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ANTIOXIDANT ACTIVITY OF SOME NATURAL ESSENTIAL OILS IN VIETNAM: COMPARISON BETWEEN QSAR SIMULATION AND EXPERIMENTAL STUDY

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Abstract. The antioxidant activity of essential oils from leaves of *Piper betle* L. (**T**) and *Cleistocalyx operculatus* L. (**V**) and aerial parts of *Ageratum conyzoides* L. (**H**), indigenously grown in Thua Thien Hue province, Vietnam, is investigated. *The quantitative structure–activity relationship* (*QSAR*) model comprising 4-hydroxy-chromene-2H-one and its 26 derivatives is used to predict the radical scavenging activity of T, V, and H. The radical scavenging activity of the oils is experimentally determined with DPPH (1,1-diphenyl-2-picrylhydrazyl) via IC_{50} values. The experimental IC_{50} values are in good agreement with those obtained from the QSAR model. The IC_{50} value of *Piper betle* L. is 3.71 µg/mL, comparable to that of the strong antioxidant ascorbic acid (3.03 µg /mL).

Keywords: essential oil, antioxidant activity, QSAR, DPPH, *Piper betle* L.

1 Introduction

Piper betle L., *Ageratum conyzoides* L., and *Cleistocalyx operculatus* L. (Fig. 1) are considered as popular component folk-medicine prescriptions [1-3]. The pharmacological studies indicate that essential oils of *Piper betle* (**T**), *Ageratum conyzoides* (**H**), and *Cleistocalyx operculatus* (**V**) from Vietnam have antioxidant, antimicrobial, anticancer, antidiarrheal, antihypertensive, antidiabetic, and anti-inflammatory properties.

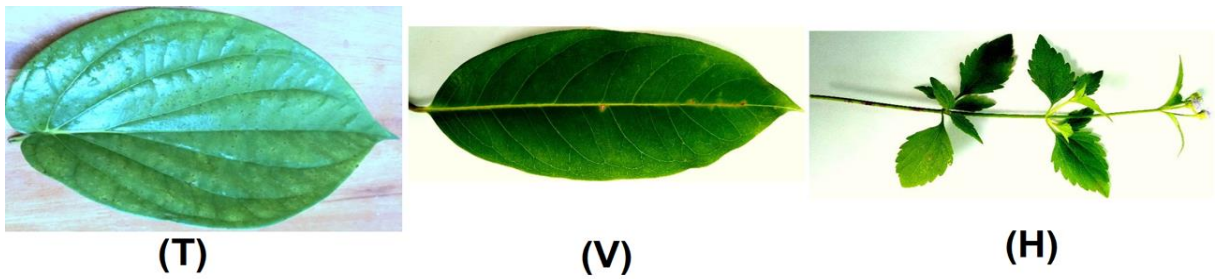

Fig. 1. Piper betle (**T**), Cleistocalyx operculatus (**V**) and Ageratum conyzoides (**H**)

In this work, the chemical composition of these three essential oils is identified by the use of GC-MS, and their antioxidant potential is determined via the stable 1,1-diphenyl 2-picrylhyrazyl (DPPH) free radical scavenging activity. The quantitative structure–activity relationship (QSAR) model is applied to predict the DPPH radical scavenging activity of the essential oils. The calculated results are compared with experimental results of DPPH radical scavenging activity to precisely determine the antioxidant bioactivity of these essential oils. The purpose of this work is to seek applications of these natural essential oils to replace antibiotics in the production of safe pharmaceutical products with high antibacterial, antifungal, and antioxidant effects.

2 Materials and methods

2.1 Sample extraction and GC-MS

The plant samples of *Piper betle (***T***), Ageratum conyzoides (***H***),* and *Cleistocalyx operculatus* (**V**) were collected in Thua Thien Hue province, Vietnam (Fig. 1). Then, they were botanically identified, and their voucher specimens were deposited at the Department of Biology, University of Sciences, Hue University. Two hundred grams of each fresh plant was subjected to steam distillation in Clevenger-type laboratory glass apparatus at 100 °C for three hours [4]. The essential oils were stored at 4 °C for further assessment after desiccating with anhydrous Na_2SO_4. The experiments were performed in triplicate [5]. The refractive index of the oils in this study was determined on a polarimeter (Reichert Cat #14003000, USA) according to the guidance of the Vietnamese Pharmacopoeia (1997).

