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Letter to the Editor

Anti-migration activity of four components from dried fruit of *Forsythia suspensa*

Dear Editor,

Breast cancer is the most common cancer and the second leading cause of cancer mortality in women (Sung et al., 2021). Tumor metastasis is the major cause of poor prognosis and survival in cancer patients, which is responsible for about 90% of cancer deaths (Gerstberger et al., 2023). Despite tremendous advances in the treatment of primary tumors, metastatic tumors remain the most difficult cancer disease to conquer (Guan, 2015). The existing clinical drugs are not satisfied for fighting against tumor metastasis. Therefore, there is an urgent need to develop novel drug candidates against metastatic breast cancer.

Forsythia suspensa (Thunb.) Vahl. is a typical heat-clearing and detoxicating herb in traditional Chinese medicine. Its dried fruits, also known as Forsythiae Fructus, have been widely used for treating cancer since ancient China. There is a great potential to develop anti-cancer drugs from *F. suspensa* dried fruit-derived compounds. It was reported that fruit extracts had anti-proliferative activity against various cancers. The underlying mechanisms include inhibiting MAPKs/Nrf2/HO-1 mediated anti-oxidation and anti-inflammation, regulating glycerophospholipid metabolism, influencing cell cycle progression and inducing apoptosis (Bao et al., 2016a; Bao et al., 2016b; Lee et al., 2017; Zhang et al., 2019). However, the anti-metastatic activity of dried fruit of *F. suspensa* and its bioactive components remains to be explored.

In this study, the growth inhibitory effect of *F. suspensa* dried fruit aqueous extract was first measured on breast cancer cell line MDA-MB-231 by MTT assay (Zhang et al., 2015; Bao et al., 2016a). The aqueous extract was prepared by adding 100 g of dried herbal piece powder to 1,000 mL of distilled water to form a slurry, bringing the subsequent mixture to a boiler and simmering at 80°C for 60 min. After the mixture cooled down, the aqueous layer was carefully decanted off of the residual solids and yielded 28.3 g lyophilized powder. The cell viability and growth inhibitory rate were calculated using the following formulas:

Cell viability (%) = Test absorbance at 570 nm / Control absorbance at 570 nm × 100

Growth inhibitory rate (%) = Control cell viability – Test cell viability

Breast cancer cell line MDA-MB-231 was obtained from ATCC and cultured in DMEM supplemented with 10% FBS. Cells were seeded at a density of 4×10^3 cells per well in a 96-well plate. After treating MDA-MB-231 cells with the extract for 48 hours, the numbers of viable breast cancer cells were significantly decreased in a dose-dependent manner (Figure 1A). The IC_{50} value of the extract was 132.1 µg/mL. When the dose of the extract was increased to 250 µg/mL, the growth inhibitory rate of MDA-MB-231 cells increased to 90.8%. This result demonstrated that *F. suspensa* dried fruit aqueous extract possessed effective anti-cancer activity against breast cancer cells.

To further explore the anti-metastatic activity of *F. suspensa* dried fruit, the wound healing assay was applied to evaluate the anti-migration effect of the extract breast cancer cells (Li et al., 2022). When evaluating the anti-migration activity of a drug *in vitro*, the ideal condition would be that its dose would only inhibit the migration of cancer cells, with no effect on growth inhibition. Its anti-metastatic activity is not contributed by growth inhibitory effect. The anti-migration activity was assessed by migration rate (%) and inhibitory rate of migration (%) using the following formulas:

Migration rate (%) = (0 hour scratch area - 48 hour scratch area) / 0 hour scratch area × 100

Migration inhibitory rate (%) = (Control migration rate - Test migration rate) / Control migration rate × 100

As shown in Figure 1B, the results showed that the extract effectively suppressed the migration of highly metastatic cancer cell line MDA-MB-231 by 31.6 and 33.4% at concentrations of 15.6 and 31.2 µg/mL, respectively. This result demonstrated the anti-metastatic activity of *F. suspensa* dried fruit against breast cancer cells.

