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Research Article

Antibacterial, antioxidant and tyrosinase inhibitor potential of new 7-(3,4-dihydroxyphenyl)-1-(4-hydroxyphenyl)-3-heptanone, a diarylheptanoid from rhizomes of *Amomum uliginosum* J. König

Nurul Syafiqah Rezali[1*] and Hasnah Mohd Sirat[2]

[1]Chemical Sciences Programme, School of Distance Education, Universiti Sains Malaysia, 11800, Minden, Penang, Malaysia
[2]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia

*Corresponding author: nsyafiqah@usm.my

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Abstract

Known as Puar Hutan or Tepus Merah by locals, the *Amomum uliginosum* J. König that belongs to the family Zingiberaceae and the genus Amomum was selected as a subject plant. Studies on the essential oil composition and some diterpenes and kawalactone from *A. uliginosum* have been reported, but information on its other chemical constituents and biological activities remains limited. Therefore, this study aims to identify additional phytochemicals from *A. uliginosum* rhizomes and evaluate their antibacterial, antioxidant, and antityrosinase activities. A new diarylheptanoid, 7-(3,4-dihydroxyphenyl)-1-(4-hydroxyphenyl)-3-heptanone **(1)**, together with two flavonoids identified as 5-hydroxy-3,7,4'-trimethoxyflavone **(2)** and 5,7,4'-trihydroxyflavanone **(3)** as well as two labdane type diterpenes, (*E*)- labda-8(17),12-dien-15,16-dial **(4)** and (*E*)-labda-8(17),12-dien-15-ol-16-al **(5)** were successfully isolated and identified from the cold extracted rhizomes of *A. uliginosum* J. König. The compounds were isolated through a combination of vacuum liquid chromatography (VLC) and successive column chromatographic techniques, while their structures were elucidated using an integrated suite of spectroscopic methods, including NMR, IR, UV, and mass spectrometry. Antibacterial properties of the ethyl acetate extract of *A. uliginosum* rhizomes (AURE) and isolated compounds were investigated against four clinical isolates of bacteria i.e. *Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa* and *Escherichia coli*. A minimum inhibitory concentration (MIC) value of 112.5 µg/mL was obtained for both AURE and compound **(1)** on *B. cereus* and *B. subtilis,* respectively. Meanwhile, antioxidant activity of AURE and five isolated compounds was determined by radical-scavenging methods. The tested samples showed varied levels of total antioxidant activity with IC_{50} value ranging from 28.2 to >1000 µg/mL. The highest activity was exerted by compound **(1)** with IC_{50} value of 28.2 µg/mL and the least activity was exerted by compound **(2)**. On the other hand, all tested samples were introduced to *in-vitro* mushroom tyrosinase inhibitory assays and gave wide-ranging levels of antityrosinase activity percentage inhibition value from 4.1±0.2% to 55.5±0.5%. AURE sample exhibited highest antityrosinase activity at 55.5±0.5%, followed by compound **(3)** and compound **(1)** at 52.6±0.6% and 51.7±0.8%, respectively.

Keywords: *Amomum uliginosum*, diarylheptanoid, antibacterial, antioxidant, tyrosinase inhibitory

Introduction

Traditional medicine (TM) has preserved its demand and has been practiced in virtually all cultures worldwide. According to the World Health Organization [1], the population in developing countries like in African region is still reliant on plant-based TM for their primary health care ranges from 60% to 90%. The continuing analysis on pharmacological potential of TM has significantly wave the discovery of plant-based modern clinical medicines. Preferably, the drugs that bearing interesting organic moieties such as flavonoids, alkaloids and terpenoids are function to derive a therapeutic effect [2]. At least a decade, number of scientists worldwide has demonstrated an antimicrobial property of natural compounds [3]. Majorly used in the treatment of infectious illness and diseases triggered by resistant microbes, this antimicrobial property has a growing demand especially from pharma industries. Other major manufacturing required supplementary significant indications of natural resources such as antioxidant and anti-tyrosinase properties [4] for food and cosmetic industries, respectively, to attest potential treatments of human health issues.

In tropical and subtropical regions, the Zingiberaceae family is among the most abundant and diverse plant

families, comprising approximately 47 widely distributed genera. Notable members include *Curcuma*, *Zingiber*, *Alpinia*, and *Kaempferia*, which have been extensively studied and reported to contain diverse secondary metabolites such as flavonoids, terpenoids, diarylheptanoids, and alkaloids, associated with biological activities including antimicrobial, antioxidant, anti-inflammatory, and enzyme inhibitory effects. Flavonoids are widely found in the rhizomes, leaves, and fruits of Zingiberaceae plants. Notable examples include cardamonin from *Alpinia* species [5], which has anti-inflammatory and anticancer activities, while quercetin, kaempferol and isorhamnetin derivatives from *Curcuma* [6] and *Alpinia* [5, 7], known for their anti-inflammatory, antioxidant and antimicrobial effects. Gingerol and shogaol derivatives from *Zingiber officinale* are well known for their potent antioxidant and anti-inflammatory activities, and recent studies have further shown that they can inhibit HDAC enzymes [8], highlighting their potential role in epigenetic regulation and cancer therapy. Sesquiterpenoids such as *α*-zingiberene, *β*-sesquiphellandrene and *β*-bisabolene in ginger (*Zingiber officinale*) not only underlies broad protective effects such as antimicrobial, antifungal, antioxidant, analgesic, anti-inflammatory, anti-ulcer, immunomodulatory and cytotoxic activities [9], but also displayed adaptogenic [10] and bronchodilatory properties, as evidenced in studies of *Zingiber officinale* [11] and *Alpinia calcarata* [12] rhizomes.

