http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2023.203.212

Research Article
Identification, Phytochemistry and Biological Activities of *Paris polyphylla* on Hepatocellular Carcinoma

[1]Ton Son Bach, [2]Viet Hong La, [1]Tran Xuan Khoi, [3]Duong Hoang Nguyen, [2]Cao Ba Cuong and [1,4]Tru Van Nguyen

[1]Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do, Cau Giay, Hanoi, Vietnam
[2]Institute for Scientific Research and Application, Hanoi Pedagogical University 2, Phuc Yen, Vinh Phuc 280000, Vietnam
[3]Center for High Technology Development, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do, Cau Giay, Hanoi, Vietnam
[4]Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do, Cau Giay, Hanoi, Vietnam

Abstract

Background and Objective: Liver cancer is the common cause of cancer death. *Paris polyphylla* is used as a traditional folk medicine in Vietnam to treat pneumonia, mastitis, bruises and fractures but no study was available regarding its ability to treat liver cancer or slow its growth. In this study, *Paris polyphylla* samples were identified and evaluated cytotoxic activity against the liver cancer cells. **Materials and Methods:** *Paris polyphylla* species were collected from various areas in Yen Bai, Vietnam, which were identified by comparative morphological method and DNA barcoding for the *18S, matK* genes and *ITS* region. *Paris polyphylla* samples were dried until constant weight, ground into a fine powder and extracted in various solvents. The bioactivity of these extracts were done by the MTT assay. **Results:** The sequences of *18S, matK* genes and *ITS* region were high similarity to sequences of *P. polyphylla* in the National Center for Biotechnology Information. The N-hexane and ethyl acetate fractions were produced from the methanol extract of *P. polyphylla*. The TLC results showed that there was a significant difference in the component of n-hexane and ethyl acetate fraction. The N-hexane fraction contains mainly low-polarity and non-polarity substances. While ethyl acetate fraction consists mainly of polar substances. In addition, ethyl acetate fraction was shown the strongest cytotoxic activity on the cancer cell lines HepG2 and Huh7 with the evaluation of IC_{50} = 115.11±2.77 μg mL^{-1} and IC_{50} = 148.11±1.78 μg mL^{-1}. **Conclusion:** The extract of *Paris polyphylla* demonstrated strong potential to inhibit the growth of the liver cancer cell line. The ethyl acetate fraction has the highest ability for cytotoxicity on the liver and cell line at a concentration of 200 μg mL^{-1} through MTT.

Key words: DNA barcoding, anticancer activities, liver cancer, *Paris polyphylla*, phytochemistry

Citation: Bach, T.S., V.H. La, T.X. Khoi, D.H. Nguyen, C.B. Cuong and T.V. Nguyen, 2023. Identification, phytochemistry and biological activities of *Paris polyphylla* on hepatocellular carcinoma. Pak. J. Biol. Sci., 26: 203-212.

Corresponding Author: Tru Van Nguyen, Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do, Cau Giay, Hanoi, Vietnam
Cao Ba Cuong Institute for Scientific Research and Application, Hanoi Pedagogical University 2, Phuc Yen, Vinh Phuc 280000, Vietnam

Copyright: © 2023 Ton Son Bach *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Liver cancer is considered an important global health problem because of the third major cause of cancer death worldwide and it is one of the top five causes of cancer death in 90 countries[1]. Cancer arises when normal cells undergo a transformation into tumor cells in multi-stage process that frequently progresses from a pre-cancerous lesion to a malignant tumor. These changes are the result of interactions between a person's hereditary factors and three types of outside forces, such as physical carcinogens (ionizing radiation and ultraviolet), biological carcinogens (infections from parasites certain, bacteria or viruses), chemical carcinogens (arsenic, tobacco smoke, asbestos, aflatoxin and alcohol)[2]. According to the World Health Organization (WHO), nearly 1 million people were diagnosed with liver cancer globally and more than 0.83 million individuals died from this disease. Egypt and Mongolia were the two countries with the highest overall rates of liver cancer in 2020, whereas, Vietnam ranks 5th with more than 26.000 cases and men accounting for about 80% (20.256 cases)[1]. This may be due to the unhealthy living habits of men when Vietnam is one of the countries that consume the most alcohol[3,4]. Significant efforts have been undertaken in recent years to enhance liver cancer treatment[5]. Treatment options for cancer include surgery, immunotherapy, radiation therapy and chemotherapy[6]. The mainstay of treatment continues to be the use of chemical medicines to slow tumor growth[7]. However, as chemotherapy treatments are used longer, tumors appear to get more resistant, which causes the killing impact of chemotherapy drugs to deteriorate progressively. Moreover, chemotherapy drugs for treating cancer have unpleasant side effects such as diarrhea, nausea, hair loss, fatigue, vomiting, fever and pain[8]. With the rising incidence of cancer-related fatalities, effective therapeutic strategies with fewer cytotoxic side effects and resistance must be developed. Natural substances have a number of advantageous properties for cancer treatment and improve health[9]. They possess selective effects against cancer cells without cytotoxic on normal cells, in addition, their chemical structures can also be used as models to create new medications. These models create medicines with benefits comparable to or superior to those currently available, with fewer side effects and resistance[10].

Paris polyphylla belongs to the family Melanthiaceae and is primarily found in Southern China and other regions of Asia, particularly in Vietnam. In Vietnam, *P. polyphylla* is used as a traditional folk medicine for treatment such as bronchitis, mastitis, bruises and fractures. The *P. polyphylla* extract has been demonstrated to have antioxidant, immunoregulatory, anti-inflammatory and anti-cancer characteristics and was also determined as having potent cytotoxicity efficiency on various cancer cell lines such as ovarian cancer, colorectal cancer, breast cancer, esophageal cancer[11-14]. The extract of *P. polyphylla* has main secondary metabolites, including flavonoids, phytosterols, polysaccharides and steroidal saponins (makeup about 80% of all active compounds) such as polyphyllin VII, II and I. Polyphyllin VII was determined to have cytotoxic activity against human lung cancer cells A549 with the value of $IC_{50} = 0.41 \pm 0.10$ μM at 24 hrs by activating the apoptosis through inhibition of the PI3K/Akt and NF-κB pathways and increased caspase-3 activity (An important enzyme of programmed cell death)[15]. Polyphyllin I was demonstrated to have the ability to prevent the development of non-small-cell lung cancer through the autophagy process by activating AMPK and inhibiting the mTOR signal[16]. The aim of the present research was to: (a) Identify *Paris polyphylla* samples from Vietnam, (b) Determination of components in the *Paris polyphylla* extract and (c) Determination of cytotoxic activity against the liver cancer cells.

