

Native Medicinal Plants (*Moringa oleifera* Lam, *Brucea javanica* (L.) Merr., *Eclipta prostrata* (L.), *Callisia fragrans* (Lindl.) Woodson, and *Zingiber zerumbet* (L.) Smith) in An Giang, Vietnam: A Preliminary Investigation for Rhabdomyosarcoma Treatments using in-vitro RD cell cytotoxicity test

Duyen Thi My Huynh¹, Minh-Ngoc T. Le¹, Van De Tran², Viet-Hung Tran^{3*}, Duy Toan Pham^{4*}

¹Department of Pharmaceutical and Pharmaceutical Technology, Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam

²Department of Health Organization and Management, Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam

³Institute of Drug Quality Control-Ho Chi Minh City (IDQC HCMC), Ho Chi Minh City, Vietnam

⁴Department of Chemistry, College of Natural Sciences, Can Tho University, Can Tho, Vietnam

ABSTRACT

Cancer, one of the deadliest diseases worldwide, is projected to affect 30.2 million people by 2040. Among the various cancer types, rhabdomyosarcoma (RMS) is a unique tumor primarily impacting the muscular system of children. The current treatment for RMS has limited efficacy and numerous side effects, emphasizing the need for novel therapeutic approaches. This study investigates the potential treatment of the RMS cell line RD using extracts from five folklore-based medicinal plants in An Giang, Vietnam. The plants—*Moringa oleifera* Lam, *Brucea javanica* (L.) Merr., *Eclipta prostrata* (L.), *Callisia fragrans* (Lindl.) Woodson, and *Zingiber zerumbet* (L.) Smith—were extracted and fractionated using three solvents: ether, ethanol, and water. These fractions underwent phytochemical screening and cytotoxicity testing on the in-vitro RMS cell line RD. The results indicate that the ether fraction of *Eclipta prostrata* (L.) and the ether and ethanol fractions of *Zingiber zerumbet* (L.) Smith exhibit moderate cytotoxic effects on RD cell lines, with IC₅₀ values of $37.08 \pm 1.23 \mu\text{g/mL}$, $23.15 \pm 1.17 \mu\text{g/mL}$, and $45.63 \pm 2.39 \mu\text{g/mL}$, respectively. These findings provide preliminary data for further in-depth research into the anticancer properties of these plants, which are widely grown in the South of Vietnam.

Keywords: rhabdomyosarcoma; *Moringa oleifera* Lam; *Brucea javanica* (L.) Merr.; *Eclipta prostrata* (L.); *Callisia fragrans* (Lindl.) Woodson; *Zingiber zerumbet* (L.) Smith; cytotoxicity; fractionation.

1. INTRODUCTION

According to the 2020 statistics from the Global Cancer Observatory (GCO), more than 19 million people worldwide have cancer, with Asia accounting for 49.3%, or about 9.5 million people¹. Predictably, by 2040, the

number of people with cancer is expected to reach 30.2 million. Currently, cancer ranks as the second leading cause of death worldwide, significantly impacting both the mental and physical lives of patients^{2,3}. Among the more than 100 types of cancers, rhabdomyosarcoma (RMS) stands out as a special and rare tumor, primarily affecting the muscular system of children, especially the skeletal (voluntary) muscles⁴. Most cases of RMS are diagnosed in children aged ≤ 6 , with risk factors and etiology remaining unknown. RMS is often sporadic, associated with familial syndromes, and can be categorized into different types:

*Corresponding authors:

Duy Toan Pham, pdtoan@ctu.edu.vn

Viet-Hung Tran, tran.viethung168@gmail.com

Received: 2/7/2023 Accepted: 10/9/2023.

DOI: <https://doi.org/10.35516/jjps.v16i4.1365>

embryonal RMS (~60%), alveolar (~20%), pleomorphic (~10%), and spindle/sclerosing (~10%)⁴. This disease has been reported as the 3rd most common cancer and the most common soft tissue sarcoma in children⁵. Moreover, it can metastasize and develop into common cancers such as uterine cancer, stomach cancer, colon cancer, lymphoma, and limb cancer⁶. The current treatments for RMS, including surgery, radiation therapy, and chemotherapy (vincristine, actinomycin D, and cyclophosphamide/ifosfamide), yield poor and inadequate outcomes, especially in patients with metastatic and/or recurrent RMS^{7,8}. For instance, the long-term event-free survival in metastatic RMS patients is <20%^{9,10}. Last but not least, these treatments often result in numerous side effects such as fatigue, hair loss, nausea/vomiting, and diarrhea¹¹. Therefore, it is crucial and urgent to search for novel treatments that offer better oncological outcomes with long-term safety for RMS patients.

To this end, a potential source for finding novel RMS chemotherapeutic treatments is the medicinal/herbal plants that grow wildly or are cultivated across ASEAN countries. Specifically, in Vietnam, a country with a rich source of medicinal plants, with over 7,000 described species, of which 3,830 species possess therapeutic properties¹². In fact, in most Vietnamese hospitals, medicinal plants have been comprehensively utilized in complement with modern medicine, with over 700 official medical products containing herbal ingredients^{13,14}. In the Mekong Delta, a green area in the South of Vietnam, nearly 1,000 medicinal plant species have been exploited, with 500-700 species originating from the forests in provinces with mountainous terrain, such as An Giang. An Giang, a frontier province bordered with Cambodia, is famous for its extremely diverse and rich vegetation, harboring numerous precious medicinal herbs^{15,16}. Among them, five particular plants have gained much interest, namely moringa (*Moringa oleifera* Lam, Moringaceae, Chum Ngay [Vietnamese], MO), Macassar kernels (*Brucea javanica* (L.) Merr., Simaroubaceae, Xoan rung

[Vietnamese], BJ), ink plant (*Eclipta prostrata* (L.), Asteraceae, Co muc [Vietnamese], EP), basket plant (*Callisia fragrans* (Lindl.) Woodson, Commelinaceae, Luoc vang [Vietnamese], CF), and shampoo ginger (*Zingiber zerumbet* (L.) Smith, Zingiberaceae, Gung gio [Vietnamese], ZZ), due to their well-known ethnopharmacology (i.e., folk remedies) in supporting cancer treatments¹⁷⁻²¹. In the literature, these medicinal plants have been widely reported for their diverse pharmacological effects, including anti-inflammation, anti-malarial, antibacterial, anti-diabetic, and anti-oxidant^{18,21-24}. Nevertheless, limited information on the chemotherapeutic properties of these plants has been published, especially for RMS treatment.

