Metabolomic Profiles of *Curcuma longa* L and *Cosmos caudatus* Extracts and Their *In-Silico* Anti-cancer Activity

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Abstract. The current work intends to analyze Curcuma longa L and Cosmos caudatus extracts using LC-HRMS study, their prospective anti-cancer activity was determined through in silico molecular-docking. Extraction of Curcuma longa L and C. caudatus was carried out first. The resulted extracts were analyzed using LC-HRMS, in the positive ion detection. Using LC-HRMS analysis, many compounds were identified in the both extracts. The target compounds for Curcuma longa L extract was curcumin, and lutein was the target compound for Cosmos caudatus. The 3D molecular structures of curcumin and lutein were downloaded from PubChem database. The protein target was caspase-8 and was retrieved from Protein Data Bank. Caspase-8 protein were docked to curcumin and lutein, performing at HEX 8.0 program and visualized using Discovery Studio v19.1.0.18287. Interaction of curcumin and lutein on caspase-8 showed different patterns. Hydrophobic interactions, formation of hydrogen bonds, and van der Waals forces were shown in the interactions between protein and ligands. The interaction between curcumin, lutein, and the mixture of lutein-curcumin resulted in the LD₅₀ values of 2000 mg/kg, 10 mg/kg, and 2000 mg/kg, respectively. These suggest that not only curcumin and lutein, but also complex of curcumin-lutein might possess capacity as anti-cancer agents.

1. Introduction
The application of natural plants for the remedy of many diseases is related to traditional medication from different parts of the world [1]. Natural products from plants, animals, bacteria, or fungi, maintain to be used in therapeutic preparations, either as extracts or the whole compounds [1].

*Curcuma longa* L. is distributed throughout tropical and subtropical regions of the world, mostly cultivated in Asian countries [2]. A natural yellow pigment derived from *Curcuma longa* L. is curcumin, and is a mixture of curcuminoids [2]. Curcuminoid is a group of phenolic compounds in the rhizome of the family of zingiberaceae. Curcumin is known to have many pharmacological and biological activities. It is known to be anti-parasitic, antioxidant, anti-fatigue, anti-inflammatory, anti-allergic, anti-microbial, anti-mutagenic, and anticancer [3, 4].
Cosmos caudatus is locally known in Indonesia as kenikir, and often consumed raw as a salad. This plant, a common and popular traditional vegetable that originated from tropical Central America, is widely grown in all regions of Indonesia. Cosmos caudatus is a rich source of bioactive compounds including proteins, minerals and vitamins, phenolics, flavonoids, and carbohydrates; and hence, increasing its nutritious significance [5]. There are a lot of interests in the nutritional and medicinal function of this herb in the last decade. Cosmos caudatus has been reported to have antioxidant, antimicrobial, and anti-cancer activities [6]. Carotenoids are a subclass of phytonutrients that contained in vegetables and fruits. Xanthophyll is a family of oxygenated carotenoids that contain hydroxyl or carbonyl, contributing to increase its solubility [7]. One of the xanthophyll pigments is lutein. Cosmos caudatus leaves extract has been reported earlier to contain lutein; and this lutein is proposed to have potential as anti-cancer agent [8].

In this current study, Curcuma longa L was extracted using ethanol, in order to obtain curcumin, and Cosmos caudatus was extracted using n-hexane in order to obtain lutein. The resultant extracts were analyzed using LC-HRMS (liquid chromatography-high resolution mass spectrometry). The mixture of Curcuma longa L and Cosmos caudatus extracts was designed into nanoparticles. The characterization of the resulted nanoparticles has been previously reported [9]. The potential as anti-cancer agent is determined through their in silico molecular docking analysis. The protein target used is caspase-8, enzyme involved in apoptosis pathway. In oncology, apoptosis proved to be the prime target for anticancer research which comprises two pathways: extrinsic death receptor-mediated pathway, and intrinsic mitochondrial-dependent pathway [10]. Caspase-8, a member of initiator caspases, mediates extrinsic apoptosis pathway [11]. The mechanism of action of nanoparticles from mixture of Curcuma longa L and Cosmos caudatus is proposed through their activation on caspase-8 protein.

