ANTIOXIDANT AND ANTICANCER ACTIVITIES OF MUSHROOM *Phellinus linteus* AND KHAAO-YEN-NUEA *Smilax* sp. EXTRACT MIXTURE

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ฤทธิ์การต้านอนุมูลอิสระและฤทธิ์ต้านเซลล์มะเร็งของส่วนผสมสารสกัดจากเห็ด *Phellinus linteus* และข้าวเย็นเหนือ *Smilax* sp.

น.ส.อัลวี จอห์ฮาโรตัส ยูคริยะ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	ANTIOXIDANT AND ANTICANCER ACTIVITIES OF MUSHROOM *Phellinus linteus* AND KHAAO-YEN-NUEA *Smilax* sp. EXTRACT MIXTURE
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อัลวี จอห์ฮาโรตัส ยูคริยะ : ฤทธิ์การต้านอนุมูลอิสระและฤทธิ์ต้านเซลล์มะเร็งของส่วนผสมสารสกัดจากเห็ด *Phellinus linteus* และข้าวเย็นเหนือ *Smilax* sp.. (ANTIOXIDANT AND ANTICANCER ACTIVITIES OF MUSHROOM *Phellinus linteus* AND KHAAO-YEN-NUEA *Smilax* sp. EXTRACT MIXTURE) อ.ที่ปรึกษาหลัก : รศ. ดร.ปฐมวดี ญาณทัศนีย์จิต, อ.ที่ปรึกษาร่วม : รศ. ดร.สีหนาท ประสงค์สุข

ในปัจจุบัน จำเป็นต้องมีการตรวจสอบแหล่งทางเลือกจำนวนมากรวมถึงสารออกฤทธิ์ทางชีวภาพจากพืชและเชื้อราเพื่อรักษามะเร็ง การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ต้านมะเร็งของสารสกัดหยาบ *Smilax* sp. และ *Phellinus linteus* แต่ละชนิด รวมถึงอัตราส่วนผสมต่าง ๆ ได้แก่ สารผสม 1 (*Smilax* sp. 50%: *P. linteus* 50%), สารผสม (*Smilax* sp. 25%: *P. linteus* 75%) และสารผสม 3 (*Smilax* sp. 75% : *P. linteus* 25%) สารสกัดแต่ละชนิดและสารผสมถูกตรวจสอบลักษณะทางเคมีกายภาพ ได้แก่ ปริมาณฟีนอลิกรวมและปริมาณพอลิแซ็กคาไรด์รวม นอกจากนี้ฤทธิ์ต้านอนุมูลอิสระของสารสกัดถูกตรวจสอบด้วยวิธี DPPH และ ABTS ฤทธิ์ต้านมะเร็งในเซลล์มะเร็งเต้านมสามชนิดที่แตกต่างกัน รวมถึง ความเป็นพิษต่อเซลล์ การต้านการเพิ่มจำนวน การก่อโคโลนี และการตายแบบอะพอพโทซิส สารสกัดจาก *Smilax* sp. มีปริมาณฟีนอลิกรวมสูง ในขณะที่สารสกัดจาก *P. linteus* มีปริมาณพอลิแซ็กคาไรด์รวมสูง ปริมาณฟีนอลิกรวมและปริมาณพอลิแซ็กคาไรด์รวมของสารผสมขึ้นอยู่กับอัตราส่วนสัมพัทธ์ของสารสกัดแต่ละชนิด ฤทธิ์ต้านอนุมูลอิสระแสดงค่าที่สูงขึ้นผ่านการทดสอบ DPPH และ ABTS คือ *Smilax* sp. และสารผสม 3 ตามด้วย สารผสม 1 สารผสม 2 และ *P. linteus* ผลความเป็นพิษของสารสกัดต่อเซลล์มะเร็งเต้านมมีความสัมพันธ์และมีแนวโน้มเดียวกันกับฤทธิ์ต้านอนุมูลอิสระ นอกจากนี้บางสารผสมแสดงผลยับยั้งการเพิ่มจำนวนของเซลล์มะเร็งเต้านมได้ดีกว่าสารสกัดแต่ละชนิดทั้งสองชนิด เช่นเดียวกับการเพิ่มจำนวนเซลล์ บางสารผสมแสดงผลที่ดีที่สุดในการลดการก่อโคโลนีเมื่อเปรียบเทียบกับสารสกัดแต่ละชนิด อย่างไรก็ตาม ผลการเหนี่ยวนำการตายแบบอะพอพโทซิสไม่มีประสิทธิภาพเนื่องจากมีค่าน้อยกว่าร้อยละ 5 โดยสรุป สารสกัดแต่ละชนิดและสารผสมมีสมบัติเป็นสารต้านอนุมูลอิสระและสารต้านมะเร็ง

สาขาวิชา เทคโนโลยีชีวภาพ ลายมือชื่อนิสิต .....

