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Antioxidant and Anti-inflammatory Activities of Diarylheptanoid from *Etlingera calophrys* Fruit

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ABSTRACT. Diarylheptanoids compounds from the genus Etlingera have attracted widespread attention due to their various structural diversity and potential pharmacological effects. This is certainly an important part to continue to study, especially from Sulawesi endemic species, one of which is Etlingera calophrys. The fruit of E. calophrys is widely utilized in the Sulawesi community. The lack of information on the content of chemical compounds and their pharmacological activities, so the study is very important. Diarylheptanoid compounds were obtained by chromatography technique, molecular structure interpretation by spectroscopy technique while pharmacological activity was tested against radical scavenging as antioxidant and inhibition of protein denaturation as anti-inflammatory. Two diarylheptanoid compounds were successfully isolated from E. calophrys fruit namely 1-(3'-methoxy-4'- hydroxyphenyl)-7-phenyl- 3-heptanone and 1-(3'-methoxy-4'- hydroxyphenyl)-7-(4"-hydroxyphenyl)- 3-heptanone. Antioxidant properties showed very strong ability with IC_{50} values of 32.21 ± 0.42 and 21.64 ± 0.63 μg/mL, respectively, while anti-inflammatory properties were strongly shown by the compound with IC_{50} values of 25.71 ± 0.54 and 15.28 ± 0.66 μg/mL, respectively. The presence of diarylheptanoid content that has good pharmacological activity can support the used of E. calophrys as a nutraceutical source.

Keywords: Anti-inflammatory, antioxidant, diarylheptanoid, *Etlingera calophrys*, fruit

INTRODUCTION

Indonesia's biodiversity is the second largest in the world, with 19,232 species of vascular plants living in nature (Darajati et al., 2016; Nash, 2022). Many biological species are commonly used by the community for various purposes, such as ornamental plants, food and medicine (Bahtiar et al., 2017). Zingiberaceae, which belongs to the ginger's family, is a flowering plant with 1300 species distributed in tropical Africa, America and Asia (Tamokou et al., 2017). The Indonesian Zingiberaceae genus, especially on Sulawesi Island, has been studied for its chemical content and pharmacology, including Meistera (Musdalipah et al., 2021), Alpinia (Yodha et al., 2023) and Etlingera (Hamsidi et al., 2024; Sahidin et al., 2022; Wahyuni et al., 2022; Jabbar et al., 2021). The genus Etlingera is easily found in humid forest areas on Sulawesi Island (Droop, 2012) with 48 endemic species in it out of approximately 200 Etlingera species spread throughout the world (Newman et al., 2004; Puspitaningrum et al., 2017).

Chemical and pharmaceutical studies of one of the Etlingera species from Sulawesi that has been successfully researched is *Etlingera calophrys*. *Etlingera calophrys* rhizomes contain nine chemical compounds namely 1,7-diphenylhept-6-en-3-one, 1,7-diphenylheptan-3-one, stigmast-4-en-3-one, β-sitosterol, stigmasterol, yakuchinone A, yakuchinone B, 7-(4-hydroxy-3-methoxyphenyl)-1-phenylhept-4-en-3-one and 1-(4-hydroxyphenyl)-7-phenylheptan-3-one (Sahidin et al., 2022). Six of them are compounds of the diarylheptanoid class characterized by a 1,7-diphenylheptane skeleton. Diarylheptanoids are known as important phenolic compounds that are currently attracting widespread attention due to their wide range of structural diversity and potential pharmacological effects. The structures of diarylheptanoids are highly diverse with a skeleton of two aromatic rings conjugated with seven carbon chains. Their forms are categorized into linear, cyclic, and diarylheptanoids with special functional groups. Several pharmaceutical studies reveal that diarylheptanoids have antioxidant (Cerulli et al., 2017), anti-inflammatory (Honmore et al., 2019), anti-tumor (Fan et al., 2019), melanogenesis (Matsumoto et al., 2013), leishmanicidal (Takahashi et al., 2004), neuroprotective (Yang et al., 2020), and hepatoprotective (Chuaicharoen et al., 2020) activities. This makes diarylheptanoid compounds interesting to continue to study, especially from *E. calophrys* species.

The most commonly utilized part of the *E. calophrys* plant is the fruit. The fruit of this species is often eaten and used in fish dishes as a flavour enhancer (Pitopang et al., 2019). Recent pharmaceutical studies explain that the methanol extract of *E. calophrys* fruit has very strong antioxidant activity. This ability is thought to be caused by the diarylheptanoid compounds contained therein (Hamsidi et al., 2024).