In contrast, some genera, such as *Amomum*, remain relatively underexplored [13]. *Amomum uliginosum* is one of the 90 recognized species within the *Amomum* genus [14] and is characterized as a flowering plant with tuberous rhizomes. It is native to Peninsular Malaysia, Borneo and Thailand, where it is locally known as 'Puar Hutan' or 'Tepus Merah'. Traditionally, it has been used in folk medicine to treat conditions such as nausea, asthma, and diarrhoea. Previously, an indane-type monoterpenoid characterized as (±)-*trans*-2,3,3a,7a-tetrahydro-1*H*-indene-4-carbaldehyde was successfully isolated from *Amomum tsao-ko* [15] and was found to produce a characteristic trigeminal sensation in the mouth. In addition, existing studies have reported the chemical composition of the essential oils extracted from *A. uliginosum*, revealing the presence of both *β*-pinene and *α*-pinene [16]. To date, only one phytochemical investigation of *A. uliginosum* has been carried out, revealing seven secondary metabolites from rhizomes collected in Chiang Rai Province, Thailand. Six of these compounds including labdane and bisnorlabdane diterpenes were reported for the first time in the *Amomum* genus, highlighting the unique chemical profile of this species [17]. Nevertheless, the scope of that study was confined to compound isolation, with no evaluation of the plant's biological activities.

Building on this preliminary research, the current study investigates the chemical composition of Malaysian *A. uliginosum* and evaluates its antibacterial, antioxidant and tyrosinase inhibitory properties. To the best of our knowledge, this work provides the first report on the bioactivities of this species and also documents, for the first time, the presence of this new diarylheptanoid in *A. uliginosum*, thereby contributing novel data on its pharmacological relevance.

Materials and Methods

Plant materials

Sample of *A. uliginosum* J. König rhizomes were collected from Hutan Simpan Bukit Hantu, Gua Musang, Kelantan. This species was identified by botanist Shamsul Khamis and the voucher specimen (SK1975/11) was deposited at the herbarium of the Institute of Bioscience, Universiti Putra Malaysia, Serdang, Malaysia.

Solvents, reagents and instrumentation

The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer using solvents chloroform-d and acetone-d6 depending on the solubility of the samples. Chemical shifts were recorded in ppm. The UV and IR spectra were measured on Shimadzu UV 1601PC spectrophotometer and Perkin Elmer FT-IR spectrometer, respectively. Mass spectrometry (MS) was obtained from Kent Mass Spectrometry Services, United Kingdom. Melting points were recorded using Leica Gallen III equipped with microscope. Deuterated solvents used in the NMR analysis, chloroform-d and acetone-d6, stabilized with silver foil, and concentrated sulphuric acid 95-98% were purchased from Merck. Thin Layer Chromatography (TLC) silica gel Kieselgel 60 F_{254} aluminium sheets 20 cm x 20 cm, silica gel 60 (0.040-0.063) for Vacuum Liquid Chromatography (230-400 mesh ASTM), silica gel 60 (0.063-0.200 mm) for Column Chromatography (CC, 70-230 mesh ASTM), silica gel 60 PF_{254} containing gypsum for preparative layer chromatography were purchased from Merck, Germany. Gradient solvent system was used for mobile phase on the chromatographic method starting from non-polar solvent, petroleum ether (PE) or *n*-hexane and then followed by combination of Et_2O, CH_2Cl_2, EtOAc, $CHCl_3$, acetone, and MeOH. PE was referred to PE with boiling point 60-80°C which was distilled prior to use. All solvents used in the isolation and purification were AR grade QRec and Merck brands.

96-round-bottom microplates was used to determine the Minimal Inhibitory Concentration (MIC) of antibacterial activity. The bacterial strains tested including *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (ATCC 11778), *Escherichia coli* (ATCC 10536) and *Pseudomonas aeruginosa* (ATCC 9027)

were purchased from the American Type Culture Collection (ATCC). Dimethyl sulfoxide was purchased from Merck, Germany and used as the solvent without further purification. Streptomycin sulfate (10 µg/disc) was purchased from Sigma-Aldrich, USA.

The materials used for antioxidant activity, including DPPH, L-ascorbic acid, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA), were purchased from Sigma-Aldrich and Fluka. Methanol, sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$), ammonium molybdate tetrahydrate, and sulfuric acid were purchased from Merck, Germany. Absorbance measurements were performed using a Perkin Elmer Lambda 25 UV-Vis spectrophotometer.

Mushroom tyrosinase, L-Dopa and kojic acid, used for tyrosinase inhibitory activity, were obtained from QRec. Sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic (NaH_2PO_4), DMSO, and methanol were obtained from Merck, Germany. Absorbance measurements were performed using a BIO-RAD ELISA plate reader.