ปีการศึกษา 2566 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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Alvi Jauharotus Syukriya : ANTIOXIDANT AND ANTICANCER ACTIVITIES OF MUSHROOM *Phellinus linteus* AND KHAAO-YEN-NUEA *Smilax* sp. EXTRACT MIXTURE. Advisor: Assoc. Prof. PATTAMAWADEE YANATATSANEEJIT, Ph.D. Co-advisor: Assoc. Prof. SEHANAT PRASONGSUK, Ph.D.

Recently, a number of alternative source including the bioactive compounds from plant and fungi are necessary to explore as therapeutic against cancer. This study aims to investigated the antioxidant and anticancer activities of *Smilax* sp. and *Phellinus linteus* (*P. linteus*) crude extract individually, also their mixture ratio variation including combination 1 (*Smilax* sp. 50%: *P. linteus* 50%), combination 2 (*Smilax* sp. 25%: *P. linteus* 75%), and combination 3 (*Smilax* sp. 75%: *P. linteus* 25%). The individually extract and its mixtures were investigated the physiochemical characterization such as total phenolic content and total polysaccharide compound. Furthermore, the antioxidant activities of extracted samples were determined by DPPH and ABTS assays. The anticancer activities on three distinct breast cancer cell lines as well as cytotoxicity, antiproliferative, colony formation, and apoptosis. The individually *Smilax* sp. extract revealed a high level of total phenolic content, while individually extract of *P. linteus* had a great amount of total polysaccharide content. The total phenolic content and total polysaccharide content of all combinations were relied on the relative ratios of individual extract content. The antioxidant activities showed the higher value through DPPH and ABTS assays were individual *Smilax* sp. and combination 3, followed by combination 1, combination 2 and *P. linteus*. The toxicity results of the extracted samples on breast cancer cell lines were related and have the same trends to its antioxidant activities. Moreover, certain combinations exhibited a greater inhibitory effects on cell proliferation against breast cancer than both individual extracts. Similar to cell proliferation, some combinations showed the greatest effect to reduce the form of colonies compare to individual extracts. However, the apoptosis induction results were not potent due to the value less than 5%. In conclusion, the individual extracts and in combination have their capabilities as antioxidant and anticancer agent.

Field of Study:	Biotechnology	Student's Signature .....
Academic Year:	2023	Advisor's Signature .....
		Co-advisor's Signature .....

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CHAPTER I

INTRODUCTION

1.1 Rationale

In 2018, new cases of cancer are expected around 18.1 million, with 9.6 million cancer related death cases. Thus, this disease continues to be leading of morbidity, mortality and increasing cost of healthcare worldwide. Breast cancer is the most prevalence among females and becomes primary cause of cancer death, estimated 2.3 million new cases globally a year. Moreover, the prevalence of breast cancer worldwide might be increased throughout the new cases of male breast cancer (Bray et al., 2018; Leone et al., 2021; Nardin et al., 2020).

In addition to genetics, various risk factors and socio-lifestyle factors such smoking, drinking, eating fast food, getting little exercise, and obesity have contributed to the development of the condition. Additionally, unfavorable environmental factors like radical exposure might disrupt a cell's biological equilibrium. One of the effects of an imbalance in cell homeostasis brought on by a lack of antioxidants and an increase in the formation of reactive oxygen species (ROS) is oxidative stress. Even though ROS may exhibit a variety of defining characteristics, like starting DNA damage and dysregulate gene expression, it is implicated in the etiology of many illnesses, including cancer. It has become evident that ROS levels play a crucial role in cancer cell growth, development, and cell proliferation as a result of cancer-associated cellular stress (Khan et al., 2021; Tufail et al., 2021).

Currently, existing therapeutic approaches such as radiotherapy and chemotherapy have failed to provide adequate relief to cancer patients due to non-selective cell death (Trachootham et al., 2009). Furthermore, drug resistance is a common side effect of cancer and a major contributor to chemotherapy failure. As a result, there is still a need to investigate more effective, safer, and inexpensive

anticancer medications from alternative sources. Several studies have recently concentrated on identifying bioactive chemicals with anticancer potential from natural sources such as plants and fungi (Lukong, 2017; Sarfraz et al., 2020).