Until now, there is no information that explains the pure compound content of *E. calophrys* fruit. Although *E. calophrys* rhizomes have been studied for chemical and pharmaceutical content, it is likely that differences in the content and levels of compounds in the fruit can be found. In another study, integrative analysis of metabolome and transcriptome to reveal the dynamic accumulation and regulatory networks of fruits and rhizomes showed that the relative content of metabolites in fruits was higher than that in rhizomes (Ning et al., 2024). Therefore, tracing the content of chemical compounds through isolation and identification of structures and evaluation of biological activity is very important to do, so that it can support its utilization in the community and can become a natural ingredient that has potential as a future drug in the pharmaceutical industry.

EXPERIMENTAL SECTION

Plant Materials and Determination

Fruit of *E. calophrys* obtained from Lamokula Village, North Moramo, South Konawe, Southeast Sulawesi Province, Indonesia 4°8'58.8 "S 122°36'31" E, 20 m. Plant samples were identified at the BRIN Biological Research Center, Indonesia. (Number 1535/IPH.1.01/If.07/VIII/2019). Ripe fruits were harvested, cleaned of impurities using running water and cut into small pieces. It was then oven dried at 40 °C and pulverized, passed through a 40-mesh sieve to obtain fruit powder (1.55 kg).

Extraction, Isolation and Identification

Etlingera calophrys fruit powder was extracted by adding 5 L of methanol solvent for 24 hours, filtered and repeated three times. The filtrate was concentrated under low pressure using a vacuum rotary evaporator (Rotavapor R-210 Buchi Germany) until free of methanol, in the form of a thick extract (21 g). The methanol extract was further fractionated by liquid vacuum chromatography using a 10 cm diameter column, silica gel 60 GF_{254} (Merk) stationary phase (250 g) and mobile phase of n-hexane:ethyl acetate mixture (9:1, 8:2, 5:5, 2:8), 100% ethyl acetate and 100% methanol resulting in 6 fractions (A-F), respectively 1.5, 3.4, 2.2, 1.9, 2.6 and 8.2 g based on Thin Layer Chromatography (TLC) monitoring of Silica gel 60 F_{254} (Merk). Fraction B was re-separated using a 5 cm column, silica gel 60 GF_{254} (Merk) stationary phase (100 g) and mobile phase of n-hexane:ethyl acetate mixture (8:2 and 5:5), 100% ethyl acetate and 100% methanol, yielding 4 subfractions B1-B4, 0.3, 1.2, 0.8 and 0.9 g, respectively. Subfraction B2 was purified using preparative centrifuges (Chromatotron model 7924T), silica gel 60 PF_{254} stationary phase containing gypsum (Merck) and n- hexane:ethyl acetate (8:2) mobile phase to give compound **1** (218 mg). Subfraction B4 was purified using preparative centrifuges (Chromatotron model 7924T), silica gel 60 PF_{254} stationary phase containing gypsum (Merck) and n-hexane:ethyl acetate (7:3) mobile phase to give compound **2** (67 mg).

Then 5 mg of each compound was dissolved in deuterated chloroform (chloroform-d, 99.8% atomic D, containing 0.03% tetramethyl silane (TMS) (v/v), Sigma-Aldrich), and measured on a Nuclear Magnetic Resonance (NMR) instrument (Jeol, 500/125 MHz). Chemical shifts (δ) are given in ppm relative (δH of 1-10 ppm and δC of 1-200 ppm) to the solvent or relative to the internal standard (tetramethyl silane), while the coupling constant (J) is given in Hz (Lo et al., 2021).

Antioxidant Properties Test

Antioxidant properties were measured with reference to previously reported methods, based on the inhibition of 2,2 diphenyl-1-picrylhydrazyl (DPPH) radicals (Yodha et al., 2022; Metasari et al., 2020) with minor modifications. A 0.05 mM DPPH (HIMEDIA) solution (1.98 mg DPPH) was prepared in 100 mL of methanol. Samples were dissolved in methanol with various concentrations (2, 4, 6, 8, 10 mg/L). A total of 2 mL of sample solution from each concentration was mixed with 2 mL of DPPH solution, then shaken and incubated for 30 minutes at room temperature in the dark. As a negative control, 2 mL of methanol was mixed with 2 mL of DPPH solution. The absorbance was determined using UV-Vis spectrophotometer (Thermo Scientific) at 517 nm against the blank (methanol). Ascorbic acid (Sigma-Aldrich) (positive control) was tested in the same way. The experiment was repeated three times, the IC_{50} value (half concentration of radical inhibition) was determined, and the results were averaged.