Agilent GC 7890B-MS 5975C instrument coupled with a HP-5MS column (30 m × 250 μm × 0.25 μm) was utilized to identify the chemical constituents of the essential oils. The compounds in the essential oils were identified by comparing their mass spectra with those in the NIST02 database. Quantification was performed by using the relative peak area percentage [6]. All reagents, solvents, and chemicals were of analytical grade and purchased from Sigma – Aldrich (USA).

2.2 QSAR simulation

Experimental data: The data set is 27 compounds comprising 4-hydroxy-chromene-2H-one and its derivatives. The in vitro DPPH radical scavenging activity (IC_{50}) is the concentration of the test compounds that reduce 50% of the initial free radical concentration [7]. The structure of 4-hydroxy-chromene-2H-one derivatives was formulated and optimized by using the PM3 method, and the molecular structure parameters were examined with molecular mechanics on the QSARIS system [8].

Development and validation of QSAR models: The linear 2D and 3D-quantitative structure-activity relationship models and DPPH activity, IC_{50}, were evaluated, and then the linear regression was used as an essential tool to develop the QSAR models. Statistical values of R^2, R^2_{pred}, absolute error, relative error (ARE, %), and mean absolute relative error (MARE, %) were used to test the predictive power of the models [8]. These models were applied to predict the IC_{50} activity of ten 4-hydroxy-chromene-2H-one compounds in the test set and 32 compounds in the essential oils of **T**, **H**, and **V**. A comparison between the IC_{50} values from the QSAR models and the experimental IC_{50} values and DPPH activity of the compounds in **T**, **H**, and **V** were performed.

2.3 DPPH free radical scavenging activity

The DPPH free radical scavenging activity of each essential oil was determined by recording the absorbance of the prospective compound in the

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extract in the DPPH solution. A Jasco V-630 Spectrophotometer was used for the measurements following the method described by Wong et al. [9] and Gan et al. [10] with certain modifications. Free DPPH radicals have strong maximum absorption at 517 nm and are purplish red. The purplish-red-to-yellow change corresponds to the decrease in DPPH's original molar absorption when DPPH's free electrons are paired with an electron from the antioxidant and a hydrogen atom (equivalent to hydride) to form DPPH-H reduction. The resultant decolorization of the equivalent amount of hydride is retained. One millilitre of each essential oil of various concentrations (details in Table 4) was dissolved in 1 mL of 100 μM DPPH in ethanol. The reaction mixture was shaken for one minute and incubated at room temperature for 30 minutes to determine the optical density (OD). The absorbance change was then measured at a wavelength of 517 nm. Ascorbic acid was used as a positive reference. The radical scavenging activity was evaluated by using the IC_{50} value calculated according to the following formula

% Inhibition = [1 – OD (DPPH + sample)/OD (DPPH)] × 100%

Determination of the inhibitory concentration 50% (IC_{50}) [11]:

+ For samples with the antioxidant activity that varies linearly with concentration: calculate the regression line from the data in the form $y = a + b \times x$, where y is the percentage of inhibition; x is the concentration.

+ For samples with the antioxidant activity that does not vary linearly with concentration: In an approximation, select the upper and lower inhibition concentrations of 50% and also draw a line of the form $y = a + b \times x$ (y is the percentage of inhibition; x is the concentration).

From the equation $y = a + b \times x$, setting $y = 50$, we can determine the value of x, which is IC_{50}.