2. Methods

2.1 Materials

The Curcuma longa L. and Cosmos caudatus dried plants were obtained from Materia Medica, Batu, East Java, Indonesia. The species of the plants was confirmed by the letter of species determination, signed by a taxonomist from the herbarium unit of Materia Medica. The dried parts of the plants crushed into powder. Other materials were purchased from Sigma-Aldrich: ethyl alcohol (pure, \( d = 0.789 \text{ g/mL} \)), isopropyl alcohol (≥99.7%, FG), n-hexane (anhydrous, 95%), sodium hydroxide (≥98%, pellets, anhydrous), potassium hydroxide (≥85%, pellets, white). Water used was demineralized water (Hydrobatt).

2.2 Extracts Preparations

Curcuma longa L. rhizome powder was extracted using maceration techniques, with 96% ethyl alcohol as solvent. The solvent was separated from the extracts by a rotary evaporator vacuum at 80 °C, 120 rpm to obtain concentrated extracts. The concentrated extracts were stored in dark glass bottles and kept at 4 °C for subsequent use.

A 100 g of Cosmos caudatus powder was extracted using n-hexane as solvent with a ratio of 1:10 and allowed to stand for 5 days. The extract was concentrated with a rotary evaporator vacuum. Concentrated extract was digested with 100 mL of isopropanol. Then, these extracts were mixed with 50% of NaOH (v/v) at 60 °C for 90 min. The KOH solution was added until the semi-solid extracts was obtained. Finally, 4× volume of distilled water was added, and the solution mixture was agitated for 4 h. The extract was separated by centrifugation.
2.3 Liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis of extracts of Curcuma Longa L and Cosmos caudatus

Liquid chromatographic separation was conducted in the LSIH Laboratory, Universitas Brawijaya. A Hypersil GOLD aQ 50 x 1 mm x 1.9 µm particle size column, the injection volume was 100 µL. Solvent A= 0.1% formic acid in water and solvent B=0.1 % formic acid in acetonitrile, were used, and the flow rate was 40 µL/min. Liquid chromatographic separation was run for 70 min, this was continued to mass spectrometry in ESI detection, in positive ion mode, using a Thermo Scientific Q Exactive mass spectrometer. The work was adjusted as follow: sheath gas (N₂) pressure=50 psi; spray voltage=4.5 kV; capillary temperature=300 K; m/z range of 50-750. The metabolites were analyzed using Compound Discoverer software with mzCloud MS/MS Library.

2.4 In silico anti-cancer activity of Curcuma longa L and Cosmos caudatus extracts

The 3D structures of Curcuma longa L and cosmos caudatus extrats were obtained from PubChem database: curcumin (CID 969516) and lutein (CID 448437). PyRx Virtual screening tool software was used to minimize their energy and convert the SDF format into PDB format. The human caspase-8 protein structure was obtained from the RCSB Protein Data Bank (PDB ID: 1QTNA), water and other ligands bound to protein were removed using Discovery studio visualizer v19.1.0.18287 program (http://3dsbiovia.com/products/). Human caspase-8 protein was docked to curcumin, lutein, and complex of curcumin-lutein. The HEX 8.0 software was used in this study to predict the interaction and energy binding of curcumin, lutein, and curcumin-lutein to caspase-8 protein. The docking results were visualized using Discovery studio visualizer v19.1.0.18287 program, 2018 version. The LD₅₀ values calculation were obtained by saving all compounds were saved in the SMILES format, and then were processed using pkSCM online tool (http://biosig.unimelb.edu.au/pkscm/prediction).

3. Result and Discussions

3.1 LC-HRMS Results

In our previous study, the phytochemical test revealed that flavonoids, phenolics, and tannins contained in the Curcuma longa L. extracts; while Cosmos caudatus extract consisted of flavonoids, steroids, and terpenoids [9]. Identification of the phytochemical components enclosed in the plant extracts is essential to predict the pharmacological and biological activities of the plants. In order to characterize further the extracts, LC-HRMS technique was performed.

LC-HRMS results from the extracts of Curcuma longa L and Cosmos caudatus are presented in Figures 1 and 2. LC-HRMS resulted in the many compounds as indicated by the Compound Discoverer software with mzCloud MS/MS Library. However, for the analysis, we excluded compounds that had Δm more than 5 ppm [12]. Therefore, analysis of Curcuma longa L and Cosmos caudatus extracts led to the tentative identification of 16 and 13 compounds, respectively (Tables 1 and 2). Most of compounds identified in the extracts were fatty acids.