Therefore, this study, for the first time, investigated the ability of these five plants to treat RMS in in-vitro cell culture settings. Prior to the cytotoxicity tests, the plants were extracted, and their phytochemical compositions were determined accordingly. We hypothesized that the plants would possess potential action on RMS, significantly contributing to the literature on novel ethnopharmacological medicinal plants in An Giang, Vietnam.

2. MATERIALS AND METHODS

2.1. Materials

The samples of MO leaves, BJ seeds, EP leaves, ZZ roots, and CF leaves were collected in Tinh Bien district, An Giang province, Vietnam, in February 2021. The plants were identified by a botanical specialist with specialized botanical documents provided by the An Giang Forest Protection Department. Voucher specimens (CTUMP-111, CTUMP-112, CTUMP-113, CTUMP-114, and CTUMP-115 for MO, BJ, EP, ZZ, and CF, respectively) were kept at the Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy. The collected plants were dried, ground, and sieved to appropriate sizes. The RD cell line (ATCC CCL-136TM) was imported from ATCC.

Chemicals for determining plant compositions (i.e.,

diethyl ether, ethanol, and acetic acid) were imported from Xilong, China; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), fetal calf serum (FCS), Eagle's minimum essential medium (EMEM), penicillin-streptomycin (Pen-Strep), trypan blue, trypsin-EDTA, L-glutamine, and dimethyl sulfoxide (DMSO) were bought from Sigma-Aldrich, Singapore. The positive control Anzatax® (paclitaxel 30 mg/5 mL) was purchased from Merck, Australia. All other chemicals were of reagent grade or higher.

2.2. Plant extraction

Fresh samples of MO leaves, BJ seeds, EP leaves, ZZ roots, and CF leaves, after being harvested, were washed, sliced, dried at ambient temperature, and finely ground to

appropriate sizes. The plant powders with a moisture content of <13% were then extracted and fractionated with three solvents of different polarity, including diethyl ether, water, and ethanol, following the process demonstrated in Figure 1. Briefly, 100 g of the plant powders were macerated with 1200 mL of ether (plant:solvent ratio of 1:12 w/v) for 24 h. The product was divided into two parts: the solution and the plant residues. The solution was condensed with a rotavapor until the moisture content reached <20%, and then used to determine phytochemical compositions and investigate cytotoxicity on cancer cells. The residues were further fractionated with ethanol and water and then divided into two fractions similar to the ether solvent.

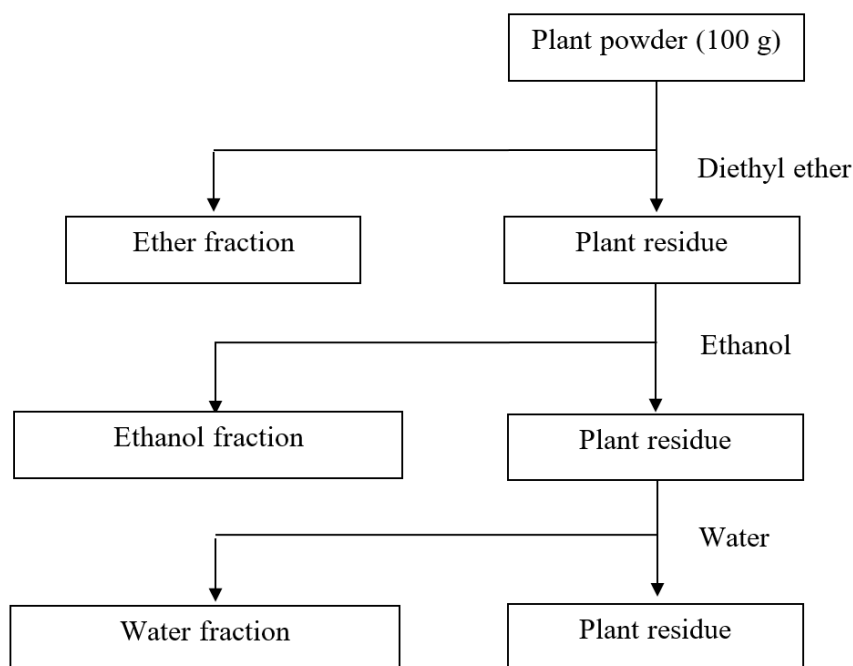


Figure 1. Plant fractionation procedure of 05 selected medicinal plants, including *Moringa oleifera* Lam (MO) leaves, *Brucea javanica* (L.) Merr. (BJ) seeds, *Eclipta prostrata* (L.) (EP) leaves, *Callisia fragrans* (Lindl.) Woodson (CF) leaves, and *Zingiber zerumbet* (L.) Smith (ZZ) roots

2.3. Phytochemical determination

The chemical constituents, in terms of the main compound groups, of the plant fractions (i.e., ether,

ethanol, and water) were determined following standard procedures described in Figure 2. Each test was repeated in triplicate to confirm the results.