Extraction and isolation of compounds

Sequential cold extraction of the air-dried rhizomes of *A. uliginosum* (480.0 g) was carried out at room temperature (28 °C) using organic solvents of increasing polarity: chloroform ($CHCl_3$, AR grade, ≥99.9%, 1500 mL), ethyl acetate (EtOAc, AR grade, ≥99.9%, 1500 mL) and methanol (MeOH, AR grade, ≥99.5%, 1500 mL). Each extraction was performed for 72 hours. After maceration, the resulting slurries were filtered, and the filtrates were concentrated at 45 °C under reduced pressure using a rotary evaporator. This yielded 12.06 g (2.49%) of a brownish $CHCl_3$ crude extract, 1.40 g (0.29%) of a dark brown EtOAc crude extract, and 7.87 g (1.62%) of a dark brown MeOH crude extract. All extracts were stored at 4 °C for further analysis.

The EtOAc extract was fractionated using by VLC over silica gel using PE (500 mL), PE-EtOAc, 4:1 (300 mL), PE-EtOAc, 3:2 (300 mL), PE-EtOAc, 2:3 (500 mL), PE-EtOAc, 1:4 (300 mL), and EtOAc (500 mL). Fractions with similar TLC profiles were combined, afforded 14 fractions, AURE1-AURE14. Further purification of fraction AURE1 by using prep-TLC eluted with *n*-hexane–Et_2O (4:1) afforded compound **(2)** (1.8 mg) and repeated crystallization of fraction AURE3 using cold PE yielded compound **(3)** (2.0 mg). Repeated purification of compound **(4)** (7.1 mg) and compound **(5)** (10.0 mg), together with compound **(1)** (234.4 mg), were obtained from repeated purification of fraction AURE2 and AURE11, respectively, using CC. Preliminary investigation of the chromatographic separation of the MeOH extract revealed that the TLC profile was similar to that of the EtOAc extract. A plant sterol identified as *β*-sitosterol **(6)** was obtained from the CH_2Cl_2 crude extract as white crystalline needle with m.p. 133-134°C (lit. 136°C). Structure of compounds (**1**-**6**) isolated from *A. uliginosum* shown in **Figure 1**.

Antibacterial activities

Antimicrobial activity assays were performed using the Minimal Inhibitory Concentration (MIC) in 96-round bottom-well sterilized microplates using a broth microdilution method [18]. All four bacterial strains used in this study were provided by the Biology Laboratory, Faculty of Biosciences and Bioengineering, Universiti Teknologi Malaysia (UTM), Skudai, Johor, and originally acquired from the American Type Culture Collection (ATCC). The bacteria were cultured in nutrient broth (NB) at 37 °C overnight. The turbidity of the bacterial suspensions was adjusted to match the McFarland standard for consistent inoculum density. Streptomycin Sulphate (SS) antimicrobial discs (10 µg/disc) were used as the positive control. Nutrient agar (NA) (20 g/L) and nutrient broth (NB) (8 g/L) were prepared using distilled water. All culture media and solutions were sterilized by autoclaving at 121.0 °C for 15 minutes under 15 psi pressure [19].

The test samples were dissolved in dimethylsulfoxide (DMSO) and their final concentrations were ranged from 14.06 to 1800 µg/mL, which were determined by 2-fold serial dilution method. The wells containing test strains and diluted samples were incubated at 37°C for 16-20 hours. The wells containing a culture suspension and DMSO were run as negative controls. All assays were carried out in three independent replicates and the results are expressed as mean ± standard deviation. The Minimal Inhibitory Concentration (MIC) was defined as the lowest antibiotic concentration that produced complete growth inhibition of the tested microorganisms.

Antioxidant activity

Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay in triplicate. The reduction of the purple DPPH radical to a yellow-coloured product was monitored spectrophotometrically at 517 nm, indicating free radical scavenging [20, 21]. Stock solutions (1.0 mg/mL) were diluted to concentrations ranging from 62.5 to 1000 µg/mL. For each assay, 0.2 mL of sample solution was mixed with 3.8 mL of 50 µM DPPH in methanol and incubated in the dark at room temperature for 30 minutes, while a control containing DPPH without sample was measured at 0 minutes. Ascorbic acid, BHT, and BHA were used as reference standards at equivalent concentrations. Methanol served as the blank for baseline correction.