3 Results and discussion

3.1 Composition of the three essential oils

The density of essential oils from **T**, **V**, and **H** is 0.989, 0.860, and 0.980 g/mL, respectively, with respective refractive index 1.52577, 1.49093, and 1.52208. Totals of 11, 19, and 10 compounds in the essential oils of T, V, and H account for 91.2, 95.4, and 87.3%, respectively (Table 1). Eugenol (63.91%), *trans*-β-ocimene (52.87%), and demethoxyageratochromene or precocene I (56.88%) are the most dominant components in the essential oil of **T**, **V**, and **H**, respectively (Table S1–S3).

Table 1. Composition of essential oils extracted from *Piper betle* (**T**), *Cleistocalyx operculatus* (**V**), and *Ageratum conyzoides* (**H**), %

Piper betle (**T**)										
T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
0.3	0.9	1.5	63.9	1.9	3.0	1.2	2.5	3.8	1.5	10.8
Cleistocalyx operculatus (**V**)										
V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
0.2	0.4	52.9	10.9	8.1	2.5	2.0	0.6	1.0	1.1	0.3
V12	V13	V14	V15	V16	V17	V18	V19			
0.4	0.8	1.0	1.1	2.1	0.8	0.7	0.5			

Ageratum conyzoides (**H**)									
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
1.0	9.0	0.2	0.5	2.2	56.9	3.6	1.8	3.8	16.3

T1-T11: 11 compounds in the *Piper betle* essential oil; **V1-V19:** 19 compounds in the *Cleistocalyx operculatus* essential oil; **H1-H10:** 10 compounds in the *Ageratum conyzoides* essential oil

Differences in the chemical composition of essential oils can be attributed to geo-ecological factors in the production of the metabolites of plants. *Cleistocalyx operculatus* (**V**) displays significant differences in *its oil composition* compared with that in previous studies [12, 13]. For example, Dung et al. report the major components in *Cleistocalyx operculatus* essential oil as follows: *cis*-β-ocimene (**V4**) (32.1%), myrcene (**V2**) (24.6%), β-caryophyllene (**V15**) (14.5%), and *trans*-β-ocimene (**V3**) (9.4%) [12]. The chemical profiles of the essential oils do not differ only in the number of molecules but also in the stereochemical types of the molecules. Karak et al. indicate that 45 constituents are identified from the leaves of *Piper betle* (T) of seven different local varieties in India [13]. This outnumbers the 11 compounds found in the **T** essential oil in this study. However, the essential oil of **H** from Nigeria [14] and India [15] has the same main constituents as those in our study, such as precocene I (**H6**) and β-caryophyllene (**H2**).

3.2 QSAR simulation

The structure and activity data of thirty-seven 4-hydroxy-chromene-2H-one derivatives were divided into a training set (27 compounds) and a test set (10 compounds). The predictive power of the QSAR model was assessed by comparing the predicted values with the activity of the compounds in the control group according to the equation $pIC_{50} = -lg(IC_{50} \times 10^{-6})$. The variability of R^2 values, predicted correlation values (R^2_{pred}), and SE (standard error) in the QSAR models include 2D and 3D descriptive parameters [8].

To develop the QSAR models, the 2D and 3D descriptive parameters are selected with the stepwise regression technique. The 2D, 3D descriptive parameters are included in the model based on the changing of R^2, SE, and R^2_{pred}. The models are cross-evaluated by using the Leave-One-Out method (LOO) to determine R^2_{pred}. The QSAR model with seven variables (k = 7), describing the molecules with the highest R^2 and R^2_{pred}, is the best one containing the 2D, 3D parameters of the molecule: log*P* (logarithm of octanol-water partition coefficient), Dipole (dipole moment of a molecule in Debyes), xc3 (connectivities Simple Cluster 3 of 2D descriptors), nelem (number of elements), MaxNeg (the largest negative charge over the atoms in a molecule), Polarizability (molecular polarizability), MaxQp (the largest positive charge over the atoms in a molecule); these parameters are typical for the polarity, bulkiness, and dispersion coefficients of molecules. These are important descriptive parameters when determining pharmacological properties of drugs [8]; therefore, the seven-variable QSAR model satisfies the appropriate statistical factors and clearly describes the nature of the molecular structure with medicinal properties, and is strictly tested for predictive power and compared with experiment.

