The Biological Function Prediction of 10-Gingerol Compound of Ginger in Inhibiting Cyclooxygenase-2 Activity

Gabriella Chandrakirana Krisnamurti1,2 and Fatchiyah Fatchiyah2,3*

1Biotechnology Program, School of Bioresources and Technology, King Mongkut’s University of Technology Thonburi, Bangkok, Thailand
2Research Center of Smart Molecule of Natural Genetics Resource, University of Brawijaya, Malang, East Java, Indonesia
3Department of Biology, Faculty of Mathematics and Natural Science, University of Brawijaya, Malang, Indonesia

*Corresponding email: fatchiya@ub.ac.id

Received 3 June 2020; Accepted 28 December 2020

ABSTRACT

Anti-inflammatory agents inhibit prostaglandin synthesis by blocking cyclooxygenases (COXs). The compounds extracted from ginger, 10-gingerol and 10-shogaol can inhibit inflammation but the mechanism of inhibition remains unclear. Here we used molecular docking to predict the molecular interactions between COXs and the three inhibitors, acetaminophen (CID1983), 10-gingerol (CID168115) and 10-shogaol (CID6442612). By using acetaminophen as a gold standard, the results demonstrated that acetaminophen, 10-gingerol, and 10-shogaol could bind catalytic domain and membrane binding domain of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). The 10-shogaol did not show significantly different binding energy to bind to COX-1 and COX-2. The 10-gingerol posed a stronger and more specific binding to the membrane-binding domain of COX-2 than acetaminophen and 10-shogaol. The specific binding of the 10-gingerol to COX-2 could prevent the binding of the natural substrate, arachidonic acid. The results provide useful information to improving activities of anti-inflammatory.

Keyword: 10-gingerol, 10-shogaol, cyclooxygenase, ginger, inflammation

INTRODUCTION

Inflammation is part of the body’s immune response to remove harmful stimuli and begin the healing process. The anti-inflammatory agents and chronic inflammation can eventually cause several diseases and conditions, including some cancers and rheumatoid arthritis. Non-steroidal anti-inflammatory drugs (NSAIDs) alleviate pain by counteracting the cyclooxygenase (COX) activity. The two isoforms, COX-1 and COX-2 use arachidonic acid as a substrate to synthesize prostaglandins, creating inflammation [1–3]. COX-1 is responsible for the baseline levels of prostaglandins modulate an initial phase of acute inflammation whereas COX-2, an essential factor or inflammation, produces prostaglandins through stimulation [1,4,5]. COX-1 and COX-2 are sharing 65% amino acid sequence identity and posting near-identical catalytic sites [6]. The classical COX inhibitors are not selective and inhibit all types of COX resulting in inhibiting prostaglandin and thromboxane synthesis. The most frequent adverse effect of NSAIDs is irritation of the gastric mucosa because prostaglandins normally have a protective role in the gastrointestinal tract. Long-term use of NSAIDs can cause gastric erosions, stomach ulcers and in extreme cases can
cause severe hemorrhage, resulting in death. Not only the COX-2 selective inhibitor drug has less side effects than the non-selective inhibitors but some of them also have antipyretic and analgesic effect [7,8].

Acetaminophen, a COX-2 selective inhibitor is commonly used as a drug to treat pain and fever due to its less side effect [9]. However, long term use of acetaminophen gives negative side effects such as causing gastrointestinal problem and promoting high blood pressure then lead to cardiovascular disease [10–13].

Herbal medicines are become more popular in today’s world because of less negative side effects [14]. Ginger has also been used for thousands of years for medicinal purposes. Possible health benefits include relieving nausea, loss of appetite, motion sickness, and pain. The number of reports has shown the evidence on ginger effects as an anti-inflammatory and anti-oxidative [15].

Ginger (Zingiber officinale) is species from Zingiberaceae family. That natural resource is widely distributed in South and Southeast Asia. Indonesia is one of the places that cultivates ginger [16,17]. The ginger rhizome is used to treat several health problems such as pain, coughs, and fever. Ginger also has other effects as anti-bacteria, anti-oxidant, and anti-inflammatory. Gingerols and shogaols are the most potent bioactive compounds in the ginger extract [18–21]. Both gingerols and shogaols exhibit biological activities, ranging from anticancer, anti-oxidant, antimicrobial, anti-inflammatory and anti-allergic to various central nervous system activities [22]. Gingerols and shogaols are more commonly found in fresh and dry rhizome, respectively [23,24]. Gingerols have four isoforms 6-gingerol, 8-gingerol, 10-gingerol, and 12-gingerol compounds. Similar to gingerols, shogaols have 4 isomers 6-shogaol, 8-shogaol, 10-shogaol, and 12-shogaol compounds [23,25]. The 10-gingerol and 10-shogaol are predicted as COXs inhibitors [26,27]. In vitro experiments have shown that 10-gingerol inhibit COX-2 and suppress synthesis of prostaglandin E (PGE) [25,27]. The 10-shogaol reduce the PGE level that produced by COX-2 [23]. The details of 10-gingerol and 10-shogaol binding to COX-1 or COX-2 enzymes remain unknown. Therefore, this study focus on identifies the binding of 10-gingerol and 10-shogaol b to COX-1 and COX-2 in comparison with acetaminophen, a common anti-inflammation drug.

EXPERIMENT

Chemicals and instrumentation

Protein structure COX-1 (PDB ID: 1EQG) and COX-2 (PDB ID: 6COX) proteins in 3D-PDB formats were downloaded from PDB database and chemical structures of acetaminophen (CID1983), 10-gingerol (CID168115), and 10-shogaol (CID6442612) were obtained from Pubchem. Using Discovery Studio Clients 3.5 prepared the proteins structure while ligands structure was use PyRx 0.8. The preparation of protein involves removing ligands and water molecules using Discovery Studio Client 3.5 (http://www.3dsbiovia.com). The ligands structures were converted in PDB format and minimized the energy using PyRx 0.8.

Docking and Bioactivities Prediction

The COX-1 and COX-2 proteins were docked with acetaminophen, 10-gingerol, and 10-shogaol by HEX 8.0.0 and the results were analyzed by using Discovery Studio Client 3.5 (http://www.3dsbiovia.com). The data for analysis showed in two dimensions and three dimensions visualization. The interaction and details were compiled in the table. The SMILES from each compound was used to predict the compound activities and inactivity. The prediction was done by PASS online (http://www.pharmaexpert.ru/passonline/).
RESULT AND DISCUSSION
Acetaminophen Bind to COXs

The analysis found that the head part of acetaminophen (phenyl group) could bind COX-1 and COX-2 in different manners (Figure 1a, Table 1, and Table 2). Acetaminophen made interactions with the catalytic domain of COX-1 with the H-bond between the hydrogen atom of the hydroxyl group and Tyr-234, and the hydrophobic interaction between the phenyl ring and Asn-236. These interactions were potentially suffered by the unfavorable steric bumps between its hydroxyl oxygen and Gly-235. Unlike binding to COX-1, acetaminophen made interactions not only with the catalytic domain but also the membrane-binding domain (MBD) of COX-2. The major interactions at the MBD comprised the H-bond between the carbonyl oxygen and Asn-144, and the Pi-anion type electrostatic interaction between a negative charge of Glu-140 and the Pi-orbital of the phenyl ring. The interaction at the catalytic domain was the hydrophobic interaction between Leu-238 and the phenyl ring (on the opposite site to Glu-140). The calculation showed that the repulsion between the 2 H-donors (the hydroxyl group and Lys-333) could encounter the binding at the catalytic domain.

Acetaminophen has not aliphatic tail as 10-gingerol and 10-shogaol. The $E_{\text{total}}$ of acetaminophen to COX-1 was -179.6kJ/mol, calculated from the conventional H-bond, electrostatic interaction and unfavorable bump. Acetaminophen bound to COX-2 stronger than COX-1. The $E_{\text{total}}$ was -192.7 kJ/mol, calculated from three the attraction, conventional H-bond, electrostatic interaction and hydrophobic interaction, and repulsion from the hydrogen donor-donor.

Combination of interactions indicates strength and selectivity of the binding of the molecules. The strength of binding, presenting in negative value, depending on types of interactions and the distance between the atoms [28]. The total binding energy ($E_{\text{total}}$) is product of the attractive and repulsive forces between the two molecules [29]. The higher $E_{\text{total}}$ indicates the more selectivity of the binding. Our results showed that acetaminophen bound to COXs weaker than 10-gingerol and 10-shogaol. Acetaminophen and 10-gingerol bound to COX-2 stronger than COX-1. The $E_{\text{total}}$ of 10-shogaol binding to COX-1 and COX-2 were not significantly different. It showed the ability of acetaminophen to bind with COX-2 [30], but might not as strong as 10-gingerol and 10-shogaol.

Ginger Bioactive Compounds (10-gingerol and 10-shogaol) with COXs Binding

The major differences between 10-gingerol and acetaminophen are the presence of a methoxy group at the head part and the long alkyl chain as a tail of the molecule. These molecular differences played an important role in binding to COXs. The main interaction with COX-1 located at the catalytic domain whereas the major interaction with COX-2 was at the MBD (Figure 1b, Figure 2b: 2e, Table 1, and Table 2). To interact with COX-1, Gln-370 and Pro-542 play crucial roles in making the H-bonds and the hydrophobic interactions with the catalytic domain. Gln-370 acted as both H-donor and H-acceptor of the carbonyl oxygen and methyl of the methoxy group, respectively. Pro542 made the hydrophobic interactions with benzyl group at the head and alkyl at the tail of 10-gingerol. Methyl group of the methoxy group made H-bond with the Asn-122 of the MBD by acting as H-donor. More interactions between 10-gingerol with COX-2 are shown in Figure 1b, Figure 2e, and Table 2. Trp-139 at the MBD made H-bonds with the hydroxyl and methoxy group at the head part of 10-gingerol. At the catalytic domain, Asp-229 made an electrostatic interaction with the phenyl ring while Leu-238 made hydrophobic interaction with the alkyl tail. A steric unfavorable between the tail and the catalytic domain was observed at Leu-224 and the carbonyl oxygen.
Similar to acetaminophen, 10-gingerol bound to COX-1 stronger than COX-2 with the Etot equal to -278.0 kJ/mol and -309.8 kJ/mol, respectively. Although 10-gingerol contains the aliphatic tail, the major interaction presented at the head part of the molecule. The 10-gingerol made more interactions to COX-1 and COX-2 than acetaminophen. The key interactions that made 10-gingerol bound to COX-2 stronger than COX-1 were the two conventional H-bonds shown in Table 1 and Table 2.

The absence of the hydroxyl group at the tail and different orientation of the methoxy group were differences between 10-gingerol and 10-shogaol. These differences played key roles in altering the interaction with COX-1 and COX-2. The methoxy group of 10-shogaol did not make interaction with COX-1 as presented in 10-gingerol, however it was able to make an H-bond with Glu236 of COX-2. The head part of 10-shogaol makes an H-bond and hydrophobic interaction with Glu-543 and Pro-542 respectively. The long alkyl tail of 10-shogaol does not interact to COX-1 but made two hydrophobic interactions with Leu-145 at the MBD and Leu-224 at the catalytic domain. There is no unfavorable interaction in the bindings between 10-shogaol and COX-1, and 10-shogaol and COX-2 (Figure 1c, Table 1, Table 2).

The 10-shogaol is slightly more polar than 10-gingerol due to the presence of the hydroxyl group at the aliphatic tail of the molecule. Although there were less pairs of interaction, an absence of repulsion made 10-shogaol bound to COX-1 and COX-2 with higher binding energies than acetaminophen and 10-gingerol. The Etotal of 10-shogaol to COX-1 and COX-2 were not significantly different, were equal to -305.7 kJ/mol and -303.5 kJ/mol respectively.

The prediction presented here has demonstrated that acetaminophen, 10-gingerol, and 10-shogaol not only bind to the catalytic sites but also the membrane-binding domain of COX-1 and COX-2. The presences of the methoxy group and the aliphatic tails in 10-gingerol and 10-shogaol play important roles in the strength and selectivity of the binding. The extension tail of 10-gingerol and 10-shogaol is a major different from acetaminophen played important roles in strength and selectivity of the molecular bindings. Together these data have revealed the strength and selectivity of the binding of acetaminophen, 10-gingerol, and 10-shogaol with COX-1 and COX-2.

Our analysis showed that 10-gingerol and 10-shogaol bound to COXs stronger than acetaminophen. The carbon hydrogen bond is known as weak hydrogen bond (C–H⋯O) and it is weaker than conventional hydrogen bond (N–H⋯O, N–H⋯N, O–H⋯O) [29]. An electrostatic interaction and hydrophobic interaction that could enhance the ligand binding efficiency are also scored in binding energy calculation [7,28,29,31]. The distance between the atoms also supports the strength of interaction. The shorter of distance indicates the stronger interaction [32,33].

The Etotal of 10-gingerol and 10-shogaol were approximate twice the amount of acetaminophen. Although 10-shogaol also can bind to COX-2 very strong comparable to 10-gingerol, The Etotal of 10-shogaol to COX-1 and COX-2 were not very different. The 10-gingerol/COX-1 interactions were dominated by carbon hydrogen bonds, whereas 10-gingerol/COX-2 interactions were dominated by conventional hydrogen bonds. These made 10-gingerol bound to COX-2 stronger than COX-1. Although 10-shogaol interacted with COX-1 and COX-2 in different manners, the binding strength was not much different because the conventional hydrogen bond and hydrophobic interaction between 10-shogaol and COX-1 were equivalent to two hydrophobic interactions combining with carbon hydrogen bond between 10-shogaol and COX-2.
Potency of 10-gingerol as anti-inflammatory inhibitor

Further analyses were conducted by using PASS online to predict the potency of ginger bioactive compounds towards COX-2. The prediction from PASS online (Table 3) showed that 10-gingerol and 10-shogaol have potency as anti-inflammatory and antipyretic agent. The 10-shogaol had high activity as anti-inflammatory, non-steroidal anti-inflammatory agent, and cyclooxygenase substrate compared to others. Whereas, 10-gingerol had the highest activity as antipyretic, this is the main function of acetaminophen.

High selectivity to COX-2 is a key property for an inflammation drug. The COXs inhibition occurred near the heme-binding or peroxidase site [6], which might indirectly prevent the prostaglandin synthesis [34]. The data presented here have showed that acetaminophen, 10-gingerol and 10-shogaol bound to COXs at the catalytic and membrane binding domain, and have provided the insights into the interactions of the three compounds to COX-1 and COX-2. The result has revealed the key interactions resulting in selectivity of acetaminophen and 10-gingerol to COX-2 but not 10-shogaol.

A phenyl ring is a common moiety of acetaminophen, 10-gingerol and 10-shogaol. The ring mainly participated in COXs binding, especially COX-1. However, the phenyl ring of 10-shogaol did not interact with COX-2. Acetaminophen and 10-gingerol showed selectivity to COX-2 but not 10-shogaol. Our result demonstrated that the selectivity to COX-2 of acetaminophen derived from the stronger interactions at the phenyl ring and at the carbonyl at the tail of the molecule. These show the selective COX-2 binding of acetaminophen.

Although the presence of an aliphatic tail in 10-gingerol is one of the differences from acetaminophen, the selectivity to COX-2 was lined at the head part of the 10-gingerol. The orientation of methoxy group and hydroxyl group at the head part of 10-gingerol made more interactions with COX-2 than COX-1, indicating the selectivity of 10-gingerol to COX-2.

Ginger has many biological activities, for example as anti-inflammatory through COX inhibition [35]. In the result, 10-gingerol posed a role as potential anti-inflammatory. The predicted mechanism of 10-gingerol inhibits prostaglandin (PG) synthesis by COX-2 is shown in Figure 3. The substrate of COX-2 is arachidonic acid (AA), locates at cell membrane. Regard to our results, the inhibition site of 10-gingerol was located at membrane binding domain and catalytic domain on the same site with AA. AA is synthesized by phospholipase A2 (PLA2) at the cell membrane could not effectively bind to COX-2. The figure shows that 10-gingerol blocks the binding site of AA on COX-2, resulting in inhibiting the PG synthesis.

The result of ginger potency prediction predicted by PASS online was consistent with the early in vitro and in vivo studies reported that either 10-gingerol can be anti-inflammatory, anti-cancer, and inhibit apoptosis [22,36]. Antipyretic is one of acetaminophen major function [37]. It also correlated with prediction score. Interestingly, the prediction has shown that anti-inflammatory score of 10-gingerol and 10-shogaol is much higher than acetaminophen (Table 3) that suggested the 10-gingerol had similar properties to acetaminophen and potentially a good anti-inflammation agent.
Figure 1. The two dimensions visualization of COX-1 and COX-2 interactions with acetaminophen, 10-gingerol, and 10-shogaol.
Figure 2. The three dimensions visualization of COXs binding with ligands: a) COX-1 with acetaminophen, b) COX-1 with 10-gingerol, c) COX-1 with 10-shogaol, d) COX-2 with acetaminophen, e) COX-2 with 10-gingerol, f) COX-2 with 10-shogaol
### Table 1. Interaction and binding energy of cyclooxygenase 1 (COX-1) with ligands

| Name              | $E_{\text{Total}}$ (kJ/mol) | Category                        | Protein                | Ligand          | Distance (Å) |
|-------------------|-----------------------------|---------------------------------|------------------------|-----------------|--------------|
|                  |                             |                                 |                        |                 |              |
| **Acetaminophen** |                             |                                 |                        |                 |              |
|                   | -179.6                      | Conventional H-bond             | Protein                | Ligand          |              |
|                   |                             | Tyr234                          | Hydroxyl oxygen (H-acceptor) | Catalytic Head | 3.0021       |
|                   |                             | Asp236                          | Amide group (positive charge) | Catalytic Head | 4.1225       |
|                   |                             | Gly235                          | Carbonyl oxygen (d-)   | Catalytic Head | 2.0398       |
| **Carbon H-bond** |                             | Gln370                          | Alkyl (H-donor)        | Catalytic Tail | 2.7152       |
| **10-gingerol**   | -278.0                      | Carbon H-bond                   | Gln370                 | Alkyl (H-donor) | 2.4644       |
|                   |                             | Asn122                          | Carbonyl oxygen (H-acceptor) | Membrane binding | 2.8582       |
|                   |                             | Gln370                          | Alkyl (H-donor)        | Catalytic Tail | 2.4644       |
|                   |                             | Pro542                          | Alkyl group (non-polar) | Catalytic Tail | 5.3721       |
| **10-shogaol**    | -305.7                      | Conventional Hydrogen Bond      | Glu534                 | Hydroxyl group (H-donor) | 2.4996       |
|                   |                             | Pro542                          | Side chain ring (non-polar) | Catalytic Head | 3.4497       |

### Table 2. Interaction and binding energy of cyclooxygenase 2 (COX-2) with ligands

| Ligand            | $E_{\text{Total}}$ (kJ/mol) | Category                        | Protein                | Ligand          | Distance (Å) |
|-------------------|-----------------------------|---------------------------------|------------------------|-----------------|--------------|
|                  |                             |                                 |                        |                 |              |
| **Acetaminophen** |                             |                                 |                        |                 |              |
|                   | -192.7                      | Conventional H-bond             | Asn144                 | Amine group (H-donor) | 2.8471       |
|                   |                             | Glu140                          | Carboxyl group (anion) | Membrane binding | 4.3765       |
|                   |                             | Leu238                          | Alkyl group (non-polar) | Catalytic Head | 4.8139       |
Table 3. Bioactive prediction from PASS Online

| Bioactive compounds | Activity           | Pa (activity score) | Pi (inhibition score) |
|---------------------|--------------------|---------------------|----------------------|
| 10-Gingerol         | Anti-inflammatory  | 0.532               | 0.048                |
| 10-Shogaol          |                    | 0.717               | 0.014                |
| Acetaminophen       |                    | 0.319               | 0.144                |
| 10-Gingerol         | Antipyretic        | 0.327               | 0.038                |
| 10-Shogaol          |                    | 0.315               | 0.040                |
| Acetaminophen       |                    | 0.675               | 0.005                |

CONCLUSION

The 10-gingerol is a ginger bioactive compound that has the potential to replace acetaminophen as an antipyretic and anti-inflammatory drug through COX-2 inhibition. The interaction data provide useful information that could be used for drug design and development. However, this study needs further investigation through toxicology and/or pre-clinical study of 10-gingerol mechanisms towards COX-2 and the complex reaction of 10-gingerol with another bioactive compound in ginger.

CONFLICT OF INTEREST

Authors declare no competing interest by submitting the manuscript for publication.
ACKNOWLEDGMENT
The authors acknowledge to Dr. Sentot Joko Raharjo for docking analysis assistance. This research was aid by national research grant (PTUPT) from Directorate General for Higher Education, Ministry of education and culture of Republic Indonesia.

REFERENCES
[1] Ricciotti, E., FitzGerald, G. A. Arterioscler. Thromb. Vasc. Biol. 2011, 31 (5), 986–1000.
[2] Arulsevan, P., Fard, M.T., Tan, W.S., Gothai, S., Fakurazi, S., Norhaizan, M.E., Kumar, S.S. Oxid. Med. Cell. Longev. 2016, 1–15.
[3] Setiawan, A., Yin, L., Auer, G., Czene, K., Smedby, K. E., Pawitan, Y. Sci. Rep. 2017, 7 (1), 1–5.
[4] Smyth, E. M., Grosser, T., Wang, M., Yu, Y., FitzGerald, G. A. J. Lipid Res. 2009, 50 (Supplement), S423–S428.
[5] Yoon, W. J., Ham, Y. M., Lee, W. J., Lee, N. H., Hyun, C. G. Turkish J. Biol. 2010, 34 (1), 25–34.
[6] Simmons, D. L., Botting, R.M., Hla, T. Pharmacol. Rev. 2004, 56, pp 387–437.
[7] Raharjo, S. J., Mahdi, C., Nurdiana, N., Kikuchi, T., Fatchiyah, F. Adv. Bioinformatics 2014, 2014 (ID850628), 1–12.
[8] Zhu, X. T., Chen, L., Lin, J. H. Medicine 2018, 97, p e11649.
[9] Hinz, B., Cheremina, O., Brune, K. FASEB J. 2007, 22 (2), 383–390.
[10] Diener, H. C., Pfaffenrath, V., Pageler, L., Peil, H., Aicher, B. Cephalalgia 2005, 25 (10), 776–787.
[11] Hinz, B., Brune, K. Ann. Rheum. Dis. 2012, 71 (1), 20–25.
[12] Turtle, E. J., Dear, J. W., Webb, D. J. Br. J. Clin. Pharmacol. 2013, 75 (6), 1396–1405.
[13] Derry, C. J., Derry, S., Moore, R. A. Cochrane Database Syst. Rev. 2014, No. 12.
[14] Karimi, A., Majlesi, M., Rafieian-Kopaei, M. J. Nephropharmacol. 2015, 4 (1), 27–30.
[15] Ghasemzadeh, A., Jaafar, H. Z. E., Rahmat, A. Molecules 2010, 15 (6), 4324–4333.
[16] Koga, A. Y., Beltrame, F. L., Pereira, A. V. Brazilian J. Pharmacogn. 2016, 26 (3), 385–391.
[17] Dhanik, J., Arya, N., Nand, V., Jyotsna Dhanik, C. J. Pharmacogn. Phytochem. 2017, 6 (3), 174–184.
[18] Ghasemzadeh, A., Jaafar, H. Z. E., Rahmat, A. BMC Complement. Altern. Med. 2015, 15 (1), 1–10.
[19] Samad, M. Bin, Mohsin, M. N. A. Bin, Razu, B. A., Hossain, M. T., Mahzabeen, S., Unnoor, N., Muna, I. A., Akhter, F., Kabir, A. U., Hannan, J. M. A. BMC Complement. Altern. Med. 2017, 17 (1), 1–13.
[20] Ali, A. M. A., El-Nour, M. E. M., Yagi, S. M. J. Genet. Eng. Biotechnol. 2018, 16 (2), 677–682.
[21] Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T., Li, H. Bin. Foods 2019, 8 (6), 1–21.
[22] Semwal, R. B., Semwal, D. K., Combrinck, S., Viljoen, A. M. Phytochem. 2015, 117, 554–568.
[23] van Breenen, R. B., Tao, Y., Li, W. Fitoterapia 2011, 82 (1), 38–43.
[24] Sharifi-Rad, M., Varoni, E. M., Salehi, B., Sharifi-Rad, J., Matthews, K. K., Ayatollahi, S. A., Kobarrfard, F., Ibrahim, S. A., Mnayer, D., Zakaria, Z. A., Sharifi-Rad, M., Yousaf, Z., Iriti, M., Basile, A., Rigano, D. Molecules 2017, 22 (12), 1–20.
[25] Dugasani, S., Pichika, M. R., Nadarajah, V. D., Balijepalli, M. K., Tandra, S., Korlakunta, J. N. J. Ethnopharmacol. 2010, 127 (2), 515–520.
[26] Nikolic, D., Habibi-Goudarzi, S., Corley, D. G., Gafner, S., Pezzuto, J. M., Van Breemen, R. B. Anal. Chem., 2000, 72 (16), 3853–3859.
[27] Lantz, R. C., Chen, G. J., Sarihan, M., Sólyom, A. M., Jolad, S. D., Timmermann, B. N. Phytomedicine 2007, 14 (2–3), 123–128.
[28] Ratih, D., Sari, T., Safitri, A., Cairns, J. R. K., Fatchiyah. J. Trop. Life Sci. 2020, 10 (1), 15-25.
[29] Ferreira De Freitas, R., Schapira, M. Medchemcomm 2017, 8 (10), 1970–1981.
[30] Krisnamurti, G. C., Fatchiyah, F. Journal of Physics: Conference Series 2019, 1146, 012004.
[31] Novoseletsky, V. N., Pyrkov, T. V., Efremov, R. G. SAR QSAR Environ. Res. 2010, 21 (1–2), 37–55.
[32] Panigrahi, S. K., Desiraju, G. R. Proteins 2007, 67 (1), 128–141.
[33] Septiadi, L., Thobibatus, N., Alfaruqi, S., Wahyudi, D., Kharisma, V. D. Bioinforma. Biomeducal Res. J. 2018, 1 (2), 33–39.
[34] Zarghi, A., and Arfaei, S. Iran. J. Pharm. Res. 2011, 10 (4), 655–683.
[35] Rahmani, A. H., Al Shabrm, F. M., Aly, S. M. Int. J. Physiol. Pathophysiol. Pharmacol. 2014. 6(2), 125-136.
[36] Desai, S. J., Prickril, B., Rasooly, A. Nutr. Cancer 2018, 70 (3), 350–375.
[37] Karbasi, S. A., Modares-Mosadegh, M., Golestan, M. J. Pediatr. (Rio. J). 2010, 86 (3), 228–232.