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EVALUATION OF THE SYNERGISTIC EFFECT OF CURCUMA AROMATICA IN COMBINATION WITH SORAFENIB ON A HEPATOBLASTOMA CELL LINE IN VITRO

Thanh-Tuan Huynh[1], Thi-Do Quyen Le[2], Thanh-Hoa Vo[3], Kieu-Minh Le[4], Thanh-Chi Hoang[5], Thi-Kim Ly Bui[6]

[1]Center for Training and Scientific Research, Tam Anh General Hospital, Ho Chi Minh City, Vietnam. tuanht@hcm.tahospital.vn

[2]Cho Ray Hospital, Ho Chi Minh City, Vietnam. drquyen2003@gmail.com

[3]College of Pharmacy, Taipei medical university, Taipei, Taiwan. 339107005@tmu.edu.tw

[4]Center for Molecular Biomedicine, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam. lkminh@ump.edu.vn

[5]Institute of Applied Technology, Thu Dau Mot University, Binh Duong Province. chiht@tdmu.edu.vn

[6]Institute of Applied Technology, Thu Dau Mot University, Binh Duong Province. lybtk@tdmu.edu.vn

ABSTRACTS

Liver cancer is one of the most common cancers, accounting for the sixth-highest number of new cases and the third-highest number of deaths. Sorafenib is a well-known targeted therapy for the treatment of liver cancer. Sorafenib's therapeutic use has also been linked to side effects such as diarrhea, hypertension, and skin toxicity. As a result, combining Sorafenib with other therapeutic agents is required to avoid unwanted side effects. In this study, we tried to test the combination effect of Sorafenib and Curcuma aromatica extract. The cytotoxic, migration, and colony assays were used to investigate the conjugated effect of C. aromatica extract and Sorafenib on liver cancer cell growth inhibition. At the same Sorafenib concentration, the more C. aromatica extract supplemented, the higher the cell death rate, with a statistically significant difference. To assess the relative potency of the combination, an isobologram model integrated into the CalcuSyn software was used. All of the data points were located in a synergistic area below the additive line, according to the schematic isobologram and CI distribution chart. The results indicated that the effect of sorafenib and C. aromatica was synergistic. C. aromatica extract could be used to help treat liver cancer when used with sorafenib.

KEYWORDS: Curcuma aromatica, Sorafenib, Hep J5, Cytotoxicity, Liver cancer.

I. INTRODUCTION

Free radicals are one of the unavoidable byproducts of normal metabolic activity [1]. When there are more free radicals present than can be kept in balance by antioxidants, the free radicals can start doing damage to fatty tissue, DNA and proteins in your body. The unavoidable result of these molecular injuries is that the body develops dangerous pathologies such as diabetes, atherosclerosis, blood vessel hardening, inflammatory conditions, high blood pressure, heart disease, neurodegenerative diseases, and even cancer [2]. The harmful effects of free radicals have sounded the alarm all over the world. Instead of looking for drugs that can treat disease at the disease-formation stage, many scientists are now screening medicinal herbs that have the function of preventing disease at the very early stages, also known as molecular level prevention. And free radicals are an excellent target that scientists have been pursuing in recent years.

Curcuma aromatica, also known as wild turmeric, is a type of curcuma. This herb is commonly used as a coloring and flavoring agent, as well as in many traditional Asian medicines. [3]. Recognizing the hidden value within C. aromatica, more and more in-depth studies are being conducted in order to fully exploit the herb's hidden value. Several studies have found that C. aromatica has a very good response to many different diseases in vitro, including anti-inflammatory, anti-platelet aggregation, and cancer cell inhibition. These diseases are unintentionally linked to free radicals [4-8]. Curcuminoids and terpenoids are the two main compound groups of C. aromatica, according to the chemical composition. Curcuminoids include a variety of potential compounds such as curcuminoid, curcumin, and bis-demethoxycurcumin, whereas terpenoids include a variety of

monoterpenes and sesquiterpenes. Secondary compounds from both of these large groups have been shown to be excellent free radical scavengers [9-12].

Many studies have been conducted to test the combination of Sorafenib and cytostatic agents, which have proven effective in the treatment of liver tumors (bevacizumab, cisplatin, irinotecan, etc.). Plant-derived compounds have been validated as a source of anticancer agents13-19. The presence of natural compounds with high content, such as terpenoids and curcuminoid, aids in the formation of bioactive compounds in Curcuma aromatica. Numerous studies have shown that this plant has antioxidant and antitumor properties, including anti-liver cancer ability. Herbs containing curcumin have recently been targeted as sources of supportive factors in cancer treatment. As a result, this study was conducted to assess the combined effect of C. aromatica and Sorafenib in the treatment of liver cancer.

