The effect of Andong (Cordyline terminalis) leave, one of the traditional plants in Bali as antioxidant and antibacterial

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Abstract. Climate change can have a direct effect (e.g. changes in air temperature, increased ultraviolet radiation, and pollution) and indirect effect (such as increased incidence infectious diseases). Infectious diseases are the main cause of high human mortality, especially in developing countries like Indonesia. Andong plant (Cordyline terminalis) is one of Bali’s local plants, which is widely used as a drug for infections due to microbes resulting from climate change. This study aimed to observe the effect of Andong leaf extract as an antioxidant and antibacterial and to identify the compounds. This research design was carried out with sample preparation including cleaning, cutting, drying, grinding and sifting to powder. Extraction was done by maceration method. Phytochemical tests were then performed with color reagents. Antioxidant test was carried out using diphenyl picryl hydrazil hydrate (DPPH) method and antibacterial activity was determined by well diffusion method. Measurement of antioxidant compounds’ levels was determined spectrophotometrically and analysis of antioxidant compounds was carried out by the LC-MS/MS method. The result showed that the Andong leaf extract contained saponins, polyphenols, flavonoids, steroids, triterpenoids, phytosterols, amino acids and alkaloids. In the testing of antioxidant compounds, Andong leaf extract contained tannins was 3324.550±0.821 mg/100 g TAE, phenol was 1398.905±0.812 mg/100 g GAE acids and alkaloids. In the testing of antioxidant compounds, Andong leaf extract contained flavonoids was 1

1. Introduction
Climate change is a change that can have a direct effect (e.g., changes in air temperature, increased ultraviolet radiation, and pollution), but most of these changes have indirect consequences (such as food availability, increased incidence of vector-borne diseases, and infectious and non-infectious diseases) [1]. The impact of climate change in Indonesia can be even more severe due to socio-economic factors such as population density, poverty, personal hygiene, availability of clean water and unequal income distribution. Indonesia is also a hotbed for infectious diseases such as dengue fever and diarrhea.

Infectious diseases are the main cause of high human mortality, especially in developing countries like Indonesia. The main pathogenic microbes that cause infectious diseases are bacteria [2]. Severe infections due to these bacteria include pneumonia, mastitis, meningitis and urinary tract infections. Staphylococcus aureus is also the main cause of nosocomial infections and food poisoning [3]. Other
bacteria that also infect humans are *Escherichia coli*. *Escherichia coli* is a gram-negative bacteria that cause diarrheal disease, urinary tract infections, nosocomial infections and can cause acute meningitis [3].

Infectious diseases caused by bacteria are often treated with antibiotics. Antibiotics have two main functions namely, to kill bacteria or slow their proliferation. Although antibiotics give satisfactory results, their use must be limited because excessive use can lead to the development of bacterial resistance. Bacterial resistance to certain antibiotics results in the use of the same antibiotics that are no longer effective in treating these bacterial infections so that the pathogenicity process due to infection continues. These events will encourage long-term use of antibiotics, which can have harmful side effects on the body [4].

An alternative method that can be done to overcome this problem is to develop herbal medicines derived from plants. The content of secondary metabolite compounds that act as antioxidants in plants plays an important role in their activity as antibacterial [5]. Andong leaf (*Cordyline terminalis* Kunth) is one of Bali’s local plants, which has benefits, namely as an ornamental plant, medicine, and religious ceremonies. The color of the leaves of this Andong plant is very influenced by the season. If the rainy season, the leaves’ color becomes slightly greenish and in summer, the color becomes bright red and it is thought that they contain lots of antioxidant compounds.

Antioxidant compounds are compounds that can capture, neutralize and stabilize free radicals. Free radicals are molecules that have one or more unpaired electron in an outer shell. These unpaired electrons cause free radicals to become highly reactive compounds to body cells by binding to electron molecule cells. In this condition, humans need antioxidant compounds obtained from foods that contain phenolics, anthocyanins, tannins, flavonoids and saponin. Various scientific evidence shows that the risk of chronic diseases due to free radical can be reduced by utilizing antioxidant compounds such as vitamins C, E, A, carotene, phenolics, and polyphenols flavonoids, steroids, tannins, and saponins [6].

