ANTI-LIPASE AND MCF-7 BREAST CANCER CELL PROLIFERATION INHIBITION *IN VITRO* OF THE EXTRACT-ENRICHED POLYPHENOLS AND SAPONINS FROM *Musa balbisiana* FRUIT

Nguyen Thi Thu Huyen, Dinh Khanh Dieu, Nguyen Huynh Tram, Hoang Thi Ngoc Nhon*

Ho Chi Minh City University of Industry and Trade

*Email: *nhonhtn@huit.edu.vn*

Received: 18 April 2023; Accepted: 8 September 2023

ABSTRACT

Musa balbisiana is one of the most widely used bananas, and its parts have been used in medicine worldwide. This study aimed to determine the anti-lipase and the inhibition of MCF-7 breast cancer cells proliferation of the polyphenol and saponin-enriched extract from *M. balbisiana* fruit. The potent anti-lipase activity of the extract was observed via an assay for pancreatic lipase, and the anticancer activity of the extract was assessed through its anti-proliferative ability on MCF-7 breast cancer cells by sulforhodamine B assay. This study concluded that the extract had high lipase and MCF-7 cell inhibition with IC_{50} values of 48.82 ± 1.21 μg/mL and 289.5 ± 8.7 μg/mL, respectively. These results suggest that the obtained extract from *M. balbisiana* fruit might have the potential to be used as an agent to support obesity reduction and inhibit breast cancer cell proliferation.

Keywords: Lipase, MCF-7 cells, *Musa balbisiana,* polyphenol, saponin.

1. INTRODUCTION

Natural products have been essential in developing drugs and disease treatment. Plants are potential sources of rich phytochemicals and secondary metabolites with beneficial medicinal properties. Plant-based medicines are still popular as a source of primary healthcare in nearly all parts of the world [1]. Among them, *Musa balbisiana* Colla belongs to the family *Musaceae* and is a herbaceous plant containing many vital compounds with biological activity, such as polyphenols, carotenoids, fibre, protein, vitamins, energy, minerals, unsaturated fatty acids, and potassium. Thus, it has been used in traditional medicine for a long time due to its nutritional value and antioxidant capacity [2]. In addition, various bioactive compounds, such as alkaloids, phenolics, flavonoids, terpenoids, etc., in plants attracted the attention of researchers to study the mechanisms of action and pharmacological properties [3]. These bioactive compounds indicate many bioactivities. Many studies have reported that parts of *M. balbisiana* possess anticancer and anti-proliferative properties [3].

Breast cancer is an uncontrolled cell growth that originates in breast tissue. In this stage, breast cells mutate, grow out of control, and form tumours - tissue masses. It can invade and grow into the tissue surrounding your breast, travel to other parts of the body and create new tumours. Surgical treatments for breast cancer can reduce breast cancer but might cause severe side effects. Thus, natural therapy using bioactive compounds from plants has attracted the attention of researchers [4]. Moreover, obesity has become a global issue due to the increasing growth of obese individuals, and it causes many metabolic diseases and serious health problems. Those who suffer from obesity are often striving to find various safe and effective

treatment methods as well as anti-obesity medicines. In addition, lipases are fat-digesting enzymes that form free fatty acids utilised for muscle energy production or are re-esterified for storage in the adipose tissue. The development of obesity is caused by a chronic imbalance between energy intake and energy expenditure, which results from changing lifestyles and eating fat-rich foods. Therefore, inhibiting digestive lipase to reduce fat absorption has been considered a significant pharmacological way to treat obesity [5]. Pancreatic lipase inhibitors have been considered in recent years because of their structural diversity, low toxicity, and wide range of sources [3]. Thus, the growing demand for finding more plant-based resources to treat cancer or obesity disorders has risen.