Anti-inflammatory Properties Test

Anti-inflammatory activity was determined by measuring protein denaturation inhibition values based on a previous report (Yodha et al., 2024; Bailey-Shaw et al., 2017) with minor modifications. Bovine serum albumin (BSA) (HIMEDIA®) protein solution (0.2%, b/v) was prepared in tris buffered saline (HIMEDIA®) pH 6.5. Samples were dissolved in methanol at various concentrations (2, 4, 6, 8, 10 μg/mL). A total of 2 mL of sample solution from each concentration was mixed with 2 mL of BSA solution. The mixture was then heated at 72 °C for 20 minutes and cooled for 30 minutes under room conditions. Turbidity was measured at 660 nm. Diclofenac sodium (Sigma-Aldrich) (positive control) was tested in the same way. The experiment was repeated three times, the IC_{50} value (half concentration

inhibiting protein denaturation) was determined, and the results were averaged.

RESULT AND DISCUSSION

Chemical Structure

The structure of the isolated compound was determined based on the interpretation of carbon NMR and proton data. The absorption peaks shown in carbon NMR (**Figure 1**) provide an overview of the structure based on the chemical shifts (δ) of various types of carbon in a compound. The number of carbons is determined based on the number of peaks formed. NMR spectroscopic data, supported by the Distortionless Enhancement by Polarization Transfer (DEPT) technique. This technique is used to distinguish between different types of carbon atoms in a molecule based on the number of hydrogen atoms (protons) bound to them. The DEPT technique involves taking multiple spectra in which the signals from different types of carbon atoms are selectively amplified or suppressed based on the number of protons bound (Ishii et al., 2019).

Compound **1** shows 18 peaks, two peaks (128.5 and 128.4 ppm) that are higher than the other peaks each indicate the presence of two carbons that appear on one chemical shift due to the influence of the same environment (carbon symmetry), so compound **1** contains 20 carbon atoms. The 20 carbons consist of 1 methyl carbon (CH_3: δC 56.0 ppm), 6 methylene carbons (CH_2: δC 44.7, 43.0, 35.8, 31.1, 29.6 and 23.5 ppm), 8 methine carbons (CH: δC 128.5 (2-carbon symmetry), 128.4 (2-carbon symmetry), 125.8, 120.8, 114.4 and 111.3 ppm) and 5 quaternary carbons (Cq: δC 210.4, 146.5, 144.0, 142.3 and 133.1 ppm). The quaternary carbon at δC 210.4 ppm is a ketone carbon (carbonyl), while methyl with a large chemical shear indicates that methyl binds to an oxygen atom to form methyl oxide (ether).

Compound **2** also shows 18 peaks, two peaks (129. 6 and 115.3 ppm) that are higher than the other peaks each indicate the presence of two carbons that appear on one chemical shift due to the influence of the same environment (carbon symmetry), so compound **2** contains 20 carbon atoms. The carbon consists of 1 methyl carbon (CH_3: δC 56.1 ppm), 6 methylene carbons (CH_2: δC 44.8, 43.1, 35.0, 31.3, 29.25 and 23.5 ppm), 7 methine carbons (CH: δC 129. 6, 129.6, 121.0, 115.3, 115.3, 114.5 and 111.3 ppm) and 6 quaternary carbons (Cq: δC 210.7, 153.8, 146.6, 144.1, 134.5 and 133.2 ppm). The quaternary carbons at δC 210.7 ppm in ketone carbons (carbonyl) while the methyl with large chemical shear indicates that the methyl binds to oxygen atoms to form methyl oxide (ether).

