Environment International 181 (2023) 108279

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Full length article

High-throughput screening of ancient forest plant extracts shows cytotoxicity towards triple-negative breast cancer

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ARTICLE INFO

Handling Editor: Adrian Covaci

Keywords:
GC-MS
LC-QTOF-MS
Phytochemicals
Apoptosis
Global goals
Planetary health

ABSTRACT

According to the World Health Organization, women's breast cancer is among the most common cancers with 7.8 million diagnosed cases during 2016–2020 and encompasses 15 % of all female cancer-related mortalities. These mortality events from triple-negative breast cancer are a significant health issue worldwide calling for a continuous search of bioactive compounds for better cancer treatments. Historically, plants are important sources for identifying such new bioactive chemicals for treatments. Here we use high-throughput screening and mass spectrometry analyses of extracts from 100 plant species collected in Chinese ancient forests to detect novel bioactive breast cancer phytochemicals. First, to study the effects on viability of the plant extracts, we used a MTT and CCK-8 cytotoxicity assay employing triple-negative breast cancer (TNBC) MDA-MB-231 and normal epithelial MCF-10A cell lines and cell cycle arrest to estimate apoptosis using flow cytometry for the most potent three speices. Based on these analyses, the final most potent extracts were from the Amur honeysuckle (*Lonicera maackii*) wood/root bark and Nigaki (*Picrasma quassioides*) wood/root bark. Then, 5×10^6 MDA-MB-231 cells were injected subcutaneously into the right hind leg of nude mice and a tumour was allowed to grow before treatment for seven days. Subsequently, the four exposed groups received gavage extracts from Amur honeysuckle and Nigaki (Amur honeysuckle wood distilled water, Amur honeysuckle root bark ethanol, Nigaki wood ethanol or Nigaki root bark distilled water/ethanol (1:1) extracts) in phosphate-buffered saline (PBS), while the control group received only PBS. The tumour weight of treated nude mice was reduced significantly by 60.5 % within 2 weeks, while on average killing 70 % of the MDA-MB-231 breast cancer cells after 48 h treatment (MTT test). In addition, screening of target genes using the Swiss Target Prediction, STITCH, STRING and NCBI-gene database showed that the four plant extracts possess desirable activity towards several known breast cancer genes. This reflects that the extracts may kill MBD-MB-231 breast cancer cells. This is the first screening of plant extracts with high efficiency in 2 decades, showing promising results for future development of novel cancer treatments.

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https://doi.org/10.1016/j.envint.2023.108279
Received 18 September 2023; Received in revised form 15 October 2023; Accepted 18 October 2023
Available online 29 October 2023

1. Introduction

According to the World Health Organization, breast cancer is responsible for most non-infectious disease mortalities before the age of 70 (WHO, 2020; Bray et al., 2018). It is among the most common cancers with 7.8 million diagnosed cases during 2016–2020 and encompasses 15 % of all cancer-related mortalities (Sung et al., 2021; WHO, 2021; Siegel et al., 2019; Ferlay et al., 2021). Breast cancer mortality in low- and middle-income countries (LMICs) is higher than in high-income countries that has decreased by 30–40 % over the past 40-years despite which countries? high breast cancer incidence (Fig. 1) (WHO, 2021). Carcinogenesis is a complex phenomenon involving many signal cascades including the interaction of tumour cells with stromal cells, immune cells, and blood vessels. Traditional oncology focuses on the application of targeted drugs (McDonald et al., 2016; Teoh et al., 2019); nevertheless, taking specific targeted drugs for a long time can easily lead to chemotherapy resistance. The development of chemotherapy resistance is multi-factorial, and possible factors include changes in drug pharmacokinetics and drug targets (Donker et al., 2014).

Current treatments of breast cancer include surgeries, and targeted immune, radiation and chemotherapy, often causing significant loss of quality of life for patients and large multi-billion socioeconomic burdens (Mirza and Karim, 2021; Zachariah et al., 2021; Lyu et al., 2019). Most available pharmaceutical drugs have several unwarranted side effects, emphasizing the need for new and more efficient treatments. This may include natural phytochemical compounds from medical plants and the search for natural plants exerting cytotoxicity towards cancer cells at low doses with minimize side effects as much as possible (Cámara-Leret and Bascompte, 2021; Holzmeyer et al., 2020).

Several medical plants possess anti-tumour secondary metabolites as reflected in a number of non-clinical trials (Table S1). Plants have been used in medicine since ancient times (Aung et al., 2017). The phytochemical diversity allows plants to produce a large variety of active chemical substances with significant biological effects (Jiang et al., 2019), of which a large number have been identified (Martin et al., 2021). Among the 370,000 plants that is scientifically described and named (Hanahan and Weinberg, 2011), 6 % has been subjected to phytochemical studies of biological activities (Holohan et al., 2013). Thus, there are still a large number of undiscovered plants with potentially useful biologically active compounds (Lu et al., 2021), and more