In Vietnam's traditional medicine, *M. balbisiana* parts have been used for various medical purposes. To isolate, characterise, and demonstrate the medical activities in this plant, we have conducted studies to find out the extraction protocol and determine bioactive compounds as well as bioactivities from *M. balbisiana* fruit. The previous report reported the polyphenols and saponins extraction conditions, antioxidant, antibacterial, antifungal, anti-inflammatory, and antidiabetic activities of the obtained extract [6]. This study aimed to study the inhibition of anti-lipase enzyme and MCF-7 breast cancer cells proliferation of extract-enriched polyphenol and saponin from *M. balbisiana* fruit.

2. MATERIALS AND METHODS

2.1. Materials

M. balbisiana fruit at ripe status was harvested in Hue Tinh commune, Chau Phu district, An Giang province, Vietnam. At the laboratory, the raw material, including peel, pulp, and seed, was washed, sliced, dried at 60 ℃ until the moisture content was ≤ 10%, and ground, then sieved through a sieve. The obtained powder was sealed in a zipper bag and used for all experiments.

Chemicals: Folin – Ciocalteu reagent, Gallic acid (Merck), Sodium carbonate (Na_2CO_3), Sulforhodamine B (SRB) (Merck), Tris-HCl (Merck), p-nitrophenyl butyrate (Merck), Lipase (Sigma Aldrich), Acetonitrile (Merck), Trichloroacetic acid (TCA) (Merck), Dimethyl sulfoxide (DMSO) (Sigma Aldrich), Camptothecin (CPT) (Sigma-Aldrich). Other chemicals and reagents were of analytical grade.

2.2. Methods

2.2.1. extract preparation

The protocol of polyphenol and saponin extraction from *M. balbisiana* fruit was followed by the results of our previous report [6]. The extraction was supported with methanol at 120 min at 65 ℃ at the material/solvent ratio of 1/36 (w/v). The obtained extract was freeze-dried to obtain power for further analysis.

2.2.2. Assay for pancreatic lipase activity

Firstly, the working, enzyme stock and p-nitrophenyl butyrate (PNPB) stock solution were prepared: Working solution (1 mg/mL): the extract enriched saponin and polyphenol (100 mg) was dissolved in 100 ml of 10% DMSO. Next, different extract concentrations (0, 20, 40, 60, 80, 100 µg/mL) were prepared. Enzyme stock solution (1 mg/mL): 20 mg lipase enzyme powder was added into 20 mL of 10% DMSO. P-nitrophenyl butyrate (PNPB) stock solution: 20.9 mg PNPB was dissolved in 2 mL of acetonitrile.

Secondly, 0.2 mL extract at different dilutions was mixed with 0.1 mL of lipase enzyme stock solution, and Tris-HCl solution was added to gain 1 mL of volume before incubating at

37 °C for 30 min. After the incubation period, 0.1 mL of PNPB solution was then added to each test tube and again incubated for 30 min at 37 °C. A similar procedure was conducted without the extract to obtain a blank solution. The positive control was orlistat. The absorbance was measured utilising a spectrophotometer (UV-Vis) at 405 nm. The lipase enzyme inhibitory was measured via the following equation [7].

$$I(\%) = \frac{A_{blank} - A_{test}}{A_{blank}}$$

Where A_{blank}= absorbance of the blank A_{test} = absorbance of substrate

2.2.3. Assay for inhibition MCF-7 breast cancer cells proliferation

The inhibition of MCF-7 breast cancer cell proliferation was determined using sulforhodamine B assay (SRB) based on measuring the content of cellular proteins [8]. The RPMI 1640 Medium (Jurkat), including L-glutamine (2 mM), HEPES (20 mm), amphotericin B (0.025 µg/mL), penicillin G (100 UI/mL), streptomycin (100 µg/mL), and 10% (v/v) fetal bovine serum (FBS) was prepared. A concentration of 10^4 cells/well was put on a 96-well plate and cultured at 37 °C for 24 h in the condition of 5% CO_2. Then, the extract-enriched polyphenols and saponins with different concentrations (25, 50, 100, 200, and 400 µg/mL) in triplicates were added to the culture cells within the plaque and cultured for 48 h. The supernatant was removed from the well, and cold TCA was added to the well and left for 1 h at 4 °C. Distilled water was used to remove TCA, and the well was dried at room temperature, and then 0.2% sulforhodamine B was added. The absorbance of the sample solution was measured by UV-VIS spectroscopy at 492 nm and 620 nm. The inhibition percentage of the cancer cell proliferation was calculated by the following equation:

$$\text{the } \%I = \left(1 - \frac{OD_{sample}}{OD_{control}}\right) \times 100\%$$

Where: OD_{sample}: the absorbance of the sample, $OD_{control}$: the absorbance of control.

