Antioxidant activity of some natural essential oils in Vietnam: Comparison between QSAR simulation and experimental study

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Abstract

Antioxidant activities of essential oils from leaves of *Piper betle* L. (**T**) and *Cleistocalyx operculatus* L. (**V**), and aerial parts of *Ageratum conyzoides* L. (**H**) natively grown in Thua Thien Hue province of Vietnam were investigated. Quantitative structure–activity relationship (QSAR) model was constructed from the 27 compounds including 4-hydroxy-chromene-2H-one and its derivatives. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was applied to estimate the antioxidant property of these essential oils through IC_{50} (μg/mL) values. QSAR model is used to predict the radical scavenging activity IC_{50} μg/mL of **T**, **V**, and **H**. The results indicate that there is a good agreement between the experimental data and the predicted values using the QSAR model. The three essential oils display the DPPH radical scavenging activities with the IC_{50} values being in the order of **T** > **H** > **V** of 3.71 μg/mL, 596.44 μg/mL and 637.03 μg/mL, respectively. The essential oil of **T** exhibits the strong DPPH radical scavenging activity that is close to the reference compound ascorbic acid (IC_{50} value of 3.03 μg /mL).

Key words: *natural essential oil, antioxidant activity, QSAR, DPPH.*

1. Introduction

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

In this work, the chemical composition of these three essential oils was identified by GC-MS and their antioxidant potential was determined through the stable 1,1- diphenyl 2-picrylhyrazyl (DPPH) free radical scavenging activity. Quantitative structure-activity relationship (QSAR) model was applied to predict DPPH radical scavenging activity of the three essential oils of *Piper betle*, *Ageratum conyzoides* and *Cleistocalyx operculatus*. The calculated results were compared to experimental results of DPPH radical scavenging activity to determine accurately the antioxidant bioactivity of investigated essential oils. Thus, there has been an orientation to use natural essential oils instead of antibiotics to produce safe pharmaceutical products with high antibacterial, antifungal and antioxidant effects.

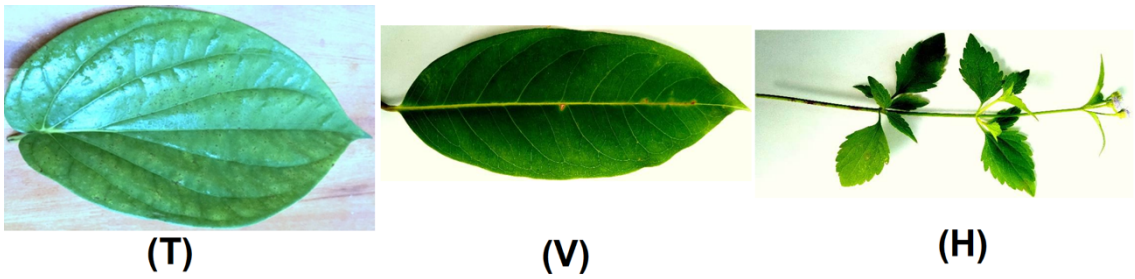

Figure 1. *Piper betle* (**T**), *Cleistocalyx operculatus* (**V**) and *Ageratum conyzoides* (**H**)

2. Materials and methods

2.1. Sample extraction and GC-MS

Plant samples including *Piper betle* (**T**), *Ageratum conyzoides* (**H**), and *Cleistocalyx operculatus* (**V**) were collected in Thua Thien Hue province, Vietnam (Figure 1). Then they were botanically identified, and their voucher specimens were deposited at the Department of Biology, University of Sciences, Hue University. 200 g of each fresh plant was obtained by steam distillation in Clevenger type laboratory glass apparatus at 100 °C for 3 hours [4]. The essential oils were stored at 4 °C for the experimental processes after desiccating by anhydrous Na_2SO_4. The experiments were performed in triplicate [5]. The refractive indices of essential oils in this study were

determined using a polarimeter (Reichert Cat #14003000, USA) based on 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 applied to indentify the chemical constituents of three essential oils. The compounds in the essential oil were identified by comparing their mass spectra with those contained in the NIST02 database. Quantification was performed using the relative peak area percentage [6]. All of the reagents, solvents and chemicals were purchased from analytical grade from Sigma - Aldrich (USA).