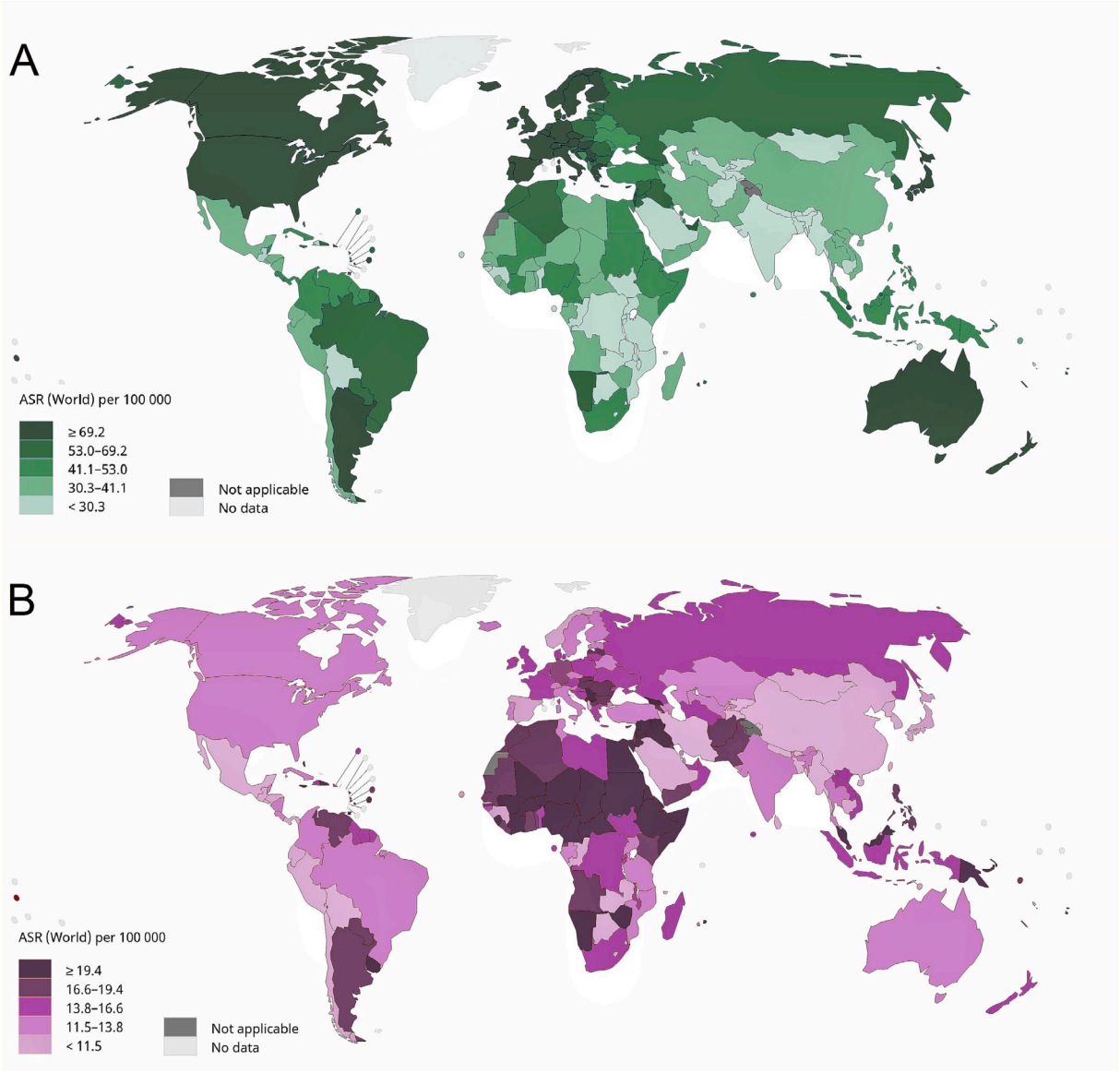

Fig. 1. Breast cancer in women in 2020. (**a**) Incidence and (**b**) mortalities of breast cancer incidence in 2020 in Each Country for Women all ages. ASR: age-standardized rate. Source: GLOBOCAN 2020.

studies are required to identify target species for screening and phytochemical evaluation in an effort to identify more chemicals with therapeutic properties (Defossez et al., 2021; Nett et al., 2021; Gardens, 2017).

To investigate the biological activity of phytochemicals further, we collected 100 plant species in the Henan Region, China scanning 479 extracts for novel bioactive compounds with cancer killing effects. We use GC–MS and LC-QTOF-MS were used to analyze compound composition and in vitro cytotoxicity and inhibition in vivo tumour growth testing of the extracts. Finally, we apply network pharmacology to study regulation of multiple gene targets, providing a basis for future development of novel forest plantbased drugs.

2. Materials and methods

2.1. Sampling and chemical extraction

A total number of 100 plant species was collected from tree forest areas in China. Seventeen plants of four seasons (spring, summer, autumn and winter) were collected from the forest area of Luanchuan County, Luoyang City while 48 plant species were collected from Biyang County, Zhumadian City. In addition, 35 species came from Lingbao City, Sanmenxia City, Henan Province (Fig. 2; see Supplementary Information for further details for further details. We divided the collected material into six sub-samples; leaf, branch, bark, wood, root and root bark resulting in 479 samples in total (see Supplementary Information for further details). These were then homogenized and extracted with ethanol, benzene/ethanol (1:1), distilled water/ethanol (1:1) and distilled water (the ratio of sample to solvent being 1:30). Amur honeysuckle wood distilled water extract and root bark ethanol extract were named LM4-4 and LM5-1. Nigaki wood ethanol extract and root bark distilled water/ethanol (1:1) extract were named PQ4-1 and PQ6-3. Finally, the extract was concentrated to 10 mL. The ethanol and benzene were obtained from Tianjin Fuyu Fine Chemical Co., Ltd. and Yantai Shuangshuang Chemical Co., Ltd., and distilled water was produced from the water plant of Henan Agricultural University (See Supplementary Information for further details).

Fig. 2. Map of the tree sampling locations of 100 plant species included in the phytochemical screening in the present study.