Traditional herb medicine is gaining popularity as a therapeutic treatment. Among this, previous study showed that phenolic compounds were largely found substances in *Smilax* sp. as medicinal plants. These compounds such as catechin, astilbin, isoastilbin, taxifolin, and smiglasides extracted by hot water from the medicinal plant *Smilax glabra* and allied species had strong growth-inhibition activities *in vitro* and *in vivo* (Nawab et al., 2011; She et al., 2015). In addition, a well-known medicinal mushroom *Phellinus linteus*, which its hot water extracts were containing polysaccharides (such as β-glucan) and polyphenols. These compounds found to be effective in reducing cancer cell viability and in scavenging the free radicals (Sarfraz et al., 2020; Wong et al., 2020).

The numerous compounds included in foods and their potential to prevent a number of chronic illnesses point to the eating of whole foods and a varied diet. According to certain investigations, using the whole herb has greater results than using only one active ingredient (Chen et al., 2022; Habtemariam, 2017). A prior investigation revealed that the polysaccharides from jujube plant and phenol compound from 6-gingerol combined had synergistic effects to improve the antioxidant and antitumor activities (Wu et al., 2021). Due to their complementary therapeutic benefits, natural polysaccharides combined with phenolic compounds are estimated to boost biological activity.

The extraction solvent is one of the most important factors concerning the effectiveness of extracting bioactive chemicals from natural sources and their consequent health benefits (Do et al., 2014). The diverse chemical properties and polarities elicited by distinct solvents extraction result in a variety of antioxidant activity. The different polarity of solvents also presented a range of cytotoxicity in

cancer cell. Cytotoxicity was shown to be reduced in the crude extract obtained using polar solvent extraction compared to a non-polar solvent extraction (Machana et al., 2012; Sultana et al., 2009).

The concept of treating with one or few active compounds is gaining recognition in the modern treatment of many diseases (Habtemariam, 2017). However, the synergy of the various components from combination of extracts may be effective in the treatment of chronic and complex disorders including cancer. Moreover, breast cancer has many types, and this study used MCF-7, MDA-MB-231, and MDA-MB-468 cell lines. MCF-7 is a common breast cancer model through the expression of estrogen and progesterone receptor, while MDA-MB-231 and MDA-MB-468 are triple negative breast cancer models that have metastatic ability (Dai et al., 2017). Thus, the use of various breast cancer types can serve as an excellent model for hormonal sensitivity and metastatic breast cancer research.

This study aimed to investigate the individually extract and interaction of *Smilax* sp. ethanol crude extract combined with *P. linteus* hot water crude extract in three distinct ratios as well as combination 1 (contained 50%:50% of *Smilax* sp.:*P. linteus*), combination 2 (25%:75% of *Smilax* sp.:*P. linteus*), and combination 3 (75%:25% of *Smilax* sp.: *P. linteus*) through antioxidant activities via DPPH and ABTS assays, also this study provided anticancer activities acquired by *in vitro* methods such as MTT, apoptosis, BrdU, and colony formation assays on three different breast cancer cell lines, which are expected to enhance these compounds potential as a chemo-preventive agent.

1.2 Objective of the study

To investigate antioxidant and anticancer activities of the *Smilax* sp. and *Phellinus linteus* crude extract individually and the mixture.

1.3 Expected beneficial outcome

The findings of this study are expected to provide the optimum mixture of bioactive compounds from Thai herbs crude extracts such as *Smilax* sp. and *Phellinus linteus*.

1.4 Research design

The research scheme of this study was shown in Figure 1.