The major metabolites contained in the Curcuma longa L extracts were tentatively identified as curcumin (RT=0.862 min), methyl palmitate (RT=10.710 min), dibutyl phthalate (RT=13.001 min), 1-stearoylglycerol (RT=16.534 min), stearamide (RT= 20.078 min), and choline (RT=26.335 min). The compounds contained in the Cosmos caudatus extracts were almost similar, which were triethanolamine (RT=1.010 min), lutein (RT=13.007 min), 1-stearoylglycerol (RT=16.586 min), stearamide (RT=20.066 min), and choline (RT=26.331 min). Nonetheless, the compounds detected using LC-HRMS are only tentatively identified, since the high-resolution MS is insufficient to confirm the exact identification of each compound. The LC-HRMS results show that curcumin and lutein as the target compound contained in Curcuma longa L and Cosmos caudatus extracts, respectively.
Figure 1. The LC-HRMS results from *Curcuma longa* L extracts: (a) chromatogram; (b) mass spectra.

Table 1. LC-HRMS results of *Curcuma longa* L extracts

| No | Proposed Compound       | RT (min) | Experimental m/z   | Calculated m/z  | Δm     |
|----|------------------------|----------|--------------------|-----------------|--------|
| 1  | Curcumin               | 0.862    | 368.12547          | 368.12599       | 0.00052|
| 2  | Bis(2-ethylhexyl) phthalate | 0.874   | 390.27613          | 390.27660       | 0.00047|
| 3  | Dibutyl phthalate      | 1.002    | 278.15105          | 278.15157       | 0.00052|
| 4  | Methyl palmitate       | 10.71    | 287.28183          | 287.28224       | 0.00041|
| 5  | Tributyl phosphate     | 11.879   | 266.16398          | 266.16454       | 0.00056|
| 6  | Dibutyl phthalate      | 13.001   | 278.15122          | 278.15163       | 0.00041|
| 7  | Ricinoleic acid methyl ester | 15.148 | 294.25510          | 294.25557       | 0.00047|
| 8  | 1-Stearoylglycerol     | 16.534   | 358.30776          | 358.30828       | 0.00052|
| 9  | Oleamide               | 17.11    | 281.27118          | 281.27165       | 0.00047|
| 10 | Hexadecanamide         | 17.36    | 255.25555          | 255.25606       | 0.00051|
| 11 | 1-Stearoylglycerol     | 18.803   | 358.30775          | 358.30827       | 0.00052|
| 12 | Bis(2-ethylhexyl)adipate | 19.19   | 370.30759          | 370.30806       | 0.00047|
| 13 | Bis(2-ethylhexyl)      | 19.639   | 390.27607          | 390.27659       | 0.00052|
**Figure 2.** The LC-HRMS results from *Cosmos caudatus* extracts: (a) chromatogram; (b) mass spectra.

**Table 2.** LC-HRMS results of *Cosmos caudatus* extracts

| No | Proposed Compound                        | RT (min) | Experimental $m/z$ | Calculated $m/z$ | $\Delta m$   |
|----|------------------------------------------|----------|--------------------|------------------|--------------|
| 1  | Triethanolamine                          | 1.01     | 117.07833          | 117.07884        | 0.00051      |
| 2  | Tributyl phosphate                       | 11.875   | 266.16407          | 266.16454        | 0.00047      |
| 3  | 3,5-di-tert-Butyl-4-hydroxybenzaldehyde  | 11.983   | 234.16149          | 234.16190        | 0.00041      |
| 4  | Lutein                                   | 13.007   | 568.87150          | 568.87094        | 0.00056      |
| 5  | $\alpha$-Eleostearic acid                | 15.4     | 278.22406          | 278.22447        | 0.00041      |
| 6  | 1-Stearoylglycerol                       | 16.586   | 358.30753          | 358.30805        | 0.00052      |
| 7  | Oleamide                                 | 17.096   | 281.27115          | 281.27162        | 0.00041      |
| 8  | Hexadecanamide                           | 17.782   | 255.25568          | 255.25609        | 0.00052      |
| 9  | 1-Stearoylglycerol                       | 18.785   | 358.30759          | 358.30806        | 0.00047      |
| 10 | Bis(2-ethylhexyl) phthalate              | 19.22    | 390.27614          | 390.27666        | 0.00052      |
| 11 | Stearamide                               | 20.066   | 283.28672          | 283.28713        | 0.00044      |
| 12 | Octyl decyl phthalate                    | 20.343   | 418.30727          | 418.30779        | 0.00052      |
Studies for the characterization of the plant extracts using LC-MS extract has been conducted before [13, 14]. LC-HRMS has been proven as a significant technique to obtain the metabolomics fingerprint. Separation by chromatography technique preceding to MS-examination is particularly important in order to maximize sensitivity and minimize ion suppression, also to separate isobaric and isomeric compounds [14].