2.2. Cytotoxicity and flow cytometry

To study the effects of the plant extracts on viability, an MTT and CCK-8 cytotoxicity assay was conducted employing triple-negative breast cancer (TNBC) MDA-MB-231, normal epithelial MCF-10A cells lines and cell cycle arrests to detect effects on cellular metabolic and mitochondrial activity. First, 479 ethanol extracts from the 100 collected plants were screened followed by another screening of 42 ethanol extract from 11 plant species (selection range: MDA-MB-23 kill rate $\geq$ 60 %, see Supplementary Information and Table S2 for further details). This was followed by a second MTT test of extracts from 11 plant species screening five extract samples from three plant species using distilled water/ethanol (1:1) extracts. These were Amur honeysuckle (*Lonicera maackii*), *Diospyros lotus* and Nigaki (*Picrasma quassioides*). These extracts had a killing rate of $\geq$ 65 % for MDA-MB-23 and $\leq$ 35 % for MCF-10A cell lines (Table S3, Fig. 3A, B and Supplementary Information for further details). The efficiency of the three plant extracts killing MDA-MB-23 breast cancer cells were verified using the Cell Counting Kit-8 (CCK-8) assay. At the end, only Amur honeysuckle (*Lonicera maackii*) extracts of wood/root bark and Nigaki (*Picrasma quassioides*) wood/root bark were screened for inhibition of MDA-MB-231 cells (Table S4, Fig. 3C; see Supplementary Information for further details).

Flow cytometry was conducted to estimate cell apoptosis. MDA-MB-231 cells with logarithmic growth and being in good condition were digested, centrifuged and finally counted. A total of 1.0×10^6 cells were added into 2 mL DMEM (Dulbecco's modified Eagle medium) containing 10 % FBS (fetal bovine serum) in each well and 6-well plates were laid. 6-well plates were incubated for 24 h and then treated with indicated concentrations of phyto extracts for an additional 20 h at 37 °C. Cells were then collected in 100ul Annexin V-FITC binding buffer and stained with Annexin V and Propidium Iodide (Dojindo, Japan). The percentage of apoptosis was then analyzed by Flowjo software and instrument model Beckman cytoflex (Fig. 5A, and Supplementary Information for further details).

2.3. Cell line derived xenografts and in vivo treatment

A total of 5×10^6 MDA-MB-231 breast cancer cells were subcutaneously injected into the right medial hind leg of 5 groups of 3 (total n = 15) nude mice to establish the xenograft model of the cell line. The tumour was then allowed to grow for seven days before treatment, and the mice were randomly divided into the 4 treatment groups wood and root bark from Amur honeysuckle and Nigaki, respectively, according to tumour volume and body weight. The mice were then treated by gavage for 2 weeks once a day with the control group given 200 μl of phosphate-buffered saline (PBS). The exposed groups received 200 μl of PBS containing plant extracts from Amur honeysuckle wood, distilled water, Amur honeysuckle root bark ethanol, Nigaki wood ethanol and Nigaki root bark distilled water/ethanol (1:1) extract. The body weight and tumour volume of the mice were measured every 3rd day, and finally the tumours were weighed after the end of treatment cycle.

2.4. Chemical analyses

We performed LC-QTOF-MS and GC/MS to analyze the extract composition of root and bark from the two most potent species Amur honeysuckle (*Lonicera maackii*) and Nigaki (*Picrasma quassioides*) (see Table S5-S11 and in Supplementary Information). First, we implemented an Agilent 7890B-5977A GC–MS equipped with a HP-5 MS (60 m × 250 μm × 0.25 μm) elasticquartz capillary column. The carrier gas used was high purity helium, with flow rate of 1.5 mL/min, and no divergence. The initial temperature program of the GC was 50 °C, increased to 250 °C at a rate of 8 °C/min. Then increased to 280 °C at a rate of 5 °C/min and maintained for 5 min. The MS program scan mass range was 30–600 amu, ionization voltage of 70 eV, ionization current