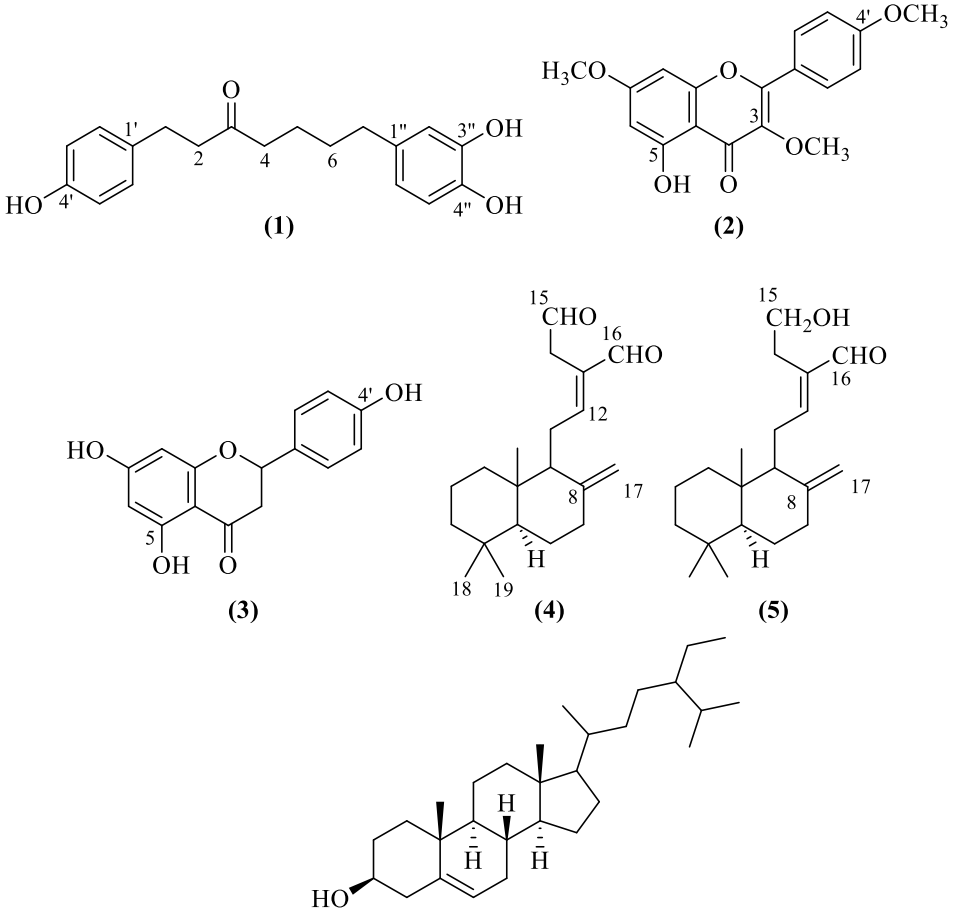


Figure 1. Structure of compounds (**1**-**6**) isolated from *A. uliginosum*

Lower absorbance values correspond to higher free radical scavenging activity. The percentage of DPPH inhibition was calculated, and IC_{50} values, defined as the concentration required to scavenge 50% of DPPH radicals, were determined from the dose-response curves.

Percent Inhibition (%) =

$$\frac{Abs(blank)-Abs\ (sample)}{Abs\ (Sample)}\ x\ 100 \qquad \text{(Eq. 1)}$$

Tyrosinase inhibitory assay

Tyrosinase inhibitory activity, a marker of skin-whitening potential, was evaluated in triplicate using a modified spectrophotometric assay [22] in a 96-well microplate format, with kojic acid used as a positive control. The test sample (2 mg) was dissolved in DMSO (2 mL) to get 0.1 mg/mL stock solution. 0.1 M phosphate buffer (pH 6.8, in d/w) (80 µL) was added to each microplate well from row A to H. The stock solution of sample (40 µL) was added to microplate wells at row A, B, C and D. Another 40 µL of buffer solution was added to well E1 and E2, followed by addition of DMSO (40 µL) into well E2 to H. Then, solution of 0.0025 M L-Dopa (40 µL) was added to all wells and incubated in the dark for 10 minutes. Tyrosinase (40 µL) was then added to all wells excluding E1 and E2 followed by a 30-minute incubation in the dark. Inhibition of tyrosinase activity was quantified by measuring absorbance at 515 nm (655 nm reference). Percentage inhibition was calculated using standard methods in Microsoft Excel.

Results and Discussion

Six compounds have been isolated from the rhizomes of *A. uliginosum* (**Figure 1**). They are identified as 7-(3,4-dihydroxyphenyl)-1-(4-hydroxyphenyl)-3-hepta none **(1)**, 5-hydroxy-3,7,4'-trimethoxyflavone **(2)**, 5,7,4'-trihydroxyflavanone **(3)**, (*E*)- labda-8(17),12-dien-15,16-dial **(4)**, (*E*)-labda-8(17),12-dien-15-ol-16-al **(5)** and *β*-sitosterol **(6)**. The structural elucidation of these compounds was based on their spectroscopic data as well as comparison of these data with the those reported [13, 20, 23-30]. The two flavonoids and two labdanes were previously isolated from Zingiberaceae family. Compound **(2)** has been isolated from *Kaempferia parviflora* Wall. Ex Baker [24] and *A. koenigii* [25] whilst compound **(3)** which

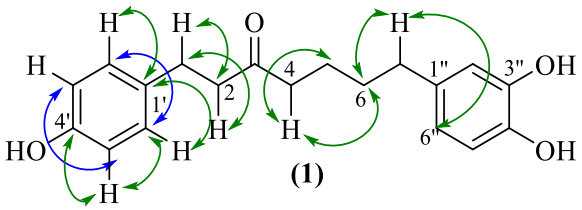
is also known as naringenin has been detected in *Curcuma longa* and *C. xanthorrhiza* [27]. The labdanes **(4)** and **(5)** were isolated for the first time from *Renealmia guianensis* [28] and *Alpinia calcarata* [29], respectively.