2.2.4. Statistical analyses

Experiments were repeated three times, and the results were presented as mean ± SD. IBM SPSS Statistics 20.0 was used to analyse experimental data, evaluate the difference between samples and optimise the extract conditions. The charts were drawn using Microsoft Excel 2016 software.

3. RESULTS AND DISCUSSION

3.1. Extract preparation

Our previous study on the extraction of polyphenols and saponins from *M. balbisiana* resulted in the obtained extract containing a lower content of steroids, tannins, and flavonoids and a high amount of polyphenols and saponins with 51.72 mg/gGAE (TPC) and 41.66 mg/g (TSC), respectively [6]. Besides the reported antioxidant, antibacterial, antifungal, anti-inflammatory, and antidiabetic activities, the anti-lipase activity and the inhibition of MCF-7 cancer cell proliferation were presented in this study.

3.2. Anti-lipase activity

The pancreatic lipase inhibitory assay was applied to determine the anti-lipase of the extract from *M. balbisiana* fruit, with the positive control being orlistat (Fig. 1).

Lipid metabolism is cleverly balanced to keep homeostasis. Without this balance, the development of obesity or hyperlipidemia could result in serious diseases such as hypertension, atherosclerosis, diabetes, and a reduction in the functionalities of specific organs. Inhibiting pancreatic lipase is a widely studied mechanism for identifying potential anti-obesity agents. Orlistat is a drug approved by the Food and Drug Administration for obesity treatment, acting through pancreatic lipase inhibition. Orlistat could inactivate the active serine site of lipase to hydrolyse dietary fat by forming a covalent bond with lipases and thus inactivating them to hydrolyse dietary fat [9]. However, orlistat also has unwanted side effects such as mental confusion, anxiety, digestive disorders, headaches, and easy flu. Thus, studies on anti-lipase activity are attracting the attention of many researchers. In this study, the anti-lipase activity of the fruit of *M. balbisiana* was investigated. From the obtained results, the IC_{50} values of the extract and the control were 18.19 ± 0.76 and 48.82 ± 1.21 µg/mL, respectively. This means that the fruit of *M. balbisiana* might have lipid-lowering activities. This potential activity may come from a significant amount of saponins and polyphenols in the extract. Pancreatic lipase shows its total catalytic activity in oil/water emulsions. As saponins can act as emulsifiers, they could stabilise the interface oil/water emulsions. By aggregating with food fat droplets to form micelles, saponins could reduce the interaction of lipase with the substrate [9].

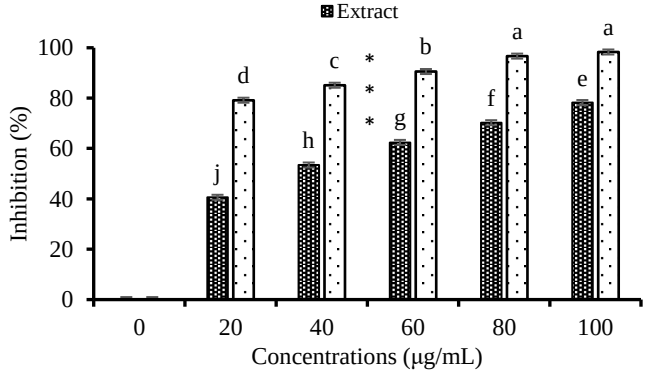

Fig. 1. The lipase inhibition activity of the extract from *M. balbisiana* fruit

*Note: Different letters in the same column represent a statistically significant difference at $p < 0.05$ according to ANOVA analysis, ***$p<0.001$ by one-way ANOVA with Tukey post hoc for each extract group with every other extract group. All differences are significant except 80 µg/ml vs. 100 µg/mL.*

3.3. The inhibition of breast cancer MCF-7 cell proliferation

The inhibition of breast cancer MCF-7 cells proliferation of extract-enriched polyphenol and saponin from *M. balbisiana* was shown as a percentage of the growth inhibition. The effects of the extract on anti-proliferative activity and its morphology are illustrated in Fig. 2 and Fig. 3.