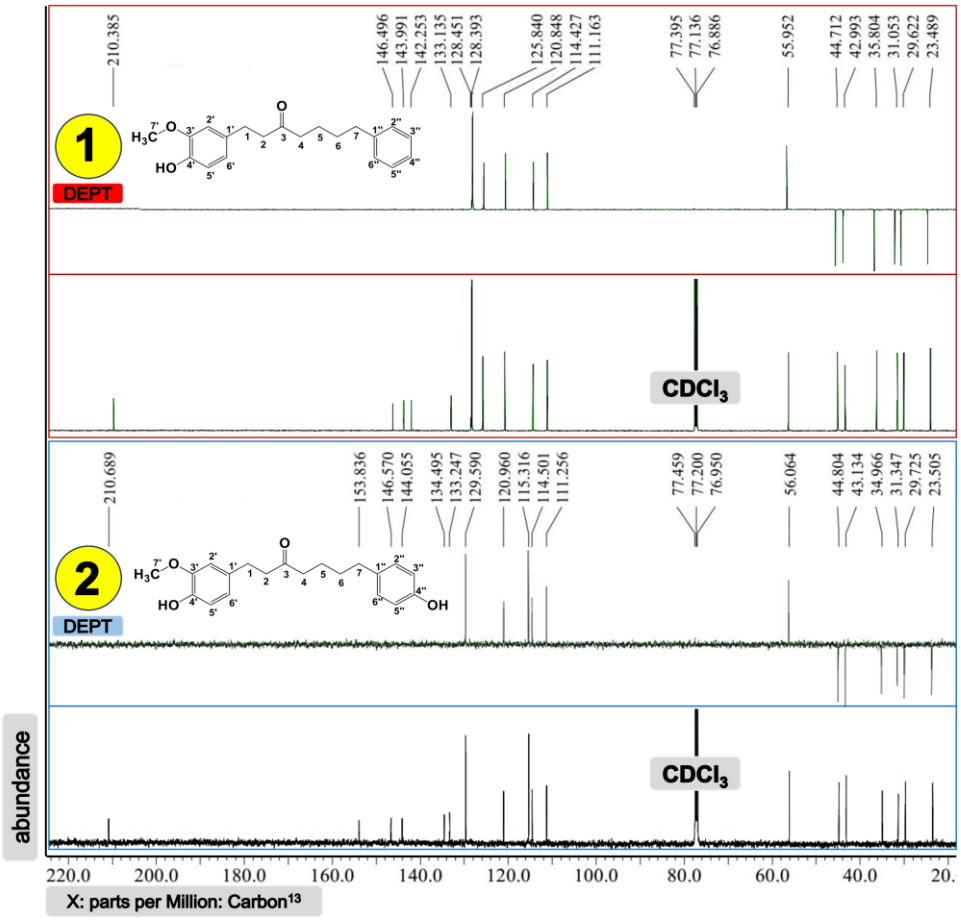

Figure 1. Spectroscopy ^{13}C NMR and DEPT (Joel, 125 MHz) compounds **1** and **2**

Proton NMR provides information related to the number of signals formed, the type of multiplicity, the number of protons in a signal (integration), the value of the chemical shift (δ), and the number of coupling constants (J) (Dona et al., 2016). Determination of compound structure by proton NMR (**Figures 2** and **3**) begins with counting the number of signals that appear from the compound being analyzed. The signals represent the different chemical environments of the hydrogen atoms in the molecule.

Based on the signal formed, the type of multiplicity can be known as an influence that describes the proton environment with other adjacent protons. Singlet multiplicity occurs when the observed protons have no neighboring nuclei that affect their resonance. As a result, they produce a single peak in the NMR spectrum. Doublet multiplicity occurs when the observed protons have one neighboring proton affecting their resonance. This pattern appears due to the coupling interaction between two protons. Doublets appear as two peaks of equal intensity in the NMR spectrum. Triplet multiplicity occurs when the observed proton has two neighboring protons that affect its resonance. In NMR spectra, this pattern consists of three peaks of different intensities, one largest peak in the center and two smaller peaks beside it. Multiplet is a general term that refers to a complex pattern in the NMR spectrum, which is produced when the observed proton has several neighboring protons that affect its resonance. Multiplets can consist of four or more peaks that are separate and differ in intensity. The doublet of doublets multiplicity is a special pattern that occurs when the observed proton has two neighboring protons that each have their own neighboring proton. This pattern consists of four peaks with the same intensity (Dona et al., 2016).

Each signal has an integration value that indicates the relative number of protons obtained from each compound (Pribitzer et al., 2021). The calculated protons are protons that are directly bound to the carbon nucleus. Some NMR proton signals such as those of hydroxyl groups (-OH) and amine groups (-NH) are not clearly formed because these two groups can undergo rapid proton exchange in solution. This phenomenon is called "exchange broadening" in NMR spectroscopy, which causes the peaks that should be formed to be widened or even not visible at all in the spectrum. This means that the number of protons counted does not fully represent all protons in a compound (Kleckner et al., 2011). Nonetheless, the integration value will correspond to the number of protons involved in the same interaction, thus providing important information about the molecular structure.

Furthermore, the chemical shift (δ) value in proton NMR of each signal measured in units of ppm (parts per million) is determined against tetramethyl silane (TMS). Chemical shifts provide information about the chemical environment observed in the molecule, which is influenced by the structure and surrounding electronic environment (Haris et al., 2002).