Forsythoside A, forsythoside I, forsythoside H, and isoforsythiaside are representative components of *F. suspensa* dried fruit, which are considered to be potential bioactive compounds (Yang et al., 2022). Forsythoside A and forsythoside I have been found with cytotoxic activity against gastric cancer, breast cancer, and melanoma (Nie et al., 2022). However, their anti-metastatic activity has not been explored. In this study, the anti-metastatic effect of forsythoside A, forsythoside I, forsythoside H, and isoforsythiaside (Chengdu Must Bio-Technology Co., Ltd., China) on breast cancer cell

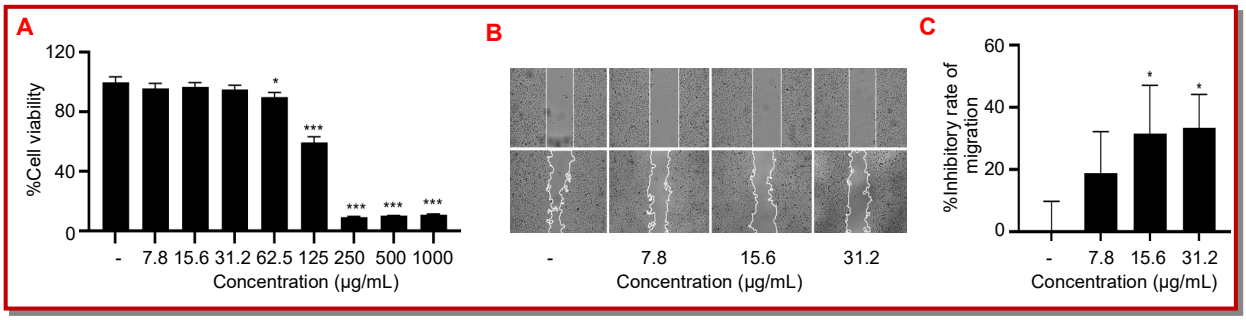

Figure 1: Cell viability (A) and migration inhibitory effect (B, C) of *F. suspensa* dried fruit aqueous extract on MDA-MB-231 cell lines incubated for 48 hours. Migration inhibitory effect was assessed by wound healing assay

line MDA-MB-231 was explored.

The cytotoxic concentrations of forsythoside A, forsythoside I, forsythoside H and isoforsythiaside were first determined, and selected drug doses without growth inhibitory effect were used to conduct their anti-migration activity. The results showed that forsythoside A, forsythoside I, forsythoside H, and isoforsythiaside at concentrations of 2.5, 5, 10, 20, and 30 µg/mL all had no growth inhibitory effects on MDA-MB-231 cells (Figure 2). So, these doses were used to perform later anti-migration activity assessment.

The wound healing assay was again applied to determine the anti-migration activity of forsythoside A, forsythoside I, forsythoside H and isoforsythiaside on MDA-MB-231 cancer cells. The results showed that forsythoside A, forsythoside I, forsythoside H, and isoforsythiaside significantly suppressed migration of cancer cells by 13.2, 4.5, 13.2, and 20.0% at the concentration of 20 µg/mL, respectively (Figure 3A,B). When their dose was increased to 30 µg/mL, their migration