MATERIALS AND METHODS

Study area: The study was performed in January, 2021 to December, 2021 at the Institute of Biotechnology.

Identification of *Paris* YB: The sample of *Paris* YB in this study was collected from Yen Bai, Vietnam in 2021. After conducting the comparative morphological method regarding several physical parameters, including plant height, leaf morphology. The plant samples were recognized using biological identification made possible by DNA barcodes for the *ITS* region[17]. Total DNA was isolated and purified from the leaves of *Paris* YB with the modified CTAB method and stored at -20°C. The PCR reactions were performed with a specific primer for *ITS* gene (*ITS*-F: 5'-ACGAATTCAT GGTCCGGTGAAGTG TTCG-3', *ITS*-R: 5'-TAGAATTCCCCGGTT CGCTCGCCGTTAC-3') in a 20 μL total containing 10 μL of Master mix 2x (Phusa, Vietnam), 1 μL of each primer (10 pmol), 5 μL of total DNA and 4 μL of dH_2O. The PCR product was electrophoresed in a 1% agarose gel and then soaked in 1% Ethidium bromide (EtBr) for 30 min. The nucleotide sequences of the *ITS* segment were sequenced using the Sanger method[18]. The *ITS* of some *Paris* species were retrieved from NCBI and aligned by Bioedit version 7.0 and constructed phylogenetic tree by MEGA 11.0 software.

Preparation of the extract of *Paris* YB: *Paris* YB fresh samples were dried at 60°C until constant weight. These dried samples were ground into a fine powder. The dried sample powder was dissolved with methanol and carried out the ultrasound-assisted extraction in an ultrasonic bath heat Elmasonic S120H (Elma Schmidbauer GmbH and Co. KG, Singen, Germany power 1000 W, frequency 50/60 Hz) at room temperature[19]. The solvent was then evaporated in the rotary evaporator RV 10 Digital V (IKA, Staufen, Germany) to create the extract. The n-hexane and ethyl acetate fractions of *Paris* YB were created based on the liquid-liquid extraction method by the modified Kwon[20]. The process of extraction was performed under low-pressure at 50°C.

Thin layer chromatography (TLC): The components of the extract were separated by the modified TLC method[21]. The TLC plates used in this study included DC-Alufolien 60 F254 (Merck 1.05715) and RP-18 F254s (Merck). Compounds or structures were detected by UV light of wavelength 254 nm or spraying with visualization reagent Vanillin to TLC plates and then dried at 100°C for some minutes.

Cell cultural: The HepG2 (liver cancer of humans), Huh7 (liver cancer of humans) and HEK 293 (Human embryonic kidney) cell lines were cultured in DMEM (Sigma-Aldrich) and supplemented with 10% FBS, penicillin/streptomycin solution (1% of 100x) and 37°C with 5% CO_2 in humid atmosphere.

MTT assay: In the MTT colorimetric experiment, the HepG2, Huh7 and HEK 293 cell line were seeded in 96-well plates containing 1×10^4 cells per well and incubated for 12 hrs. Then the cells were treated with various concentrations of *Paris* YB extracts for 24 hrs. Next, each well received 15 µL of MTT solution (5 mg mL^{-1}) and kept at 37°C for 4 hrs. The medium was carefully discarded. The formazan crystal was dissolved in 150 µL DMSO and then measured at an absorbance of 562 nm. The experiment was performed three times. The DMSO was used as the negative control. The following formula will be used to calculate cell viability and death[22]:

$$\text{Cell death (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Statistical analysis: Data were evaluated statistically using SPSS 20.0 software and Microsoft Excel version 2016. The results were presented as the Mean±Standard deviation of three repeats. A p-value of less than 0.05 was considered to be significant based on t-test.

RESULTS

Identification of *Paris* YB species through morphological comparison and DNA barcoding: Using the comparative morphological approach, the samples taken from Yen Bai, Vietnam were identified by using DNA barcode markers. The morphological comparison has been one of the most reliable ways to determine plant species through physical taxonomic criteria, such as stem, roof and leaf (Fig. 1a-c). However, when knowledge is inadequate and external features were harmed as a result of inappropriate specimen management, morphological identification might be difficult.

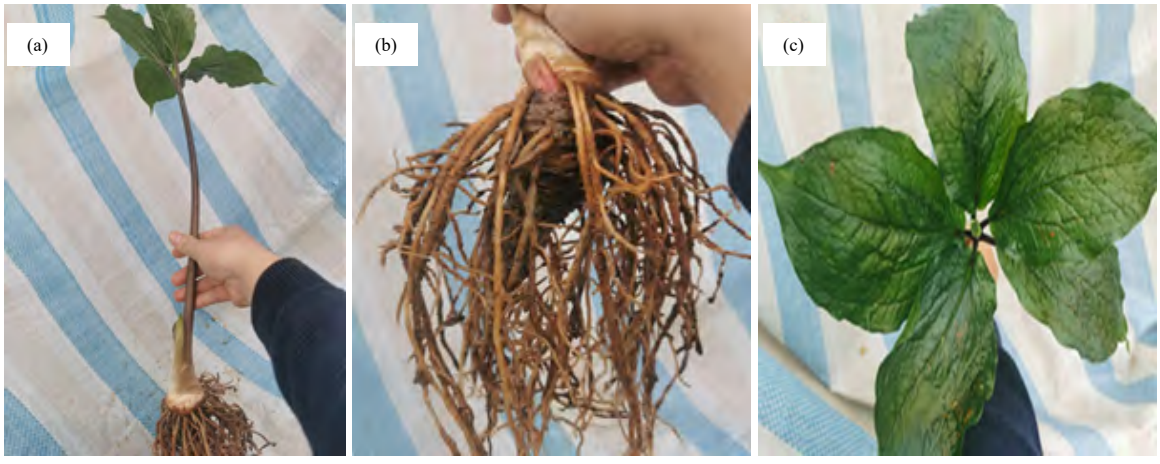

Fig. 1(a-c): Morphological characterisation of *Paris* YB, (a) Stem, (b) Root and (c) Leaf