Several studies on antioxidant compounds’ activity have been carried out on Andong plant, namely Andong leaf saponins as antiobesity and anticholesterol [7][8]. Andong leaf extract has also been able to act as an antidiabetic in obese Wistar rats [9]. These activities indicate that Andong leaves contained antioxidant compounds that are thought to also act as antimicrobials.

This study aimed to observe the effect of Andong leaf extract as an antioxidant and antibacterial and to identify the compounds contained in the plant leaf extract.

2. Material and methods

2.1. Materials and tools
The research material was Andong leaf obtained in Tampaksiring, Gianyar, Bali, Indonesia; 2,2 - Diphenyl-1-picrylhydrazyl (DPPH) ex. Sigma; ethanol solvent for Spectrophotometer ex. E. Merck; Heidolph freeze dryer; Hitachi U1800 spectrophotometer, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Shigella flexneri*.

2.2. Preparation and extraction of andong leaves
Andong leaves powder of 350 g was extracted by maceration using methanol solvent for 24 hours at room temperature and the extraction process was carried out 5 times. The extract obtained was dried with the freeze-drying technique.

2.3. Phytochemical test
According to Hossain and Nagooru [10], the phytochemical test was carried out qualitatively.

2.4. Determination of total flavonoids content
The content of total flavonoid was determined by AlCl₃ method using quercetin as a standard compound. The absorbance of the mixture was measured at 415 nm. The total flavonoid content is expressed in quercetin equivalent (mg/100 g QE) [11].
2.5. Determination of total phenol content
The content of total phenol was carried out using the Folin-Ciocalteu method. The samples were measured spectrophotometrically at 765 nm. The total phenolic amount was determined as gallic acid and expressed as mg/100 g GAE [12].

2.6. Determination of total tannin content
Total tannin of leaf extract was determined using the spectrophotometry method, according to Saranya et al [12].

2.7. The activity of 1,1-diphenyl-2-picrylhydrazylhydride (DPPH) radical scavenging
The activity of DPPH radical scavenging of Andong leaf extract was determined based on Bogoriani et al [9]. The DPPH radical concentration was calculated using the following equation (1).

\[
\% \text{ inhibition} = \frac{A_{\text{Control}} - A_{\text{test}}}{A_{\text{Control}}} \times 100
\]

(1)

2.8. Analysis of microbiological
Microbiological analysis of Andong leaf extracts was performed according to Mahuruk et al [13]. The antimicrobial activity was characterized by forming clear zones around the wells and measured for its diameter (mm) [13].

2.9. Analysis of Andong leaf extract with LC-MS/MS method
LC-MS/MS analysis uses the ACQITY UPLC * H-Class System (water, USA) LC, and Xevo G2-S Qtof Mass Spectrometer (water, USA). All mass spectrometer (MS) spectra were extracted using masslynx v 4.1 software.

2.10. Analysis of statistic
The data were tested by one-way ANOVA followed by the least-significant-difference test (LSD) and Tamhan’s test for determined the value difference between treatment groups. The criterion for significance differences was set at p < 0.05.

3. Result and discussion

3.1 Andong leaf methanol extract with maceration method
Maceration results from 350 grams of dried powder of red Andong leaf using 96% methanol solvent obtained as much as 65 grams of powder extract which was dark green and had a distinctive aroma. The yield of powder extract was 18.57 ± 0.671%. The yield of powder extract was calculated according to the extraction yield formula given by Tahar et al. [14].