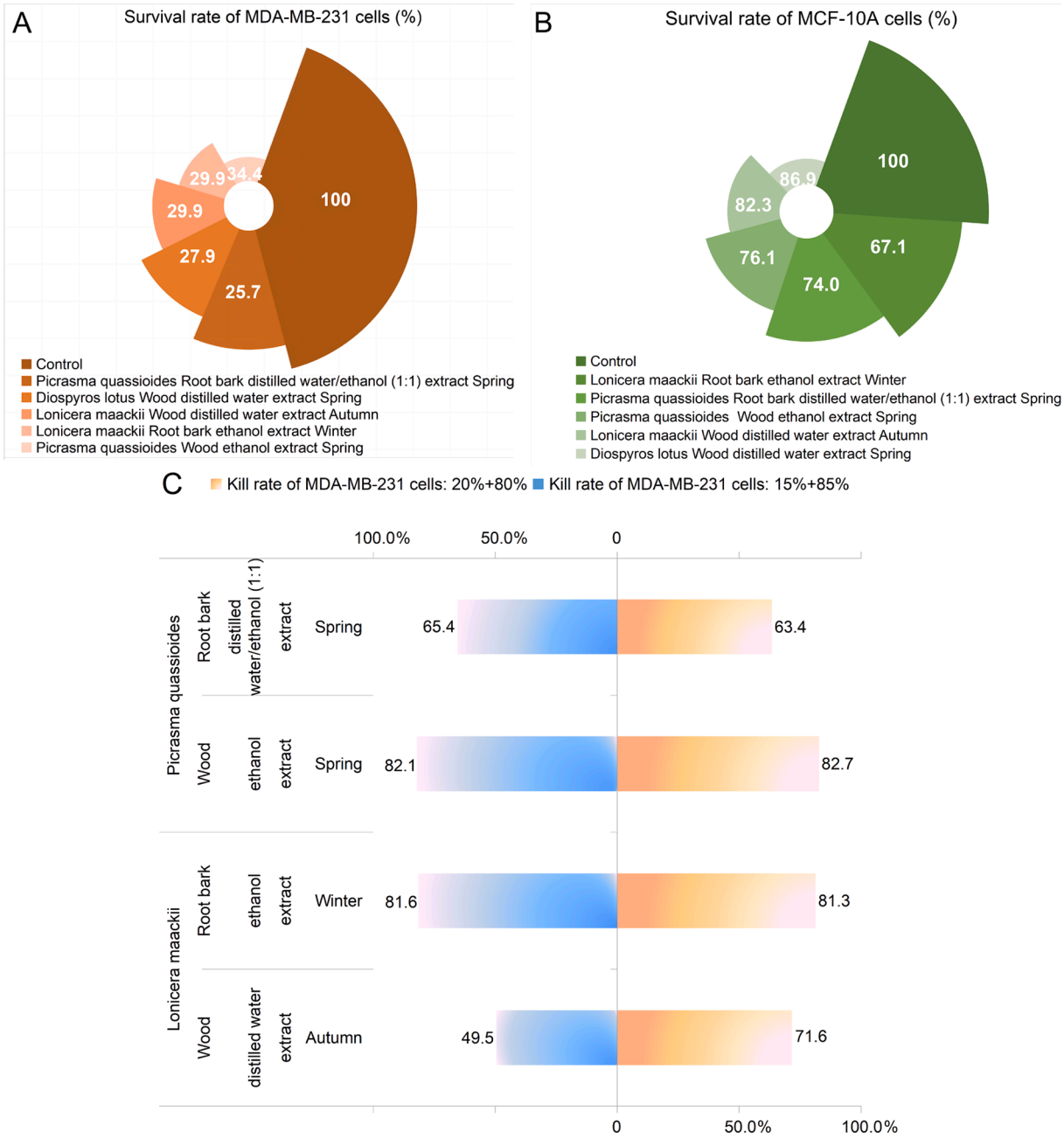

Fig. 3. (A and B) Killing rates of triple-negative breast cancer (TNBC) MDA-MB-231 cells (A) and healthy breast (B, MCF-10A) cells of *Amur honeysuckle* and *Nigaki* extracts detected by MTT. (C) Killing rates of cancer cells by Amur honeysuckle and Nigaki detected by CCK-8.

of 150 μA electron ionization (EI). The ion source and the quadrupole temperature were set to 230 °C and 150 °C, respectively. Then, we used Liquid Chromatography-Quadrupole Time of Flight-Mass Spectrometry (LC-QTOF-MS) analysis. LC: The chromatographic column was ACQUITY UPLC I-Class-Xevo G2-XS (2.1 × 50 mm, 1.7 μm). Mobile phase: 0.10 % (v/v) formic acid (A), acetonitrile with 0.10 % (v/v) formic acid (B). Flow rate: 0.30 mL/min. Column temperature: 30 °C. Post time: 5 min. Gradient elution: [Time (min), B (%)] was [0, 5], [2, 5], [20, 100], [25, 100] in turn. MS: Ion source: AJS ESI. Detection mode: positive ion mode. Capillary voltage: 3.0 kV. Sample cone: 40v. Source temperature: 100 °C. Desolvation temperture: 400 °C. Cone gas: 50L/h. Desolvation gas: 800L/h. Database Screening: Traditional Chinese Medicine Database in Waters UNIFI software. Scan mass range program: 50–1200 *m/z*. Reference ion: 121.0509 (64.0158), 922.0098 (Positive ion mode).

2.5. Modelling of target genes

https://www.chemicalbook.comSupplementary InformationWe conducted a screening of target genes associated with breast cancer using the Swiss Target Prediction, STITCH, STRING and NCBI-gene database. An interaction network was constructed using network pharmacology to analyze the complex relationship between these active components and their targets. All network maps were visualized and analyzed using Cytoscape 3.8.2 (http://www.cytoscape.org/).

2.6. Statistical analyses

Statistical analyses were conducted using Graph Pad Prism9.0 software. All data were tested for normality and a paired subject *t*-test was used to test for differences between control and sample group. In cases of