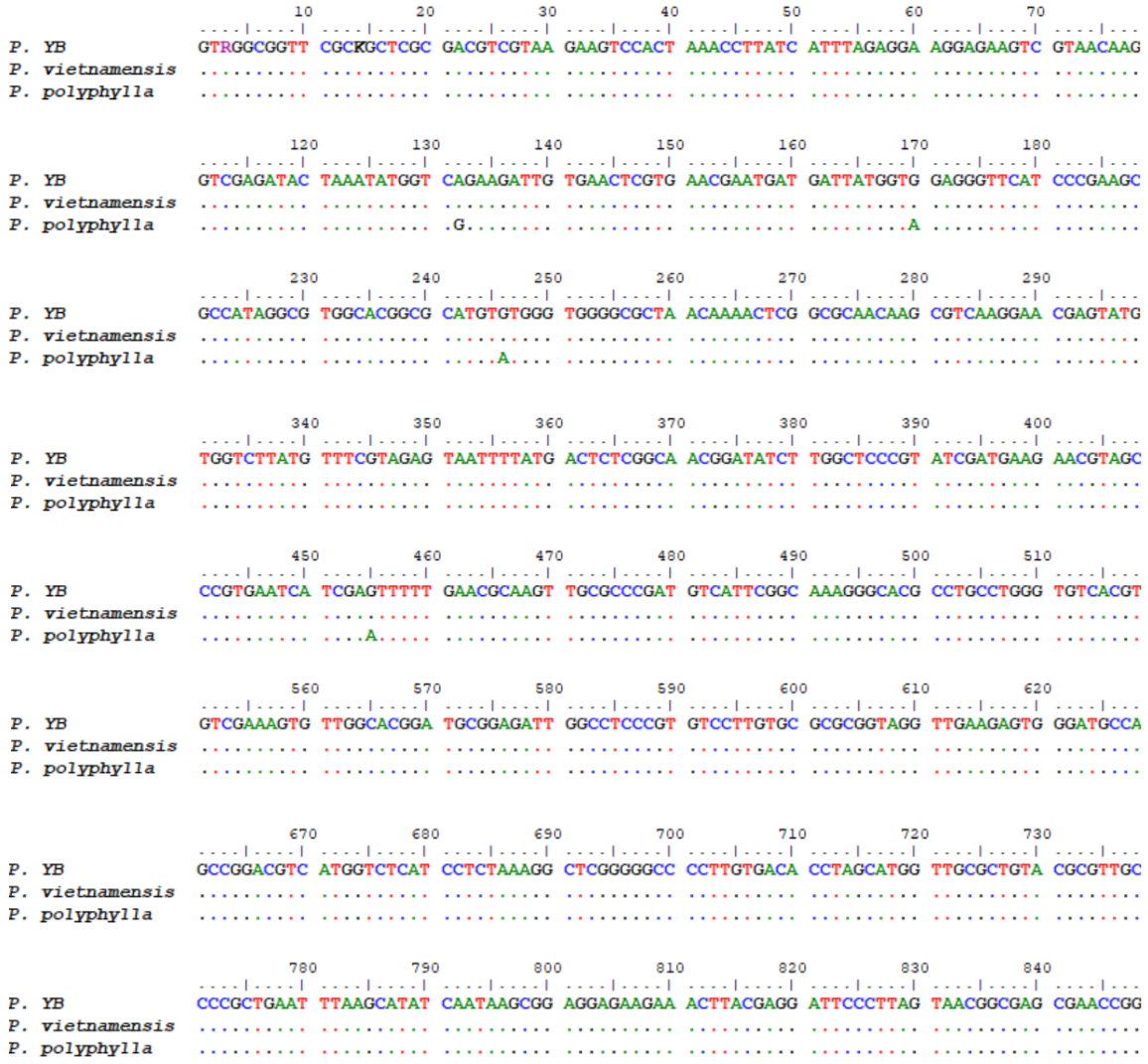

Fig. 2: Alignment of *ITS* from *Paris* YB, *P. vietnamensis* (OP021455) and *P. polyphylla* (OK103639)

Table 1: Yield of *P. vietnamensis* methanol extract and fractions were obtained in this research

Solvent	Dried mass (g)	Concentration (g)	
Methanol	30	1.52±0.04[a]	5.07±0.13%[a]
N-hexane	30	0.31±0.02[b]	1.03±0.07%[b]
Ethyl acetate	30	0.32±0.03[b]	1.07±0.10%[b]

[a,b]Substantial difference between fractions at p<0.05

Another method was also performed to identify *Paris* YB species using DNA barcoding based on the *ITS* genes. The length of *ITS* from *Paris* YB was 849 bp and was equal to the length of *ITS* genes from *P. vietnamensis* and *P. polyphylla*. The result was then analyzed by Bioedit and BLAST showed that the sequence of *ITS* region collected in Yen Bai shared 100% similar to the *ITS* sequence of *P. vietnamensis* in NCBI (Accession number: OP021455) (Fig. 2). Phylogenetic tree analysis has also shown that the example from Yen Bai belonged to the same group with *P. vietnamensis* (Fig. 3a-b). Therefore, we have concluded that samples collected in Yen Bai are steadily *P. vietnamensis* species.

Components in the *P. vietnamensis* extracts: The dried *P. vietnamensis* power (30 grams) was extracted with methanol. The extract on the *P. vietnamensis's* n-hexane, ethyl acetate fractions was produced using liquid-liquid extraction. The results of the extraction process were shown in Table 1. From 30 g dried samples, 1.52 g of pure methanol extracts were obtained, yielding a 5.07% rate. By using the liquid-liquid method, n-hexane and ethyl acetate fractions were recovered at similar concentrations 0.31 g and 0.32 g. The components of each fraction were then separated by the TLC method.