3.2 Phytochemical test
Table 1 shows positive reactions to all test reagents. The methanol extract of Andong leaf contained all tested compounds like polyphenols, flavonoids, saponins, alkaloids, steroids, tannins, glycosides, and triterpenoids, phytosteroid and amino acids. These compounds have been reported to have medicinal benefits like anti glycemic, antioxidant, anticancer, antilipidemic, antiobesity, antimicrobial and immunomodulatory activities [7][8][11]. Methanol extracts of Andong leaf were quite an effective solvent in the extraction of antioxidant compounds. Methanol extracts of Andong leaf exhibited a positive reaction in all the assays. The phytochemical analysis results were almost the same as a previous study by Hossain and Nagooru [10].
Table 1. Result of phytochemical test from Andong leaf methanol extract

| No | Phytochemical compound | Test                          | Result |
|----|------------------------|-------------------------------|--------|
| 1  | Polyphenols            | FeCl₃ test                    | +      |
| 2  | Flavonoids             | Mg powder dan concentrated HCl | +      |
| 3  | Saponins               | Frothing test                 | +      |
| 4  | Alkaloids              | Mayer’s reagent test/Drageordoff’s test | + |
| 5  | Steroids               | Liebermann-burchard test      | +      |
| 6  | Tannins                | Ferric Chloride test          | +      |
| 7  | Glycosides             | Borntrager test               | +      |
| 8  | Triterpenoids          | Liebermann-burchard test      | +      |
| 9  | Phytosterols           | Liebermann-burchard test      | +      |
| 10 | Amino acids            | Ninhydrin reagent             | +      |

+ = presence; - = absence

3.3. Determination of total phenolic compounds content

Table 2 shows that Andong leaf’s extract contained the highest tannins with a significant difference (p<0.05) compared to phenols and flavonoids. Total phenol was 1399±0.81 mg/100 g GAE; tannin was 3324.55±0.82 mg/100 g TAE; and flavonoids was 1373.07±1.28 mg/100g QE. Andong leaf extract exhibited a good radical scavenging activity on DPPH (88.26±0.015 ppm). Generally, phenolic compounds’ antioxidant activity can scavenge free radicals, chelate metal cations, or donate hydrogen atoms or electrons [5]. The antioxidant capacity of phenolic compounds is also attributed to their ability to chelate metal ions involved in free radicals production [6].

Table 2. The contents of the flavonoids, tannins, phenolic compounds and IC₅₀ of Andong leaf extract

| Sample          | Flavonoids mg/100g QE | Tannins mg/100g TAE | Phenolic mg/100g GAE | IC₅₀ ppm |
|-----------------|-----------------------|---------------------|----------------------|----------|
| Andong leaf     | 1373.07±1.28b,d       | 3324.55±0.82a,c,d    | 1398.92±0.92b,d      | 88.26±0.015a,b,c |

The mean ± standard deviation (n = 5) followed by a superscript in the same row shows a significant difference p < 0.05. The letters (a,b,c,d) indicated the non-homogeneous subsets of Tamhane’s test. QE = quercetin equivalents; TAE = tannic acid equivalent; GAE = gallic acid equivalents.

Tannins as a secondary metabolite have been reported to have a role in reducing feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in test animals. The antioxidant properties of tannins have the potential to be anti-carcinogenic and antimutagenic and can protect cell damage due to lipid peroxidation processes [15].

Flavonoids, a group of hydroxylated phenolic substances known to be potent free radical scavengers, have attracted tremendous interest in therapeutics against free radical-mediated diseases, particularly diabetes mellitus and their different pharmacological effects are mostly structured dependent. Flavonoids of plants are most commonly known for antioxidant and antimicrobial biological activities [6].