II. MATERIALS AND METHODS

Sample and extract preparation

Samples were collected from An Giang province, Vietnam and identified as describe in our previous paper [13]. The percolation method is used to extract *C. aromatica*. The dried powder was ground into a coarse powder, moistened with 96 % ethanol, and kept stable for 2 hours. Then, open the extraction vessel, place the moistened medicinal herbs in the extraction flask, slowly add a certain amount of 96 % ethanol until the ethanol comes out of the faucet, close the faucet, let the ethanol cover the medicinal herbs by about 5 cm, and soak overnight. Take the extract out at a rate of 2-3 ml/min. It was possible to obtain a 96 % ethanol extract. To get 50 and 30 percent ethanol extracts, repeat the process with 50 and 30 % ethanol.

DPPH radical scavenging activity

The radical scavenging activity was determined using a modified version of the method described by Vuong *et al.* [14]. A stock solution was made by dissolving 24mg DPPH in 100ml ethanol and storing it at -20°C. The working solution was then prepared fresh by combining 10ml stock solution with 45ml methanol to achieve an absorbance of 1.1 ± 0.02 at 515nm. 750µl of working solution was added to 250µl of sample at various concentrations and then left in the dark at 37°C for 30 minutes before measuring the absorbance at 517nm with a UV spectrophotometer. As positive and negative controls, 250µl of ascorbic acid (vitamin C) and ethanol were substituted for 250µl of sample, respectively.

The following equation was used to calculate the DPPH scavenging effect.:

$$\text{DPPH scavenging effect (\%)} = \left[1 - \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}}\right] \text{x} 100$$

Where $OD_{control}$ is the absorbance of negative control (0.004% DPPH solution) and OD_{sample} is the absorbance in presence of extract or ascorbic acid.

ABTS radical scavenging activity

The radical scavenging activity was measured based on the method described by Thaipong et al. (2006)), with some modifications [15]. The stock solutions included 7.4 mM $ABTS^+$ solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml $ABTS^+$ solution with methanol to obtain an absorbance of 1.0 ± 0.02 units at 734 nm using the spectrophotometer. Fresh $ABTS^+$ solution was prepared for each assay. 250 µl of ethanol extracts at different concentrations were allowed to react with 750 µL of the $ABTS^+$ solution at 37°C for 15 minutes in a dark condition. 250 µl of ascorbic acid (vitamin C) and ethanol was replaces for 250 µl sample as positive and negative controls, respectively. Then the absorbance was taken at 734 nm using the spectrophotometer.

ABTS scavenging effect was calculated using the following equation:

$$\text{ABTS scavenging effect (\%)} = \left[1 - \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}}\right] \text{x} 100$$

Where $OD_{control}$ is the absorbance of negative control and OD_{sample} is the absorbance in presence of extract or ascorbic acid.

Statistical Analysis

Statistical analysis was done using Graphpad prism. Every experiment was repeated at least 3 times. Results obtained were expressed as means + SE. Significant differences in treatments were accepted at P <0.05.

III. RESULTS

DPPH free radical scavenging capacity

The antioxidant capacity of various ethanol extracts was determined using the DPPH method and measured at 517 nm. Table 1 summarizes the results of the non-linear representation of the percentage of DPPH scavenged.

Table 1. DPPH free radical scavenging activities of vitamin C and different *C.aromatica* ethanol extracts

Concentration (*μ*g/mL)	**DPPH free radical scavenging**			
	Ethanol 96%	**Ethanol 50%**	**Ethanol 30%**	**Vitamin C**
1000	86.43 ± 1.74	75.19 ± 0.69	63.98 ± 0.77	93.97 ± 1.13
500	76.72 ± 0.31	70.08 ± 3.87	61.52 ± 0.85	90.11 ± 0.21
250	65.35 ± 1.55	64.00 ± 0.93	41.82 ± 1.05	89.83 ± 0.35
125	56.51 ± 2.71	42.02 ± 2.06	28.37 ± 2.88	89.50 ± 0.57
62.5	20.54 ± 2.47	23.15 ± 4.02	16.90 ± 6.90	89.48 ± 0.82
31.25	8.68 ± 4.32	14.68 ± 4.58	11.39 ± 0.90	87.33 ± 0.32
15.625	3.77 ± 3.92	5.14 ± 1.22	5.74 ± 2.48	61.59 ± 0.31
7.812	3.62 ± 3.91	2.59 ± 1.20	3.77 ± 2.18	28.59 ± 2.07
3.906	2.19 ± 2.36	1.92 ± 1.76	1.74 ± 2.29	13.40 ± 0.58