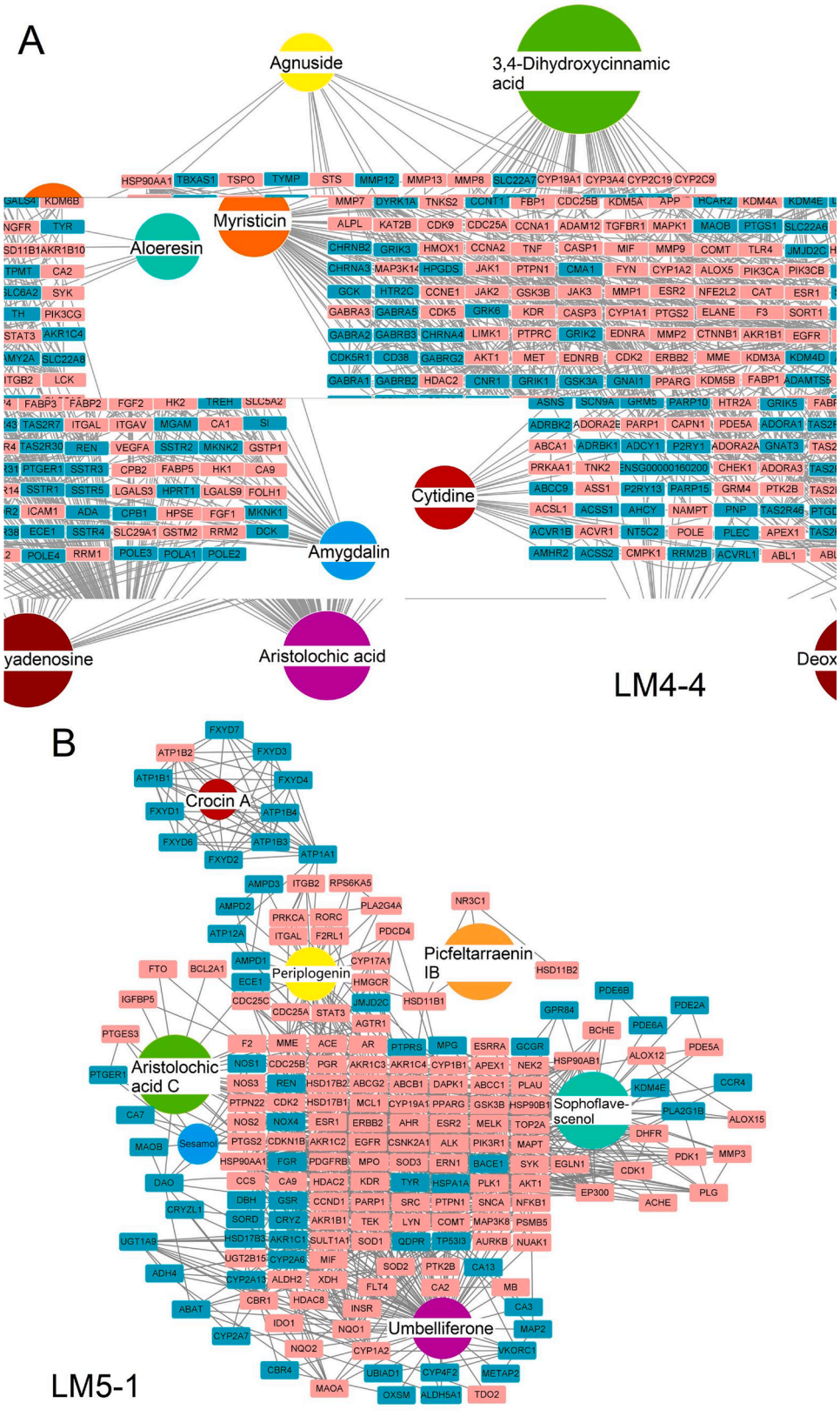

Fig. 4. Gene target network model of the chemical components of four samples acting on triple-negative breast cancer (TNBC) MDA-MB-231 cells. Red represents gene targets consistent with breast cancer while blue is not consistent. (**A**) Amur honeysuckle wood distilled water extract (LM4-4). (**B**) Amur honeysuckle root bark ethanol extract (LM5-1). (**C**) Nigaki wood ethanol extract (PQ4-1). (**D**) Nigaki root bark distilled water/ethanol (1:1) extract (PQ6-3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