A prominent blue spot with strong intensity was observed on the TLC plate with R_f = 0.55 suggesting compound **(1)** containing phenol ring moiety [31]. The presence of benzene ring in compound **(1)** was then supported by the UV spectrum which showed maximum absorption bands at 283 nm and 227 nm when it was dissolved in MeOH. Analysis of its ^{1}H and ^{13}C NMR revealed the existence of seven aromatic protons and carbons, respectively. The expansion of ^{1}H NMR spectrum showed an *ortho*-coupled aromatic proton signals observed as a doublet A_2B_2 pattern with *J* values of 8.4 Hz at δ 6.76 and δ 7.04 integrating for two protons each. Signals at δ 6.61, δ 6.77 and δ 6.56 were assigned to H-2" (1H, d, *J* = 2.0 Hz), H-5" (1H, d, *J* = 8.0 Hz) and H-6" (1H, dd, *J* = 8.0, 2.0 Hz) displayed an ABX spin system. Analysis of the ^{1}H NMR spectrum also showed four sets of triplet integrating for two protons each, resonated at δ 2.84 (H-1, *J* = 7.2 Hz), δ 2.69 (H-2, *J* = 7.2 Hz), δ 2.36 (H-4, *J* = 7.0 Hz) and δ 2.44 (H-7, *J* = 7.4 Hz) which were attributable to four sets of methylene protons, as summarized in **Table 1**. The ^{1}H-^{1}H COSY NMR spectra clearly showed that H-2'/H-6' and H-3'/H-5' were coupled to each other (**Figure 2**). There were also correlations between aromatic protons H-2", H-5" and H-6". Besides, a long range ^{1}H-^{13}C HMBC showed the correlations between H-1 to C-2, C-1' and C-2'/C-6', H-2 to C-1, H-4 to C-5 and C-6, H-7 to C-6 and C-6", as well as H-3'/H-5' to C-4' and C-2'/C-6' (**Figure 2**). These data revealed that compound **(1)** have two different benzene ring systems.

The EIMS spectrum, displaying a molecular ion peak at *m/z* 314, was consistent with the molecular formula $C_{19}H_{22}O_4$. The principal fragment ions were observed at *m/z* 164, 147, 107, 91, and 77. The presence of a base peak at *m/z* 107 was due to the β-cleavage of C-1 and C-2 as benzylic carbocation which then delocalized and expand the ring to form the hydroxyl tropylium ion. Fragment ions at *m/z* 91 and *m/z* 77 were due to the loss of OH and CH_2 radicals from the hydroxyl tropylium ion. The fragment ions at *m/z* 147 and *m/z* 164 were attributed to α-cleavage, a common electron ionization fragmentation behaviour observed in benzylic and alkoxy-substituted systems. In compound (**1**), this α-cleavage occurs between the carbonyl group and C2 or C4, producing two resonance-stabilized cationic species. After all, compound **(1)** was elucidated as 7-(3,4-dihydroxyphenyl)-1-(4-hydroxyphenyl)-3-heptanone which was isolated from *A. muricarpum* [13].

The other compounds were identified based on NMR, MS, and literature comparison. The spectral data of compound **(2)** corresponded to 5-hydroxy-3,7,4'-trimethoxyflavone [24, 25], while compound **(3)** showed chemical shifts and fragmentation consistent with 5,7,4'-trihydroxyflavanone [27]. The labdane diterpenoids **(4)** and **(5)** exhibited NMR and MS features in agreement with previous reports [28, 29]. Overall, the spectroscopic data confirmed the identities of all isolated compounds. The antibacterial, antioxidant and anti-tyrosinase activities of the key crude extract and five isolated compounds were determined. AURE was evaluated to assess the overall bioactivity of the plant, as all isolated compounds were derived from this extract, allowing for the assessment of potential combined or synergistic effects.

The antibacterial activities of AURE and selected isolated compounds are showed in **Table 2**. The MIC values of the tested samples were in the range of 112.5-1800 µg/mL. No significant antimicrobial activity was observed for most of the tested samples, except for AURE and compound **(1)**. Both samples exhibited measurable antibacterial activity, with the most notable effect against both *Bacillus* species (MIC = 112.5 µg/mL). Although this activity is lower than that of the standard antibiotic streptomycin (MIC = 14.1 µg/mL), it is likely due to the presence of phenolic hydroxyl groups in the compound, which can interact with microbial proteins and inhibit enzyme function [14].



(1)

Figure 2. ^{1}H-^{1}H COSY (blue) and HMBC ^{1}H-^{13}C (green) correlations of compound **(1)**

Table 1. ^{1}H NMR and ^{13}C NMR of Compound **(1)**

Position	^{1}H NMR	^{13}C NMR
1	2.84 (2H, t, *J* = 7.2 Hz)	29.0
2	2.69 (2H, t, *J* = 7.2 Hz)	44.4
3	-	211.3
4	2.36 (2H, t, *J* = 7.0 Hz)	43.0
5	1.52 (2H, m)	23.3
6	1.43 (2H, m)	30.8
7	2.44 (2H, t, *J* = 7.4 Hz)	34.9
1'	-	132.9
2'/6'	6.76 (2H, d, *J* = 8.4 Hz)	115.2
3'/5'	7.04 (2H, d, *J* = 8.4 Hz)	129.5
4'	-	153.9
1"	-	134.8
2"	6.61 (1H, d, *J* = 2.0 Hz)	115.0
3"	-	143.6
4"	-	141.9
5"	6.77 (1H, d, *J* = 8.0 Hz)	115.4
6"	6.56 (1H, dd, *J* = 8.0, 2.0 Hz)	120.5

Table 2. Antibacterial activities of EtOAc crude extract and selected isolated compounds from *A. uliginosum* J. König (MIC: µg/ml)