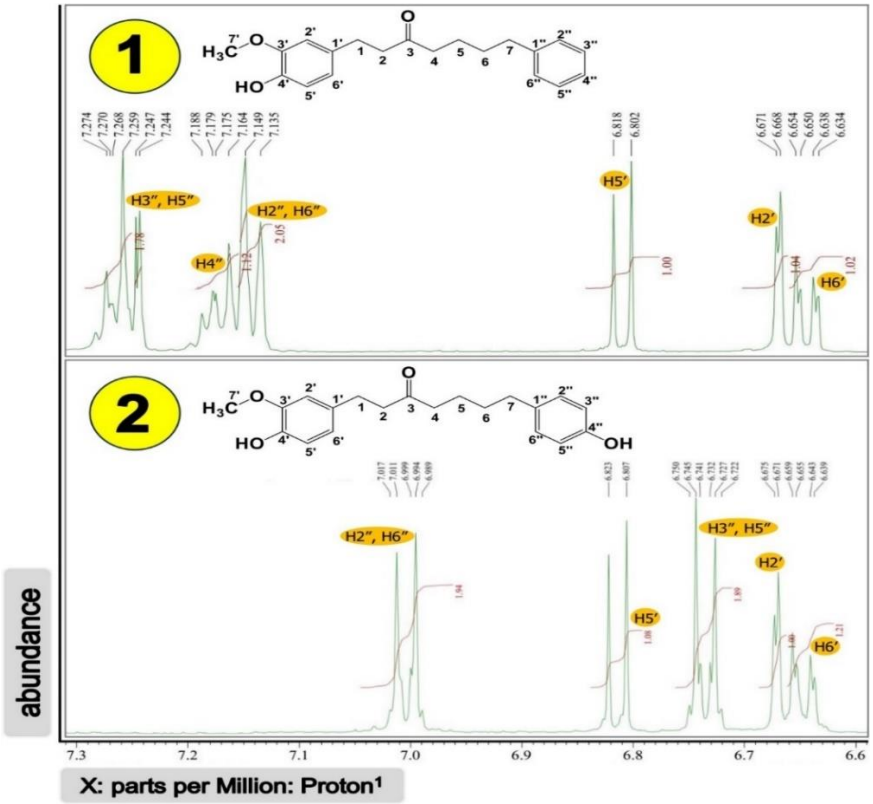

Figure 2. Spectroscopy ^{1}H NMR (Joel, 500 MHz) compounds 1 and 2 (6.6 – 7.3 ppm)

Finally, the value of the coupling constant (J) in the NMR spectrum, which is a measure of the strength of the interaction between neighboring proton nuclei (splitting), is determined by the resolution of the instrument. The coupling constant is a numerical quantity that describes the distance between peaks in the splitting pattern. The coupling constant provides information about the chemical environment and conformation in the molecule, and determines the bonding relationships in the compound structure. Analysis of the coupling constant together with chemical shifts gives a more complete picture of the molecular structure (Wu et al., 2021).

Compound **1** shows 12 proton signals consisting of 1 singlet multiplicity signal (s), 1 doublet signal (d), 4 triplet signals (t), 4 multiplet signals (m) and 1 doubedoublet signal (dd). Based on the identified integration values, compound 1 shows 23 protons. Protons at chemical shifts above 5 ppm (δH 7.26, 7.17, 7.14, 6.81, 6.67, and 6.64 ppm) were deshielded by the anisotropy effect of the alkene group identified in the carbon signal δC 100-160 ppm. Proton signals with singlet multiplicity (s) at chemical shear δH 3.84 ppm each consisting of three protons indicate methyl protons that are deshielded by the presence of atoms that have large electronegativity (oxygen). This oxygen-bound methyl forms a methoxy group bound to the quaternary carbon. Proton signals with triplet multiplicity (t) and multiplets at chemical shear δH 1.4 - 2.9 ppm indicate that these protons are close together and around their chemical environment there are other protons.

Compound **2** shows 11 proton signals consisting of 1 singlet multiplicity signal (s), 4 doublet signals (d), 4 triplet signals (t), 1 multiplet signal (m) and 1 doubedoublet signal (dd). Compound 2 contains 22 integrated protons. Protons above 5 ppm (δH 7.00, 6.82, 6.74, 6.67 and 6.65 ppm) were deshielded by the anisotropy effect of alkene groups. The singlet proton signal (s) at δH 3.85 ppm consists of three protons indicating methyl protons that are deshielded by the presence of atoms that have a large electronegativity (oxygen). This oxygen-bound methyl forms a methoxyphenyl group. Proton signals with triplet multiplicity (t) and multiplets at δH 1.4 - 2.9 ppm indicate chemical influence with other protons indicating that the protons are derived from an aliphatic skeleton.

The results of carbon NMR analysis get information about the number and type of bonds of carbon atoms. Furthermore, proton NMR analysis gets the number and type of protons present in the compound structure. The structure of a compound is determined by combining the results of carbon NMR and proton NMR (**Table 1** and **2**) which are compared with the literature. Based on spectra data, compound **1** is identical to yakuchinone A (Sahidin et al., 2022), while compound **2** is 1-(3'-methoxy-4'-hydroxyphenyl)-7-(4"-hydroxyphenyl)- 3-heptanone (Zeng et al., 2007).