The sulforhodamine B assay resulting in the sample showed that the inhibition of MCF-7 cancer cell proliferation increased with an increase in concentration on the sample with the IC50 value of 289.6 ± 8.8 µg/mL. The results of the sulforhodamine B assay showed trends toward more significant inhibition of MCF-7 cells with an increasing concentration of extract. The IC_{50} value was 289.5 ± 8.7 µg/mL. Several studies on parts of Musa sp. indicated that this material possesses antioxidant and anticancer activity. The survey on fruit peel and core of Musa acuminata showed that 70% ethanol extract of fruit peel and core expressed by inhibiting concentration of 50% proliferation (IC_{50}) of MCF-7 cancer cells were 115.001 µg/mL and 338.469 µg/mL, respectively. The cytotoxic activity could be attributed to the flavonoids, saponins, triterpenes, and tannins present in the extracts [10].

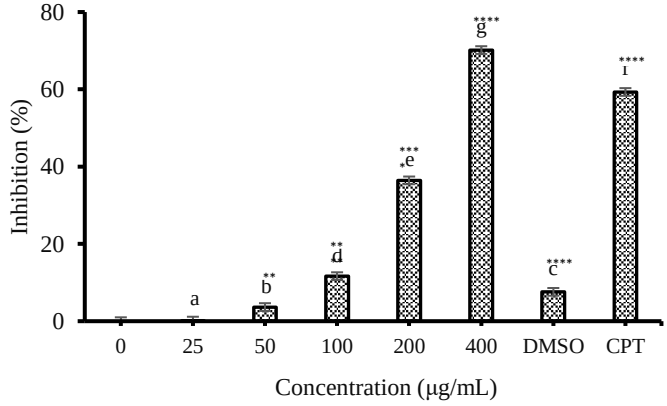

Fig. 2. The inhibition activity of the extract against MCF-7 cancer cell proliferation (CPT: Camptothecin, DMSO: Dimethyl sulfoxide)

*Note: Different letters in the same column represent a statistically significant difference at p < 0.05 according to ANOVA analysis, ** p<0.01, ****p<0.0001 by one-way ANOVA with Tukey post hoc test except 0 µg/mL vs 25 µg/mL.*

In addition, the cytotoxicity activity on carcinoma of cervix (HeLa) cells of the ethanolic extract rhizome from *M. acuminata* was investigated by Adinarayana K. indicated that the test for cytotoxicity at the highest concentration of the tested dose (256 µg/mL) had the maximum rate of inhibition of 50.32% [11]. Another experimental study by Roobha J. revealed that anthocyanin extracts from *M. acuminata* bract had strong anticancer activity against MCF-7 cells [12]. The findings of Revadigar V. suggested that the ethanolic extract of the inflorescence of *M. balbisiana* had promising cytotoxicity on colorectal human cancer cells of HT-29 and HCT-116 and moderate cytotoxicity on MCF-7 breast cancer cells. These activities might come from the high total phenolics in the extract with 22 compounds [13].

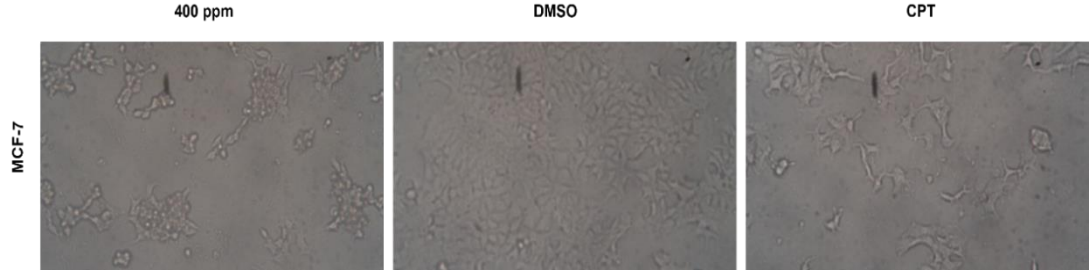

Fig. 3. The morphology of the MCF-7 cancer cells treated by CPT 0.05 ppm and the extract