2.2. QSAR Simulation

Experimental data: The data set comprised of 27 compounds including 4-hydroxy-chromene-2H-one and its derivatives; and *in vitro* DPPH radical scavenging activity IC_{50} μg/mL posted the concentrations of the test compounds that reduce 50 % of the initial free radical concentration which have been reported by Mladenović et al. [7]. The structures of 4-hydroxy-chromene-2H-one derivatives were formulated and optimized using PM3 method. The molecular structure parameters were examined by molecular mechanics on 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 QSAR models. Statistical values of R^2, R^2_{pred}, absolute error and relative error (ARE, %), 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} μg/mL 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 IC_{50} calculation resulting from QSAR models with IC_{50} μg/mL experimental values and DPPH activity of compounds in **T**, **H**, and **V** were analyzed.

2.3. DPPH free radical scavenging activity

The DPPH free radical scavenging activity of each essential oil was determined by recording absorbance of prospective compound in extract is mixed with DPPH solution. Jasco V-630 Spectrophotometer is used according to the method described in Wong et al. [9] and Gan et al. [10] with certain modifications. Free DPPH radicals have a strong maximum absorption at 517 nm and are purplish red. The purplish red to yellow process corresponds to the decrease in DPPH's original molar absorption when DPPH's free electrons are paired with an electron from an

antioxidant and a hydrogen atom (equivalent to hydride) to form DPPH-H reduction. The resultant decolorization of the equivalent amount of hydride is retained. 1 mL of each essential oil of various concentrations (details presented in Table 4) were dissolved in 1 mL of 100 μM DPPH in ethanol. The reaction mixture was shaken for 1 minute and incubated at room temperature for 30 minutes to determine the optical density (OD). Absorbance changes were then measured at a wavelength of 517 nm. Ascorbic acid was used as positive control (reference standard). Radical scavenging activity was evaluated using the IC50 value calculated by the formula as follows:

$$\% \ Inhibition = [1 - OD\ (DPPH + sample)/OD\ (DPPH)] \times 100\%$$

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

+ For samples has antioxidant activity that varies linearly with concentration: make the regression line through all points of the form y = a + b.x (y is the percentage of inhibition; x is the concentration).

+ For samples has antioxidant activity that did 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.x (y is the percentage of inhibition; x is the concentration).

From the equation y = a+ b.x, substituting y = 50, we can infer that the concentration value of x is IC_{50}.

3. Results and discussion

3.1. Composition of the three essential oils

The densities of essential oils from **T**, **V** and **H** were 0.989 g/mL, 0.860 g/mL and 0.980 g/mL, respectively. The refractive indices of these oils corresponded to 1.52577 g/mL, 1.49093 g/mL and 1.52208 g/mL. The presence of active compounds and their content in three essential oils of **T**, **V**, and **H** were determined by GC- MS analysis, and the data were recorded in Table S1–S3 and Figure S2–S4 in the supporting information. Totals of 11, 19 and 10 compounds in the essential oils of **T**, **V**, and **H** were 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. Percentages of compounds **T1 – T11**, **V1–V19**, **H1–H10** in the essential oils of *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	

The difference in chemical composition of essential oils can be attributed to the importance of geo-ecological factors in the production of metabolites of the plant. In fact, the chemical constituents of essential oil of *Cleistocalyx operculatus* (**V**) (Table 1 and Table S2) revealed significant differences in *the percentage of* components compared to those in previous studies [12, 13]. The major components in *Cleistocalyx operculatus* essential oil obtained were *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 did not differ only in the number of molecules but also in the stereochemical types of molecules in their ingredients. Karak et al. indicated that 45 constituents in essential oils were identified from seven different local varieties in leaves of *Piper betle* (**T**) from India [13], much more than only 11 compounds found in **T** essential oil collected from Thua Thien Hue, Vietnam in this study. However, the essential oil of **H** from Nigeria and India [14, 15] had the same main constituents with our study including precocene I (**H6**) and β-caryophyllene (**H2**).