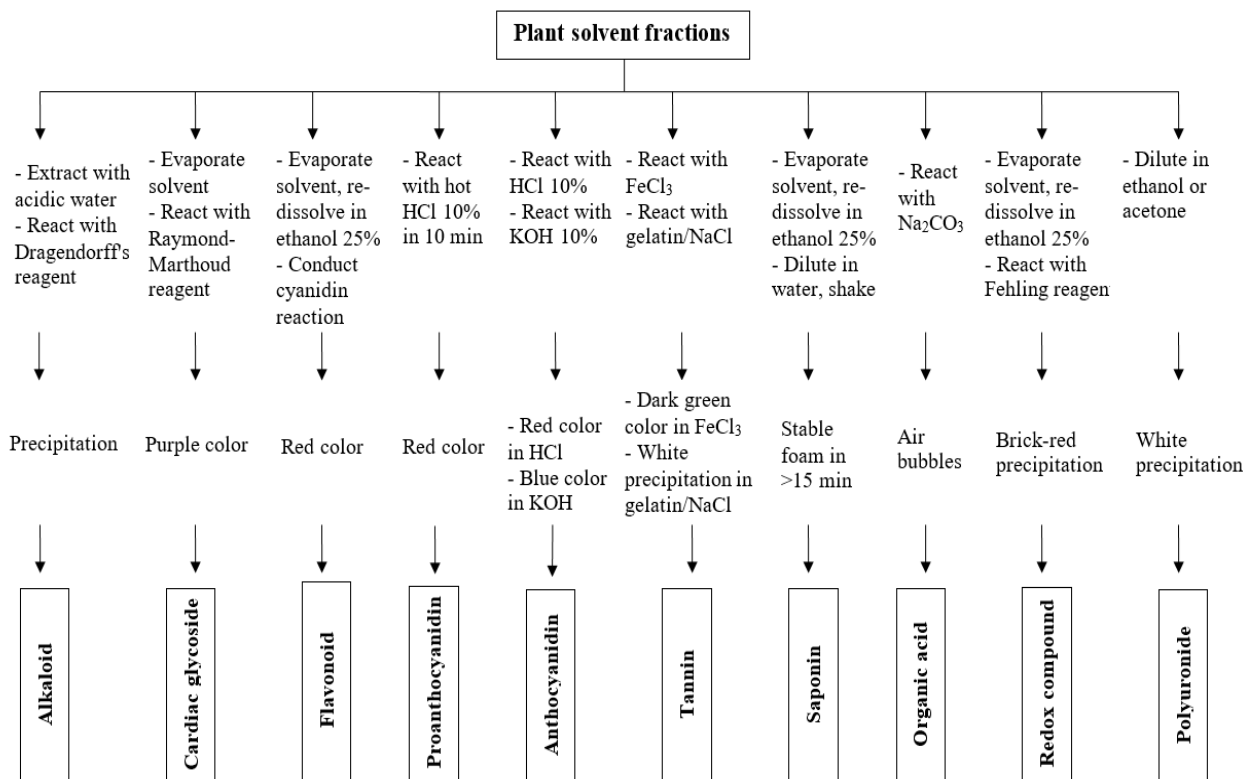


Figure 2. Phytochemical determinations procedures of the fractions (ether, ethanol, and water fractions) of 05 medicinal plants, *Moringa oleifera* Lam, *Brucea javanica* (L.) Merr., *Eclipta prostrata* (L.), *Callisia fragrans* (Lindl.) Woodson, and *Zingiber zerumbet* (L.) Smith.

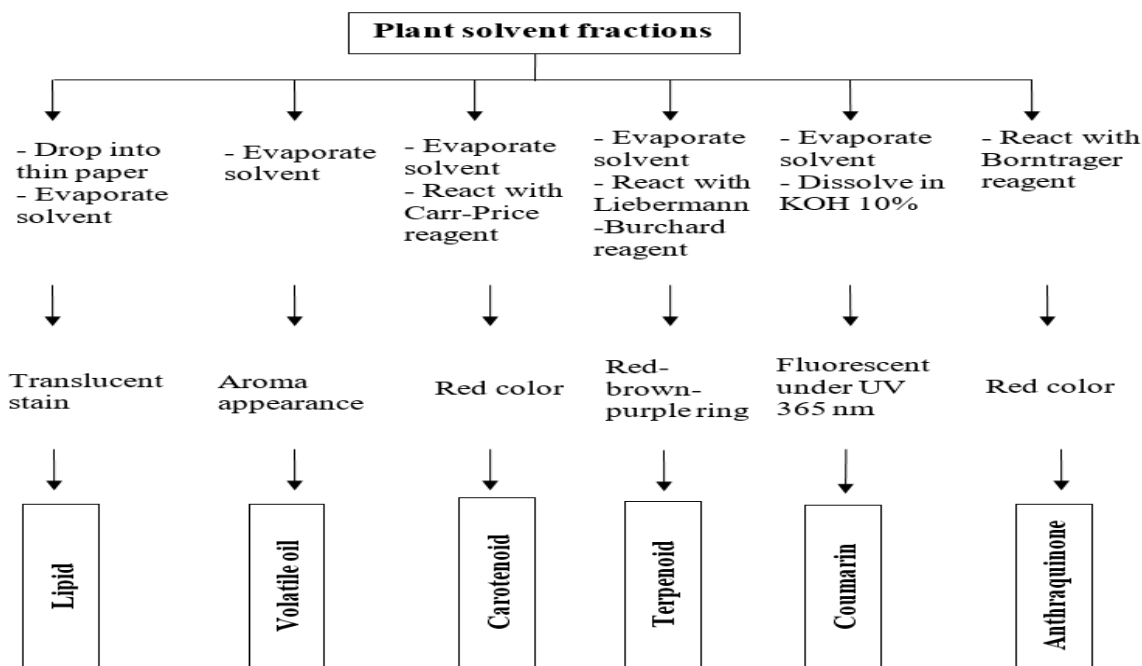


Figure 2 (Continued)

2.4. In-vitro cytotoxicity MTT assay

The cytotoxicity of the plant fractions on RMS was tested using RD cell line. The cells were grown in EMEM medium, supplemented with 10% FCS, 2 mM L-glutamine, and 100 IU/mL + 100 µg/mL PenStrep. Cells were cultured in a 75-cm² flask, incubated at 37 °C with 5% CO₂ in humidified atmosphere, and the medium was changed every even day. Confluent cells (70-80% flask coverage) were washed with PBS, trypsinized with trypsin-EDTA, counted with trypan blue 0.4%, and the cell suspension was transferred into 96-well plates with a density of 12.5 x 10⁴ cells/mL (100 µL/well) for the testing experiments.