In Fig. 3, the control MCF-7 cells that were untreated cells appeared healthy with characteristic morphology and attached to the substrate in the disc. Nevertheless, opposite appearances were observed with cells treated with the extract 400 ppm and CPT 0.05 ppm. These treated cancer cells had distinct cellular morphology that indicated their unhealthy status, with lots of cells that tend to shrink and become spherical shape. The others were broken with unclear borders because of the absorption and reaction with the extract or CPT. It was concluded that programmed cell death - apoptosis, which occurred via cell shrinkage and nuclear fragmentation. Therefore, it could be suggested that the obtained extract-enriched polyphenol and saponin from *M. balbisiana* fruit could promote the apoptosis of the cancer cells, which might have a potential therapeutic activity on MCF-7 breast cancer cells.

4. CONCLUSION

In summary, the findings of the present study suggest that the methanolic extract-enriched polyphenol and saponin from the fruit of *M. balbisiana* initially reveal a potential anti-lipase activity and promising cytotoxicity on the MCF-7 breast cancer cells. The obtained extract can be very helpful in addition to being the primary medicine used in treating obesity or breast cancer disorders. Further, *in vivo* works should be undertaken to obtain the full bioactivities of the extract from *M. balbisiana* fruit for functional food or pharmaceutical applications.

Acknowledgement: We would like to thank Ho Chi Minh City University of Industry and Trade (HUIT) for the support for this study. This work has been sponsored and funded by Ho Chi Minh City University of Industry and Trade under Contract No.47/HD-DCT.

REFERENCES

1. Taylor J., Rabe T., McGaw L., Jäger A., and Van Staden J. - Towards the scientific validation of traditional medicinal plants. Plant growth regulation **34** (2001) 23-37. https://doi.org/10.1023/A:1013310809275
2. Valsalam S., Agastian P., Esmail G.A., Ghilan A.K.M., Al-Dhabi N.A., and Arasu M.V. - Biosynthesis of silver and gold nanoparticles using *Musa acuminata* colla flower and its pharmaceutical activity against bacteria and anticancer efficacy. Journal of Photochemistry and Photobiology B: Biology **201** (1029) 111670. https://doi.org/10.1016/j.jphotobiol.2019.111670
3. Swargiary A., Boro H., Roy M.K., & Akram M. - Phytochemistry and Pharmacological Property of *Musa balbisiana* Colla: A Mini-Review. Pharmacognosy Reviews **15** (29) (2021). https://doi.org/10.5530/phrev.2021.15.11
4. Waks A. G., & Winer E. P. - Breast cancer treatment: a review. Jama, **321** (3) (2019), 288-300. https://doi.org/10.3390/molecules24142612
5. De La Garza A.L., Milagro F.I., Boque N., Campión J., & Martínez J. A. - Natural inhibitors of pancreatic lipase as new players in obesity treatment. Planta medica **77** (08) (2011) 773-785. https://doi.org/10.1055/s-0030-1270924
6. Nhon Hoang T.N., Lien Phan T.K., Van Nguyen N.H., Dao Dong T.A., & Anh Le T.H. - Phytochemical Screening, Extraction, and Determination of the Bioactivities of the Extract-Enriched Polyphenols and Saponins from *Musa balbisiana* Fruit. Journal of Food Processing and Preservation **2023** (2023). https://doi.org/10.1155/2023/2581641
7. Jaradat N., Zaid A.N., Hussein F., Zaqzouq M., Aljammal H., & Ayesh O. - Anti-lipase potential of the organic and aqueous extracts of ten traditional edible and medicinal plants in Palestine; a comparison study with orlistat. Medicines **4** (4) (2017) 89. https://doi.org/10.3390/medicines4040089
8. Spyridopoulou K., Fitsiou E., Bouloukosta E., Tiptiri-Kourpeti A., Vamvakias M., Oreopoulou A., ... & Chlichlia K. - Extraction, chemical composition, and anticancer potential of *Origanum onites* L. essential oil. Molecules **24** (14) (2019) 2612. https://doi.org/10.3390/molecules24142612
9. Lunagariya N.A., Patel N.K., Jagtap S.C., & Bhutani K.K. - Inhibitors of pancreatic lipase: state of the art and clinical perspectives. EXCLI Journal **13** (2014) 897.
10. Sutjiatmo A.B., Widowati W., Sumiati I., Priestu T., Arumwardana S., Kusuma H.S.W., & Azizah A.M. - Antioxidant and anticancer potential of raja bulu banana peel and heart