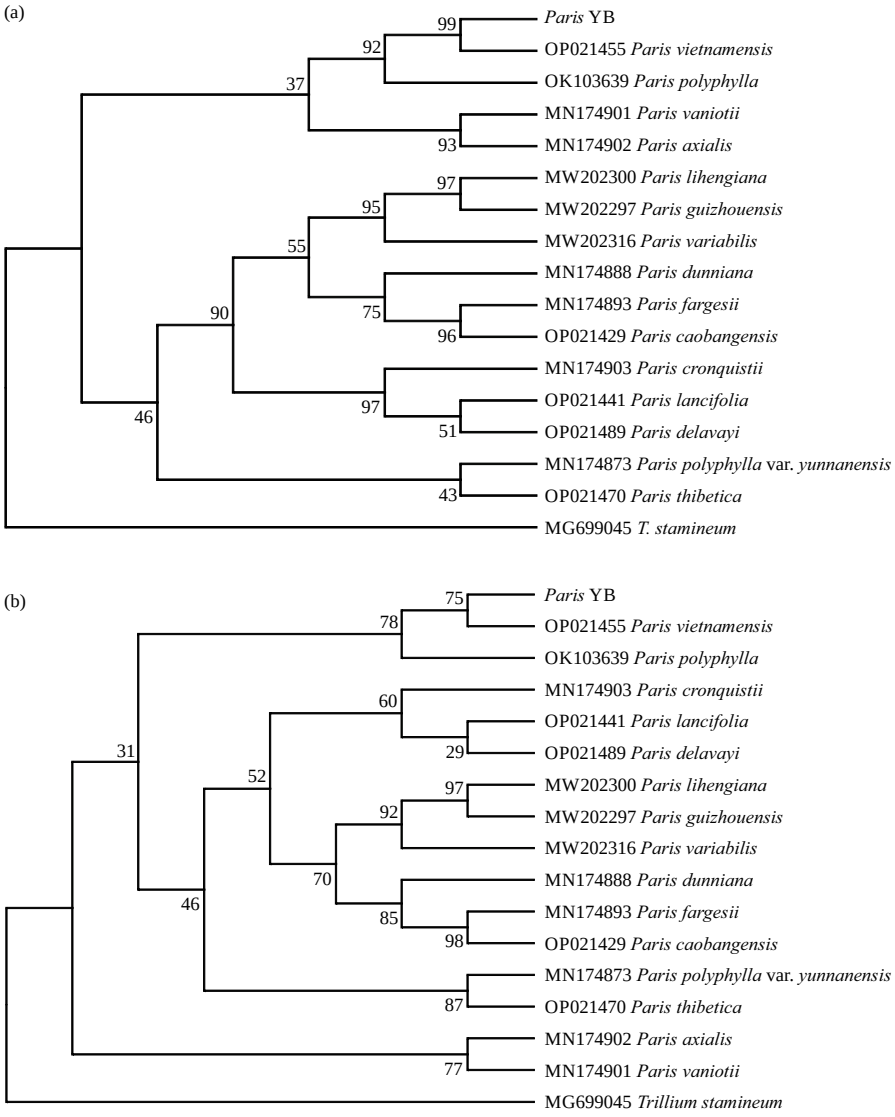

Fig. 3(a-b): Phylogenetic tree among the *Paris* genus samples based on *ITS* sequence by (a) Maximum-likelihood and (b) Neighbor-Joining

Branches value of the phylogenetic tree was a bootstrap of 1000 replications and *T. stamineum* was used as outgroup

TLC analysis: The TLC is a kind of chromatography method that separates substances according to their polarity by using a thin glass plate that has either silica gel or aluminum oxide as the solid phase. A solvent is selected for the mobile phase based on the polarity of the components in the mixture. The distribution of compounds between a liquid mobile phase that is moving over a solid fixed phase that has been placed on a glass or plastic plate creates separate layers of compounds. The composition of the extracts was quantified by thin-layer chromatography and the results were shown in Fig. 4a-b.

The analysis of the *P. vietnamensis* sample's whole extract revealed the presence and high separation of numerous groups of secondary metabolites with different polarities. The solvents dissolved groups of substances based on polarity and the difference was visible when comparing n-hexane and ethyl acetate extracts. The bands of the n-hexane fraction tended to move far up the plate, whereas the bands of the ethyl acetate fractions were still concentrated below the plate. Due to the process performed fractionation by liquid-liquid method, substances with low polarity and non-polarity were dissolved in n-hexane solvent and ethyl acetate dissolved substances with higher polarity. Next that, the total and fraction extracts were evaluated for their cytotoxic activity and damage recovery ability on liver cancer cell lines.

Cytotoxic activity of *P. vietnamensis* extracts: The extract of *P. vietnamensis* at all concentrations was evaluated for cytotoxic activity on HepG2, Huh7(cancer cells) and Hek293 (normal cell line). The results were shown in Fig. 5a-d.

The cytotoxicity demonstrated the correlation between sample concentration and cell viability, indicating that the samples are biological activity. The results showed that the cell density of liver cancer lines decreased significantly

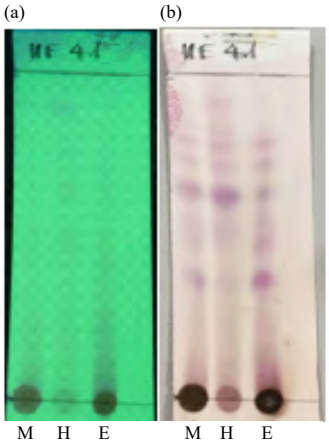

Fig. 4(a-b): TLC analysis of *P. vietnamensis* extract using solvent chloroform-methanol (8:2), (a) Under UV light and (b) With Vanillin oil
M: Methanol extract, H: n-hexane extract and E: Ethyl acetate extract