The plant fraction test samples were prepared in a DMSO solution at an initial concentration of 10 mg/mL and diluted in medium to reach the investigated concentrations of 100, 50, and 10 µg/mL. The negative control was DMSO at the same concentrations in the test samples (1%, 0.5%, and 0.1%, respectively). The positive

control was the reference drug paclitaxel. All samples were filtered through a 0.22-µm membrane prior to cell treatments. The samples were subjected to the cells, incubated for 24 h, and the cytotoxic MTT assay was then conducted following the manufacturer's protocol^{25,26}. The formed formazan crystal was dissolved in isopropanol, and the solutions were UV-Vis spectroscopically measured at 570 nm with a microplate reader (Multiskan). All experiments were repeated four times. The %Cell inhibitory was calculated based on equation (1).

$$\% \text{Cell inhibitory} = 100\% - \frac{\text{OD sample} - \text{OD blank}}{\text{OD negative control} - \text{OD blank}} \times 100\% \quad (1)$$

2.5. Statistical analysis

The results were processed using Microsoft Excel software and presented as mean ± standard deviation (SD). For statistical significance, the Mann-Whitney test was utilized on SPSS 20.0 software, with p<0.05 for meaningful comparisons.

3. RESULTS

3.1. Phytochemical determination

The phytochemical constituents of the five investigated medicinal plants (MO, BJ, EP, CF, and ZZ) are presented in Table 1. Each fraction contained different chemical groups, dependent on their polarity. Overall, the MO leaves contain major components of lipids, carotenoids, volatile oils, flavonoids, tannins, and polyuronides. The BJ seeds mainly

possess lipids, alkaloids, coumarins, anthraquinones, and tannins. The EP leaves have volatile oils, terpenoids, alkaloids, flavonoids, saponins, and tannins as the main constituents. The ZZ roots mostly comprise volatile oils, alkaloids, anthraquinones, flavonoids, saponins, tannins, and polyuronides. The CF leaves contain terpenoids, alkaloids, coumarins, flavonoids, proanthocyanidins, saponins, tannins, and polyuronides.

Table 1. Phytochemical constituents of the ether fraction, ethanol (EtOH) fraction, and water fraction of the five investigated medicinal plants, *Moringa oleifera* Lam (MO) leaves, *Brucea javanica* (L.) Merr. (BJ) seeds, *Eclipta prostrata* (L.) (EP) leaves, *Callisia fragrans* (Lindl.) Woodson (CF) leaves, and *Zingiber zerumbet* (L.) Smith (ZZ) roots. (–): absent; (+): present with limited amount; (++): present with moderate amount; (+++): present with high amount

Phytochemical group	<i>Moringa oleifera</i>			<i>Brucea javanica</i>			<i>Eclipta prostrata</i>			<i>Callisia fragrans</i>			<i>Zingiber zerumbet</i>		
	Ether	EtOH	Water	Ether	EtOH	Water	Ether	EtOH	Water	Ether	EtOH	Water	Ether	EtOH	Water
Lipids	+++	–	–	+++	–	–	–	–	–	+	–	–	+	–	–
Carotenoids	++	–	–	–	–	–	–	–	–	+	–	–	–	–	–
Volatile oils	++	–	–	–	–	–	++	–	–	–	–	–	+++	++	–
Terpenoids	+	–	–	–	–	–	++	–	–	++	–	–	–	–	–
Alkaloids	+	+++	+++	++	+	+	+	+++	+++	++	++	++	++	++	++
Coumarins	–	++	–	–	++	–	–	–	–	++	++	–	–	–	–
Anthraquinones	–	–	–	++	–	–	–	–	–	–	–	–	++	–	–
Flavonoids	–	+++	+++	+	+	+	+	++	++	++	+++	++	++	++	++
Cardiac glycosides	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–
Anthocyanidins	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Proanthocyanidins	–	+	+	–	+	–	–	+	+	–	++	++	–	–	–
Tannins	–	+++	+++	–	+++	+++	–	+++	+++	–	++	++	–	+++	+++
Saponins	–	–	–	–	–	–	–	++	++	–	++	++	–	+++	+++
Organic acids	–	++	++	–	+	+	–	++	+	–	++	+	–	+++	++
Redox compounds	–	++	++	–	+	+	–	+	+	–	+	+	–	+++	+++
Polyuronides	–	–	+++	–	–	–	–	–	+	–	–	+++	–	–	+++

3.2. In-vitro cytotoxicity MTT assay

The in-vitro cytotoxicity MTT assay on the RMS cell line RD of the ether fraction, the ethanol fraction, and the water fraction, at concentrations of 100, 50, and 10 µg/mL, of the five medicinal plants (MO, BJ, EP, CF, and ZZ) are presented in Table 2, in comparison with the reference compound, paclitaxel. The results demonstrated that the MO leaves, BJ seeds, and CF leaves did not possess significant

cytotoxic effects on the RD cells (i.e., $IC_{50} > 100$ µg/mL). On the other hand, the EP and ZZ fractions showed high efficacy. Specifically, the EP ether fraction, the ZZ ether fraction, and the ZZ ethanol fraction, had an IC_{50} of 37.08 ± 1.23 µg/mL, 23.15 ± 1.17 µg/mL, and 45.63 ± 2.39 µg/mL, respectively. Compared to the well-known drug paclitaxel with an IC_{50} of 6.96 ± 0.72 µg/mL, these fractions show potential efficacy on the RD cell lines.