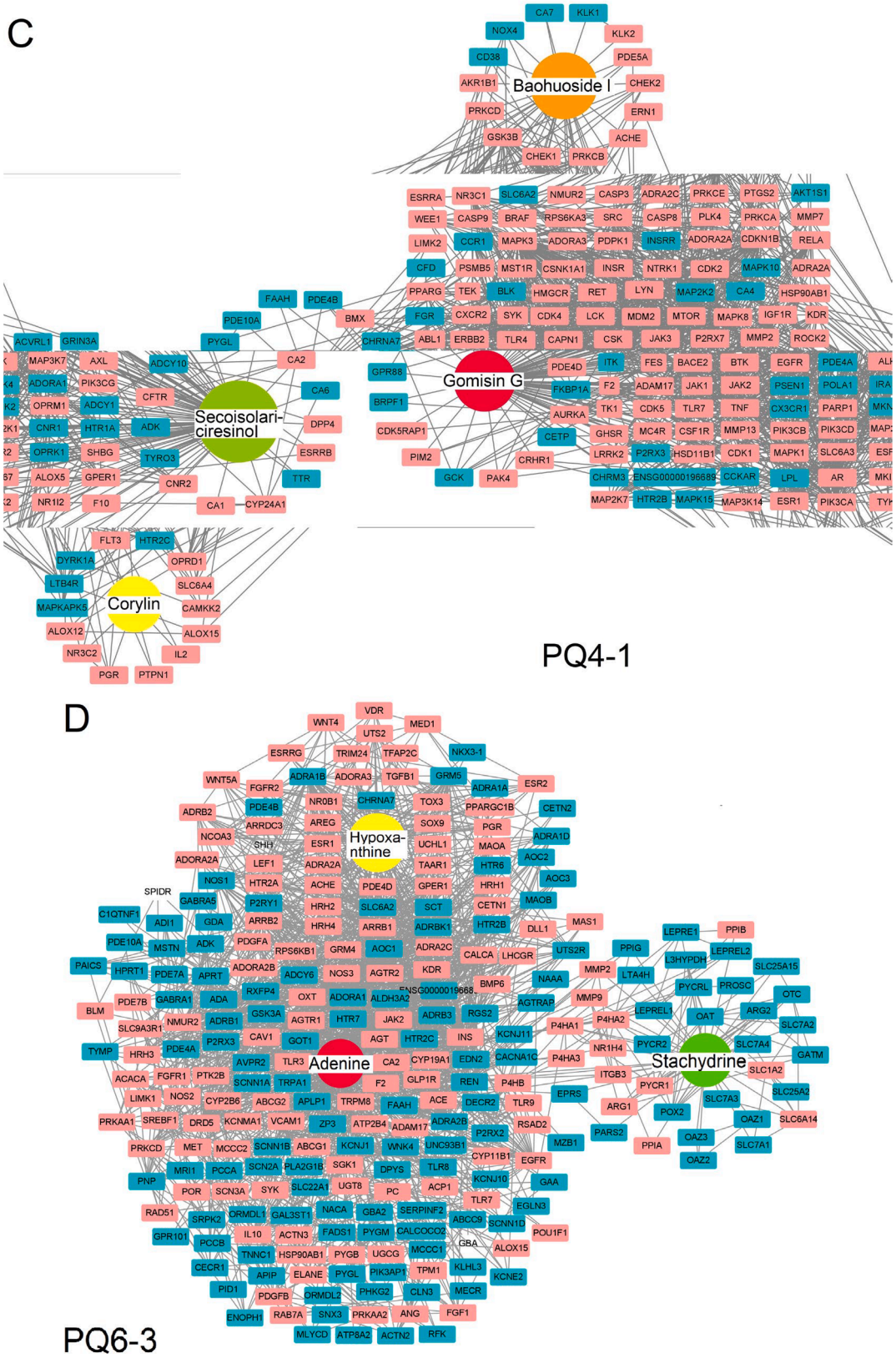

Fig. 4. (*continued*).

multiple groups, an ANOVA was applied followed by a Tukey Post Hoc test. The statistical significance was set to $p < 0.05$.

3. Results

3.1. Cell line MTT and CCK-8 treatment

In MCF-10A normal breast epithelial cells, we found the apoptosis rate between 13.1–32.9 % (Fig. 3A, B), while the apoptosis rate of MDA-MB-231 breast cancer cells was between 65.7–74.3 % (Fig. 3A, B, Table S3). The results of MTT cytotoxicity showed that extracts from plants effectively killed triple-negative breast cancer cells. We used the Cell Counting Kit-8 (CCK-8) detection method for confirmation, showing that extracts from Amur honeysuckle and Nigaki synergistically inhibited the growth of MDA-MB-231 cells up to 82.7 % (Fig. 3C, Table S4).

3.2. Chemical composition

The GC/MS analyses of Amur honeysuckle root bark and Nigaki wood ethanol extracts showed 64 and 48 compounds, respectively (Table S5 and 6). These compounds mainly included alcohols, esters, acids, aldehydes, ketones, phenols and ethers (Fig. S4). Based on this, we performed LC-QTOF-MS analyzis to identify the extracted compounds, revealing that esters, acids and ketones were the dominant compounds present in the wood and root bark extracts of the Amur honeysuckle and Nigaki (Table S7-10). For Amur honeysuckle, esters were the most abundant, while ketones were in the highest proportion for Nigaki. Among the high concentrations of active substances detected in Amur honeysuckle and Nigaki; 3,4-dihydroxycinnamic acid, myristicin, genipin, esculentoside A, secoisolariciresinol, baohuoside I, canthin-6-one, crocetin and sophoflavescenol possess anti-cancer and anti-tumour effects (Table S11). The toxicity analysis of components detected by GC/MS and QTOF-LC-MS analyses fell into low and high toxicity categories including side effects (Table S12). In addition, the percentage of cancer cells killed was significantly higher than that of normal breast cells ($p < 0.01$) (Fig. 3A and B).

3.3. Modelling target genes

The modelling of potential target genes is shown in Fig. 4. The red boxes represent several potential cancer target genes while the blue boxes indicate non-targets, which reflect potential undesirable side effects. These multi-target genes reflect anti-inflammatory and anti-proliferative endpoints, likely due to the contents of various flavonoids, alkaloids, and polyphenols (Table S13). The active compounds in the four plant extracts possess desirable activity towards several breast cancer genes, and thereby potentially indicating multi-target treatment endpoints (Fig. 4; see materials and methods). One of the effective ways to obtain new anti-cancer drugs is to synthesize a series of derivatives, and further studies are needed to identify individual bioactive compounds only affecting target genes (red boxes) shown in Fig. 4.

3.4. Cell viability

Flow cytometry analysis, cell apoptosis and cell line xenografts showed that the wood and root bark extracts of the Amur honeysuckle and Nigaki were the most potent extracts, significantly reducing the viability and proliferation of cancer cells but also the weight of the mice indicating that the extracts are toxic ($p < 0.01$). All four plant extracts significantly induced cell apoptosis and cytotoxicity on MDA-MB-231 breast cancer cells (Fig. 5A). These extracts were therefore used in the cell line derived xenografts and in vivo treatment of nude mice.

3.5. In vivo treatment and tumour growth

Tumours were dissected from the sacrificed animals two weeks after the injection of the four extracts (Fig. 5B). The tumour weight of treated tumour-bearing nude mice reduced significantly by approximately 60.5 % within 2 weeks ($p < 0.01$) (Fig. 5D). The tumour volume of animals treated with Amur honeysuckle and Nigaki extracts was reduced by approximately 63.2 % within 2 weeks, except for Amur honeysuckle root bark ethanol extract (Fig. 5B and C). For Amur honeysuckle root bark ethanol extracts, tumour weight was reduced by 59.8 % (Fig. 5C). The plant extract killed about 70 % of the MDA-MB-231 breast cancer cells within 48 h in the MTT test (Fig. 3A). Furthermore, the breast cancer tumour weight was reduced significantly by 56.9–64.2 % during