Samples	*Bacillus subtilis*	*Bacillus cereus*	*Pseudomonas aeruginosa*	*Escherichia coli*
AURE	>128	**112.5**	>128	>128
(1)	**112.5**	>128	>128	>128
(2)	>128	>128	>128	>128
(3)	>128	>128	>128	>128
(4)	>128	>128	>128	>128
(5)	>128	>128	>128	>128
Standard: SS	14.1	14.1	14.1	14.1

These findings are consistent with the general bioactive profile of the Zingiberaceae family. Members of this family typically exhibit stronger antibacterial activity against Gram-positive bacteria than against Gram-negative strains. This selective activity is often associated with the presence of representative phenolic compounds [34] which decrease the compound's lipophilicity and restrict passage through the cell membrane, resulting in diminished cellular bioavailability in Gram-negative bacteria [35].

For the antioxidant assay, compound **(1)** displayed the most significant activity with 88% percentage inhibition at the lowest concentration (62.5 µg/mL) and an IC_{50} value of 28.2 µg/mL. This high activity is attributed to the presence of a catechol (ortho-dihydroxyl) moiety [20], which is a key structural determinant for free radical scavenging [18]. Other tested compounds showed lower activity or negligible activity, likely due to the absence of hydroxyl groups or ortho-dihydroxylation, which reduces radical-scavenging potential. The importance of the catechol group is well established. Flavonoids bearing an ortho-catechol group (3',4'-OH) in the B-ring exhibit higher antioxidant capacity, as they can effectively stabilize free radicals and chelate metal ions [36]. This general principle is further supported within the Zingiberaceae family, for instance, flavonoids isolated from *Amomum koenigii* show little to no antioxidant activity when the B-ring lacks a catechol (3',4'-dihydroxyphenyl) structure [25]. Meanwhile, alcoholic extracts of *Curcuma longa* [37] and *Etlingera elatior* [38] exhibited strong antioxidant activity, which has been attributed to the presence of ortho-dihydroxy functional groups in their active compounds.

In the present study, compound (**2**) exhibited weak antioxidant activity, which is consistent with

previous reports. For compound **(3)**, although it possesses three hydroxyl groups, its antioxidant activity remains limited, likely because effective radical scavenging requires not only multiple hydroxyl groups but also specific substitution patterns such as two hydroxyl groups accompanied by two methoxy groups on ring A as displayed by *Prunus cerasus* from Rosaceae family [39]. **Table 3** summarizes the DPPH radical scavenging activity of the tested samples and standards.

The *in-vitro* mushroom tyrosinase inhibitory activity for AURE and selected isolated compounds from *A. uliginosum* J. König was successfully conducted. **Table 4** shows the percent of inhibition activity against tyrosinase. Results show that the AURE possessed highest percentage inhibition which is 55.5±0.5%. This revealed that AURE contained bioactive compounds and has been proven by the isolation of diarylheptanoid **(1)**, flavone **(2)**, flavanone **(3)**, two diterpene labdanes **(4)** and **(5)**, as discussed earlier. The inhibition of tyrosinase ability might depend on the hydroxyl groups of the phenolic compounds of the mushroom extracts that able to form a hydrogen bond at active site of the enzyme leading to changed conformation [18].

Among the tested compounds, compounds **(3)** and **(1)** showed significant ability to act as tyrosinase activity inhibitors with percentage inhibition of 52.6±0.6% and 51.7±0.8%, respectively. The study on structure-activity relationship revealed that the 4-substituted resorcinol (1,3-benzenediol) moiety, catechol and polyhydroxyl substituents are important criteria for the tyrosinase inhibitor against tyrosinase mushroom [18, 20]. Compound **(3)** showed the presence of resorcinol moiety in A ring and hydroxyl substituent in B ring which contributed to the significant of tyrosinase inhibitory activity [18]. Furthermore, **(1)** possessed a catechol moiety at H-3" and H-4", and hydroxyl substituent at H-4' which associated to be potent tyrosinase activity inhibitor.

Other members of the Zingiberaceae family have also been reported to exhibit significant tyrosinase inhibitory activity. For instance, extracts from *Curcuma amada* [40], *Alpinia galanga* [41], *Zingiber montanum* [40], *Curcuma zedoaria* [42], *Curcuma heyneana* [43], as well as *Hedychium coronarium* [44], and a peptide derived from *Zingiber cassumunar* [45], demonstrated similar effects. These findings highlight the chemotaxonomic relevance of phenolic and catechol-containing compounds within the family and suggest that the activity observed in AURE and its isolated compounds is consistent with the general bioactive profile of Zingiberaceae.