3.2 In silico molecular docking analysis
Recently, content variation, and hence safety of herbal medicaments or formulations enforced researchers to seek for alternative methodology to standardize, isolate, and formulate herbal drugs [15]. Old-fashioned approach used mixture of multiple herbal components as a drug [1], but modern medical science shifted the approach to isolate single component and establish the pharmacological significance of it. In this current work, in silico testing was conducted to in order to investigate further potential anti-cancer activity of curcumin, lutein, and mixture of curcumin-lutein.

The ligands-protein interaction was presented by the binding sites on amino acid residues and the types of formation of chemistry bond are shown in Table 3. There were 5 interactions between curcumin and caspase-8 protein, consist of 2 hydrogen bonds, 2 hydrophobic interactions, and 1 unfavorable interaction (Figure 3). The amino acid residue bound to the enzyme through hydrogen bonds was Gln366. The amino acid residues, Lys461 and Met463, involved in hydrophobic interactions. Van der Waals forces formed between Lys367, Ile369, Val371, Thr373, Asp374, Asn452, Tyr488, Ser451, and Gln462 and caspase-8. The binding energy between curcumin and caspase-8 was -291.8 cal/mol, with an LD_{50} value of 2000 mg/kg.

![Figure 3](image-url)

**Figure 3.** In silico molecular docking results of interactions between human caspase-8 protein with curcumin: (a) overview of curcumin-caspase-8 complex; (b) the 3D structure of curcumin-caspase-8 complex; and (c) the 2D structure of curcumin-caspase-8 complex.
**Figure 4.** *In silico* molecular docking results of interactions between human caspase-8 protein with lutein: (a) overview of curcumin-caspase-8 complex; (b) the 3D structure of lutein-caspase-8 complex; and (c) the 2D structure of lutein-caspase-8 complex.

Results of lutein interactions with caspase-8 are shown in Figure 4. There were more interactions between lutein and caspase-8. Those interactions consisted of 1 hydrogen bond, 6 hydrophobic interactions, and 4 unfavorable interactions. The amino acid residues involved in interactions through hydrogen bonds was Asp308. The other amino acid residues: Ala349, Lys231, Ile393, and Pro346, had hydrophobic interactions with caspase-8. Finally, van der Waals forces formed between the enzyme and Try392, Arg391, Ser347, Lys351, Ser305, Gln227, Lys229, Ser230, Asn306, Gly350, Pro394, Asp395, Leu343, and Thr341. The binding energy between lutein and caspase-8 was -377.9 cal/mol, with LD$_{50}$ value of 10 mg/kg.

The interaction between the curcumin-lutein complex and caspase-8 is illustrated in Figure 5. There was formation of 11 interactions, 5 hydrophobic interactions and 6 interactions that are unfavorable. The binding energy between lutein-curcumin and caspase-8 was -425 cal/mol, with an LD$_{50}$ value of 2000 mg/kg. The interaction of complex of curcumin-lutein with caspase-8 enzyme was the strongest, as indicated from the lowest binding energy. This suggest that the mixture of curcumin and lutein had the highest potential as anti-cancer agent.

However, the LD$_{50}$ value of complex of curcumin-lutein has the same LD$_{50}$ value of that of curcumin (2000 mg/kg). This may be caused by using *in silico* molecular modelling need the 3D structures of the identified compound. There is no established 3D structure of the complex between curcumin and lutein. The structure of mixture of curcumin and lutein used in this work is virtual prediction. The 3D crystal structure of mixture of curcumin-lutein need to be established.