3.2. QSAR Simulation

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

To develop the QSAR models, 2D and 3D descriptive parameters were selected by stepwise regression technique. The 2D, 3D descriptive parameters were included in the model based on the changing of statistical values of R^2, SE and R^2_{pred}. The models were cross-evaluated using the Leave-One-Out method (LOO) to determine the R^2_{pred}. The QSAR model with k = 7 variables

describing the molecules with the highest R^2 and R^2_{pred}, is the best model including the 2D, 3D parameters of the molecule: LogP (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 polarity, bulkiness and dispersion coefficients of molecules. These are important descriptive parameters when determining pharmacological properties of the drug [8], therefore QSAR model k = 7 satisfies the appropriate statistical factors, describes the nature of the molecular structure with medicinal properties, and is strictly tested for predictive power and compared with experiment.

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

Test set	pIC_{50}exp	pIC_{50}cal	*ARE*, %
1d (Mladenović et al., 2011)	3.46	3.537	2.230
2d (Mladenović et al., 2011)	2.06	2.057	0.150
3d (Mladenović et al., 2011)	2.86	2.881	0.726
4d (Mladenović et al., 2011)	3.46	3.467	0.191
5d (Mladenović et al., 2011)	4.26	4.318	1.354
6d (Mladenović et al., 2011)	2.21	2.227	0.758
7d (Mladenović et al., 2011)	1.13	1.046	7.395
8d (Mladenović et al., 2011)	2.14	2.223	3.863
9d (Mladenović et al., 2011)	4.33	4.356	0.611
10d (Mladenović et al., 2011)	3	2.951	1.626
MARE,%			1.890

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 k = 7 was used to predict resistance activity via the equation: $pIC_{50} = - \lg(IC_{50}.10^{-6})$ with the IC_{50} investigated compounds for the test set and the compounds in the essential oils of **T**, **H**, and **V**.

QSAR model with k = 7.

pIC_{50} = 0.1504 - 0.525LogP + 0.042Dipole + 1.359xc3 - 0.399nelem -13.866MaxNeg + 0.520Polarizability - 9.646MaxQp

n = 27 compounds in 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 value < 0.05.

Testing the ability to predict the pIC_{50} activity using the QSAR model, k = 7, for 10 derivatives in the test set (1d-10d) [7], and comparing with experimental values. The obtained results are shown in Table 2 with a relative average error of MARE,% = 1,890%. ANOVA analysis of single factor comparing pIC_{50exp} và pIC_{50cal} exhibited the similar trend with $F_{cal} = 0.001 < F_{\alpha} = 4.414$. Testing the results using the QSAR model, k = 7, showed the ability to predict pIC_{50} activity. The errors were considered within the tolerance of experimental measurements.

As shown in Table 3, the predicted pIC_{50} values, the activity of compounds in the essential oil of **T** was in the following order: **T11 > T3 > T4 > T5 > T1 > T8 > T6 > T10 > T9 > T7 > T2**; and the order of activity in the essential oil of **H** was: **H10 > H6 > H2 > H1 > H5 > H3 > H4 > H7 > H8 > H9**, while the essential oil of **V** exhibited the order: **V15 > V19 > V18 > V17 > V16 > V10 > V7 > V5 > V13 > V8 > V6 > V11 > V1 > V2 > V9 > V12 > V14 > V3 > V4**; From the calculated pIC_{50} data and the percentage of compounds in the three essential oils, the average pIC_{50} of each essential oil was calculated in the following equation:

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

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

Then the average pIC_{50} was converted to the average IC_{50} for the 3 essential oils of **T**, **H**, and **V**. The results are presented as follows: $IC_{50\ (T,\ cal)} = 3.713 < IC_{50\ (H,\ cal)} = 547.470 < IC_{50\ (V,\ cal)} = 677.708$ with a mean absolute relative error *MARE*, % = 3.486%.

Table 3. The prediction about activity pIC_{50} 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} :			$pIC_{50\ (T,\ cal)} = 5.430$ or $IC_{50\ (T,\ cal)} = 3.713$				
			$pIC_{50\ (H,\ cal)} = 3.262$ or $IC_{50\ (H,\ cal)} = 547.470$				
			$pIC_{50\ (V,\ cal)} = 3.169$ or $IC_{50\ (T,\ cal)} = 677.708$				

Interestingly, the same trend of pIC_{50} values of **H** and **V** was found in this study compared to the previous results where the value of $IC_{50\,(H,\,exp)} = 570.000 < IC_{50\,(V,\,exp)} = 806.720$ and $IC_{50\,(H,\,exp)} = 570.000 < IC_{50\,(V,\,exp)} = 806.720$ were respectively reported by Dung et al. [17] and Patil et al. [14].