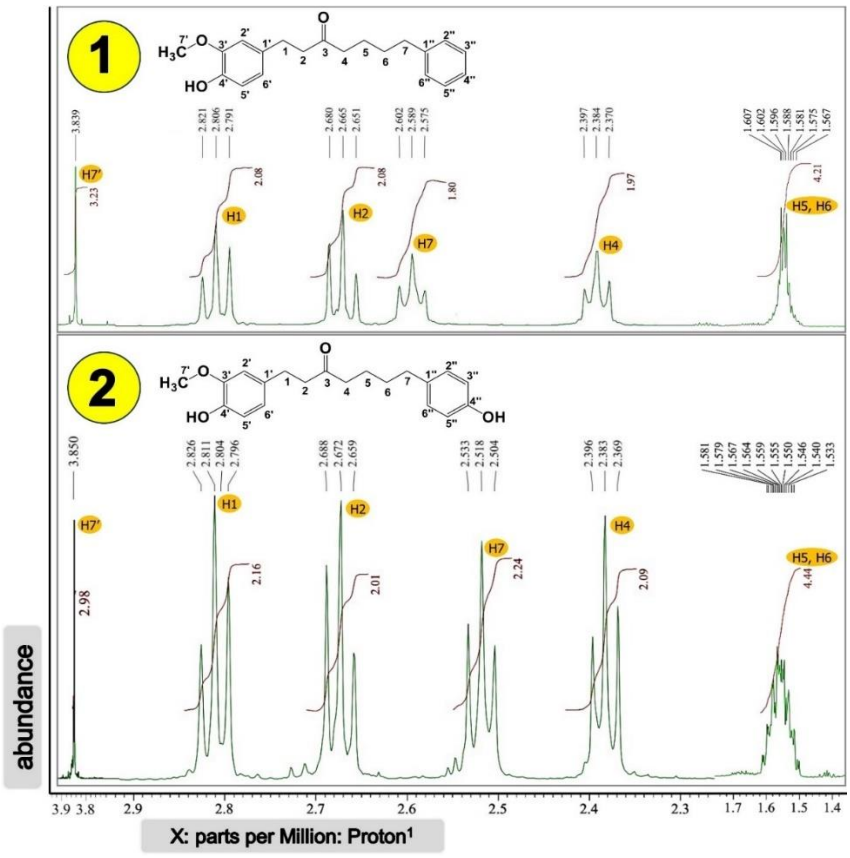

Figure 3. Spectroscopy ^{1}H NMR (Joel, 500 MHz) compounds **1** and **2** (1.4 – 4.0 ppm)

Table 1. The ^{13}C and ^{1}H NMR spectra of compounds **1** and literature

No	Compound 1			Yakuchinone A (Sahidin et al., 2022)	
	δ C (ppm)	DEPT	δ H (ppm) (ΣH, m, J (Hz))	δ C (ppm)	δ H (ppm) (ΣH, m, J (Hz))
1	29.6	CH_2	2.81 (2H, t, 7.5)	29.6	2.83 (2H, t, 7.79)
2	44.7	CH_2	2.67 (2H, t, 7.5)	44.8	2.69 (2H, t, 7.79)
3	210.4	C=O	-	210.7	-
4	43.0	CH_2	2.38 (2H, t, 7.0)	43.0	2.41 (2H, t, 6.87)
5	23.5	CH_2	1.59 (2H, m)	23.5	1.61 (2H, m)
6	31.1	CH_2	1.59 (2H, m)	31.1	1.61 (2H, m)
7	35.8	CH_2	2.59 (2H, t, 7.0)	35.9	2.61 (2H, t, 6.87)
1'	133.1	Cq	-	133.2	
2'	111.2	CH	6.67 (1H, d, 2.0)	111.3	6.70 (1H, d, 1.83)
3'	146.5	Cq	-	146.6	
4'	144.0	Cq	-	144.0	
5'	114.4	CH	6.81 (1H, d, 8.0)	114.0	6.84 (1H, d, 7.79)
6'	120.8	CH	6,64 (1H, dd, 2.0 & 8.0)	120.9	6.67 (1H, dd, 1.83 & 7.79)
3'-OCH_3	56.0	CH_3	3.84 (3H, s)	56.0	3.84 (3H, s)
1"	142.3	Cq	-	142.3	-
2"	128.4	CH	7.14 (1H, m)	128.4	7.19 (1H, m)
3"	128.5	CH	7.26 (1H, m)	128.5	7.29 (1H, m)
4"	125.8	CH	7.17 (1H, m)	125.9	7.17 (1H, m)
5"	128.5	CH	7.26 (1H, m)	128.5	7.29 (1H, m)
6"	128.4	CH	7.14 (1H, m)	128.4	7.19 (1H, m)