Table 2. In-vitro cytotoxicity on the rhabdomyosarcoma RD cell lines of the ether fraction, ethanol (EtOH) fraction, and water fraction, at concentrations of 10, 50, and 100 µg/mL, of the 05 investigated medicinal plants, *Moringa oleifera* Lam (MO) leaves, *Brucea javanica* (L.) Merr. (BJ) seeds, *Eclipta prostrata* (L.) (EP) leaves, *Callisia fragrans* (Lindl.) Woodson (CF) leaves, and *Zingiber zerumbet* (L.) Smith (ZZ) roots. The results are expressed in terms of %Cell inhibitory (mean ± SD) and IC₅₀ (mean ± SD) (n = 4). Note: the concentrations of the positive control (paclitaxel) were 10, 5, and 1 µg/mL, correspondingly.

Concentration (µg/mL)	%Cell inhibitory								
	<i>Moringa oleifera</i>			<i>Brucea javanica</i>			<i>Eclipta prostrata</i>		
	Ether	EtOH	Water	Ether	EtOH	Water	Ether	EtOH	Water
100	3.4 ± 0.5	10.8 ± 1.3	27.8 ± 2.4	14.2 ± 1.3	5.2 ± 0.8	10.5 ± 1.9	37.8 ± 4.5	13.6 ± 2.3	0.9 ± 0.2
50	7.3 ± 2.1	7.7 ± 1.8	29.7 ± 3.1	12.8 ± 1.7	13.0 ± 1.1	20.5 ± 2.1	62.7 ± 4.1	18.1 ± 3.0	0.2 ± 0.1
10	0.9 ± 0.1	6.3 ± 1.6	20.7 ± 3.4	14.2 ± 2.0	12.6 ± 1.5	12.6 ± 1.4	31.7 ± 2.8	18.1 ± 3.1	5.7 ± 1.2
IC ₅₀ (µg/mL)	> 100	> 100	> 100	> 100	> 100	> 100	37.08 ± 1.23	> 100	> 100
Concentration (µg/mL)	<i>Callisia fragrans</i>			<i>Zingiber zerumbet</i>			<i>Paclitaxel (concentration)</i>		
	Ether	EtOH	Water	Ether	EtOH	Water			
100	29.0 ± 2.5	22.2 ± 3.1	11.3 ± 1.7	84.2 ± 5.5	76.3 ± 7.9	25.0 ± 1.7	51.7 ± 5.8 (10 µg/mL)		
50	27.7 ± 3.4	21.3 ± 3.0	7.8 ± 1.5	71.9 ± 6.7	59.2 ± 4.3	29.2 ± 3.3	46.9 ± 4.1 (5 µg/mL)		
10	19.0 ± 2.8	18.0 ± 2.7	4.1 ± 0.8	38.7 ± 4.0	27.0 ± 3.2	18.1 ± 2.6	44.5 ± 4.6 (1 µg/mL)		
IC ₅₀ (µg/mL)	> 100	> 100	> 100	23.15 ± 1.17	45.63 ± 2.39	> 100	6.96 ± 0.72		

4. DISCUSSION

RMS, one of the most common cancers in children, has gained increasing attention due to its treatment difficulty¹¹. In fact, current RMS treatments involving surgery, radiation therapy, and chemotherapy possess poor outcomes with numerous side effects^{7,8}. Thus, novel chemotherapeutic agents are necessary. To this end, this study preliminarily investigated the efficacy of ether, ethanol, and water fractions of five potential medicinal plants, based on local folk remedies in An Giang, Vietnam, in the in-vitro cytotoxicity assay on the RD cell line. Ethanol and water were selected due to folklore wisdom (i.e., these plants are ethnopharmacologically used with ethanol and water as maceration solvents)^{19,21,22}, whereas ether was chosen because it is a non-polar solvent that could possibly extract the non-polar therapeutic compounds in these plants.

Firstly, the MO plant has been ethnopharmacologically utilized for a long time in An Giang ethnic groups as an antioxidative, anti-inflammatory, antihypertensive, and

immuno-regulatory agent. In addition, MO leaves are used in folk remedies for cancer treatments. In terms of phytochemical constituents, the main compounds in MO leaves are lipids, alkaloids, flavonoids, coumarins, and tannins, in agreement with a previous study²⁷. Interestingly, the phytochemical compounds in our research were different than the MO leaves in Nigeria²⁸, which could be due to geographical variations such as soil conditions, weather, and plant growth stages. Regarding its anticancer effect, previous studies have confirmed that the compound niaziminin (a water-soluble thiocarbamate glycoside) in MO leaves possesses high anticancer properties^{17,29,30}. Nevertheless, in all three fractions, with different polarities, the MO leaves did not show significant cytotoxicity on the RD cell line, indicating that this plant might not be potential for RMS treatment.

Secondly, the BJ plant is commonly used as food ("goi Sau dau" [Vietnamese] – a Vietnamese salad with a mixture of various vegetables and herbs).

Ethnopharmacologically, the BJ seeds could be ground, and its aqueous extract (i.e., tea) is used to treat diarrhea, appendicitis, and malaria. In BJ seeds, the main components are lipids, terpenoids, alkaloids, flavonoids, saponins, and tannins³¹. Its anticancer property is acknowledged to be based on the compound bruceantin (C₂₈H₃₆O₁₁), a quassinoid. Bruceantin has been proven to have high anticancer activity in various cancers such as lung cancer, myeloma, and gastric cancer^{32–35}. Furthermore, in clinical tests with 68 lung cancer patients, BJ seeds extract demonstrates a complementary effect, in conjunction with radiotherapy, in enhancing the patients' quality of life and prolonging their lifespan from 10 months to 15 months compared to radiotherapy alone³². However, in our study, the BJ seeds fractions did not show adequate action on the RD cell line.