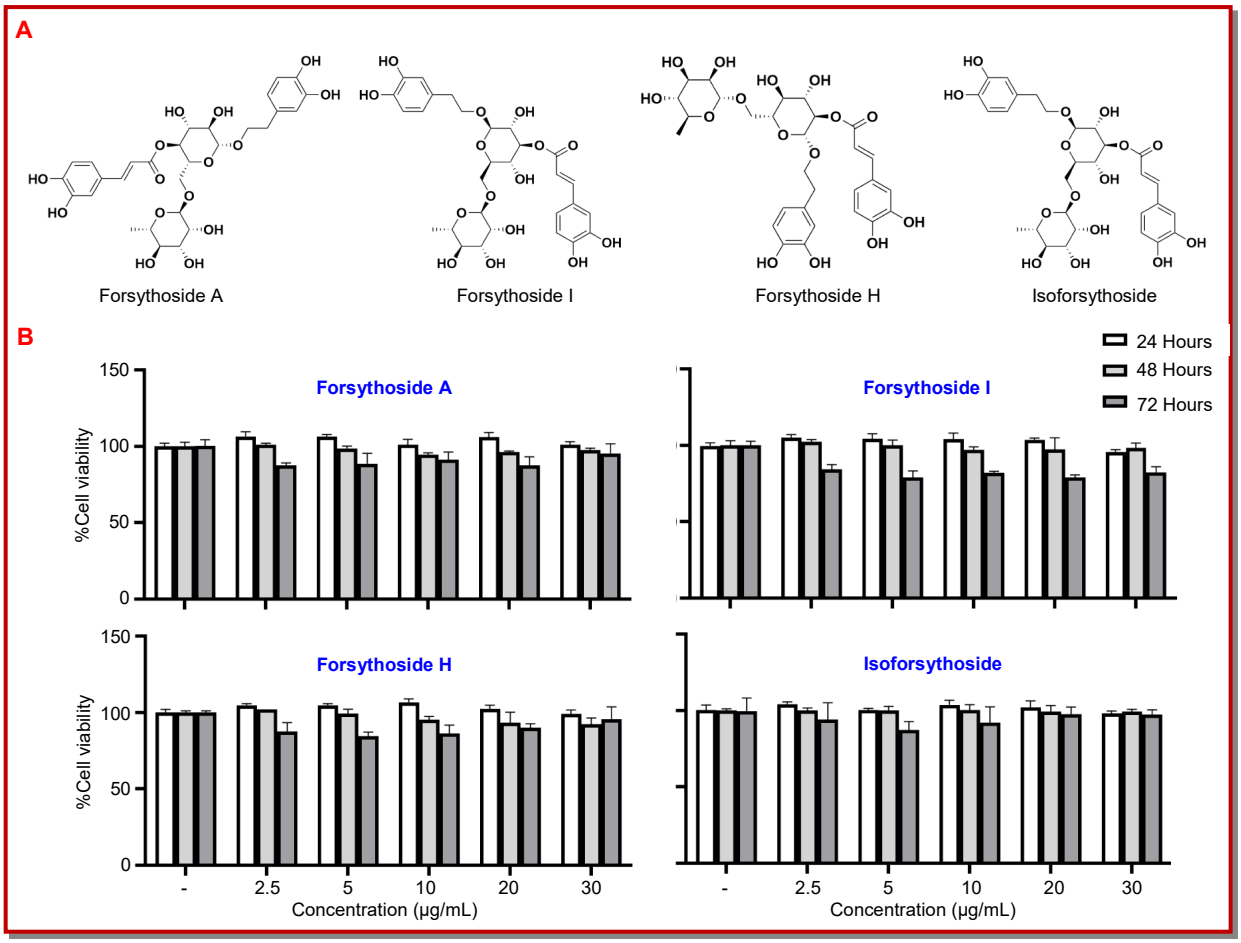

Figure 2: Growth inhibitory effect of forsythoside A, forsythoside I, forsythoside H and isoforsythiaside. (A) Chemical structures of forsythoside A, forsythoside I, forsythoside H and isoforsythiaside. (B) Growth inhibitory effect of FA, FI, FH and IFA on MDA -MB- 231 cells. Cells were incubated with indicated compounds for 24, 48 and 72 hours