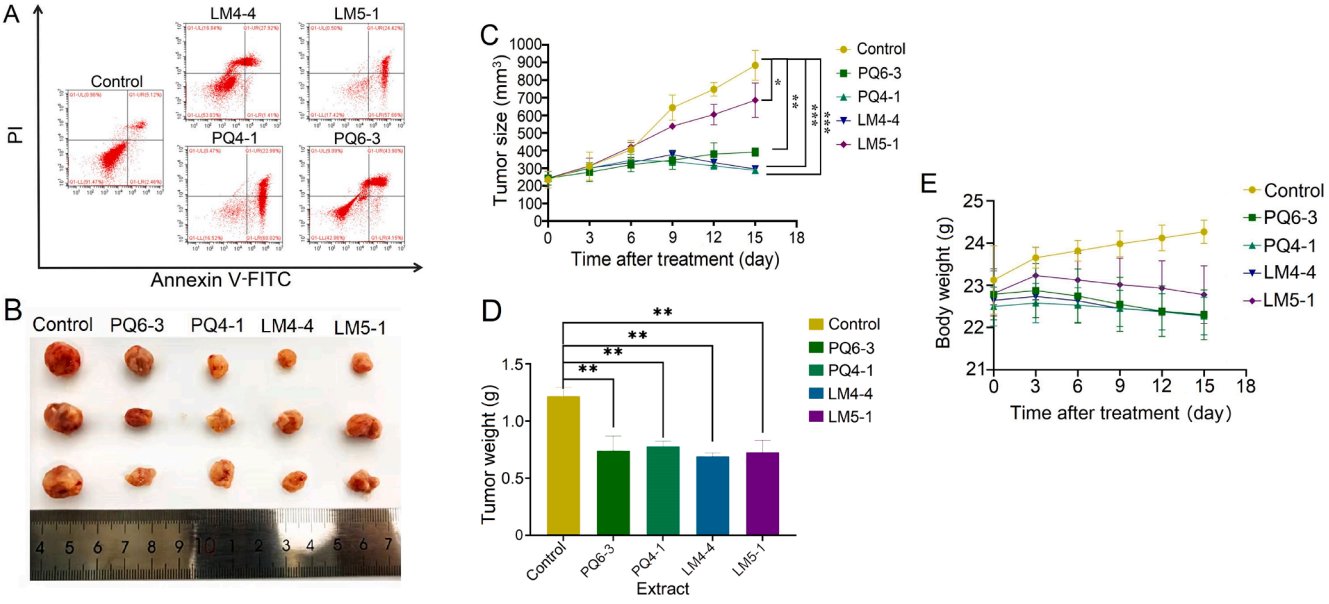

Fig. 5. (**A**) Apoptosis rate of triple-negative breast cancer (TNBC) MDA-MB-231 cells detected by flow cytometry. (**B**) Tumour size images measured two weeks after treatment using four kinds of extracts. (**C**) Average tumour volume following tumour growth kinetics-treatment. (**D**) Average tumour weight after two weeks of treatment. (**E**) The average weight change of tumour-bearing mice within two weeks.

the 2-week treatment period (all $p < 0.01$) (Fig. 5D). Body weight in the four treatment groups decreased marginally compared to the control group and this was not significant (all $p > 0.05$) (Fig. 5E).

4. Discussion

The plant extract mixture in our study contained a high number of potential anti-cancer and anti-tumour phytochemicals. This reflects the prospects for Amur honeysuckle and Nigaki extracts to treat TNBC with several endpoint targets that could potentially target more than just one type of breast cancer cells. This is the first study demonstrating how Amur honeysuckle and Nigaki extracts inhibit tumour growth and kill MDA-MB-231 breast cancer cells and is a major first step forward showing anti-cancer efficacy. Compared with for example *Taxus wallichiana* (Iqbal et al., 2020); Amur honeysuckle and Nigaki plant extracts are relatively easy to obtain and possess similar activity due to their phytochemical content including 3,4-dihydroxycinnamicacid and myristicin (Table S12). 3,4-dihydroxycinnamic acid has anti-mutagenic and anti-cancer pharmacological effects (Rahaman et al., 2018), while myristicin shows cytotoxic and apoptotic effects towards cancer in human cell lines (P., 2019). Likewise, baohuoside I is a novel potential anticancer compound reducing the proliferation of a variety of cancers (Guo et al., 2020). The ethanol extracts of Amur honeysuckle root bark and Nigaki wood are rich in active chemical components containing flavonoids. Flavonoids are plant-derived polyphenols, and part of the normal human diet, that may affect health both positively and negatively depending on dose–response relationships (Bondonno et al., 2019). Previously, preclinical studies have shown how flavonoids may reduce the risk of cancer (Carocho and Ferreira, 2013).

The triple-negative breast cancer (TNBC) MDA-MB-231 cell line represents the most malignant, and lethal breast cancer type with poor prognosis and treatment options (Chonghaile et al., 2011; Kim et al., 2021; Balko et al., 2014; Denkert et al., 2017). It does not overexpress Human Epidermal Growth Factor Receptor 2 nor is this cell line expressing the estrogen and progesterone receptors (Kim et al., 2021). Therefore, TNBC cases suffer from a lack of effective targeted therapy making the majority of the cases with lethal outcomes (Chonghaile et al., 2011). Research efforts have been put into the exploration of phyto extracts for cancer treatment (Khan et al., 2021). Polyphenols for example, are a broad class of plant metabolites possessing anti-tumour activity reducing metastasis through cell survival and proliferation (Hasitha and Dharmesh, 2018). Currently, chemotherapy using anthracyclines and taxane-based drugs is still of the first choice for treating TNBC patients (Park et al., 2018). Nevertheless, long-term use of a single targeted therapy could undesirably lead to drug resistance in up to 70 % of TNBC patients (Dowling et al., 2021).

Our study shows that extracts from Amur honeysuckle and Nigaki affect multiple target genes related to tumour growth and cancer. Compounds detected in the extracts include 3,4-dihydroxycinnamic acid, myristicin, genipin, esculentoside A, secoisolariciresinol, baohuoside I, canthin-6-one, crocetin and sophoflavescenol, all showing bioactivity towards anti-cancer and anti-tumour effects (Table S12). Of these, esculentoside A and secoisolariciresinol inhibit the proliferation of breast cancer stem cells and induce breast cancer stem cell apoptosis and show strong anti-oxidative properties in laboratory animals and cell lines, respectively (Liu et al., 2018; Bowers et al., 2019). This reflects that extracts of Amur honeysuckle and Nigaki contain a high number of bioactive ingredients of which some show potential to be developed into authorized drugs over the coming decades.