Table 3. DPPH radical scavenging activity of EtOAc crude extract and selected isolated compounds from *A. uliginosum* J. König

Samples	Percent Inhibition at 62.5 µg/mL (%)	IC_{50} (µg/mL)
AURE	83	32.4
(1)	88	28.2
(2)	10	> 1000
(3)	31	189.3
(4)	inactive	inactive
(5)	inactive	inactive
Standard: A. Acid	97	16.7
Standard: BHT	29	124.4

Table 4. Tyrosinase inhibitory activities of EtOAc crude extract and selected isolated compounds from *A. uliginosum* J. König

Samples	Inhibitory Activity against Tyrosinase at 0.1 mg/mL (% ± SEM)
AURE	55.5 ± 0.5
(1)	51.7 ± 0.8
(2)	4.1 ± 0.2
(3)	52.6 ± 0.6
(4)	47.4 ± 0.2
(5)	38.6 ± 0.5
Standard: Kojic Acid	81.8 ± 0.6

7-(3,4-dihydroxyphenyl)-1-(4-hydroxyphenyl)-3-heptanone **(1)**
Brown gum, (234.4 mg, 16.7%); R_f = 0.55 (*n*-hexane:EtOAc, 2:3); IR (ATR) υ_{max} cm^{-1}: 3352 (OH), 1700 (C=O), 1641 and 1446 (C=C aromatic) and 1228 (C-O); UV (MeOH) λ_{max} nm: 283, 227; ^{1}H NMR ($CDCl_3$): δ 7.04 (2H, d, *J* = 8.4 Hz, H-3'/H-5'), 6.77 (1H, d, *J* = 8.0 Hz, H-5"), 6.76 (2H, d, *J* = 8.4 Hz, H-2'/H-6'), 6.61 (1H, d, *J* = 2.0 Hz, H-2"), 6.56 (1H, dd, *J* = 8.0, 2.0 Hz, H-6"), 2.84 (2H, t, *J* = 7.2 Hz, H-1), 2.69 (2H, t, *J* = 7.2 Hz, H-2), 2.44 (2H, t, *J* = 7.4 Hz, H-7), 2.36 (2H, t, *J* = 7.0 Hz, H-4), 1.52 (2H, m, H-5) and 1.43 (2H, m, H-6); 13C NMR (CDCl3): δ 211.3 (C-3), 153.9 (C-4'), 143.6 (C-3"), 141.9 (C-4"), 134.8 (C-1"), 132.9 (C-1'), 129.5 (C-3'/C-5'), 120.5 (C-6"), 115.4 (C-5"), 115.2 (C-2'/C-6'), 115.0 (C-2"), 44.4 (C-2), 43.0 (C-4), 34.9 (C-7), 30.8 (C-6), 29.0 (C-1), 23.3 (C-5); and EIMS *m/z* (rel. int): 314 (0.5), 164 (71), 147 (30), 107 (100), 91 (46), 77 (35).

5-Hydroxy-3,7,4'-trimethoxyflavone **(2)**
Yellow crystalline needles, (1.8 mg, 0.28%); R_f = 0.48 (*n*-hex: $CHCl_3$/2:3); m.p. 156-157°C (lit. [18] 157-158°C); IR (ATR) υ_{max} cm^{-1}: 3518 (OH), 1709 (C=O), 1602 and 1461 (C=C aromatic), and 1217 (C-O); UV (MeOH) λ_{max} nm: 345, 267; UV (MeOH+$AlCl_3$) λ_{max} nm: 344, 284; ^{1}H NMR ($CDCl_3$): *δ* 12.68 (s, 5-OH), 8.01 (2H, d, *J* = 8.8 Hz, H-3'/H-5'), 7.04 (2H, d, *J* = 8.8 Hz, H-2'/H-6'), 6.46 (1H, d, *J*= 2.0 Hz, H-6), 6.37 (1H, d, *J* = 2.0 Hz, H-8), 3.91 (3H, s, 3-OCH_3), 3.89 (3H, s, 4'-OCH_3) and 3.87 (3H, s, 7-OCH_3); ^{13}C NMR ($CDCl_3$): *δ* 178.8 (C-4), 165.4 (C-7), 162.1 (C-2), 161.7 (C-5), 156.8 (C-4'), 155.8 (C-8a), 138.9 (C-3), 130.2 (C-3'/C-5'), 122.6 (C-1'), 114.1 (C-2'/C-6'), 105.9 (C-4a), 97.8 (C-8), 92.2 (C-6), 60.1 (7-OCH_3), 55.8 (4'-OCH_3) and 55.4 (3-OCH_3).

5,7,4'-Trihydroxyflavanone **(3)**
White powder, (2.0 mg, 0.31%); R_f = 0.73 (*n*-hexane:EtOAc, 2:3); m.p. 249-251°C (lit. [20] 247-250°C); IR (ATR) υ_{max} cm^{-1}: 3276 (OH), 1708 (C=O), 1588 and 1458 (C=C aromatic), and 1025 (C-O); UV (MeOH) λ_{max} nm: 321, 286; ^{1}H NMR ($CDCl_3$): *δ* 12.20 (s, 5-OH), 8.61 (br, 7-OH), 8.15 (br, 4'-OH), 7.41 (2H, d, *J* = 8.6 Hz, H-3'/H-5'), 6.91 (2H, d, *J* = 8.6 Hz, H-2'/H-6'), 5.97 (1H, d, *J* = 2.0 Hz, H-6), 5.96 (1H, d, *J* = 2.0 Hz, H-8), 5.47 (1H, dd, *J* = 12.8, 3.0 Hz, H-2), 3.19 (1H, dd, *J* = 17.2, 12.8 Hz, H-3*β*) and 2.74 (1H, dd, *J* = 17.2, 3.0 Hz, H-3*α*); ^{13}C NMR ($CDCl_3$): *δ* 196.4 (C-4), 166.6 (C-8a), 164.4 (C-7), 163.5 (C-5), 157.9 (C-4'), 128.1 (C-3'/C-5'), 115.3 (C-2'/C-6'), 102.3 (C-4a), 95.9 (C-6), 94.9 (C-8), 79.1 (C-2) and 42.6 (C-3); and EIMS *m/z* (rel. int): 272, 179, 153, 120.