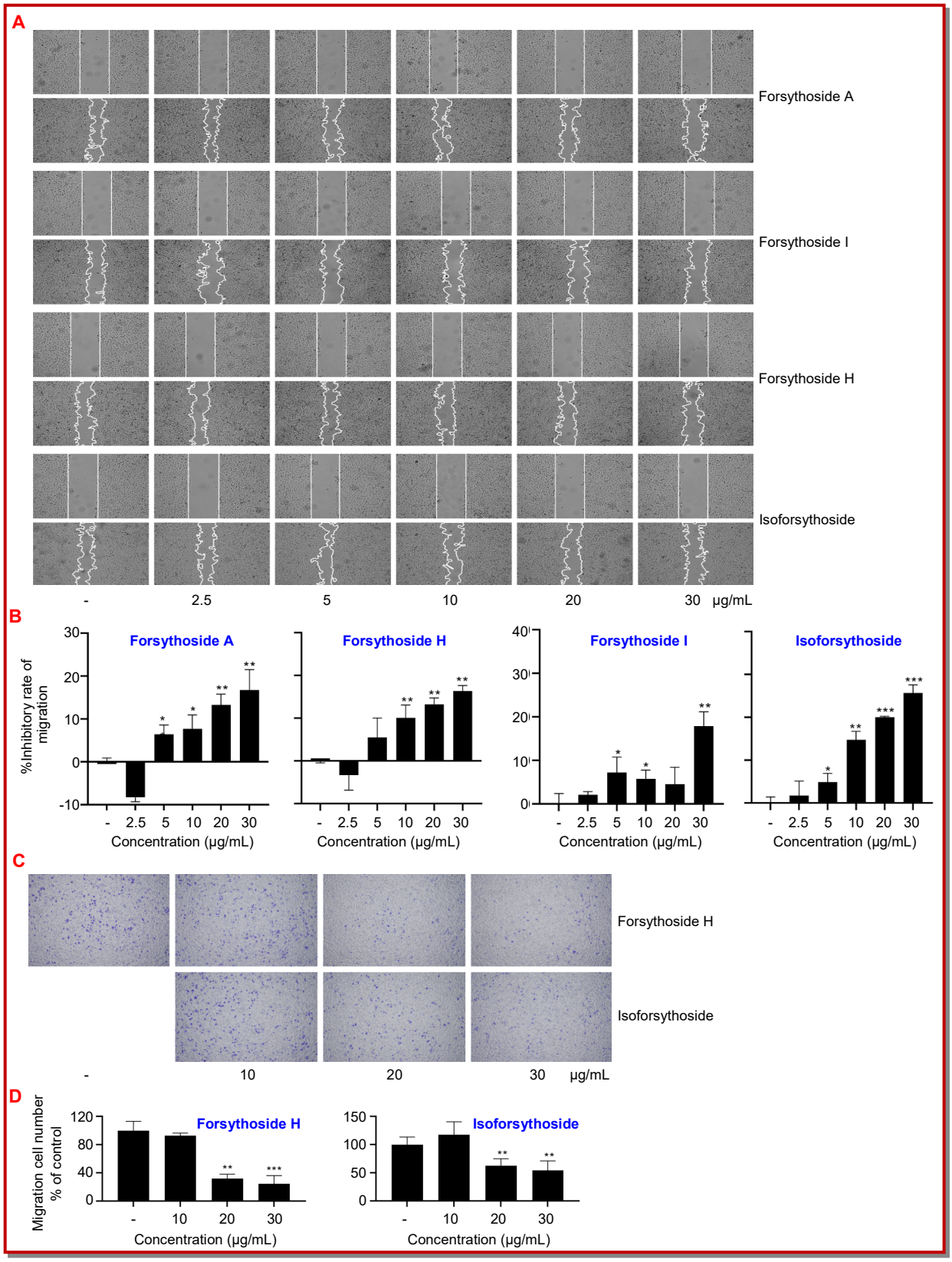

Figure 3: Migration inhibitory effect of forsythoside A, forsythoside I, forsythoside H and isoforsythiaside. (A) Wound healing assay to evaluate anti-migration activity of compounds at doses of 0, 2.5, 5, 10, 20 and 30 μg/mL. Cells were incubated with indicated compounds for 48 hours. (B) The statistics of inhibitory rate of migration for indicated compounds assessed by wound healing assay. (C) Transwell assay to evaluate anti-migration activity of forsythoside H and isoforsythiaside at doses of 0, 10, 20 and 30 μg/mL. Cells were incubated with forsythoside H and isoforsythiaside for 8 hours. (D) The statistics of inhibitory rate of migration for indicated compounds assessed by transwell assay

inhibitory rate increased to 16.8, 17.9, 16.3, and 25.7%, respectively (Figure 3A,B). In general, forsythoside A, forsythoside I, forsythoside H, and isoforsythiaside suppressed cancer cell migration in a dose-dependent manner. Isoforsythiaside and forsythoside H exhibited the best migration inhibitory effect at doses of 20 and 30 µg/mL compared to forsythoside A and forsythoside I.

The transwell assay was next used to further evaluate anti-migration activity of forsythoside H and isoforsythiaside (Zaki et al., 2019). MDA-MB-231 cells were seeded in the upper chamber of transwell inserts containing serum-free medium and exposed to varying concentrations of forsythoside H and isoforsythiaside. The transwell inserts were placed into 24-well plates and 500 µL culture medium containing 10% FBS was added to each lower chamber. The membrane pore size between upper and lower chambers was 8.0 µm, so that invasive cancer cells could migrate from upper chambers to lower chambers. After drug incubation for 12 hours, the migrated cells were fixed by 4% paraformaldehyde for 30 min, then stained the cells with 0.1% crystal violet for 30 min. Migrated cells were visualized by microscope as shown in Figure 3C. The results of the transwell assay revealed that forsythoside H and isoforsythiaside at concentrations of 20 and 30 µg/mL exhibited effective migration inhibitory activity on MDA-MB-231 cancer cells (Figure 3C,D), which is consistent with previous findings in wound healing assay (Figure 3A,B).

