tooth and gum diseases. Several medicinal properties such as antimicrobial, antibacterial, antioxidant, antiulcer, antimutagenic, and anticancer can be attributed to different compounds isolated from different parts of this plant.

Chemical studies of C. rotunda rhizomereported several monoterpenoids, i.e. geranial and neral; chalcones, i.e. boesen bergin A, boesen bergin B, cardamonin, pinochromin, panduratin A, and dihydro methoxy chalcone; and flavanones, i.e. pinocembrin, alpinetin, and 5-hydroxy-7-methoxyflavanone. Chalcone and flavonone derivatives were suggested to play an important role as antioxidant agents. Bioassay-guided isolation of the active compounds from CH2Cl2: MeOH 1:1 of C. rotunda extract lead to the isolation of panduratin A, 4-hydroxy-panduratin A, 5-hydroxy-7-methoxyflavanone, 5, 7- dihydroxyflavanone, 2′, 6′- dihydroxy-4′-methoxychalcone, and 2′, 4′-dihydro-
xy-6’-methoxychalcone. Among them, panduratin A and 4-hydroxypanduratin A might also contribute on antioxidant activity.

Studies on isolation and bioactivity-tested of plant-origin antioxidants were significantly increased in recent years. To the best of our knowledge, there is very little information available regarding the antioxidant activity of *C. rotunda* rhizome. We were thus encouraged to investigate and evaluate antioxidant activity of Indonesian *C. rotunda* rhizome. Antioxidant activity was carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The DPPH test is general bioassays for pharmacological activities of active phytochemical compounds derived from medicinal plants. It is a well-established assay for the *in-vitro* examination of antioxidant activity in medicinal plant extracts.

**MATERIAL AND METHODS:**

**Plant Materials:** The rhizome of *C. rotunda* was purchased from Tropical Biopharmaca Research Center, Bogor Agricultural University, West Java, Indonesia.

**General Experimental Procedures:** Vacuum liquid chromatography (VLC) was performed using Si 60 G (Merck) for column packed and Si 60 (0.2-0.5 mm) (Merck) for sample adsorbed. Column chromatography (CC) was performed using Si 60 (70-230 mesh) (Merck). For TLC analysis, pre-coated silica gel plates (Merck Si 60 GF254, 0.25 mm thickness) and Ce(SO₄)₂·4H₂O 1.5% in H₂SO₄ 2N as appariition stain reagent were used. LC-MS analysis was performed using LC-MSXevo G2-S QTOF with time of flight (TOF) as mass analyses on low power at 4 V, and high power at 25-70 V in duration time 23 min and Quadrupole-time of flight (Q-TOF) was used for mass analysis. The LC system consisted of a C18 column (particle size 2.1 m x 15.0 cm x 1.8 µm) equipped with a guard column containing the same material. The LC was run under gradient system on 0-2 min to 95% water and 5% methanol, isocratic on 2-3 min to 75% water and 25% methanol, gradient system on 14-15 min with 100% water, and isocratic on 19-23 min with 95% water and 5% methanol with 0.2 mL/min flow rate. Chromatogram obtained was analyzed by Masslynx V4.1 software, and mass spectrum was analyzed by ChemSpider, Massbank, and Human Metabolome Database (HMDB) software.

**Extraction, Fractionation, and Purification:** Dried rhizome of *C. rotunda* (1.2 kg) was ground and macerated three times with acetone at room temperature gained 161.02 g of crude extract. The crude extract (30 g) then was separated using VLC using various components of *n*-hexane: EtOAc yielded 8 major fractions (Fr. A-H). Fr. F (466.4 mg) was further fractionated using CC with *n*-hexane: EtOAc (9:1 to 1:9) resulted in 8 subfractions (Fr. F1-F8). Fr. F2 exhibited 1 spot on TLC using *n*-hexane: EtOAc 7:3 with RF value of 0.71 while Fr. F3 showed 2 spots with RF values of 0.71 and 0.53, respectively on the same eluent. Both fractions then was analyzed using LC-MS.

**Antioxidant Activity Assay:** Antioxidant activity was examined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid as a positive control. The correlation between concentration and activity was determined, and IC₅₀ was measured using interpolation. Antioxidant activity appears in the value of IC₅₀.

**RESULTS AND DISCUSSION:** Chromatogram of both Fr. F2 and F3 showed many peaks in range retention time of 0-22 min. Fr. F2 showed 1 peak with high abundance at a retention time of 13.30 minute, while Fr. F3 exhibited 2 peaks at retention time of 11.30 and 13.39 min *Fig. 1.*

Based on Masslynx software, the peak with a retention time of 13.30 min (Fr.F2) showed similarity with a peak at a retention time of 13.39 min (Fr 3) indicated a compound with the C₁₆H₁₅O₄ molecular formula. The compound had a 99.66% resemblance to the [M+H]+271.0975 m/z *Fig. 2.* According to HMDB, Massbank, and ChemSpider databases, these compounds were classified as chalcone and flavonoids, *i.e.* pinostrobin (1), cardamonine (2), eqinatine (3), (2E)-1-(4-hydroxy-2-methoxyphenyl)-3-(4-hydroxyphenyl)-prop-2-ene-1-one (4), and 7-hydroxy-8-methoxy-2-phenyl-3,4-dihydro-2H-1-benzopyrane-4-one (5) *Fig. 3.*

Compounds 1 and 2 had been isolated from CHCl₃ extract of *C. rotunda* cultivated in Bangkok, Thailand. Also, compound 1 was also reported from CH₂Cl₂ extract of *C. rotunda* cultivated in Pahang, Malaysia, while compound 2 was also isolated from the methanol extract of *C. Rotunda* cultivated in other Thailand regions. However,
compounds 1 and 2 had not yet reported from other Curcuma species, and compounds 3, 4, and 5 had not yet been reported both in C. rotunda and other Curcuma species.