**Figure 5.** *In silico* molecular docking results of interactions between human caspase-8 protein with complex of curcumin-lutein: (a) overview of curcumin-lutein-caspase-8 complex; (b) the 3D structure of curcumin-lutein-caspase-8 complex.
Studies on the plants extracts compounds acting as activators for caspase-8 enzyme through molecular docking approach have been conducted previously [16, 17]. Results from these studies showed that triterpenoids [16] and coumarin derivatives [17] have apoptogenic potential through stimulation of caspase-8. Thus, these compounds are reported to have anticancer activity. Another study using in silico molecular docking approach has shown biological activities on the flavonoids compounds (acarbose, rosmarinic acid, and sinensetin) acting as alpha-amylase proteins inhibitors, i.e. showing anti-diabetic activity [18]. These mean that in silico molecular docking is an effective and rapid

| Compound       | Interaction*                      | Chemistry bond | Types          | Energy (cal/mol) | Compound |
|----------------|-----------------------------------|----------------|----------------|-----------------|----------|
| Curcumin       | A:GLN366:HE21 - :LIG1:O           | Hydrogen Bond  | Conventional   | -291.8          | 2000 mg/kg |
|                | A:GLN366:HE21 - :LIG1:O           | Hydrogen Bond  | Conventional   |                 |          |
|                | :LIG1 – B:LYS461                  | Hydrophobic    | Pi-Alkyl       |                 |          |
|                | :LIG1 – B:MET463                  | Hydrophobic    | Pi-Alkyl       |                 |          |
|                | A:GLN366:CG - :LIG1:O             | Unfavourable   | Unfavourable   |                 |          |
| Lutein         | :LIG1:H – A:ASP308:OD1            | Hydrogen Bond  | Carbon         | -377.9          | 10 mg/kg |
|                | A:ALA349 - :LIG1:C                | Hydrophobic    | Alkyl          |                 |          |
|                | A:ALA349 - :LIG1:C                | Hydrophobic    | Alkyl          |                 |          |
|                | A:ALA349 - :LIG1:C                | Hydrophobic    | Alkyl          |                 |          |
|                | :LIG1:C – A:LYS231                | Hydrophobic    | Alkyl          |                 |          |
|                | :LIG1:C – A:PRO346                | Hydrophobic    | Alkyl          |                 |          |
|                | A:GLY342:CA - :LIG1:C             | Hydrophobic    | Alkyl          |                 |          |
|                | A:GLY342:CA - :LIG1:H             | Unfavourable   | Unfavourable   |                 |          |
|                | A:AL349:CB - :LIG1:C              | Unfavourable   | Unfavourable   |                 |          |
|                | A:AL349:CB - :LIG1:H              | Unfavourable   | Unfavourable   |                 |          |
| Curcumin-lutein| A:ALA349 - :LIG1                  | Hydrophobic    | Alkyl          | -425            | 2000 mg/kg|
|                | B:ILE393 - :LIG1                  | Hydrophobic    | Alkyl          |                 |          |
|                | :LIG1:C – B:ARG391                | Hydrophobic    | Alkyl          |                 |          |
|                | :LIG1:C – B:ILE393                | Hydrophobic    | Alkyl          |                 |          |
|                | :LIG1 – A:LYS351                  | Hydrophobic    | Pi-Alkyl       |                 |          |
|                | A:GLN227:NE2 - :LIG1:C            | Unfavourable   | Unfavourable   |                 |          |
|                | A:GLN227:HE22 - :LIG1:C           | Unfavourable   | Unfavourable   |                 |          |
|                | A:GLN227:HE22 - :LIG1:H           | Unfavourable   | Unfavourable   |                 |          |
|                | A:SER305:O - :LIG1:O              | Unfavourable   | Unfavourable   |                 |          |
|                | B:ARG391:O - :LIG1:C              | Unfavourable   | Unfavourable   |                 |          |
|                | B:ARG391:O - :LIG1:H              | Unfavourable   | Unfavourable   |                 |          |
method for investigating molecular interaction and mode of action of for bioactive compounds targeting enzyme activity.

4. Conclusion
This study suggests that the use of LC-HRMS tentatively identified compounds contained in Curcuma longa L and Cosmos caudatus extracts. Curcumin, from Curcuma longa L extracts, and lutein from Cosmos caudatus extracts had the potential as anti-cancer agent, as well as mixture of curcumin and lutein. These have been shown by computational molecular docking of these compounds to caspase-8 protein, apical caspase which initiates programmed cell death. From the in silico studies, interactions of caspase-8 protein to curcumin, lutein, and complex of curcumin-lutein resulted in the binding energy of -291.8 cal/mol, -377.9 cal/mol, and -452 cal/mol. Additional in vitro and in vivo approaches are needed to corroborate this current work results.

Acknowledgement
This research was funded by Hibah Doktor grant from Brawijaya University, grant number 45/UN10.F09/PN/2020 for Anna Safitri.

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