Table 2. The ^{13}C and ^{1}H NMR spectra of compounds **2** and literature

No	Compound 2			1-(3'-methoxy-4'- hydroxyphenyl)-7-(4"- hydroxyphenyl)- 3-heptanone (Zeng et al., 2007)	
	δ C (ppm)	DEPT	δ H (ppm) (ΣH, m, J (Hz))	δ C (ppm)	δ H (ppm) (ΣH, m, J (Hz))
1	29.7	CH_2	2.81 (2H, t, 7.5)	29.5	2.80 (2H, m)
2	44.8	CH_2	2.67 (2H, t, 8.0)	44.6	2.67 (2H, m)
3	210.7	C=O	-	211.2	-
4	43.1	CH_2	2.38 (2H, t, 7.0)	42.9	2.39 (2H, t, 6.8)
5	23.5	CH_2	1.59 (2H, m)	23.2	1.57 (2H, m)
6	31.3	CH_2	1.54 (2H, m)	31.1	1.51 (2H, m)
7	35.0	CH_2	2.52 (2H, t, 7.5)	34.7	2.50 (2H, t, 7.5)
1'	133.2	Cq	-	132.9	-
2'	111.3	CH	6.67 (1H, d, 1.5)	111.1	6.66 (1H, d, 1.5)
3'	146.6	Cq	-	146.4	-
4'	144.1	Cq	-	143.8	-
5'	114.5	CH	6.82 (1H, d, 8.0)	114.3	6.81 (1H, d, 8.0)
6'	121.0	CH	6.65 (1H, dd, 2.0 & 8.0)	120.7	6.62 (1H, d, 8.0)
3'-OCH_3	56.1	CH_3	3.85 (3H, s)	55.8	3.82 (3H, s)
1"	134.5	Cq	-	134.0	-
2"	129.6	CH	7.00 (1H, d, 8.5)	129.3	6.97 (1H, d, 8.4)
3"	115.3	CH	6.74 (1H, d, 9.0)	115.1	6.74 (1H, d, 8.4)
4"	153.8	Cq	-	153.8	-
5"	115.3	CH	6.74 (1H, d, 9.0)	115.1	6.74 (1H, d, 8.4)
6"	129.6	CH	7.00 (1H, d, 8.5)	129.3	6.97 (1H, d, 8.4)

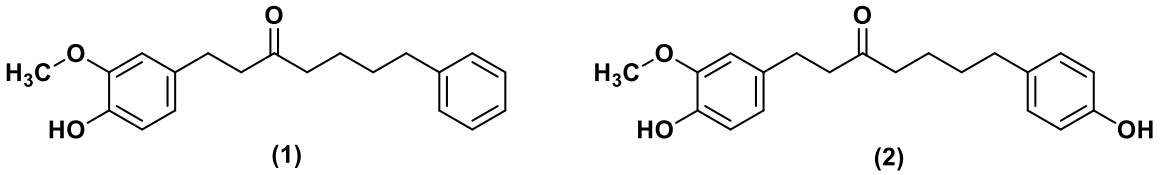

Figure 4. Structures of compounds **1** and **2**

Antioxidant and Anti-inflammatory Properties

Compounds **1** and **2** from *E. calophrys* fruit were measured for their antioxidant and anti-inflammatory properties (**Table 3**). Antioxidant properties were measured against DPPH through its ability to reduce radical compounds, while anti-inflammatory properties were measured against BSA through its ability to inhibit protein denaturation.

The main principle of antioxidant testing is to measure the ability of compounds to stop redox reactions with DPPH compounds. DPPH compounds are organic compounds containing one nitrogen atom that have free radical properties. This DPPH radical is dark purple in solution. The compound will transfer hydrogen atoms or electrons to DPPH to reduce this free radical. The occurrence of the reaction between DPPH and the compound is characterized by a reduction in the intensity of the purple color or even turns clear depending on how effective the antioxidant stops the free radical reaction. The color change is determined spectrophotometrically by measuring the absorbance at the maximum wavelength of DPPH (usually around 517 nm). The higher the antioxidant activity, the greater the decrease in absorbance measured (Musa et al., 2013; Dawidowicz et al., 2012). Furthermore, the antioxidant activity of the tested compound can be calculated based on the comparison of the absorbance of the solution after treatment with and without antioxidant, which is then calculated as the IC_{50} value.

Compounds 1 and 2 show values of IC_{50} <50 μg/mL (**Table 3**). IC_{50} value >200 μg/mL is no activity, >150-200 μg/mL is weak, >100-150 μg/mL is quite strong, >50-100 μg/mL is strong and <50 μg/mL is very strong (Molyneux, 2004). Based on these criteria, compounds 1 and 2 have a very strong ability to provide antioxidant activity.