The biological activity prediction results are consistent with the experimental data as evidenced by the predicted R^2 and R^2_{Pred} values [7, 8]. The QSAR model with seven variables is used to predict resistance activity via the equation $pIC_{50} = -lg(IC_{50} \times 10^{-6})$ with the compounds investigated IC_{50} for the test set and the compounds in the essential oil of **T**, **H**, and **V**.

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QSAR model with seven variables

$pIC_{50} = 0.1504 - 0.525 \times \log P + 0.042 \times \text{Dipole} + 1.359 \times c3 - 0.399 \times \text{nelem} - 13.866 \times \text{MaxNeg} + 0.520 \times \text{Polarizability} - 9.646 \times \text{MaxQp}$

The statistics are as follows: n = 27 compounds in the training set [16]; R^2 = 0.960; R^2 for prediction = 0.862; standard error = 0.167; F = 27.72; $F_\alpha = 5.3223.10^{-5}$; $p < 0.05$.

The resistance ability was tested to predict the pIC50 activity using the QSAR model, k = 7, for 10 derivatives in the test set (1d–10d) [7], and compared with experimental values. The obtained results of test set (1d-10d) are shown in Table 2 with a MARE% of 1.89%. The ANOVA analysis of a single factor comparing $pIC50_{exp}$ and $pIC50_{cal}$ exhibits the same trend with $F_{cal} = 0.001 < F_\alpha = 4.414$. Testing the results by using the QSAR model, k = 7, shows the resistance ability to predict pIC_{50} activity. The errors are within the tolerance of experimental measurements.

Table 3 shows the predicted pIC_{50} values in the following order: **T11** > **T3** > **T4** > **T5** > **T1** > **T8** > **T6** > **T10** > **T9** > **T7** > **T2**; **H10** > **H6** > **H2** > **H1** > **H5** > **H3** > **H4** > **H7** > **H8** > **H9**; **V15** > **V19** > **V18** > **V17** > **V16** > **V10** > **V7** > **V5** > **V13** > **V8** > **V6** > **V11** > **V1** > **V2** > **V9** > **V12** > **V14** > **V3** > **V4.** The average pIC_{50} of each essential oil is calculated according to the following formula

$$pIC_{50} = \frac{1}{100}\sum_{i=1}^{n} a_i x_i$$

where a_i is the content of substance i in the essential oil; x_i is the calculated value pIC_{50} of substance i in the essential oil; n is the total number of substances in the essential oil.

The average IC_{50} for the oils is as follows: $IC_{50\ (T,\ cal)}$ = 3.713 μg/mL, $IC_{50\ (H,\ cal)}$ = 547.470 μg/mL, and $IC_{50\ (V,\ cal)}$ = 677.708 μg/mL with a MARE, % of 3.486%.

Table 2. Values via equation $pIC_{50} = -\lg(IC_{50} \times 10^{-6})$ calculated of 4-hydroxy-chromene-2H-one derivatives from QSAR model, k = 7

Test set	$pIC50_{exp}$	$pIC50_{cal}$	ARE, %
1d [7]	3.46	3.537	2.230
2d [7]	2.06	2.057	0.150
3d [7]	2.86	2.881	0.726
4d [7]	3.46	3.467	0.191
5d [7]	4.26	4.318	1.354
6d [7]	2.21	2.227	0.758
7d [7]	1.13	1.046	7.395
8d [7]	2.14	2.223	3.863
9d [7]	4.33	4.356	0.611
10d [7]	3	2.951	1.626
MARE,%			1.890

Table 3. The prediction about activity pIC_{50} (μg/mL) of derivatives in the essential oils of **T**, **H**, and **V**