Thirdly, the EP leaves are popularly utilized to make "tea" by the local people in An Giang. According to folklore, EP tea is believed to possess numerous effects, including antimicrobial, antiviral, pain relief, and anticancer properties. Phytochemically, the EP leaves in An Giang have similar constituents compared to those in other regions¹⁹. In terms of cytotoxicity activity, dasyscyphin C (C₂₈H₄₀O₈), a saponin in EP, has been confirmed to possess various anticancer effects³⁶ such as cervical carcinoma (IC₅₀ = 50 µg/mL on HeLa cells). Interestingly, in our study, the ethanol and water fractions, which contain lots of saponin, did not yield significant effects on RD cells (IC₅₀ > 100 µg/mL). On the other hand, the ether fraction, with no saponin, exhibited potential cytotoxicity on the RD cell line (IC₅₀ = 37.08 ± 1.23 µg/mL). Further study is necessary to investigate in-depth the biological activities of the components present in the EP leaves ether fraction. Moreover, it is worth noting that the high concentration of 100 µg/mL of the ether extract significantly reduced the EP leaves' cytotoxicity on the RD cells (Table 2). This could be attributed to the fraction's cell proliferation effect. Therefore, the optimal concentration, in this case, was 50 µg/mL. Conclusively, it

is necessary to investigate the suitable dose of herbal extracts to ensure their effectiveness in cancer treatment.

Fourthly, the CF leaves are commonly extracted with ethanol to produce a traditional pharmaceutical dosage form called herbal wine. According to the folklore, this wine is a good complementary medicine in treating liver cirrhosis, liver cancer, and other liver-related diseases, as well as acne, joint pain/inflammation, and gout²⁰. CF phytochemicals consist of alkaloids, flavonoids, glycosides, coumarins, and saponins, which are in well agreement with previous studies^{20,37,38}. In terms of the anticancer effects, to the best of our knowledge, no report (up to 2022) has been published on the CF extract action on the cancerous cells/tissues. Our results, for the first time, showed that CF leaves fractions might not be potent on RD cell line (IC₅₀ > 100 µg/mL). Nevertheless, its anticancer effects need further analysis and evaluation on other cell lines, to fill in the literature gap.

Finally, the ZZ roots, which is generally used as "ginger tea" for various therapeutic effects, contain mostly volatile oils, flavonoids, saponins, and alkaloids, which are in correlation with previous work³⁹. Among these chemicals, zerumbone (C₁₅H₂₂O), a sesquiterpene volatile oil with a humulan-based carbon framework, possesses outstanding anticancer activity⁴⁰. For example, the anticancer activity of zerumbone against the human HeLa cell line was confirmed with an IC₅₀ of 2.5 µg/mL⁴¹. In our work, the IC₅₀ values of the ether fraction and ethanol fraction of ZZ roots were 23.15 µg/mL and 45.63 µg/mL, respectively, indicating the potential effects of this plant on RMS cell line RD. The fact that the ether fraction was more potent than the ethanol fraction could be contributed to the higher amount of volatile oils (i.e., zerumbone) in the former (Table 1)^{42,43}.

5. CONCLUSION

This study investigated the potency of five common medicinal plants (MO, BJ, EP, CF, and ZZ) in An Giang, Vietnam, for the treatment of RMS using RD cell line. The EP ether fraction, the ZZ ether fraction, and the GIN

ethanol fraction, possess moderate cytotoxic effects on RD cell lines, with an IC₅₀ of 37.08 ± 1.23 µg/mL, 23.15 ± 1.17 µg/mL, and 45.63 ± 2.39 µg/mL, respectively. These results provide preliminary data for further in-depth research on the RMS anticancer properties of these plants, especially the EP and ZZ plants, which are widely grown in the South of Vietnam.

ACKNOWLEDGEMENTS

The authors would like to thank Can Tho University and Can Tho University of Medicine and Pharmacy for supporting this research.

REFERENCES

1. Global Cancer Observatory.
2. Pham, D. T., Saelim, N., and Tiyafoonchai, W., Alpha mangostin loaded crosslinked silk fibroin-based nanoparticles for cancer chemotherapy. *Colloids Surf. B. Biointerfaces*. 2019; 181, 705–713.
3. Pham, D. T., Saelim, N., and Tiyafoonchai, W., Paclitaxel loaded EDC-crosslinked fibroin nanoparticles: a potential approach for colon cancer treatment. *Drug Deliv. Transl. Res*. 2020; 10, 413–424.
4. Kaseb, H., Kuhn, J., and Babiker, H. M., Rhabdomyosarcoma. *StatPearls*. 2022.
5. Miwa, S., Yamamoto, N., Hayashi, K., Takeuchi, A., Igarashi, K., and Tsuchiya, H., Recent Advances and Challenges in the Treatment of Rhabdomyosarcoma. *Cancers (Basel)*. 2020; 12.
6. Skapek, S. X., Ferrari, A., Gupta, A. A., et al., Rhabdomyosarcoma. *Nat. Rev. Dis. Prim*. 2019; 5, 1.
7. Dantonello, T. M., Int-Veen, C., Schuck, A., et al., Survival following disease recurrence of primary localized alveolar rhabdomyosarcoma. *Pediatr. Blood Cancer*. 2013; 60, 1267–1273.
8. Malempati, S. and Hawkins, D. S., Rhabdomyosarcoma: review of the Children's Oncology Group (COG) Soft-Tissue Sarcoma Committee experience and rationale for current COG studies. *Pediatr. Blood Cancer*. 2012; 59, 5–10.
9. Oberlin, O., Rey, A., Lyden, E., et al., Prognostic factors in metastatic rhabdomyosarcomas: results of a pooled analysis from United States and European cooperative groups. *J. Clin. Oncol*. 2008; 26, 2384–2389.
10. Weigel, B. J., Lyden, E., Anderson, J. R., et al., Intensive Multiagent Therapy, Including Dose-Compressed Cycles of Ifosfamide/ Etoposide and Vincristine/Doxorubicin/ Cyclophosphamide, Irinotecan, and Radiation, in Patients With High-Risk Rhabdomyosarcoma: A Report From the Children's Oncology Group. *J. Clin. Oncol*. 2016; 34, 117–122.
11. Board, P. P. T. E., Childhood Rhabdomyosarcoma Treatment (PDQ®). *PDQ Cancer Inf. Summ*. 2022.
12. Do, T. L. and Nguyen, X. D., Native drugs of Vietnam: which traditional and scientific approaches? *J. Ethnopharmacol*. 1991; 32, 51–56.