Andong leaf extract could neutralize free radicals from DPPH (Table 2). This test is one of the most widely used in vitro tests to determine extracts’ effect on free radicals from DPPH. With Andong leaf extract, which can donate hydrogen atoms, DPPH free radicals are lost and the purple color turns yellow (diphenyl picryl hydrazine) [7]. This study indicated that Andong leaf extract had IC₅₀ = 88.26 ± 0.015 ppm, which means that the extract had strong antioxidant activity. It was proven that the extracts showed the ability to donate hydrogen atoms so that the extracts can function as a free radical scavenger [7].
3.4. Antibacterial activity of Andong leaf extracts

Antibacterial activity of Andong leaf was determined against three bacteria viz. *Staphylococcus aureus*, *Shigella flexneri*, and *Escherichia coli* by diffusion method. Table 3 indicates that all the test bacteria were found to be susceptible to Andong leaf extracts. Extract of Andong leaf 100 ppm was more effective against *E. coli* compared to *S. aureus* and *S. flexneri*. The inhibitory power of extract with 100 ppm was the highest against *E. coli* and the lowest was against *S. aureus*. Inhibitory extracts of Andong leaf was higher than tetracycline with a significant difference (p < 0.05). Pavithra et al [5] reported that medicinal plants have been used as remedies for centuries of diseases. Antimicrobials of plant origin have enormous therapeutic potential due to the presence of certain metabolites [5].

| Test bacteria   | *Escherichia coli* | *Shigella flexneri* | *Staphylococcus aureus* |
|-----------------|--------------------|---------------------|------------------------|
| Control (methanol) | 0.79±0.19<sup>b,c</sup> | 0.82±0.16<sup>b,c</sup> | 0.77±0.23<sup>b,c</sup> |
| Extract 100 ppm  | 8.43±0.55<sup>a,c</sup> | 7.44±0.55<sup>a,c</sup> | 6.84±0.54<sup>a,c</sup> |
| Tetracycline     | 6.02±0.24<sup>a,b</sup> | 5.31±0.61<sup>a,b</sup> | 6.40±0.52<sup>a,b</sup> |

The mean of inhibition zone (mm) ± standard deviation (n = 5) followed by a superscript in the same row shows a significant difference at p < 0.05. The letters (a,b,c,d) indicated the non homogeneous and homogeneous subsets of Tamhane and LSD’ test.

3.5 The analysis of Andong leaf extract with LC-MS/MS

The chromatograms and compounds of Andong leaf extract analysis using methanol and dichloromethane (DCM) are presented in Figures 1 and 2, respectively (see Appendix Table 4 and Table 5).

**Figure 1.** Chromatogram of extraction result sample with methanol solvent
4. Conclusion
Andong leaf extract had antioxidant and antibacterial activity due to secondary metabolites namely tannins, phenolic, flavonoid, steroids, alkaloids, and saponins the potential to treat infectious diseases caused by climate change.

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### Appendix table

**Table 4. Identification of compounds of Andong leaf extract with methanol solvent**

| Time retention (minute) | m/z (M+H)<sup>+</sup> | Formula (+H<sup>+</sup>) | Compound name | Structure |
|-------------------------|------------------------|--------------------------|---------------|-----------|
| 0.69                    | 144.1021              | C₇H₁₄NO₂                 | 1-Aminocyclohexanecarboxylic acid | ![Structure](image1) |
| 1.72                    | 154.0745              | C₇H₆O₄                   | Protocatechuic acid | ![Structure](image2) |
| 1.72                    | 353.1475              | C₁₄H₂₅O₁₀                | Methyl 4-O-{[(5R)-5-vinyl-α-L-arabinopyranosyl]-α-D-galactopyranoside | ![Structure](image3) |
| 3.73                    | 154.0745              | C₇H₆O₄                   | Protocatechuic acid | ![Structure](image4) |
| 4.06                    | 191.0809              | C₁₀H₁₂N₂O₄               | 4-Cyanophenylalanine | ![Structure](image5) |
| 4.64                    | 636.2259              | C₃₉H₆₅NO₂₄              | N-[2-{[(4-O-(α-D-Glucopyranosyl)-β-D-glucopyranosyl)oxy]ethoxy}ethyl]-9-oxo-9H-fluorene-2-carboxamide | ![Structure](image6) |
| 5.38                    | 611.1625              | C₂₃H₂₂N₂O₁₂              | 4-{[(4-Benzoyloxy)-1H-benzimidazol-6-yl]carbamoyl}oxy]methyl]-2-nitrophenyl hexopyranosiduronic acid | ![Structure](image7) |
| 5.63                    | 625.3586              | C₂₃H₂₇O₁₆                | 5,7-Dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-chromeniumyl 2-O-[β-D-glucopyranosyl-α-D-mannopyranoside | ![Structure](image8) |
| 6.0                     | 1029.5291             | C₁₄H₂₃N₃O₅S             | | |