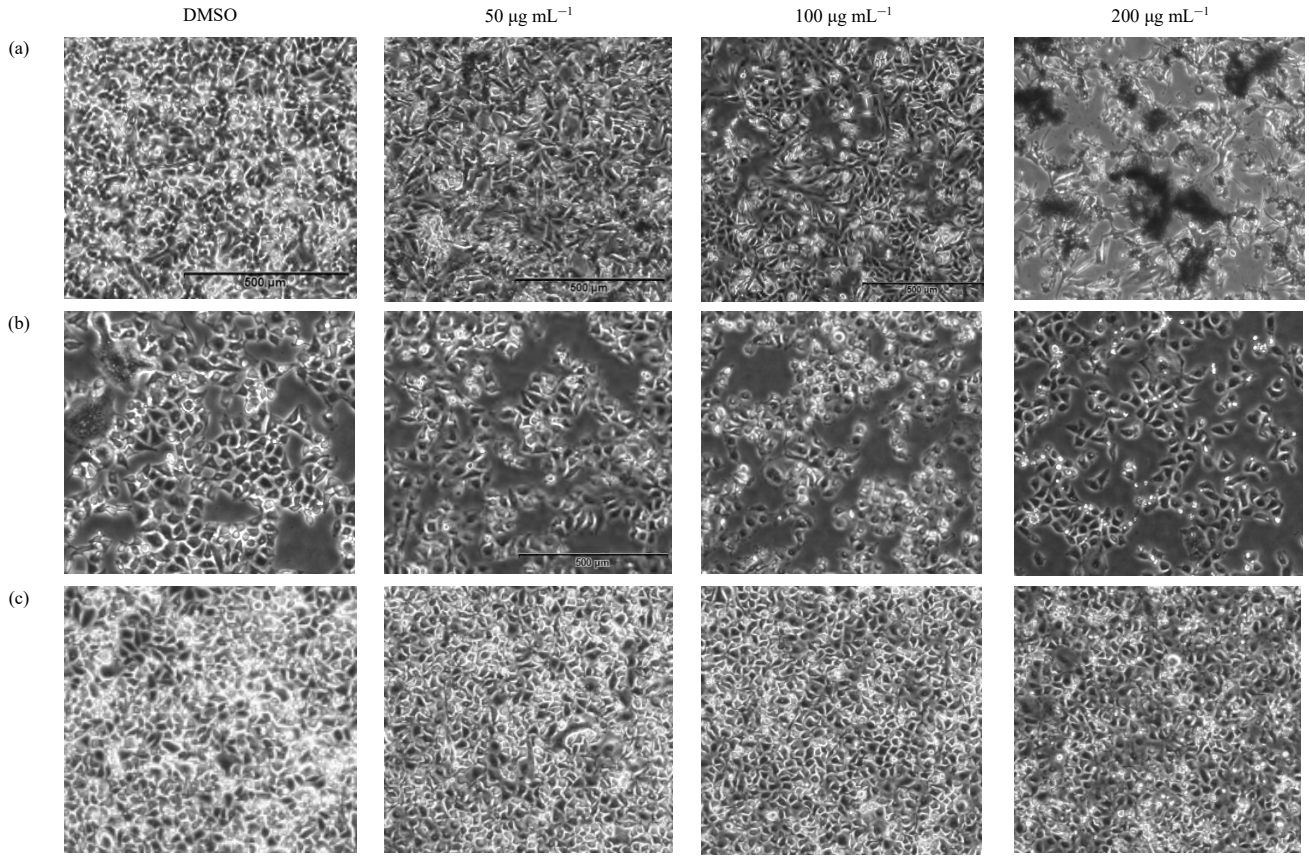

Fig. 5(a-d): Continue

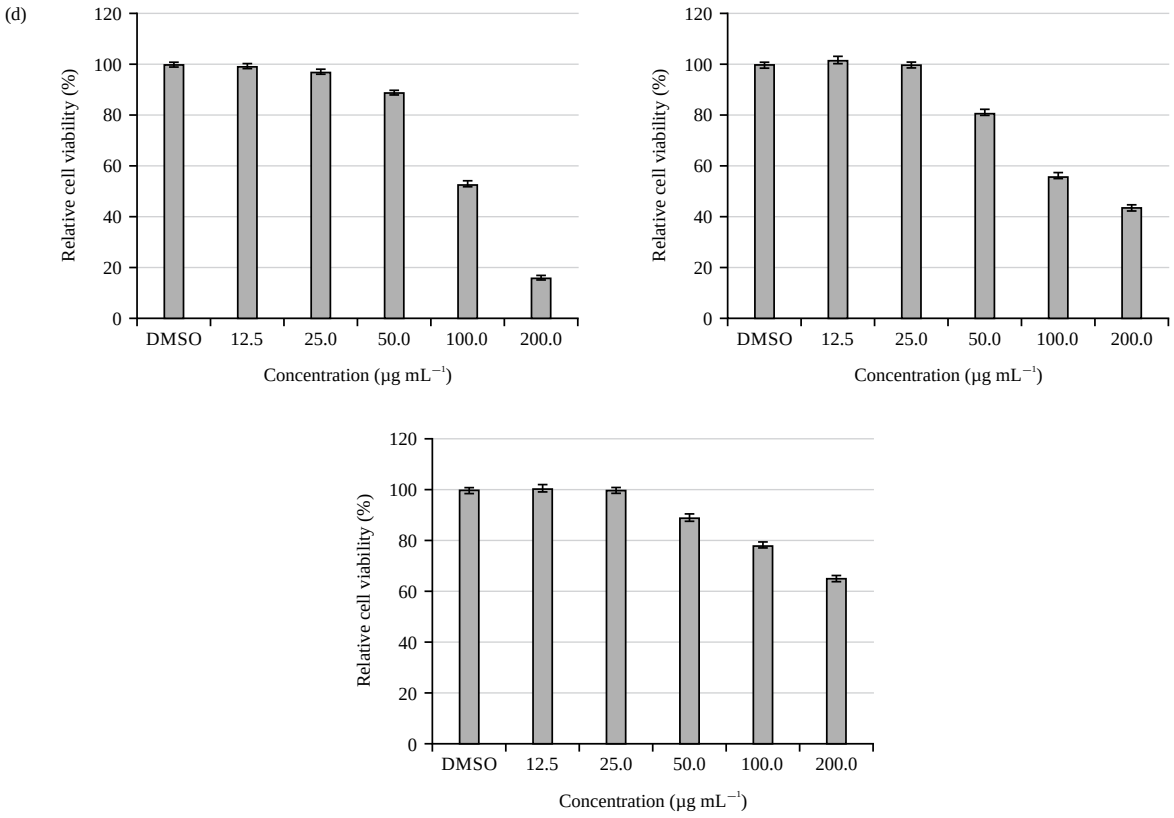

Fig. 5(a-d): Density of (a) HepG2, (b) Huh7, (c) Hek293 cells when treated with the ethyl acetate extract at experiment concentrations and (d) Percentage of cell viability when treated with the ethyl acetate extract at experiment concentrations