Funding

None to declare.

Conflict of interest

None to declare.

Ethical Approval

Ethical Approval is not applicable for this article.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

13. Nguyen, P. H., Tran, V. D., Pham, D. T., Dao, T. N. P., and Dewey, R. S., Use of and attitudes towards herbal medicine during the COVID-19 pandemic: A cross-sectional study in Vietnam. *Eur. J. Integr. Med.* 2021; 44, 101328.
14. Tran, V. D., Pham, D. T., Cao, T. T. N., et al., Perspectives on COVID-19 prevention and treatment using herbal medicine in Vietnam: A cross-sectional study. *Ann. Ig.* 2021.
15. Pham, D. T., Thao, N. T. P., Thuy, B. T. P., Tran, V. De, Nguyen, T. Q. C., and Nguyen, N. N. T., Silk fibroin hydrogel containing *Sesbania sesban* L. extract for rheumatoid arthritis treatment. *Drug Deliv.* 2022; 29, 882–888.
16. Huynh, D. T. M., Le, M. T., Tran, V. D., and Pham, D. T., Antibacterial hydrogel containing Piper Betel L. extract for acne treatment, an ex vivo investigation. *Pharm. Sci. Asia.* 2022; 49, 372–380.
17. Krishnamurthy, P. T., Vardarajalu, A., Wadhvani, A., and Patel, V., Identification and characterization of a potent anticancer fraction from the leaf extracts of *Moringa oleifera* L. *Indian J. Exp. Biol.* 2015; 53, 98–103.
18. Chen, M., Chen, R., Wang, S., et al., Chemical components, pharmacological properties, and nanoparticulate delivery systems of *Brucea javanica*. *Int. J. Nanomedicine.* 2013; 8, 85.
19. Timalisina, D. and Devkota, H. P., *Eclipta prostrata* (L.) L. (Asteraceae): Ethnomedicinal Uses, Chemical Constituents, and Biological Activities. *Biomolecules.* 2021; 11.
20. El Sohafy, S. M., Nassra, R. A., D'Urso, G., Piacente, S., and Sallam, S. M., Chemical profiling and biological screening with potential anti-inflammatory activity of *Callisia fragrans* grown in Egypt. *Nat. Prod. Res.* 2021; 35, 5521–5524.
21. Zakaria, Z. A., Yob, N. J., Jofrry, S. M., Affandi, M. M. R. M. M., Teh, L. K., and Salleh, M. Z., *Zingiber zerumbet* (L.) Smith: A Review of Its Ethnomedicinal, Chemical, and Pharmacological Uses. *Evid. Based. Complement. Alternat. Med.* 2011; 2011.
22. Paikra, B. K., Dhongade, H. K. J., and Gidwani, B., Phytochemistry and Pharmacology of *Moringa oleifera* Lam. *J. Pharmacopuncture.* 2017; 20, 194.
23. Yarmolinsky, L., Zaccari, M., Ben-Shabat, S., and Huleihel, M., Anti-Herpetic Activity of *Callisia fragrans* and *Simmondsia chinensis* Leaf Extracts In Vitro. *Open Virol. J.* 2010; 4, 57.
24. Feng, L., Zhai, Y. Y., Xu, J., et al., A review on traditional uses, phytochemistry and pharmacology of *Eclipta prostrata* (L.) L. *J. Ethnopharmacol.* 2019; 245, 112109.
25. Freshney, R. I., Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications: Sixth Edition. *Cult. Anim. Cells A Man. Basic Tech. Spec. Appl. Sixth Ed.* 2011.
26. Gerlier, D. and Thomasset, N., Use of MTT colorimetric assay to measure cell activation. *J. Immunol. Methods.* 1986; 94, 57–63.
27. Rani, N. Z. A., Husain, K., and Kumolosasi, E., *Moringa* genus: A review of phytochemistry and pharmacology. *Front. Pharmacol.* 2018; 9, 108.
28. Aja, P. M., Aja, P. M., Nwachukwu, N., et al., Chemical Constituents of *Moringa oleifera* Leaves and Seeds from Abakaliki, Nigeria. *Am. J. Phytomedicine Clin. Ther.* 2014; 2, 310–321.
29. Tiloke, C., Phulukdaree, A., and Chuturgoon, A. A., The antiproliferative effect of *Moringa oleifera* crude aqueous leaf extract on cancerous human alveolar epithelial cells. *BMC Complement. Altern. Med.* 2013; 13.
30. Khor, K. Z., Lim, V., Moses, E. J., and Abdul Samad, N., The In Vitro and In Vivo Anticancer Properties of *Moringa oleifera*. *Evid. Based. Complement. Alternat. Med.* 2018; 2018.