Amino acid

Phenolic

Glycoside

Phenolic

Amino acid

Phenolic

Glycoside

Phenolic

Flavonoid
| Time retention (minute) | r/m (M+H)+ | Formula (+H)+ | Compound name | Structure |
|------------------------|------------|---------------|---------------|-----------|
| 5.74                   | 609.3636   | C_{27}H_{29}O_{16} | 3-[(2-O-Hexopyranosyl)hexopyranosyl]oxy]-5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-olate | Flavonoid |
| 6.21                   | 611.3766   | C_{13}H_{10}O_{10} | (3β,5α,16ξ,22S,23S,25R,26S)-3,22-Dihydroxy-23,26-epoxyfurostan-26-yl β-D-glucopyranoside | Saponin |
| 6.29                   | 648.2014   | C_{13}H_{12}O_{10} | 1(R,3S,4R,5S)-3-Hydroxy-1,4,5-tris(3,4,5-trihydroxybenzoyl)oxy)cyclohexancarboxylic acid | Polyphenolic |
| 7.27                   | 181.1220   | C_{11}H_{10}O | 2-(5-Hexen-1-yl)-5-hydroxy-3,4-dihydropyranium | |
| 7.72                   | 192.1832   | C_{12}H_{10}NO | N-(3-Hydroxybenzyl)-N,N-dimethyl-2-propen-1-aminium | Alkaloid |
| 7.80                   | 721.4144   | C_{34}H_{61}N_{10}OS_{3} | Ethyl4-(((3β,5α)-cholestan-3-yloxy)carbothioyl)sulfanyl)-3-oxobutanoate | Steroid |
| 8.19                   | 331.2258   | C_{21}H_{31}O_{3} | 11α-hydroxy-Progesterone | Steroid |
| 8.55                   | 225.1958   | C_{21}H_{22}NO | Ethyl (4S)-5-cyclohexyl-2,2-difluoro-4-[(2S)-2-[[N-(4-morpholinsulfonyl)]-L-phenylalanyl]amino]-4-pentenoyl]amino)-3-oxopentanoate | Amino acid |
| 9.00                   | 403.2011   | C_{26}H_{29}N_{2}O_{3} | 4-[[E]-2-[(3-Ethoxy-4-[(2-[4-methylphenyl]amano)-2-oxoethoxy]phenyl)vinyl]-1-methylpyridinium | Alkaloid |
| Time retention (minute) | m/z (M+H)^+ | Formula (+H^+) | Compound name | Structure |
|-------------------------|-------------|----------------|---------------|-----------|
| 9.32                    | 312.2535    | C_{19}H_{20}O_{4} | 1-Oxo-1-phenyl-2-propanyl (4-methoxyphenyl)propanoate |
| 9.65                    | 314.2694    | C_{19}H_{22}O_{4} | 4-Methoxyphenyl (2-isopropyl-5-methylphenox)acetate |
| 10.28                   | 286.2739    | C_{19}H_{20}O_{4} | Kaempferol |
| 10.78                   | 314.2689    | C_{19}H_{20}O_{4} | 4-Methoxyphenyl (2-isopropyl-5-methylphenox)acetate |
| 11.12                   | 318.3003    | C_{19}H_{20}O_{4} | 5,7,8-Trihydroxy-2-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one |
| 11.48                   | 884.5023    | C_{40}H_{71}O_{17} | Osladin |
| 11.70                   | 884.5013    | C_{40}H_{71}O_{17} | |
| 12.11                   | 886.5179    | C_{40}H_{71}O_{17} | (3β,5β,9α,14α,25S)-Spirostan-3-yl 6-deoxy-a-L-mannopyranosyl(1->4)[β-D-glucopyranosyl(1->2)]β-D-glucopyranoside |
| 13.07                   | 721.4172    | C_{27}H_{41}N_{2}O_{8}S | |