Com.	$pIC_{50\ cal.}$	Com.	$pIC_{50\ cal.}$	Com.	$pIC_{50\ cal.}$	Com.	$pIC_{50\ cal.}$
T1	4.764	**T11**	7.526	**H10**	3.806	**V10**	4.241
T2	3.491	**H1**	3.615	**V1**	3.564	**V11**	3.574
T3	7.262	**H2**	3.692	**V2**	3.546	**V12**	3.511
T4	6.152	**H3**	1.334	**V3**	3.491	**V13**	3.679
T5	5.157	**H4**	1.190	**V4**	3.491	**V14**	3.494
T6	3.692	**H5**	3.597	**V5**	3.692	**V15**	5.554
T7	3.597	**H6**	3.696	**V6**	3.597	**V16**	4.893
T8	3.716	**H7**	1.068	**V7**	3.716	**V17**	5.028
T9	3.658	**H8**	0.981	**V8**	3.615	**V18**	5.056
T10	3.679	**H9**	0.669	**V9**	3.516	**V19**	5.402
The average pIC_{50} (μg/mL): $pIC_{50\ (T,\ cal)}$ = 5.430 or $IC_{50\ (T,\ cal)}$ = 3.713 μg/mL)							
				$pIC_{50\ (H,\ cal)}$ = 3.262 or $IC_{50\ (H,\ cal)}$ = 547.470 μg/mL)			
				$pIC_{50\ (V,\ cal)}$ = 3.169 or $IC_{50\ (T,\ cal)}$ = 677.708 μg/mL)			

Interestingly, the same trend of pIC_{50} values of H and V in this study is found as that reported by Dung et al. [17]: $IC_{50\ (H,\ exp)}$ = 570.000 < $IC_{50\ (V,\ exp)}$ = 806.720 and by Patil et al. [14]: $IC_{50\ (H,\ exp)}$ = 570.000 < $IC_{50\ (V,\ exp)}$ = 806.720.

Notably, these data indicate that the accuracy of prediction of the QSAR model, k = 7, for predicted IC_{50} errors and experimental results is within an allowable range with a *MARE*, % of less than 5%. Basing on the predicted pIC_{50} results of each molecule and the average pIC_{50} value of each essential oil, we found that the predicted DPPH free radical scavenging activity is in the following order: **T** > **H** > **V** (Fig. 2).

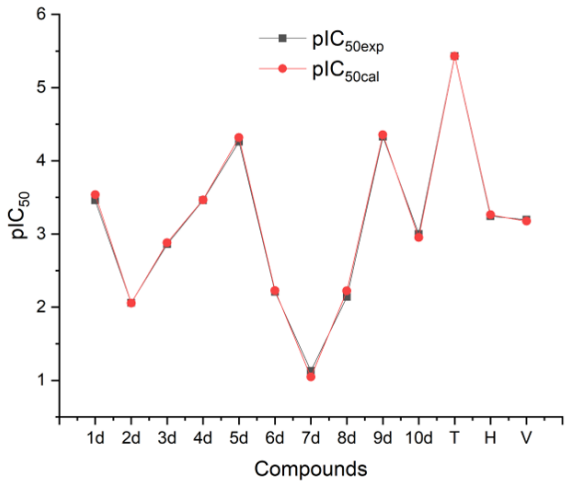

Fig. 2. Experimental and calculated pIC_{50} values: 1d–10d for selected compounds in the test set (10 compounds) and **T**, **H**, **V** for the studied oils

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3.3 DPPH radical scavenging activity

The change of colour from purple to yellow confirms the antioxidant activity of the constituents in the three essential oils. Table 4 indicates that the higher the concentrations of the essential oils of **T**, **V**, **H** are, the better the DPPH inhibition will take place. The essential oil of **T** has the highest DPPH radical scavenging activity with an IC_{50} value of 3.71 μg/mL, close to the IC_{50} value of ascorbic acid (3.03 μg/mL). The high antioxidant activity of the essential oil of **T** could be due to the presence of a large amount of eugenol (63.9%) and eugenol acetate (10.8%) as the main constituents in the oil. The IC_{50} values of the essential oils of **H** and **V** are 569.44 μg/mL and 637.03 μg/mL, respectively, indicating that the antioxidant activity of these two essential oils is much lower than that of the essential oil of **T** and ascorbic acid.