The results showed that the DPPH free radical scavenging efficiency of C. aromatica extract was directly proportional to the extract concentration; as the extraction concentration increased from 3.906 μg/ml to 1000 μg/ml, the free radical scavenging efficiency of ethanol extract increased gradually 2.19, 1.92, 1.74 % to 86.43 (ethanol 96%), 75.19 (ethanol 50%) and 63.98% (ethanol 30%) respectively.

For the vitamin C sample, the percentage value of DPPH scavenged increased from 13.4 % at a concentration of 3.9 μg/ml to 89.45% at a concentration of 62.5 μg/ml.

At the concentration of 1000 μg/ml, the free radical scavenging efficiency of 96% ethanol extract reached 86.43%, nearly equal to the free radical scavenging effect of vitamin C at the same concentration investigated (93.97 ± 1.13%).

3.1.1 ABTS free radical scavenging capacity

The antioxidant capacity of *C. aromatica* extract at various extraction concentrations was determined spectrophotometrically at 734 nm using the ABTS method. Table 2 shows the percentage of captured ABTS results.

Table 2. ABTS free radical scavenging ability of vitamin C and ethanol *C. aromatica* extract

Concentration (*μ*g/mL)	ABTS free radical scavenging			
	Ethanol 96%	Ethanol 50%	Ethanol 30%	Vitamin C
1000	76.58 ± 0.38	56.22 ± 0.80	30.83 ± 0.03	96.57 ± 0.03
500	44.02 ± 0. 74	42.93 ±1.06	29.41 ± 0.34	96.21 ± 0.14
250	27.74 ± 1.17	35.37 ± 1.02	18.83 ± 2.54	96.03 ± 0.18
125	16.54 ± 0.76	24.38 ± 5.34	10.83 ± 2.35	84.00 ± 0.28
62.5	9.66 ± 1.85	14.49 ± 1.17	6.73 ± 0.74	55.63 ± 0.28
31.25	7.89 ± 0.54	7.29 ± 0.64	5.58 ± 1.25	17.24 ± 0.66
15.625	7.36 ± 0.49	5.11 ± 0.52	4.21 ± 0.32	10.16 ± 1.38
7.8125	6.53 ± 0.98	4.17 ± 0.83	3.21 ± 0.58	7.39 ± 0.67
3.90625	5.79 ± 0.48	2.89 ± 0.90	1.28 ± 0.69	3.81 ± 0.66

Similar to the DPPH method, ABTS free radical scavenging capacity of *C. aromatica* extract increased gradually by concentration.

The free radical scavenging capacity of the extract increased on the order of 30% ethanol extraction to 96% ethanol extraction and lower than vitamin C.

The percentage of ABTS free radical scavenging capacity of 90% ethanol extract, 50% ethanol extract and 30% ethanol extract increased proportionally from 5.79, 2.89, 1.28 % to 76.58, 56.22 and 30.82%, respectively.

3.1.2 The DPPH and ABTS free radical scavenging efficiency of C. aromatica extract

The raw data of DPPH and ABTS methods will be statistically processed through Graphpad prism software, thereby deducing non-linear regression equations.