| 13.28                   | 413.3060    | C_{7}H_{11}O_{3} | 3-Oxandrostan-4-en-17-yl 3-cyclopentylpropanoate |
| 13.43                   | 415.3211    | C_{7}H_{11}O_{3} | Diosgenin |
| Time retention (minute) | m/z (M+H)^+ | Formula (+H)^+ | Compound name | Structure |
|------------------------|-------------|----------------|---------------|-----------|
| 14.01                  | 522.3565    | C_{24}H_{26}O_{13} | 5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,6-dimethoxy-4-oxo-4H-chromen-7-y1-D-glucopyranoside | ![Structure Image](image1) |
| 14.31                  | 217.1586    | C_{15}H_{21}O     | Turmerone     | ![Structure Image](image2) |
| 15.11                  | 324.2900    | C_{18}H_{12}O_{6} | 2-[(Methoxycarbonyl)phenyl]-2-oxo-2H-chromene-3-carboxylate | ![Structure Image](image3) |
| 15.36                  | 341.2836    | C_{19}H_{17}O_{6} | 4-[(2E)-3-(3-Ethoxy-4-hydroxyphenyl)-2-propenoyl]phenoxy]acetate | ![Structure Image](image4) |
| 15.69                  | 397.384     | C_{21}H_{17}O_{6} | 6-Carboxy-2-[2-(5-carboxy-2-hydroxy-3-methoxyphenyl)vinyl]-8-methoxychromenium | ![Structure Image](image5) |
| 16.24                  | 776.5663    | C_{45}H_{74}O_{10} | 1S,5'R,6R,6'R,7S,8R,10S,11R,12R,14R,15R,16S,22S,25R,27S,28R,29S-[22-Ethyl]-7,11,15-trihydroxy-6-[(2S)-2-hydroxypropyl]-5,6,6,10,12,14,16,28,29-nonamethyl-3,4,5,6-tetrahydro-3H-6H,13H-spiro[2,26-dioxabicyclo[23.3.1]nonacosa-3,9,13-trione | ![Structure Image](image6) |
| 16.64                  | 397.3821    | C_{23}H_{18}     | Stigmasta-3,5-diene | ![Structure Image](image7) |
| 17.08                  | 504.4414    | C_{24}H_{12}O_{2} | 1,4-Dioxo-1,4-dihydro-2-naphthalenyl-2-acetyl-α-D-galactopyranoside | ![Structure Image](image8) |
| 17.82                  | 411.3254    | C_{22}H_{12}O_{2} | Ergosta-4,24(28) diene-3,6-diene | ![Structure Image](image9) |
| Time retention (minute) | m/z (M+H)* | Formula (+H*) | Compound name | Structure |
|-------------------------|------------|---------------|---------------|-----------|
| 17.94                   | 494.5659   | C_{26}H_{38}O_{9} | (1S,4S,9R,12S,13R,16S,17R)-17-(Hydroxymethyl)-12-methyl-7-oxo-8-oxapentacyclo[14.2.1.0\{1,13\}.0\{4,12\}.0\{5,9\}]nonadec-5-en-17-yl β-D-glucopyranoside | Glycoside |
| 17.99                   | 522.5964   | C_{24}H_{26}O_{13} | Iridin | Flavonoid |
| 19.74                   | 550.6291   | C_{25}H_{26}O_{14} | 5,8-Dihydroxy-3-methyl-1,4-dioxo-1,4-dihydro-2-naphthalenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside | Phenolic |
| Time retention (minute) | m/z (M+H)<sup>+</sup> | Formula (+H<sup>+</sup>) | Compound name | Structure |