Notably, these data indicate that the accuracy of prediction of the QSAR model, k = 7, predicted IC_{50} errors and experimental results are within the allowable range with a mean absolute relative error *MARE*, % < 5%. Based on the predicted pIC_{50} results of each molecule and calculating the average pIC_{50} value of each essential oil, we found that the predicted DPPH free radical scavenging activity is **T** > **H** > **V** (Figure 2).

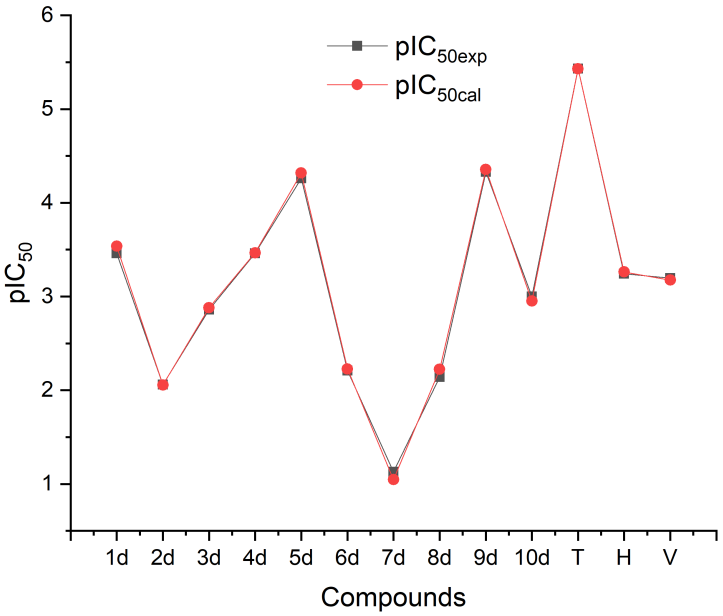

Figure 2. pIC_{50} values via experimental and QSAR model calculation: 1d – 10d are selected compounds in the test set (10 compounds); the three essential oils of **T**, **H**, **V**

3.3. DPPH radical scavenging activity

The purple to yellow process confirms the antioxidant activity of the constituents in the three essential oils. Table 4 indicates that the higher the concentrations of the essesstial oils of **T**, **V**, **H** were, the better the DPPH inhibitions are. The essential oil of **T** have the strongest DPPH radical scavenging activity with the IC_{50} of 3.71 µg/mL, quite close to the IC_{50} value of ascorbic acid (3.03 µg/mL). The high antioxidant activity of the essential oil of **T** is possibly explained by the presence of a large amount of eugenol (63.9 %) and eugenol acetat (10.8 %) as main constituents in this essential oil. The IC_{50} results of the essential oils of **H** and **V** are respective 569.44 µg/mL and

637.03 μg/mL, recording the antioxidant activitiy of these two essential oils are much lower than that of the essential oil of **T** and ascorbic acid.

Table 4. The 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**		
The **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**		
The **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**		
The **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 $IC_{50, cal}$ calculated by QSAR simulation and IC_{50} of DPPH radical scavenging activity

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

The highly active compounds in **T** are **T11** and **T3**; in **H** are **H10** and **H6**; and in **V** are **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 production of drugs that are capable of causing toxicity on bacterial and fungal cells but safe for human body at 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 has been clarified that highly reactive molecules are important

for pharmacists in drug research, and this is a potential, safe, 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** by QSAR model and DPPH radical scavenging activity proves that the theories regarding the structural nature of the compounds, the interaction nature and the calculated pIC_{50} activity data are completely consistent with the experiment.

4. Conclusions

The experimental results indicate the strongest DPPH radical scavenging activity of the essential oil of *Piper betle* (**T**) with its IC_{50} value was not much significant difference compared with the IC_{50} value of the reference compound as ascorbic acid. The QSAR study allowed to predict DPPH radical scavenging activity of compounds in investigated essential oils in the same trend to those of 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** had 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.

Acknowledgements

This research is funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.06-2017.303 (Nguyen Thi Ai Nhung).

Conflict of interest

The authors declare no conflict of interest.

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