when treated with the ethyl acetate extract and was in a dose-dependent manner. The fraction of the n-hexane extract did not exhibit cytotoxic activity against the cancer cell line HepG2 and Huh7 at experiment concentrations. The ability of growth inhibition with HepG2 cells when treated with the ethyl acetate extract was measured with value IC_{50} = 115.11±2.77 μg mL^{-1} at 24 hrs. The half -inhibition concentration value of the Huh7 cell line with ethyl acetate fraction of *P. vietnamensis* was measured with value IC_{50} = 148.11±1.78 μg mL^{-1} at 24 hrs. A lower IC_{50} value suggested that the essence has higher cytotoxic activity so the cytotoxicity of the *P. vietnamensis* ethyl acetate fraction was stronger in the HepG2 cell line than in the Huh7 cell line. There was a substantial difference in cell proliferation inhibition in the range of concentrations from 50 to 200 μg mL^{-1} of *P. vietnamensis* ethyl acetate fractions. The Huh7 cell lines decreased in their ability to grow by 43.96 and 56.13% respectively when exposed to ethyl acetate extracts at concentrations of 100 and 200 g mL^{-1}. In addition, the extract of *P. vietnamensis* was also determined for slight cytotoxic activity against the normal cell line Hek293 even at the highest concentration (200 μg mL^{-1}), the inhibition of Hek293 cell growth was 36.17%, significantly lower than the cancer cell lines HepG2 and Huh7.

DISCUSSION

In this study, the *Paris polyphylla* species (origin in Yen Bai, Vietnam) were identified through the comparative morphological method and biological identification method. The *ITS* region was gained from *Paris polyphylla* in Yen Bai, Vietnam, which shared 100% similar to the *ITS* region in NCBI (ID: OK103639). This result demonstrated that the *ITS* region has been highly conserved among individuals of the same species. In addition, bioactive of *P. polyphylla* extracts were also examined in a dose-dependent manner. The bioactivity test was performed to determine the extract's short-term negative effects when treated with cancer cells for 24 hrs. The Half-maximal Inhibitory Concentration (IC_{50}) value was defined as the compound concentration at which the percent inhibition equaled 50[23]. In treating cancer, administering certain medications at the IC_{50} concentration means that the

tumor's development is reduced by half. Suppose the IC_{50} was discovered at a lower concentration while testing for IC_{50} curves. In that case, it expressed that the drug will be highly effective at lower concentrations and cause less systemic toxicity when given to patients for therapy[24]. The IC_{50} concentration can express the destruction of cancer cells and preventing cancer cell proliferation while having a lower harmful effect on the healthy cells[25]. The current MTT assay result has shown effective of *P. polyphylla* extract in preventing the growth of liver cancer cell lines at concentration between 50 to 200 μg mL^{-1}, the fraction of ethyl acetate extract was highest bioactivity.

Several *Paris* species have been reported to have existed in Vietnam, including *P. polyphylla* var. *Yunnanensis*, *P. fargesii,* Franch, *P. caobangensis*, *P. delavayi*, *P. cronquistii*, *P. xichuensis*, *P. polyphylla* Sm and *P. polyphylla* var. *chinensis*[26], *Paris vietnamensis* (Takht) H. Li[27]. However, the *Paris* species share morphological similarities when employing the laborious and inaccurate comparative morphological approach. Hence, identifying the sample before extracting it is critical so biological identification based on DNA barcodes is considered the solution. Lan and colleagues identified several species of *Paris* such as *Paris polyphylla* var *chinensis*, *Paris vietnamensis* and *Paris fargesii* through specific DNA barcodes for the *ITS* region and *matK* gene[28]. The *ITS* (internal transcribed spacer) region and *matK* gene have been commonly used in identification species research due to the highly conserved and availability of its structural information, examination at a higher taxonomic level are possible. Additionally, the standard DNA barcodes from the *ITS* region may be beneficial for identifying medicinal plants because of their advantageous traits, such as the simplicity of its amplification, the availability of conserved areas for developing universal primers and adequate diversity to distinguish even closely related species[29]. Therefore, the *ITS* region and *matK* gene are now thought to be the most valuable markers for identifying and analyzing plant phylogeny[30].

The methanol solvent is commonly used in the extraction of polar compounds. In the previous study, it was discovered that aqueous methanol recovered the highest concentrations of phenolic compounds, polyphenols and antioxidants more efficiently[31]. Therefore, methanol has been used in this study of *P. vietnamensis* and has exhibited its effectiveness in the extraction. The concentration of *P. vietnamensis* methanol extract and each fraction in our research were significantly different from the samples were collected in Sapa, Vietnam[32]. Although the yield of methanol extract obtained in the current study is slightly lower than the yield of ethanol extract in Duyen's research, the concentration of ethyl acetate and n-hexane fractions is considerably higher than the correlative solvents. A previous pharmacological research indicated that the overall phenolic content of *Plectranthus rotundifolius* tuber methanol extracts were substantially lower than the *P. polyphylla*[33]. In addition, there have been several pieces of literature investigating the impact of environmental factors on the content of compounds present in *P. vietnamensis*. For example, the total phenolic contents of *P. polyphylla* were about more than 3 times that of lower-altitude samples[33] and the growing areas were also a factor affecting the total saponin content in *P. polyphylla*[34].

The cytotoxicity of methanol extracts and solvent fractions were investigated through the MTT assay on liver cancer cell lines and human embryonic kidney cell lines, which exhibited that the ethyl acetate fraction of *P. vietnamensis* exhibited the strongest cytotoxicity on the cancer cell line HepG2 in the concentration of 200 μg mL^{-1}. In contrast, the n-hexane fraction did not exhibit cytotoxic activity on the cancer cells in a dose-dependent manner. The N-hexane is a non-polar solvent and has been frequently utilized in the extraction of oil, but the substances in *P. polyphylla* are mostly polar compounds (steroid accounting for about 80%)[15,35]. Some previous research regarding the extract of *P. polyphylla* has shown the ability to inhibit the development of various cancer cell lines. Kshetrimayum *et al.*[36] demonstrated that the extract of *P. polyphylla* contains high saponin, flavonoid, phenol and steroidal saponin content and selective cytotoxic activity against the HCT-116 colon cancer cell line with value $IC_{50} = 8.72 \pm 0.71$ μg mL^{-1} while having negligible effects in the CCD-841 cell line. In a pharmacological report, authors determined the ability in turn of the *Lantana camara* and *Eucomis autumnalis* ethyl acetate extract to cytotoxicity cancer cell lines Huh7 with value $IC_{50} = 44.1$ μg mL^{-1} and value $IC_{50} = 24.8$ μg mL^{-1} at 72 hrs[37]. Hence, species could have different cytotoxic potentials for the cancer cell lines and cell viability also depends on the time incubation[38]. The current study results have several similarities with previous report, the cytotoxicity of the *P. polyphylla* ethanol extract to the cancer cell line HepG2 was still more effective than Huh7 and lightly damaged to the control cell line[39]. Thus, current study findings regarding *P. polyphylla*'s cytotoxic properties were highly expected and *P. polyphylla* extract has great potential in drug preparation, which can be cytotoxic to cancer cells without affecting normal cells. The results of this research could use to develop the medicine to support cancer treatment in more effective, cheaper and prevent side effects of chemical medicines.