Table 4. DPPH radical scavenging activity rates of the three essential oils of **T**, **H**, and **V**

Ascorbic acid					
Concentrations (μg/mL)	1.25	2.5	5	7.5	10
Inhibited DPPH (%)	25.8	42.3	78.4	89.3	93.5
IC_{50} (μg/mL)			**3.03**		
T essential oil					
Concentrations (μg/mL)	1.25	2.5	5	7.5	10
Inhibited DPPH (%)	20.8	40.3	60.4	71.5	75.5
IC_{50} (μg/mL)			**3.71**		
V essential oil					
Concentrations (μg/mL)	300	400	500	600	700
Inhibited DPPH (%)	12.3	26.7	36.9	42.3	63.2
IC_{50} (μg/mL)			**637.03**		
H essential oil					
Concentrations (μg/mL)	300	400	500	600	700
Inhibited DPPH (%)	15.3	29.3	42.8	53.2	68.2
IC_{50} (μg/mL)			**569.44**		

3.4 Comparison of IC_{50} calculated with QSAR simulation and IC_{50} of DPPH radical scavenging activity

The single factor ANOVA is also applied to compare the predicted IC_{50} ($IC_{50,\ cal}$) from QSAR simulation with experimental IC_{50} of **T**, **V**, and **H** essential oils. There is not a significant difference at a confidence level of 95% between $IC_{50,\ cal}$ and IC_{50} of **T**, **V**, and **H** essential oils ($F_{cal} = 3.86 < F_{crit\ (0.05,1,4)} = 7.71$). The calculated pIC_{50} and IC_{50} indicate that **T**, **V**, and **H** have strong antioxidant activities with the order **T** > **H** > **V**.

Highly active compounds in the oils are **T11**, **T3**, **H10**, **H6**, **V15**, and **V19**. This confirms that the activity of an essential oil compound depends on the nature of the molecular structure and its content in the essential oil. However, the structure plays a crucial role. A highly active compound, sometimes even in a small quantity can be used in research and for the production of drugs that are capable of causing toxicity on bacterial and fungal cells but safe for the human body at the therapeutic dose [18, 19]. The boundary between toxicity and good medicinal properties is of particular interest to scientists. Therefore, the concentration threshold factor is investigated very strictly. Here, it is obvious that highly reactive molecules are important for pharmacists in drug research, and this is a potential, safe, and natural drug approach that can substitute certain types of antibiotics at present time.

The above data show the results of analyzing the activity of compounds in the essential oils of **T**, **H**, and **V** with the QSAR model, and the DPPH radical scavenging activity proves that the theories regarding the structural nature of the compounds, the nature of the interaction, and the calculated pIC_{50} activity data are completely consistent with the experiment.

4 Conclusions

The experimental results indicate the strong DPPH radical scavenging activity of the essential oil of *Piper betle* (**T**). Its IC_{50} value is not significantly different from the IC_{50} value of ascorbic acid. The QSAR study allows predicting the DPPH radical scavenging activity of the compounds in the investigated essential oils in the same trend as those of the experimental study. The compounds **T3**, **T11**, **H6**, **H10**, **V15**, and **V19** are also predicted to have higher DPPH radical scavenging activity than other compounds available in the corresponding essential oils (**T**, **H**, and **V**). Both experimental study and QSAR simulation indicate that the essential oil of **T** has the best antioxidant activity, followed by the essential oil of **H**, which possessed the greater potency than the essential oil of **V**. This open the door to apply these natural and safe essential oils as natural anti-microbial and antioxidant remedies.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Aishwarya J, Chauhan ES, Singh A, Tiwari AJAJoP, Therapeutics C. A Review: Nutraceuticals Properties of Piper betel (Paan). 2016;4:028-41.
2. Bhargava P, Uppoor A, Pralhad S, Nayak DGJNjoirim. An Invitro Study Of Determination Of Anti-Bacterial, Antioxidant, Anti- Inflammatory Potential Of Piper Betel Essential Oil. 2015;6:37-44.
3. Row LM, Ho J. The Antimicrobial Activity, Mosquito Larvicidal Activity, Antioxidant Property

pISSN 1859-1388
eISSN 2615-9678

and Tyrosinase Inhibition ofPiper Betle. Journal of the Chinese Chemical Society. 2009 06;56(3):653-658.