31. Helmi, H. and Susanti, I., Phytochemical tested and in vitro screening antimalaria activity of Belilik Brucea javanica L Merr against Plasmodium falcifarum. *J. Biol. Res.* 2015; 19, 1–4.
32. Su, S. Y., [Treatment of lung cancer with brain metastasis using an intravenous drip of a 10% emulsion of Brucea javanica seminal oil]. *Zhong xi yi jie he za zhi = Chinese J. Mod. Dev. Tradit. Med.* 1985; 5, 66-67,86-88.
33. Cuendet, M. and Pezzuto, J. M., Antitumor activity of bruceantin: an old drug with new promise. *J. Nat. Prod.* 2004; 67, 269–272.
34. Wang, X., Wang, H., Cao, L., et al., Efficacy and Safety of Brucea javanica Oil Emulsion Injection in the Treatment of Gastric Cancer: A Systematic Review and Meta-Analysis. *Front. Nutr.* 2021; 8.
35. Issa, M. E., Berndt, S., Carpentier, G., Pezzuto, J. M., and Cuendet, M., Bruceantin inhibits multiple myeloma cancer stem cell proliferation. *Cancer Biol. Ther.* 2016; 17, 966–975.
36. Khanna, G. and Krishnan, K., Anticancer-cytotoxic activity of saponins isolated from the leaves of Gymnema sylvestre and Eclipta prostrata on HeLa cells. *Int. J. Green Pharm.* 2009; 3.
37. Olennikov, D. N., Ibragimov, T. A., Zilfikarov, I. N., and Chelombit'ko, V. A., Chemical composition of Callisia fragrans juice 1. Phenolic compounds. *Chem. Nat. Compd.* 2008; 44, 776–777.
38. Olennikov, D., Stolbikova, A., Rokhin, A., Ibragimov, T., and Zilfikarov, I., Chemical composition of Callisia fragrans juice. II. Carbohydrates. *Chem. Nat. Compd. - CHEM NAT COMPD.* 2010; 46, 273–275.
39. Tian, M., Wu, X., Hong, Y., Wang, H., Deng, G., and Zhou, Y., Comparison of Chemical Composition and Bioactivities of Essential Oils from Fresh and Dry Rhizomes of *Zingiber zerumbet* (L.) Smith. *Biomed Res. Int.*, (ed. Rinaldo, S.). 2020; 2020, 9641284.
40. Dũng, N. X., Chĩnh, T. D., and Leclercq, P. A., Chemical Investigation of the Aerial Parts of *Zingiber zerumbet* (L.) Sm. from Vietnam. *J. Essent. Oil Res.* 1995; 7, 153–157.
41. A.B.H, A., Al-Zubairi, A., Nirmala Devi, T., et al., Anticancer Activity of Natural Compound (Zerumbone) Extracted from *Zingiber zerumbet* in Human HeLa Cervical Cancer Cells. *Int. J. Pharmacol.* 2008; 4.
42. Sowndhariya, S. S., Ravi, S., Dharani, J. D., and Sripathi, R. S., Chemical Constitution, In-silico Molecular Docking Studies and Antibacterial Activity of Flower Essential Oil of *Artabotrys hexapetalus*. *Jordan J. Pharm. Sci.* 2022; 15, 341–354.
43. Osanloo, M., Yousefpoor, Y., Alipanah, H., Ghanbariasad, A., Jalilvand, M., and Amani, A., In-vitro Assessment of Essential Oils as Anticancer Therapeutic Agents: A Systematic Literature Review. *Jordan J. Pharm. Sci.* 2022; 15, 173–203.

النباتات الطبية المحلية (Moringa oleifera Lam)، Eclipta، Brucea javanica (L.) Merr.، Callisia fragrans (Lindl.) Woodson، prostrata (L.) و Zingiber zerumbet (L.) في آن جيانج، فيتنام: تحقيق أولي لعلاج الساركوما العضلية المخططة باستخدام اختبار السمية الخلوية لخلايا RD في المختبر

دوين ثي ماي هوينه¹، مينه نجوك تي لي¹، فان دي تران²، فيت هونج تران³، دوي تون فام⁴*

¹قسم الصيدلة والتكنولوجيا الصيدلانية، كلية الصيدلة، جامعة كان ثو للطب والصيدلة، كان ثو، فيتنام.

²قسم تنظيم وإدارة الصحة، جامعة كان ثو للطب والصيدلة، كان ثو، فيتنام.

³معهد مراقبة جودة الدواء، مدينة هوشي منه (IDQC HCMC)، مدينة هوشي منه، فيتنام.

⁴قسم الكيمياء، كلية العلوم الطبيعية، جامعة كان ثو، كان ثو، فيتنام.

ملخص

يُقدر أن السرطان، وهو أحد أكثر الأمراض فتكًا في جميع أنحاء العالم، سيؤثر على 30.2 مليون شخص بحلول عام 2040. ومن بين أكثر من 100 نوع من السرطان، يعد الساركوما العضلية المخططة (RMS) نوعًا خاصًا من الأورام التي تؤثر في الغالب على الجهاز العضلي للأطفال. يمتلك علاج RMS الحالي فعالية محدودة والعديد من الآثار الجانبية. وبالتالي، فإن العلاجات الجديدة ضرورية. هنا، بحثت هذه الدراسة في إمكانات علاج خط خلايا RMS لخمس نباتات طبية قائمة على الفولكلور في آن جيانج، فيتنام. تم استخلاص النباتات (Moringa oleifera Lam)، Brucea javanica (L.)، Eclipta prostrata (L.)، Callisia fragrans (Lindl.) Woodson، و Zingiber zerumbet (L.) في المختبر. أظهرت النتائج أن جزء إيثر Eclipta prostrata (L.) و Zingiber zerumbet (L. Smith ether) وجزء الإيثانول، لهما تأثيرات سامة للخلايا معتدلة على خطوط خلايا RD، مع IC50 يبلغ 1.23 ± 37.08 ميكروغرام / مل، و 1.17 ± 23.15 ميكروغرام/مل، و 2.39 ± 45.63 ميكروغرام/مل، على التوالي. توفر هذه النتائج بيانات أولية لإجراء مزيد من الأبحاث المتعمقة حول خصائص RMS المضادة للسرطان لهذه النباتات، والتي تنمو بشكل كبير في جنوب فيتنام.

الكلمات الدالة: الساركوما العضلية المخططة. المورينغا أوليفيرا لام؛ بروسيا جافانিকা (L.) مير؛ إكليبتا بروستراتا (L.)؛ عطر كاليسيا (ليندل) وودسون؛ زنجبير زيرومبيت (L.) سميث؛ السمية الخلوية. تجزئة.

* المؤلف المراسل:

فيت هونج تران، pdtoan@ctu.edu.vn

دوي تون فام، tran.viethung168@gmail.com

تاريخ استلام البحث: 2023/7/2 وتاريخ قبوله للنشر 2023/9/10.