CONCLUSION

Based on the bioactivity test, the extract of *Paris polyphylla* was demonstrated the effective for inhibiting liver cancer cell lines without damage to normal cells. The ethyl acetate fraction has the highest ability for cytotoxicity on the liver and cell line at a concentration of 200 μg mL^{-1} through MTT assay. The *P. polyphylla* plant has the potential to be utilized medicinally. However, more research is still required to confirm the activity and provide more comprehensive evidence of this activity.

SIGNIFICANCE STATEMENT

Liver cancer is the common cause of cancer death. However, chemotherapy drugs for treating cancer have unpleasant side effects such as diarrhea, nausea, hair loss, fatigue, vomiting, fever and pain. Natural substances have a number of advantageous properties for cancer treatment and improve health. *Paris polyphylla* is used as a traditional folk medicine in Vietnam to treat pneumonia, mastitis, bruises and fractures but no study was available regarding its ability to treat liver cancer or slow its growth. This study has demonstrated that the *Paris polyphylla* has effective in inhibiting liver cancer cell lines without damage to normal cells. The *P. polyphylla* plant has the potential to be utilized medicinally. The results of our research could use to develop the medicine to support cancer treatment in more effective, cheaper and prevent side effects of chemical medicines.

ACKNOWLEDGMENT

This research is funded by the Institute of Biotechnology, Vietnam Academy of Science and Technology for Tru Van Nguyen's young researchers project. (No: DTCS-CNSH 01/2021).

REFERENCES

1. Rumgay, H., M. Arnold, J. Ferlay, O. Lesi and C.J. Cabasag *et al*., 2022. Global burden of primary liver cancer in 2020 and predictions to 2040. J. Hepatol., 77: 1598-1606.
2. Torre, L.A., F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent and A. Jemal, 2015. Global cancer statistics, 2012. CA: Cancer J. Clinicians, 65: 87-108.
3. Thi, H.V., M.L. Nguyen, L.T. Tran, A.D. Ngo, K.H. Nguyen, T.M.N. Thi and D.T. Chu, 2023. Food poisoning: A case study in Vietnam. Case Stud. Chem. Environ. Eng., Vol. 7. 10.1016/j.cscee.2022.100295.
4. Giang, K.B., H. van Minh and P. Allebeck, 2013. Alcohol consumption and household expenditure on alcohol in a rural district in Vietnam. Global Health Action, Vol. 6. 10.3402/gha.v6i0.18937.
5. Wu, J., Y. Li, Q. He and X. Yang, 2023. Exploration of the use of natural compounds in combination with chemotherapy drugs for tumor treatment. Molecules, Vol. 28. 10.3390/molecules28031022.
6. Abbas, Z. and S. Rehman, 2018. An Overview of Cancer Treatment Modalities. In: Neoplasm, Shahzad, H. (Ed.), IntechOpen, London, ISBN: 978-1-78923-778-8.
7. Ndagi, U., N. Mhlongo and M.E. Soliman, 2017. Metal complexes in cancer therapy-an update from drug design perspective. Drug Des. Dev. Ther., 11: 599-616.
8. Anand, U., A. Dey, A.K.S. Chandel, R. Sanyal and A. Mishra *et al*., 2023. Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. Genes Dis., 10: 1367-1401.
9. Ji, H.F., X.J. Li and H.Y. Zhang, 2009. Natural products and drug discovery: Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? EMBO Rep., 10: 194-200.
10. Darnell Jr., J.E., 1997. STATs and gene regulation. Science, 332: 1630-1633.
11. Chen, M., K. Ye, B. Zhang, Q. Xin and P. Li *et al*., 2019. Paris Saponin II inhibits colorectal carcinogenesis by regulating mitochondrial fission and NF-κB pathway. Pharmacol. Res., 139: 273-285.
12. Li, Y., X. Wang, S. Cheng, J. Du and Z. Deng *et al*., 2014. Diosgenin induces G2/M cell cycle arrest and apoptosis in human hepatocellular carcinoma cells. Oncol. Rep., 33: 693-698.
13. Sun, J., B.R. Liu, J. Wei, X.P. Qian and L.X. Yu *et al*., 2011. The extract of *Paris polyphylla* exerts apoptotic induction and synergic antiproliferative effect with anticancer drugs in SMMC-7721 human liver cancer cells. Biomed. Preventive Nutr., 1: 186-194.
14. Wang, C.W., C.J. Tai, C.Y. Choong, Y.C. Lin, B.H. Lee, Y.C. Shi and C.J. Tai, 2016. Aqueous extract of *Paris polyphylla* (AEPP) inhibits ovarian cancer via suppression of peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1alpha. Molecules, Vol. 21. 10.3390/molecules21060727.
15. He, H., C. Xu, L. Zheng, K. Wang, M. Jin, Y. Sun and Z. Yue, 2020. Polyphyllin VII induces apoptotic cell death via inhibition of the PI3K/Akt and NF-κB pathways in A549 human lung cancer cells. Mol. Med. Report., 21: 597-606.
16. Wu, Y., Y. Si, Y. Xiang, T. Zhou and X. Liu *et al*., 2020. Polyphyllin I activates AMPK to suppress the growth of non-small-cell lung cancer via induction of autophagy. Arch. Biochem. Biophys., Vol. 687. 10.1016/j.abb.2020.108285.