4. Vietnamese Pharmacopoeia. 5th ed. Hanoi: Medical Publishing House; 1997. p. 134.
5. Boukeria S, Kadi K, Kalleb R, Benbott A, Bendjedou D, Yahia A. Phytochemical and physicochemical characterization of Allium sativum L. and Allium cepa L. Essential oils. Journal of Materials and Environmental Science. 2016;7(7):2362-2368.
6. Diao W, Hu Q, Zhang H, Xu J. Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (Foeniculum vulgare Mill.). Food Control. 2014;35(1):109-116.
7. Mladenović M, Mihailović M, Bogojević D, Matić S, Nićiforović N, Mihailović V, et al. In Vitro Antioxidant Activity of Selected 4-Hydroxy-chromene-2-one Derivatives—SAR, QSAR and DFT Studies. International Journal of Molecular Sciences. 2011;12(5):2822-2841.
8. Tat PV. Development of new anticancer agents from Leaf of plants in Viet Nam. Hanoi: LAP Lambert Academic Publishing; 2017. 80 p.
9. Wong S, Leong L, Williamkoh J. Antioxidant activities of aqueous extracts of selected plants. Food Chemistry. 2006;99(4):775-783.
10. Gan R-Y, Xu X-R, Song F-L, Kuang L, Li H-B. Antioxidant activity and total phenolic content of medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases. Journal of medicinal plant research. 2010;4:2438-2444.
11. Alexander B, Browse D, Reading S, Benjamin I. A simple and accurate mathematical method for calculation of the EC50. Journal of Pharmacological and Toxicological Methods. 1999;41(2-3):55-58.
12. Dung NX, Van Luu H, Khoi TT, GC and GC/MS analysis of the leaf oil of Cleistocalyx operculatus Roxb. Merr. et Perry (Syn. Eugenia operculata Roxb.; Syzygicum mervosum DC.). Journal of Essential Oil Research. 1994;6(6):661-662.
13. Karak S, Acharya J, Begum S, Mazumdar I, Kundu R, De B. Essential oil of Piper betle L. leaves: Chemical composition, anti-acetylcholinesterase, anti-β-glucuronidase and cytotoxic properties. Journal of Applied Research on Medicinal and Aromatic Plants. 2018;10:85-92.
14. Patil RP, Nimbalkar MS, Jadhav UU, Dawkar VV, Govindwar SP. Antiaflatoxigenic and antioxidant activity of an essential oil from Ageratum conyzoides L.. Journal of the Science of Food and Agriculture. 2010;90(4):608-614.
15. Usman L, Olawore ZM, Muhammad NO, M'Civer FA, Ismaeel RO. Chemical constituents of flower essential oil of Ageratum conyzoides growing in Nigeria. Elixir Organic Chemistry. 2013;54:12463-12465.
16. Rani N, Kumar P, Singh R, Sharma A. Molecular docking evaluation of imidazole analogues as potent candida albicans 14α-Demethylase inhibitors. Current Computer Aided-Drug Design. 2015;11(1):8-20.
17. Dung NT, Kim JM, Kang SC. Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of Cleistocalyx operculatus (Roxb.) Merr and Perry buds. Food and Chemical Toxicology. 2008;46(12):3632-3639.
18. Liao SY, Chen JC, Qian L, Shen Y, Zheng KC. QSAR, action mechanism and molecular design of flavone and isoflavone derivatives with cytotoxicity against HeLa. European Journal of Medicinal Chemistry. 2008;43(10):2159-2170.
19. Wang T, Chen I, Lu P, Wong C, Liao C, Tsiao K, et al. Synthesis, antiproliferative, and antiplatelet activities of oxime- and methyloxime-containing flavone and isoflavone derivatives. Bioorganic & Medicinal Chemistry. 2005;13(21):6045-6053.