To sum up, the anti-cancer growth and anti-migration activities of *F. suspensa* dried fruit aqueous extract against breast cancer cells were studied, of which the anti-migration activities of *F. suspensa* dried fruit was reported. Forsythoside H and isoforsythiaside were identified as important anti-metastatic compounds in *F. suspensa* dried fruit responsible for its anti-metastatic activity, which was evidenced by wound healing assay and transwell assay. Forsythoside H and isoforsythiaside demonstrated effective migration inhibitory activity on MDA-MB-231 cancer cells at doses 20 and 30 µg/mL. From these results, forsythoside H and isoforsythiaside could be potential drug candidates for developing novel anti-metastatic treatments.

The anti-metastatic activities of *F. suspensa* dried fruit aqueous extract and its bioactive compounds were only assessed by *in vitro* assays using wound healing and transwell approaches, further validation studies on evaluating their anti-metastatic activities in animals, such as mice, were deserved in the future.

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Ethical issue: The development, acquisition, authentication, cryopreservation, and transfer of cell lines between laboratories were followed according to the guidelines published in British Journal of Cancer, 2014.

Conflict of interest: The authors declare no competing interests.

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Wanrong Wang, Zongmao He and Jiaolin Bao

Key Laboratory of Tropical Biological Resources of Ministry of Education, School of Pharmaceutical Sciences, Collaborative Innovation Center of One Health, Hainan University, Haikou, 570228, China.

Corresponding author: Jiaolin Bao

email: baojiaolin@hainanu.edu.cn

References

Bao J, Ding R, Zou L, Zhang C, Wang K, Liu F, Li P, Chen M, Wan JB, Su H, Wang Y. *Forsythiae fructus* inhibits B16 melanoma growth involving MAPKs/Nrf2/HO-1 mediated anti-oxidation and anti-inflammation. Am J Chin Med. 2016; 44: 1043-61.

Bao J, Liu F, Zhang C, Wang K, Jia X, Wang X, Chen M, Li P, Su H, Wang Y, Wan JB. Anti-melanoma activity of *Forsythiae Fructus* aqueous extract in mice involves regulation of glycerophospholipid metabolisms by UPLC/Q-TOF MS-based metabolomics study. Sci Rep. 2016: 6: 39415.

Geraghty RJ, Capes-Davis A, Davis JM, Downward J, Freshney RI, Knezevic I, Lovell-Badge R, Masters JR, Meredith J, Stacey GN, Thraves P, Vias M. Guidelines for the use of cell lines in biomedical research. Br J Cancer. 2014; 6, 1021-46.

Gerstberger S, Jiang Q, Ganesh K. Metastasis. Cell 2023; 186: 1564-79.

Guan X. Cancer metastases: Challenges and opportunities. Acta Pharm Sin B. 2015; 5: 402-18.

Lee SE, Lim C, Ahn SC, Cho S. A study of the anti-cancer effects of the hexane fraction of the methanol extract of *Forsythiae fructus*. Pharmacogn Mag. 2017;13: 719.

Li R, Li H, Lan J, Yang D, Lin X, Xu H, Han B, Yang M, Su B, Liu F, Jiang W. Damnacanthal isolated from morinda species inhibited ovarian cancer cell proliferation and migration through activating autophagy. Phytomedicine. 2022; 100: 154084.

Nie CD, Deng B, Liu Y, Huang YF, Zhang SS, Yan XJ, Wen J. Chemical constituents of phenylethanoid glycosides from *Forsythiae Fructus* and their antitumor activities. Zhongguo Zhong Yao Za Zhi. 2022; 47: 6641-46.

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021; 71: 209-49.

Yang HX, Liu QP, Zhou YX, Chen YY, An P, Xing YZ, Zhang L, Jia M, Zhang H. Forsythiasides: A review of the pharmacological effects. Front Cardiovasc Med. 2022; 9: 971491.

Zhang WG, Liu Q, Lei CP. *Forsythia suspensa* extract has inhibitory effect on proliferation and apoptosis of A549 lung cancer cells. Trop J Pharm Res. 2019; 18: 1949-54.

Zaki SSO, Kanesan L, Leong MYD, Vidyadaran S. The influence of serum-supplemented culture media in a transwell migration assay. Cell Biol Int. 2019; 10: 1201-04.