17. Kress, W.J. and D.L. Erickson, 2012. DNA Barcodes: Methods and Protocols. 1st Edn., Humana Press, Totowa, New Jersey, ISBN: 978-1-61779-591-6, Pages: 470.
18. Men, A.E., P. Wilson, K. Siemering and S. Forrest, 2008. Sanger DNA Sequencing. In: Next Generation Genome Sequencing: Towards Personalized Medicine, Janitz, M. (Ed.), Wiley-VCH Verlag GmbH & Co. KGaA, Germany, ISBN: 9783527625130, pp: 1-11.
19. Şahin, S. and R. Şamlı, 2013. Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology. Ultrason. Sonochem., 20: 595-602.
20. Kwon, J.H., J.M.R. Belanger and J.R.J. Pare, 2003. Optimization of microwave-assisted extraction (MAP) for ginseng components by response surface methodology. J. Agric. Food Chem., 51: 1807-1810.
21. Cai, L., 2014. Thin Layer Chromatography. In: Current Protocols Essential Laboratory Techniques, Cai, L. (Ed.), Wiley, Hoboken, New Jersey, pp: 6.3.1-6.3.18.
22. Salim, A., S. Abdul Rahman, S. Khamis, H.A. Masalli, M.Z.A.M. Amiroudine and D.J. Arapoc, 2022. Antioxidant property and mtt assay screening of local and imported punica granatum. Eur. Chem. Bull., 11: 6-10.
23. MarÉchal, E., 2011. Measuring Bioactivity: KI, IC50 and EC50. In: Chemogenomics and Chemical Genetics: A User's Introduction for Biologists, Chemists and Informaticians, MarÉchal, E., S. Roy and L. Lafanechère (Eds.), Springer, Berlin Heidelberg, Germany, ISBN: 978-3-642-19615-7, pp: 55-65.
24. McDermott, M., A.J. Eustace, S. Busschots, L. Breen and J. Crown *et al*., 2014. *In vitro* development of chemotherapy and targeted therapy drug-resistant cancer cell lines: A practical guide with case studies. Front. Oncol. Vol. 4. 10.3389/fonc.2014.00040.
25. Bocci, G., K.C. Nicolaou and R.S. Kerbel, 2002. Protracted low-dose effects on human endothelial cell proliferation and survival *in vitro* reveal a selective antiangiogenic window for various chemotherapeutic drugs. Cancer Res., 62: 6938-6943.
26. Thuy, V.T.T., 2017. The biological characteristics and pharmacological values of species seven leaves a flower of genus *Paris* [In Vietnamese]. TNU J. Sci. Technol., 171: 49-54.
27. Dũng, N.T., N.Q. Nga, T.N. Lân, N.T. Thu and N.T. Phíp *et al*., 2018. Morphological characteristics and DNA barcodes of *Paris vietnamensis* (Takht.) H.Li in Vietnam. Vietnam J. Agri. Sci., 16: 282-289.
28. Lan, N.T.N., N.H. Quân, V.T.T. Th y and C.H. M u, 2020. Identification of three species, *Paris fargesii*, *Paris polyphylla*, *Paris vietnamensis* collected in Vietnam and phylogenetic inference in the genus *Paris* [In Vietnamese]. TNU J. Sci. Technol., 225: 395-402.
29. Yao, H., J. Song, C. Liu, K. Luo and J. Han *et al*., 2010. Use of ITS2 region as the universal DNA barcode for plants and animals. PLoS ONE., Vol. 5. 10.1371/journal.pone.0013102.
30. Mishra, P., A. Kumar, V. Rodrigues, A.K. Shukla and V. Sundaresan, 2016. Feasibility of nuclear ribosomal region ITS1 over ITS2 in barcoding taxonomically challenging genera of subtribe Cassiinae (Fabaceae). PeerJ, Vol. 4. 10.7717/peerj.2638.
31. Sultana, B., F. Anwar and M. Ashraf, 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules, 14: 2167-2180.
32. Duyên, N.T., Đ.T. Hà, T.V. Th ng, N.Q. Chi n and P.Q. Long, 2017. Chemical composition of the ethyl acetate extract of the heptapod (*Paris polyphylla* var. chinensis Franchet) grown in Vietnam [In Vietnamese]. Vietnam J. Chem., 55: 367-371.
33. Lepcha, D.L., A. Chhetri and D.R. Chhetri, 2019. Antioxidant and cytotoxic attributes of *Paris polyphylla* smith from Sikkim Himalaya. Pharmacogn. J., 11: 705-711.
34. Hai-Tao, L., L. Xian-Wen, G. Yan-Hong, Z. Li-Xia and G. Wei-Wei, 2014. Comparison between content of saponins of *Paris polyphylla* var. yunnanensis in different areas of Yunnan Province. China J. Chin. Mater. Med., 39: 803-806.
35. Kumar, S.P.J., S.R. Prasad, R. Banerjee, D.K. Agarwal, K.S. Kulkarni and K.V. Ramesh, 2017. Green solvents and technologies for oil extraction from oilseeds. Chem. Cent. J., Vol. 11. 10.1186/s13065-017-0238-8.
36. Kshetrimayum, V., R. Heisnam, O.S. Keithellakpam, P. Radhakrishnanand, S.J. Akula, P.K. Mukherjee and N. Sharma, 2023. *Paris polyphylla* sm. induces reactive oxygen species and caspase 3-mediated apoptosis in colorectal cancer cells *in vitro* and potentiates the therapeutic significance of fluorouracil and cisplatin. Plants, Vol. 12. 10.3390/plants12071446.
37. Bisi-Johnson, M.A., C.L. Obi, T. Hattori, Y. Oshima and S. Li *et al*., 2011. Evaluation of the antibacterial and anticancer activities of some South African medicinal plants. BMC Complementary Altern. Med., Vol. 11. 10.1186/1472-6882-11-14.
38. Chen, T., J. Lin, D. Tang, M. Zhang, F. Wen, D. Xue and H. Zhang, 2019. Paris saponin H suppresses human hepatocellular carcinoma (HCC) by inactivation of Wnt/β-catenin pathway *in vitro* and *in vivo*. Int. J. Clin. Exp. Pathol., 12: 2875-2886.
39. Ziyi, A.N., H.E. Peiyan, G. Wang, G. Liu and J. Jiang, 2022. PP-10 induces apoptosis via JNK/SPAK activation and STAT3 inhibition in Hepatocarcinoma cells *in vitro*. Food Sci. Technol., Vol. 42. 10.1590/fst.102921.