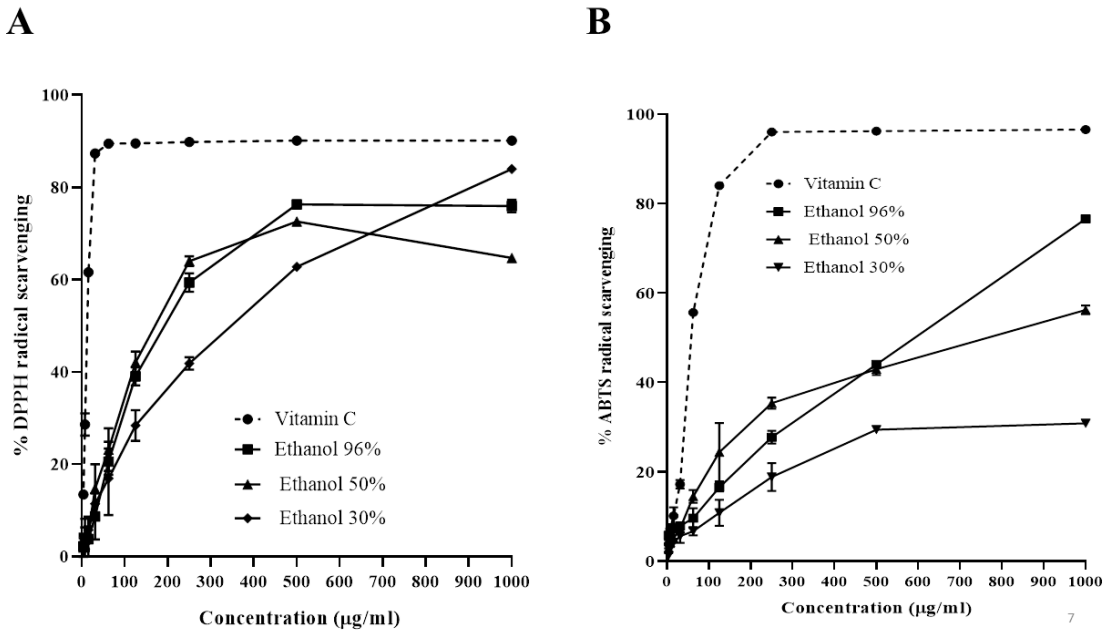

Fig 1. The chart shows the percentage of DPPH (A) and ABTS (B) free radicals captured

From the non-linear regression equation at Fig 1, the EC50 values of *C. aromatica* extract 96%, 50%, 30% and the EC50 values of vitamin C are illustrated in Table 3.

Table 3. EC50 values of DPPH and ABTS of *C. aromatica* Extract and Vitamin C

	(EC_{50}) µg/mL	
	DPPH	ABTS
96% ethanol extract	147.97 ± 8.421	501.35 ± 30.8045
50% ethanol extract	190.41 ± 10.161	682.08 ± 36.5610
30% ethanol extract	376.68 ± 19.729	>1000
Vitamin C	12.34 ± 0.637	57.71 ± 1.6895

Through the results in Table 3, it can be seen that *C. aromatica* extract has much better DPPH free radical scavenging ability than ABTS free radical scavenging ability. EC50 value for DPPH method is higher than EC50 value for ABTS method about 3 times.

IV. DISCUSSION

According to Yu-Ling Lee et al. (2006), the DPPH free radical scavenging activity of the ethanol extract from the roots of C. aromatica was 27.2 percent at a concentration of 0.1 mg/mL[16]. And according to findings of this study, all three ethanol extracts (30%, 50%, and 96%) provided an equivalent DPPH free radical scavenging percentage.

In another study conducted by Ammayappan Rajam Srividya, the 50% free radical capture DPPH results of *C. aromatica* extracted by Soxhlet method in petroleum ether, toluene, chloroform, ethyl acetate, acetone, ethanol and water were at 229.5 ± 1.12, 50.62 ± 0.998, 235.56 ± 0.634, 118.75 ± 0.667, 150.55 ± 1.345, 132.5 ± 1,876 µg/ml and 427.75 ± 1,436 µg/ml, respectively [17]. Thus, *C. aromatica* ethanol extract of the Indian research

group had stronger DPPH radical scavenging activity than the ethanol extract 96% in this study which gave the best EC50 index of 147,97 ± 8,421 µg/ml. In addition, the results of the Indian group also showed that the *C. aromatica* toluene extract gave optimum results with the IC50 value equal to that of vitamin C. Also in same study, the Indian group measured the ABTS free radical scavenging capacity of *C. aromatica* extracts in different solvents. However, the results of ABTS cannot be compared with this study because the two groups used different standards to construct the standard curve, the Indian group used Trolox while in this study, Vitamin C was used.

Also when comparing the EC50 value of vitamin C of ABTS assay with other previously published studies also showed similarity with EC50 value about 75 µg/ml [18]. The ABTS results were also similar to those of DPPH showing that the 96% extract had the best free radical scavenging effect among the three investigated extracts.

V. CONCLUSION

The use of curcumin as an adjuvant in cancer treatment to reduce drug side effects has been studied. The results of the cytotoxic, clonogenic, and migration assays revealed a synergistic effect of Sorafenib and C. aromatica extract. The synergism suggested that the C. aromatica extract could be used to help treat liver cancer.

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