The strength of activity shown by compounds 1 and 2 is due to the presence of phenol groups. Compounds with phenol structures in them will inhibit oxidation and reduce radical compounds. Radical compounds will capture hydrogen atoms from OH groups to form oxygen radicals and then undergo electron delocalization through resonance to form stable radicals (Chaimaa et al., 2023; Katia et al., 2023).

By comparing the IC_{50} values of other diarylheptanoid compounds (Li et al., 2012), it is clear that the activity of compounds 1 and 2 also depends significantly on the introduction of electrondonating groups (hydroxyl and methoxy) in the ortho position of the 4-hydroxyl group, and the phenoxyl group makes somewhat a bigger contribution to DPPH activity than methoxyphenoxyl group. So compound 2 with three electrondonating groups (two phenoxyl groups and one methoxyphenoxyl group) showed the most activity, and compound 1 with two electron groups (one phenoxyl groups and one methoxyphenoxyl group) showed relatively weaker activity compared to compound 2. Based on the analysis of charge conducted by Liu and Guo (2006), the presence of methoxyphenyl group causes the oxygen atom (O) of diarylheptanoid at the 4'-OH position to have a larger negative charge. This is due to the formation of hydrogen bonds between 4'-OH with 3'-OCH3. From the negative charge on the oxygen atom, diarylheptanoid compounds such as compounds 1 and 2 showed higher antioxidant activity based on the introduction of electron donating groups.

In anti-inflammatory testing, compounds are measured based on their ability to inhibit protein denaturation. Proteins undergo denaturation when they lose their secondary and tertiary structures through the application of external stress or compounds such as heat, strong acids, or bases. Protein denaturation leads to autoantigen production in conditions such as rheumatoid arthritis, cancer, and diabetes which are inflammatory conditions. Therefore, by inhibition of protein denaturation, inflammatory activity can be recognized so any agent that can inhibit protein denaturation is worth considering for anti-inflammatory drug development (Bailey-shaw et al., 2017).

Table 3. The IC_{50} values of antioxidant and anti-inflammatory activity

Sample	IC_{50} (μg/mL)	
	Antioxidant activity	Anti-inflammatory activity
Compound 1	32.21 ± 0.42	25.71 ± 0.54
Compound 2	21.64 ± 0.63	15.28 ± 0.66
Ascorbic Acid	7.39 ± 0.35	-
Diclofenac sodium	-	5.42 ± 0.46

In the process, Bovin Serum Albumin (BSA) as a protein is interacted with compounds and given heat. Heat will affect non-polar hydrophobic interactions, causing the molecules that make up the protein to move very quickly, disrupting hydrogen bonds. In addition, heat will result in the breaking of non-covalent interactions that exist in the natural structure of the protein but does not break its covalent bonds in the form of peptide bonds. Inhibition of protein denaturation is known by measuring the uptake of BSA with UV-Vis spectrophotometry (Verma et al., 2011). The higher the anti-inflammatory activity, the greater the decrease in measured uptake which indicates that no denaturation occurs.

Compounds 1 and 2 showed IC_{50} values of 10-30 μg/mL (**Table 3**). Specifically, a substance is declared as a very strong anti-inflammatory if IC_{50} is less than 10 μg/mL, strong if IC_{50} is 10-30 μg/mL, moderate if IC_{50} is 31-50 μg/mL, weak if IC_{50} is 51-100 μg/mL and inactive if IC50 is more than 100 μg/mL (Musdalipah et al., 2023). Based on these criteria, compounds 1 and 2 have a strong ability to provide anti-inflammatory activity.

The strength of anti-inflammatory activity shown by compounds 1 and 2 is due to the presence of carbonyl groups and phenol groups. The carbonyl group on the diarylheptanoid interacts with the H atom on the amino acid residue in the protein chain, while the phenol group can interact with albumin in the form of hydrogen bond interactions formed between the H atom of the hydroxyl group on phenol with the N atom on the amino acid residue thus making the protein structure more stable and not easily denatured (Zinellu et al., 2015). The presence of two phenol groups in compound 2, makes the compound have a better ability than compound 1.

CONCLUSIONS

In this study, two diarylheptanoid compounds were obtained from *E. calophrys* fruit. The structural interpretation results showed that the compounds were yakuchinone A and 1-(3'-methoxy-4'- hydroxyphenyl)-7-(4"-hydroxyphenyl)- 3-heptanone. Both compounds showed very strong properties in inhibiting DPPH free radicals and strongly inhibited BSA protein denaturation. This ability strongly supports its utilization as a nutraceutical product.

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