![Image 1: THE Fr. F2 (A) AND F3 (B) FRACTIONS CHROMATOGRAM ON LC-MS OF INDONESIAN C. ROTUNDA](image1)

**FIG. 1:** THE Fr. F2 (A) AND F3 (B) FRACTIONS CHROMATOGRAM ON LC-MS OF INDONESIAN C. ROTUNDA

![Image 2: THE MS SPECTRUM FROM 13.30 min (Fr. F2) AND 13.39 min (Fr. F3) RETENTION TIME (A) BASIC SPECTRUM (B) MS FRAGMENTATION SPECTRUM](image2)

**FIG. 2:** THE MS SPECTRUM FROM 13.30 min (Fr. F2) AND 13.39 min (Fr. F3) RETENTION TIME (A) BASIC SPECTRUM (B) MS FRAGMENTATION SPECTRUM

![Image 3: PROSPECTIVE COMPOUNDS WITH 13.30 min (Fr. F2) AND 13.39 min (Fr. F3) RETENTION TIME BASED ON HMDB, MASSBANK, AND CHEMSPIDER DATABASE](image3)

**FIG. 3:** PROSPECTIVE COMPOUNDS WITH 13.30 min (Fr. F2) AND 13.39 min (Fr. F3) RETENTION TIME BASED ON HMDB, MASSBANK, AND CHEMSPIDER DATABASE

![Image 4: FRAGMENTATION PATTERN OF COMPOUND 1](image4)

**FIG. 4:** FRAGMENTATION PATTERN OF COMPOUND 1

Fig. 2 showed the [M+H]+ of 271.0975 m/z Fig. 2(a) and fragmented peak of 179.0346, 167.0350, 131.0499, and 103.0550 m/z Fig. 2(b). The fragmented peak of a compound corresponding to the values of 167.0350, 131.0499, and 103.0550 m/z was thought to originate from the release of radical ions [C₈H₇], [C₇H₈O₃], and [C₈H₇O₄], respectively. The presumptive compounds according to the fragmentation pattern were compounds 1 and 2 Fig. 3 with fragmentation patterns showed in Fig. 4.

According to Masslynx software, the peak with a retention time of 11.30 min (Fr.F3, Fig. 1b) could have C₁₅H₁₅O₄ molecular formula that 99.88% resemblance with [M+H]+ of 257.0821 m/z Fig. 5. Based on HMDB, Massbank, and ChemSpider database, the predicted compounds were pinocembrin (6) and liquiritigenin (7) as flavonoid derivatives, chalcone pinocembrin (8) and
isoliquiritigenin (9) as chalcone derivatives, and dihydrodaidzein (10) as isoflavonoid derivative. Fig. 6. Compound 6 had been reported in CHCl₃: methanol 9:1 extract of C. ecalcarata cultivated in India, 11 EtOAc extract of C. rotunda cultivated in Surabaya, Indonesia, 12 and CHCl₃ extract of C. rotunda cultivated in Bangkok, Thailand. Compound 8 had also been reported in the methanol extract of C. rotunda cultivated in Kuala Lumpur, Malaysia. 13 Compounds 6 and 8 had also been recorded on diethyl ether extract of C. rotunda cultivated in Thailand. 14 However, compound 8 had not been reported yet in other Curcuma species and compounds 7, 9, and 10 had also not been reported yet in both C. rotunda and other Curcuma species.

Fig. 5 showed the values of [M+H]⁺ of 257.0821 m/z Fig. 5(A) and fragmented peak of 179.0343, 167.0346, 153.0191, 131.0499, and 103.0549 m/z Fig. 5(B). The fragmented peak of a compound corresponding to the values of 179.0343, 153.0191, 131.0499, and 103.0549 m/z were thought to arise from the release of successive radical ions [C₆H₅], [C₈H₇], [C₆H₅O₃], and [C₇H₅O₄]. The alleged compounds according to the fragmentation pattern were compounds 6 and 8. Fig. 7 showed fragment ions from compound 6.

According to Tanjung et al., 12 compounds 2 showed the IC₅₀ value of 21.54 μg/mL against DPPH. The difference was presumably due to several factors, such as plant genetic factors, growing areas, environmental stresses such as heavy metals or ultraviolet exposure, and physical factors such as temperature, humidity, and climate.16 Phenolic and flavonoid in all accessions of C. rotunda rhizome, ascorbic acid, Fr. F2, and F3 showed that none of the fractions was found to be more active than ascorbic acid (IC₅₀ = 4.62 ppm). The acetone extract exhibited a moderate antioxidant activity with IC₅₀ value of 125.75 ppm. However, this value was greater than Pratiwi, 16 which reported the IC₅₀ value of 100.24 μg/mL. Fr. F2 and F3 could not be determined for their antioxidant activity because their inhibition value was less than 50%.
rotunda were not responsible for their antioxidant activity, or alternatively, that is radical scavenging activity was due to specific phenol or flavonoid compounds. In other medicinal plants, the results of that study are in agreement with other reports.¹⁷

CONCLUSION: The secondary metabolites had been isolated from acetone extract of Indonesian C. rotunda rhizomes. Separation and identification used various chromatography methods and LC-MS, respectively, showed a dominant peak on the retention time of 13.30 and 11.30 min suspected pinostrobin (chalcone) and pinocembrin (flavanone), respectively. Antioxidant activity towards DPPH of crude extract showed moderate activity with IC₅₀ of 125.75 ppm. However, the antioxidant activities from both isolated fractions could not be determined due to less than 50% of its inhibition percentage.

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CONFLICT OF INTEREST: Nil

REFERENCES: