In Silico Study of Selected Volatile Active Compounds from *Hydrilla verticillata* on MMP-2 and MMP-9 Breast Cancer Proteins

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Abstract. *Hydrilla verticillata* is an aquatic plant that contains various volatile active compounds that are potential candidates for breast cancer inhibitors. Matrix Metalloprotein Proteins (MMP) are important factors in the inhibition and invasion of breast cancer cells via the mechanism of metastasis and angiogenesis. The docking of volatile active compounds in *Hydrilla verticillata* on MMP-2 and MMP-9 proteins has been carried out to determine the binding affinity and type of binding between the ligand and the receptor. Six volatile active compounds *from Hydrilla verticillata*, potentially inhibiting breast cancer cell growth, were taken from the PubChem database. Molecular docking of these compounds to MMP-2 and MMP-9 receptors (3AYU and 4H1Q) was carried out using PyRx Virtual Screening Tool and visualized using BIOVIA Discovery Studio Visualizer. The results showed that phytol, 14-methylpentadecanoic acid, 1,2-benzene dicarboxylic acid butyl octyl ester, cis-10-octadecenoic acid, trans-10-Octadecenoic acid and ergost-5-en-3-ol, 22,23-dimethyl-acetate had binding affinities of -6.3, -6.2, -7.0, -6.4, -5.7 and -8.6 kcal/mol towards 3AYU MMP-2 receptor and -4.5, -5.2, -5.7, -4.1 and -8,5 kcal/mol towards 4H1Q MMP-9 receptor. While the native ligand has a binding affinity of -8.0 and -7.8 kcal/mol. Ergost-5-en-3-ol, 22,23-dimethyl-acetate has a better binding affinity than native ligand and, according to drug-likeness analysis, fulfill Lipinski's and Veber's rules with bioavailability score of 0.55. So, it can be concluded that these compounds have the potential as anti-cancer.

INTRODUCTION

In the last few decades, progress in the health sector has shown significant improvement concerning the prevention and treatment of cancer [1]. However, several cancer treatment options, whether surgery, radiation, or chemotherapy, have limitations such as toxicity of chemotherapy drugs, drug resistance, and lack of cell cycle specificity [2]. This poses a severe challenge in eradicating cancer. Researchers are busy working on alternative treatments for cancer or developing new molecules [3]. Cancer research can be directed at the development and identification of novel therapeutic candidates from natural products that have the potential to inhibit the activity of cancer cells specifically and do not affect normal cells [4].

Indonesia is a tropical country that is rich in plants, both land plants, and aquatic plants. One of the good and valuable plants is *Hydrilla verticillata* (L.f) Royle. *H. verticillata*, which was initially not expected to be present, turns out to be very potential to be researched and explored as a source of active compounds that have the potential to be used in the fields of pharmacology (as medicine) and treatment of environmental pollution, as well as alternative energy sources. *H. verticillata* has benefits, including anti-malarial [5], anti-microbial [6], anti-adipogenesis [7], and has anti-oxidant activity [8-10].

Bioactivity is strongly influenced by the content of chemical compounds contained in it. Several studies have shown that *H. verticillata* contains several volatile components. The extract of *H. verticillata* from maceration with PE: acetone as solvent showed the presence of Pythol and 3,5,11,15-tetramethyl-1-hexadecen-3-ol compounds [11, 12]. Soxhletation extract with ethanol solvent contains diterpene compound (Phytol), sesquiterpene compound (coryan-17-ol-18,19-didehydro-10-methoxy-acetate), steroid compound (ergost-5-en-3-ol, 22,23-dimethyl-acetate) Stearic compound (pentadecanoic acid-14-methyl, methyl ester), linoleic compound (10-octadecenoic acid, methyl ester), and plasticizer compound (1,2-Benzene dicarboxylic acid butyl octyl ester) [13].

Phytol and 9-12-octadecenoic acid are often used for pharmacological purposes [13]. Substances that promote ROS, such as phytol, represent a new class of drugs for treatment of chronic inflammatory diseases, such as rheumatoid arthritis [14]. Phytol is a recommended compound that may act as an anti-inflammatory, diuretic, anti-microbial, and anti-cancer [15-19]. 9-12-octadecenoic acid has anti-arthritis and anti-inflammatory properties [20,21].

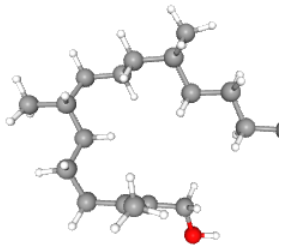
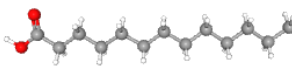
Matrix metallo proteinases (MMPs) play an important role in tumor initiation and development. MMPs play a role in angiogenesis, cell proliferation, immune surveillance, and apoptosis [22]. MMPs can degrade the extracellular matrix component [23] and set other proteases, chemokines, growth factors, cytokines, and receptor activity [24]. Important members of the MMPs family are MMP-2 and MMP-9.

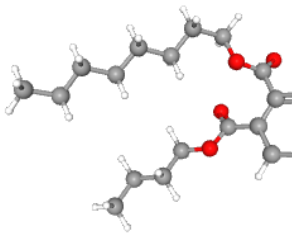
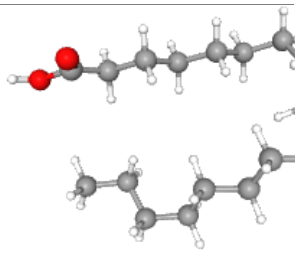
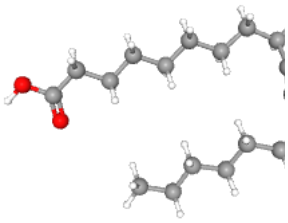
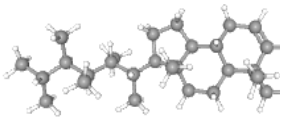
Based on the description above, to obtain compounds that are thought to have breast anti-cancer activity, an in-silico study was carried out on steroid compounds from *Hydrilla verticillata* using MMP-2 receptor with PDB ID 3AYU and MMP-9 receptor with PDB ID 4H1Q. It is hoped that steroid compounds can potentially be breast anti-cancer from this in silico test.

MATERIAL AND METHODS

The six volatile active compounds in this study were obtained from *Hydrilla verticillata*. These compounds consist of diterpene, plasticizer, fatty acid, and steroid compounds [13], as shown in **Table 1.** The structure of the six volatile active compounds was obtained from the PubChem format in SMILE and SDF. The MMP-2 receptor with PDB ID 3AYU and MMP-9 receptor with PDB ID 4H1Q taken from the Protein Data Bank were visualized using the BIOVIA Discovery Studio Visualizer. Molecular docking was carried out using AutoDock Vina embedded in the PyRx Virtual Screening Tool software. The grid box was determined at center X = 1.2320; Y = - 10.9627; Z = - 4.1593; dimension (Á) at X = 50.0234, Y = 50.0552, and Z = 50.0548. The interaction between the receptor molecules and the bioactive compounds using the BIOVIA Discovery Studio Visualizer. The Physicochemical Properties, Pharmacokinetics, Drug likeness, and Bioavailability were determined using SWISS ADME.

TABLE 1. The Volatile Active Compounds Identified from *Hydrilla verticillata.*

No.	Compounds	ID	Molecule Formula	SMILE	Chemical Structure
1.	Phytol	PubChem CID: 5280435	$C_{20}H_{40}O$ 296.5	CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C	
2.	14-methyl pentadecanoic acid	PubChem CID: 36247	$C_{16}H_{32}O_2$ 256.42	CC(C)CCCCCCCCCCCCC(=O)O	

No.	Compounds	ID	Molecule Formula	SMILE	Chemical Structure
3.	1,2-Benzenedicarboxylic acid butyl octyl ester	PubChem CID: 66540	$C_{20}H_{30}O_4$ 334.4	CCCCCCCCOC(=O)C1=CC=CC=C1C(=O)OCCCC	
4.	cis-10-Octadecenoic acid	PubChem CID: 5282759	$C_{18}H_{34}O_2$ 282.5	CCCCCCCC=CCCCCCCCCC(=O)O	
5.	trans-10-Octadecenoic acid	PubChem CID: 5282760	$C_{18}H_{34}O_2$ 282.5	CCCCCCCC=CCCCCCCCCC(=O)O	
6.	Ergost-5-en-3-ol, 22,23-dimethyl-acetate	PubChem CID: 91703800	$C_{32}H_{54}O_2$ 470.8	CC(C)C(C)C(C)C(C)C(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)OC(=O)C)C)C	

RESULT AND DISCUSSION

Docking Results of the MMP-2 and MMP-9 receptors and Bioactive Compounds

Docking simulations carried out using PyRx Virtual Screening Tool software between MMP-2 receptor (3AYU) and MMP-9 receptor (4H1Q) with phytol; 14-methyl pentadecanoic acid; 1,2-benzene dicarboxylic acid butyl octyl ester; cis-10-Octadecenoic acid; trans-10-Octadecenoic acid and Ergost-5-en-3-ol, 22,23-dimethyl-acetate are shown in **Table 2**.

TABLE 2. Docking result of *H. verticillata* active compounds with MMP-2 Receptor and MMP-9 Receptor

Compound Name	Binding Affinity (kcal/mol)	
	MMP-2 Receptor	MMP-9 Receptor
Phytol	-6.3	-4.5
14-Methylpentadecanoic acid	-6.2	-6.4
1,2-Benzene dicarboxylic acid butyl octyl ester	-7.0	-5.7
cis-10-Octadecenoic acid	-6.4	-4.6
trans-10-Octadecenoic acid	-5.7	-4.1
Ergost-5-en-3-ol, 22,23-dimethyl-acetate	-8.6	-8.5
2-Anilino-6-cyclohexyl methoxypurine	-8.0	-7.8

Based on the analysis data from the docking of volatile active compounds of *Hydrilla verticillata* with MMP-2 receptor with PDB ID 3AYU and MMP-9 receptor with PDB ID 4H1Q (**Table 2**), it was found that ergost-5-en-3-ol, 22,23-dimethyl-acetate had a lower binding affinity than 2-Anilino-6-cyclohexyl methoxypurine as references. Low binding affinity indicates stability and compatibility in binding to MMP-2 and MMP-9 receptors. This compound shows good potential as a candidate for breast cancer compounds.

Ergost-5-en-3-ol, 22,23-dimethyl-acetate interacts with the MMP-2 receptor 3AYU via Hydrogen bonding with Tyr142 with a bond length of 193 Å; Pi-Sigma interaction with Tyr73 (3.74Å), His42 (3.89Å), Phe86 (3.53Å); Pi-alkyl interaction with Phe4 (5.47Å), His84 (3.89Å), Phe86 (4.01Å) His120 (5.04Å), His124 (4.39Å), His130 (4.88Å); alkyl interaction with Leu81(5.01Å), Leu84(4.46Å), Leu82(5.06Å) and Van der Walls interactions with Ala83, Ala85, Pro140, Pro141 (**Fig. 1**). Meanwhile, interaction with MMP-9 receptor 4H1Q through Pi-alkyl with Phe304 with a bond length of 4.66, 4.84, and 4.87 Å, alkyl interaction with Lys56 (5.41 Å), Val69 (3.71 and 3.77 Å), Ile70 (4.30 and 4.94 Å), Hys71 (5.09 Å) and Lys300 with length 3.65, 4.36, 4.61, 4.71 and 5.24 Å (**Fig. 2**).

Ergost-5-en-3-ol, 22,23-dimethyl-acetate contained in *Hydrilla verticillata* has the potential to inhibition of MMP-2 and MMP-9 enzymes. This compound belongs to the class of steroids. Steroid compounds have been extensively studied for their cytotoxic activity [26-28]. Ten compounds reported throughout the *H. verticillata* ethanol extract, steroid compounds, sesquiterpene compounds, plasticizer compound, stearic acid, linoleic compounds, and diterpene compounds (phytol) anti-bacterial, have anti-fungal, anti-rheumatic, antioxidant, anti-inflammatory, anti-diabetic, anti-cancer properties and improve immunity [13].

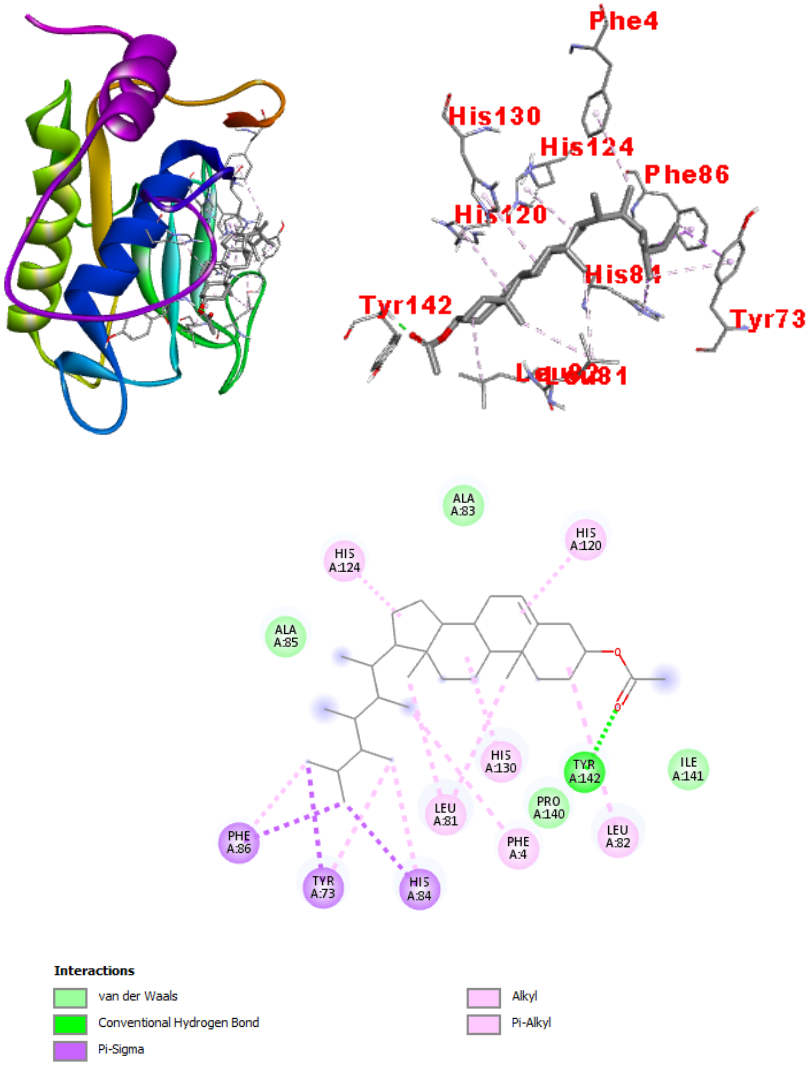


FIGURE 1. Ergost-5-en-3-ol, 22,23-dimethyl-acetate interacts with the MMP-2 receptor 3AYU

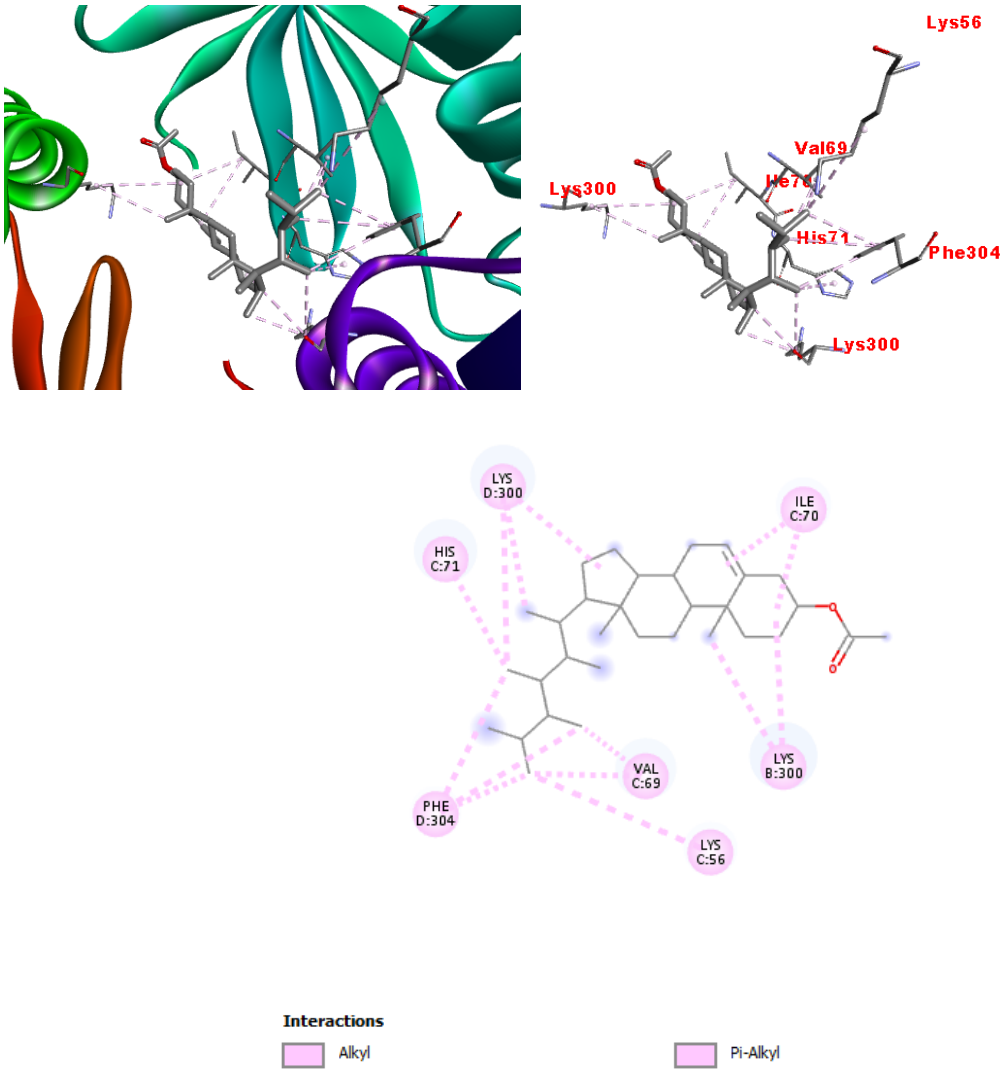


FIGURE 2. Ergost-5-en-3-ol, 22,23-dimethyl-acetate interacts with the MMP-9 receptor 4H1Q.

Physicochemical Properties, Pharmacokinetics, Drug-likeness, and Bioavailability

Druglikeness and bioavailability were personalized by six aspects: molecular size, lipophilicity, polarity, solubility, saturation, and flexibility. The bioavailability radar of Ergost-5-en-3-ol, 22,23-dimethyl-acetate are shown in **Fig. 3** and **Table 3**. This compound fulfills the suitable physicochemical space for oral bioavailability with molecular size 470.77 g/mol (150 - 500 g/mol), polarity (TPSA) 26.30 $Å^2$ (20 - 130 $Å^2$), in saturation (Fraction Csp3) 0.91 (between 0.25 until 1.0), and flexibility with the number of rotatable bonds 8 (no more than 9). while lipophilicity and insolubility outside the zone with XLOGP3 10.02 and log S (ESOL) -8.54. However, from the drug-likeness analysis, it still meets Lipinski's rule (just one violation MLOGP > 4.15) and meets Veber's rule (no violations). The bioavailability score was 0.55.

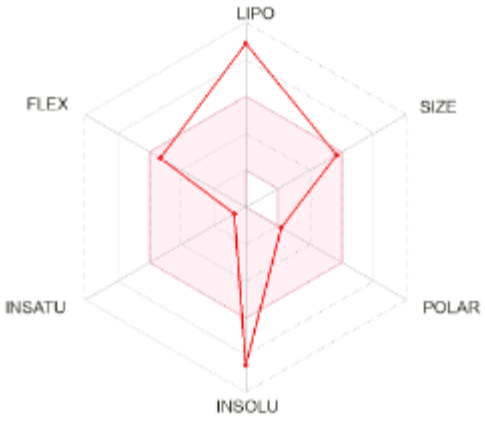


FIGURE 3. The bioavailability radar of ergost-5-en-3-ol, 22,23-dimethyl-acetate

TABLE 3. Druglikeness of Ergost-5-en-3-ol,22,23-dimethyl-acetate

Ligand	Lipinski's Rules*				Veber's rule**	
	MW	HBA	HBD	LogP	RB	TPSA
Ergost-5-en-3-ol, 22,23-dimethyl-acetate	470.77 g/mol	2	0	7.08	8	26.30 20 Å^2

* Lipinski's rule: MW ≤ 500g/mol; HBA ≤ 10; HBD ≤ 5, LogP ≤ 5
**Veber's rule: RB ≤ 9, TPSA 20 - 130 Å^2 [29]

CONCLUSIONS

Phytol, 14-methylpentadecanoic acid, 1,2-benzene dicarboxylic acid butyl octyl ester, cis-10-octadecenoic acid, trans-10-octadecenoic acid and Ergost-5-en-3-ol, 22,23-dimethyl-acetate had binding affinities of -6.3, -6.2, -7.0, -6.4, -5.7 and -8.6 kcal/mol towards 3AYU MMP-2 receptor and -4.5, -5.2, -5.7, -4.1 and -8,5 kcal/mol towards 4H1Q MMP-9 receptor. While the native ligand has a binding affinity of -8.0 and -7.8 kcal/mol. Some volatile active compounds from *Hydrilla verticillata* have a better binding affinity than native ligand and, from drug-likeness analysis, fulfill Lipinski's and Veber's rules with bioavailability score of 0.55. So, it can be concluded that these compounds have the potential for anti-cancer, especially MMP-2 and MMP-9 Breast Cancer Proteins.

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REFERENCES

1. I. A. Burney and M. S. Al-Moundhri, *Sultan Qaboos Univ. Med. J.* **8**. 137–148 (2008).
2. V. J. Vishnuvarthan, K.S. Laksmi, and A. R. Srividya, *J. Young Pharm.* **9**, 168-71, (2017)
3. C. G. Da Silva, G. J. Peters, F. Ossendorp, and L. J. Cruz, *Cancer Chemother. Pharmacol.* **80**, 881–894 (2017).
4. B. E. Oyinloye, T. A. Adekiya, R. T. Aruleba, O. A. Ojo, and B. O. Ajiboye, *Curr. Drug Discov. Technol.* **16**, 406–416 (2019).
5. S. W. Annie, R. Raveen, M. G. Paulraj, T. Samuel, and S. Arivoli, *Screening* **1**. 43–48 (2016).
6. P. Prabha and J. Rajkumar, **7**. 1809–1815 (2015).
7. S. P. Prabha, S. Sadhana, C. Karthik, and D. G. Caroline, *Pharmacogn. Mag.* **16**, 498 (2020).
8. D. Pal and S. B. Nimse, *Nat. Prod. Radiance* **5**. 108–111 (2006).
9. A. G. Fasya, B. Purwantoro, L. H. Ulya, and M. Ahmad, *ALCHEMYJournal Chem.* **8**, Art. no. 1 (2020).

10. A. G. Fasya, S. Amalia, D. S. Megawati, F. Salima, V. A. Kusuma, and B. Purwantoro, *IOP Conf. Ser. Earth Environ. Sci.* **456**, 012009 (2020).
11. B. Ponnana, V. Anuradha, E. Rosmine, N. Kumar, and N. S.M., *J. Plant Biochem. Biotechnol.* **22**, (2012).
12. B. Das, D. Pal, and A. Haldar, *Int. J. Res. Pharm. Sci.* **1**. 1–5 (2015).
13. V. Kensa and R. Neelamegum, *Asian J. Biol.* **1**, 1–6 (2016).
14. G. K. Ogunlesi, R. P. Singh, and K. K. Sakariah, *Food Chem*.**73**, 285-290 (2009)
15. P.A. Praveen, F.K. Okwuasaba, L.G. Binda, and J. Ethnopharm. **72**, 421-427 (2000)
16. M. Sermakkani, and V. Thangapandian, *Asian J Pharm Clin Res*. **5** no. 2, 90-94 (2012)
17. M. Alagammal, P.S. Tresina, and V.R. Mohan, *Int J of Curr Pharm Res.* **4** no. 2, 42-4 (2012)
18. S.Gopinath, G.Sakthidevi, S.Muthukumaraswamy, and V.R.Mohan, *J. Curr. Chem. Pharm. Sci*. **3** no. 1, 6-15 (2013)
19. V. Prabhadevi, S.Sathish, M. Johnson, B.Venkatramani, and N. Janakiraman, *Asian Pac J Trop Biomed*, **2** no. 2, 550-4 (2012).
20. J. Lalitharani, C. Stushnoff, E. Locke, and J. M. Vivanco, *Food Chem*. **83**, 547-550 (2003)
21. A. Kala, M. Dagila, C. Aceti, M. Quaglia, C. Gregotti, and G. Grazzani, J. Agric. *Food Chem*. **54**, 1209-1216 (2006)
22. H. Mohammadian, R. Sharifi, A. S. Rezanezhad, E. Taheri, and B. A. Babazadeh, *Gene Rep*. **21**, 100906 (2020)
23. K. Kessenbrock, C. Y. Wang, and Z. Werb, *Matrix Biol,* **44**, no. 46, 184-90 (2015)
24. S. Mondal, N. Adhikari, S. Banerjee, S.A. Amin, and T. Jha, *Eur J Med Chem*, **194**, 112260 (2020)
25. Rollando, Warsito, Masruri, and Widodo. *Trop J Nat Prod Res*., **5** no.1, 113-121 (2021)
26. G.F. Chi, R.V.T. Sop, A.T. Mbaveng, O. J. Omollo, G.W. Fotso, G.S. Nguenang, V. Kuete, T. Efferth, B.T. Ngadjui, Steroids, 163, 108724 (2020)
27. J.N. Wang, Z.Y. Zhang, P. Sun, D.H. Cao, Y.D. Xiao, X.C. Shi, C.F. Xiao, H.B. Hu, Y. Xu, Fitoterapia, 146, 104696 (2020)
28. J.H. Yu, S.J. Yu, K.L. Liu, C. Wang, C. Liu, J.Y. Sun, and H. Zhang, Steroids, 165, 108767 (2021).
29. A. Daina, O.Michielin, and V. Zoete, *Sci Rep*., **7**, no. 1, 1–13 (2017)