Compared to the one-drug and one-target model implemented in classic drug development, plant extracts are characterized by having multiple active ingredients and target points, which is a challenge concerning drug development (Singh et al., 2016). For example, the phytochemicals detected by QTOF-LC-MS act on several genes including ABCA1, ABL1, SHBG, STAT3 CDK2 CYP1A1 FYN and TYR due to the content of secoisolariciresinol, aristolochic acid, myristicin and 3,4-dihydroxycinnamic acid (Seo et al., 2011; Messina et al., 2011; Bolvig et al., 2016; Leung et al., 2017; Liu et al., 2020; Zhu et al., 2019; Kang et al., 2009; Rezg et al., 2015). Further elucidations on chemical-target interactions require preparative HPLC, NMR and structure identification to recognize specific compounds, receptors and pathways including dose–response studies (Fu et al., 2021). Previous studies showed that extracts from *Withania somnifera* inhibit xenograft MDA-MB-231 breast cancer cell proliferation in nude mice reducing tumour size by 60 % within 8 weeks of treatment (Khazal and Hill, 2015). Likewise, 20 μg of total phenolics from muscadine grapes (*Vitis rotundifolia*) inhibited the proliferation of MDA-MB-231 breast cancer cells by 44 % (Collard et al., 2020), and rosemary extract show a maximum inhibition rate at 35 % when applied at a concentration of 50–100 ug/mL (Jaglanian and Tsiani, 2020). In comparison, *Lippia origanoides* extract reduce the viability of MDA-MB-231 breast cancer cells to about 2 % at the highest dose of 0.15 mg/mL within 48 h while the viability of normal MCF-10A cells reduces to about 10 % within 72 h (Raman et al., 2018). This is in contrast to our study where the extract kills 70 % of the MDA-MB-231 breast cancer cells within 48 h and reduce tumour weight by 60 % within 2 weeks. Compared to other phytochemicals, the current extracts from Amur honeysuckle and Nigaki possess superior properties, but further studies are required to establish causal relationships between individual compounds in the extracts and the targets resulting in the observed anti-cancer effects.

To our knowledge, the present study is the first to report the effective medicinal effects of Amur honeysuckle and Nigaki on TNBC. These studies are all laboratory studies on extracts and do not pinpoint one-drug one-target and therefore call for further studies that recognize the specific compounds, their receptors and safety measures through classical non-clinical and clinical trials. More work is therefore needed, taking Paclitaxel (taxol) being a natural secondary metabolite from the bark of gymnosperm *Taxus wallichiana*, currently used to treat primary and metastatic tumours, as an example (Adams and Molinero, 2019; Scribano Christina, 2021; Rathaur et al., 2021). This study provides evidence that finding novel plant extract shows great promise for the future development of new TNBC pharmaceutical cancer drugs supporting the WHO Global Breast Cancer Initiative (GBCI) to reduce global breast cancer mortality year by year in the future (WHO, 2021).

5. Conclusions

The selected wood and root bark extracts possess significant cytotoxic properties to breast cancer cells. *In vivo* and *in vitro* experiments of extracts from Amur honeysuckle and Nigaki demonstrated induced apoptosis and cell death of triple-negative MDA-MB-231 breast cancer cells with only a few side effects on normal breast epithelial cells (MCF-10A). The tumour weight of treated animals was reduced by approximately 61 % within 2 weeks, while killing 70 % of the MDA-MB-231 breast cancer cells at 48 h treatment. This is the first study in two decades on the identification and application of highly efficient plant candidates, showing great efficiency for future treatment of breast cancer. Among others, it may pave forward novel cancer treatments for better livelihood and lower socioeconomic burdens, but further identification of active ingredients, along with clinical and non-clinical trials, to identify targets and non-targets, are required.

CRediT authorship contribution statement

Yiyang Li: Investigation, Visualization, Writing – original draft. **Nyuk Ling Ma:** Methodology, Writing – original draft. **Huiling Chen:** Investigation, Visualization. **Jiateng Zhong:** Investigation, Visualization, Writing – original draft. **Dangquan Zhang:** Investigation, Visualization. **Wanxi Peng:** Conceptualization, Methodology, Investigation, Visualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Su Shiung Lam:** Writing – review & editing. **Yafeng Yang:** Investigation, Visualization.

Xiaochen Yue: Investigation, Visualization. **Lijun Yan:** Investigation, Visualization. **Ting Wang:** Investigation, Visualization. **Bjarne Styrishave:** Writing – review & editing. **Tomasz Maciej Ciesielski:** Writing – review & editing. **Christian Sonne:** Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The assistance provided by Dongfang Zheng, Jianqi Li, Yaoming Wang, Shuaiwei Dong, Hongqi Yang, Dongfeng Yan, Yuqiang Sang, and Huitao Bi in collecting samples. Ruirui Guan, Quan Zhou, Zihan Wei, Yacheng Wang, Yuhao Peng, Chaofan Suo, and Cheng Li are acknowledge for assistance the laboratory work and testing. The authors would also like to thank Yuan Ze University and Saveetha Institute of Medical and Technical Sciences for the facilities and support provided. Competing interests: Authors declare no competing interests. Data and materials availability: Read raw data is in Figures, Tables and extended data in Supplementary Information. Program for Innovative Research Team (in Science and Technology) in University of Henan Province Grant No. 21IRTSTHN020 (WP), Central Plain Scholar Funding Project of Henan Province Grant No. 212101510005 (WP).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.108279.

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