|------------------------|------------------------|--------------------------|----------------|-----------|
| 5.48                   | 227.1550              | C<sub>15</sub>H<sub>19</sub>N<sub>2</sub> | 1-(1-Naphthylmethyl)piperazine | ![Alkaloid](image) |
| 9.50                   | 273.1492              | C<sub>15</sub>H<sub>13</sub>O<sub>5</sub> | 3,5-Dihydroxy-4-[3-(4-hydroxyphenyl)propanoyl]phenolate | ![Phenolic](image) |
| 9.87                   | 396.2477              | C<sub>21</sub>H<sub>32</sub>O<sub>7</sub> | 5α,1,2-O-Isopropylidene-3-O-[4-(pentyloxy)benzyl]-α-D-xylo-hexofuranose | ![Phenolic](image) |
| 12.06                  | 301.1418              | C<sub>18</sub>H<sub>21</sub>O<sub>4</sub> | (S)-2-(3,4-Dimethoxyphenyl)-7-methoxychroman 3',4',7-Trimethoxyflavan | ![Phenolic](image) |
| 10.24                  | 139.9886              | C<sub>2</sub>H<sub>4</sub>O<sub>4</sub> | p-Benzoquinone, 2,5-dihydroxy | ![Phenolic](image) |
| 14.44                  | 378.2224              | C<sub>2</sub>H<sub>2</sub>O<sub>3</sub> | Methyl4-[2-(4-phenylmethoxyphenoxy)ethoxy]benzoate | ![Phenolic](image) |
| 15.07                  | 639.2832              | C<sub>34</sub>H<sub>54</sub>O<sub>11</sub> | 1-O-[1α,3β,11β,22R,24E-1,3,11,22-Tetrahydroxy-26-oxoergosta-5,24-dien-26-yl]-β-D-glucopyranose | ![Saponin](image) |
| 15.44                  | 653.2996              | C<sub>31</sub>H<sub>39</sub>N<sub>8</sub>O<sub>3</sub> | 1-[4-(2-[1-Methyl-2-oxo-2,3-dihydro-1H-indol-5-yl]amino)-4-pyrimidinyl]oxy]-1-naphthyl]-3-[1-(4-methylphenyl)]-3-(2-methyl-2-propanyl]-1H-pyrazol-5-yl]urea | ![Alkaloid](image) |
| 16.24                  | 494.5655              | C<sub>26</sub>H<sub>22</sub>O<sub>3</sub> | Methyl 3-[2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4-oxo-4H-chromen-8-yl]-3-(4-methoxyphenyl)propanoate | ![Flavonoid](image) |
| Time retention (minute) | m/z (M+H)^+ | Formula (+H^+) | Compound name | Structure |
|------------------------|-------------|----------------|---------------|-----------|
| 16.64                  | 629.5655    | C_{31}H_{32}O_{14} | 4-[(3R)-1-Oxo-8-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)oxy]-3,4-dihydro-1H-isochromen-3-yl]phenyl acetate | ![Flavonoid](image1.png) |
| 17.19                  | 522.5969    | C_{24}H_{26}O_{13} | 5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,6-dimethoxy-4-oxo-4H-chromen-7-yl α-D-glucopyranoside | ![Flavonoid](image2.png) |
| 16.64                  | 550.6291    | C_{25}H_{26}O_{14} | 5,8-Dihydroxy-3-methyl-1,4-dioxo-1,4-dihydro-2-naphthalenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside | ![Phenolic](image3.png) |
| 17.82                  | 394.3482    | C_{23}H_{44}N     | Cholesta-3,5-diene-3-carbonitrile | ![Steroid](image4.png) |