(E)- labda-8(17),12-dien-15,16-dial **(4)**
Brownish liquid, (7.1 mg, 0.89%); R_f = 0.42 (*n*-hexane–$CHCl_3$, 1:1); IR (NaCl) υ_{max} cm^{-1}: 3080 (=CH), 2929 (sp^3 C-H), 2846, 2718 (CH-aldehyde), 1727 (non-conjugated C=O), 1683 (conjugated C=O), 1643 (C=C); ^{1}H NMR ($CDCl_3$): *δ* 9.64 (t, *J* = 1.4 Hz, 15-CHO), 9.41 (s, 16-CHO), 6.78 (1H, t, *J* = 6.4 Hz, H-12), 4.88 and 4.38 (2H, s, H-17), 3.45 and 3.42 (2H, dd, *J* = 1.4, 16.8 Hz, H-14), 2.49 to 1.07 (14H, m, H-11, H-7, H-5, H-6, H-1, H-2, H-3, H-9), 0.90 (3H, s, H-18), 0.83 (3H, s, H-19) and 0.74 (3H, s, H-20); ^{13}C NMR ($CDCl_3$): δ 197.3 (15-CHO), 193.6 (16-CHO), 159.9 (C-12), 148.0 (C-8), 134.9 (C-13), 107.8 (C-17), 56.5 (C-9), 55.4 (C-5), 41.9 (C-3), 39.6 (C-10), 39.4 (C-14), 39.3 (C-1), 37.9 (C-7), 33.6 (C-4, C-18), 24.7 (C-11), 24.1 (C-6), 21.7 (C-19), 19.3 (C-2), 14.4 (C-20); and EIMS *m/z*: 302, 137.

(E)-labda-8(17),12-dien-15-ol-16-al **(5)**
Yellow liquid (10.0 mg, 1.02%); R_f = 0.60 (*n*-hex: Et_2O/1:3); IR (ATR) υ_{max} cm^{-1}: 3425 (OH), 2864 (CH-aldehyde), 1711 (conjugated C=O), 1644 (C=C); ^{1}H NMR ($CDCl_3$): *δ* 9.39 (s, 16-CHO), 6.72 (1H, t, *J* = 6.4 Hz, H-12), 4.87 and 4.40 (1H, s, H-17), 3.35 and 3.41 (2H, br d, *J* = 16.4 Hz, H-15), 2.58 and 2.56 (2H, m, H-14), 2.62 and 2.61 (2H, m, H-11), 1.12 to 2.46 (12H, m, H-7, H-5, H-6, H-1, H-2, H-3, H-9), 0.90 (3H, s, H-18), 0.84 (3H, s, H-19) and 0.76 (3H, s, H-20); and ^{13}C NMR ($CDCl_3$): *δ* 193.7 (16-CHO), 159.4 (C-12), 148.1 (C-8), 135.7 (C-13), 107.9 (C-17), 56.4 (C-9), 55.4 (C-5), 42.0 (C-3), 39.6 (C-10), 39.2 (C-1), 37.9 (C-7), 33.6 (C-4, C-18), 29.7 (C-14), 29.5 (C-15), 24.6 (C-11), 24.1 (C-6), 21.7 (C-19), 19.3 (C-2) and 14.4 (C-20).

Conclusion
This study represents the first detailed report on the chemical composition of rhizomes of *Amomum uliginosum* J. König. Six compounds were successfully isolated, including a new diarylheptanoid, 7-(3,4-dihydroxyphenyl)-1-(4-hydroxyphenyl)-3-heptanone **(1)**, two flavonoids, 5-hydroxy-3,7,4'-trimethoxyflavone **(2)** and 5,7,4'-trihydroxyflavanone **(3)**, and two labdane-type diterpenes, (*E*)-labda-8(17),12-dien-15,16-dial **(4)** and (*E*)-labda-8(17),12-dien-15-ol-16-al **(5)** as well as a plant sterol **(6)**. The compounds were isolated using a combination of vacuum liquid chromatography and column chromatographic techniques. The combination of spectroscopic data, including NMR, IR, UV, and mass spectrometry, along with comparison to literature values, validates the identification of the new diarylheptanoid and other isolated compounds. Biological evaluation revealed that **(1)** consistently showed the highest activity across multiple assays, including potent antibacterial effects (MIC = 112.5 µg/mL against *B. subtilis* and *B. cereus*), antioxidant activity (IC_{50} = 28.2 µg/mL) and substantial tyrosinase inhibitory activity (51.7 ± 0.8%). In comparison, the other compounds exhibited lower or selective activity depending on the assay. These results suggest a clear

correlation between the presence of hydroxylated phenyl rings and biological activity, as the hydroxyl groups in diarylheptanoid **(1)** enhanced both antioxidant and tyrosinase inhibitory properties. Our findings also support previously reported correlations between *p*-hydroxy substituted amides and anti-tyrosinase activity [46]. Overall, the combined chemical and biological data support the potential of compound **(1)** as a promising candidate for further development in functional food and pharmaceutical applications, highlighting the importance of specific structural features in modulating activity.

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