(*Musa acuminata* Colla (AAB group)) Ethanol Extracts in MCF-7 Cell Lines. Majalah Obat Tradisional **26** (1) (2021) 49-58. https://doi.org/10.22146/mot.55333

11. Adinarayana K.P.S., & Babu A.P. - Antioxidant activity and cytotoxicity of ethanolic extracts from the rhizome of *Musa acuminata*. Natural Science **3** (4) (2011) 291-294. http://dx.doi.org/10.4236/ns.2011.34037
12. Jenshi Roobha J., & Aravindhan M.S.K. - *In vitro* evaluation of anticancer property of anthocyanin extract from *Musa acuminate* bract. Research in Pharmacy **1** (4) (2015).
13. Revadigar V., Al-Mansoub M.A., Asif M., Hamdan M.R., Majid A.M.S.A., Asmawi M.Z., & Murugaiyah V. - Anti-oxidative and cytotoxic attributes of phenolic rich ethanol extract of *Musa balbisiana* Colla inflorescence. Journal of Applied Pharmaceutical Science **7** (5) (2017) 103-110. https://doi.org/10.7324/JAPS.2017.70518.

TÓM TẮT

HOẠT TÍNH ỨC CHẾ ENZYM LIPASE VÀ CHỐNG TĂNG SINH DÒNG TẾ BÀO UNG THƯ VÚ MCF-7 CỦA CHIẾT XUẤT GIÀU SAPONIN, POLYPHENOL TỪ QUẢ CHUỐI HỘT *Musa balbisiana* Colla

Nguyễn Thị Thu Huyền, Đinh Khánh Diệu,

Nguyễn Huỳnh Trâm, Hoàng Thị Ngọc Nhơn*

Trường Đại học Công Thương Thành phố Hồ Chí Minh

*Email: *nhonhtn@huit.edu.vn*

Chuối hột *Musa balbisiana* là một trong những loại chuối phổ biến trên thế giới và các bộ phận của nó đã được sử dụng rộng rãi trong y học. Nghiên cứu này nhằm xác định khả năng ức chế enzyme lipase và ức chế sự tăng sinh dòng tế bào ung thư vú MCF-7 của chiết xuất giàu polyphenol và saponin từ quả chuối hột *M. balbisiana*. Hoạt tính kháng lipase cao của dịch chiết được đánh giá thông qua xét nghiệm lipase tụy và hoạt tính chống tăng sinh dòng tế bào ung thư vú MCF-7 bằng xét nghiệm SRB. Kết quả cho thấy dịch chiết thu được có khả năng ức chế lipase và ức chế tăng sinh dòng tế bào MCF-7 với giá trị IC_{50} lần lượt là 48,82 ± 1,21 µg/mL và 289,5 ± 8,7 µg/mL. Điều này cho thấy chiết xuất thu được từ quả chuối hột có thể có tiềm năng được sử dụng như một tác nhân hỗ trợ giảm béo phì và ức chế sự phát triển của dòng tế bào ung thư vú.

Từ khóa: Lipase, *Musa balbisiana*, polyphenol, saponin, tế bào MCF-7.