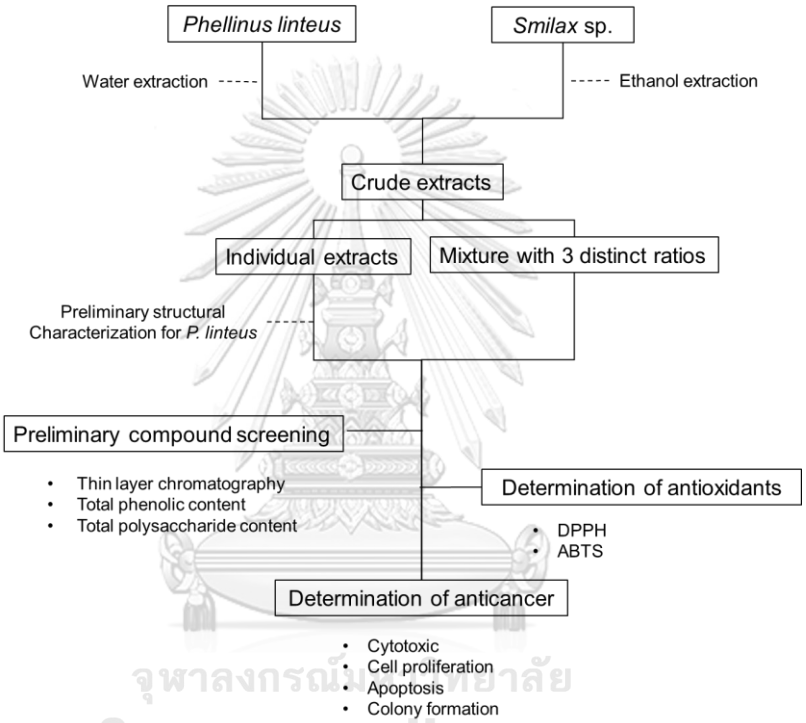


Figure 1. Research design for antioxidant and anticancer activities investigation of *Smilax* sp. and *Phellinus linteus* crude extracts.

CHAPTER II

LITERATURE REVIEW

2.1 *Smilax* sp.

The medicinal *Smilax* sp. from family Smilacaceae, Liliales, popularly called as sarsaparilla. It can be found in several areas such as Brazil, China, Himalayas, and Indochina including Thailand (Lee et al., 2018; Martins et al., 2014). This genus is one of the best-selling medicinal plants in traditional pharmacies in Thailand and has been globally recognized drugs in pharmacies throughout Brazil (Itharat et al., 2004; Martins et al., 2014). Smilacaceae contains advantageous phytochemical, which are mainly from phenolic compounds (Lu et al., 2014). In traditional medication, the extract from some parts including leaf and root are used to treat health problem such as rheumatism, asthma, and toothaches (Itharat et al., 2004; Raúl et al., 2017; Winterbottom, 2015).

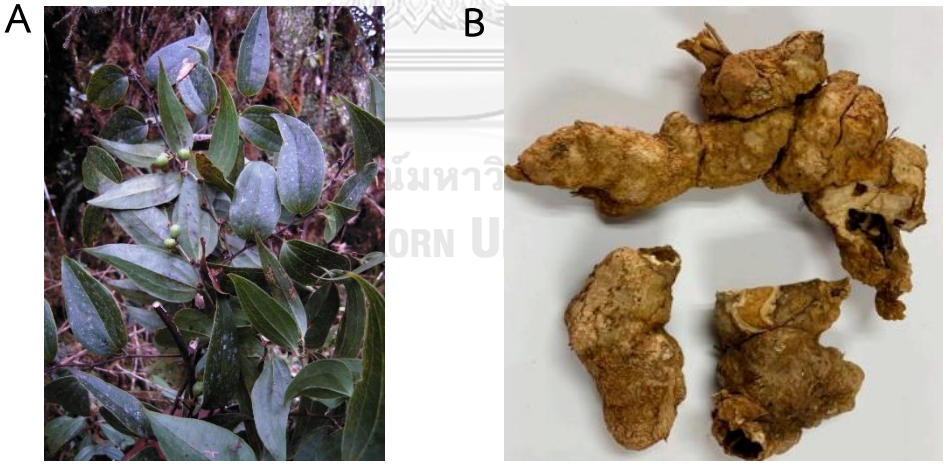


Figure 2. The appearance of *Smilax* sp. (*Smilax corbularia*). A: Aerial parts (leaves, stems, fruits) (source: https://worldfloraonline.org/images/fullsize/145823.jpg), B: Root parts (source: Jeeno et al., 2022).

Smilax glabra Roxb (*S. glabra*) and *Smilax corbularia* Kunth (*S. corbularia*) are most quoted species that distributed all over eastern and southeast regions of Asia

(Jeeno et al., 2022; Koyama, 1984). These species can be found in evergreen forest, sparse woodlands, and mixed deciduous forest. *S. corbularia* is a medicinal plant native to Cambodia, China Southeast, Hainan, Laos, Myanmar, Vietnam and Thailand. It has some identified characteristics such as a climber plant that has dark red flesh, delicate texture, and a buttery flavor (Jeeno et al., 2022; Kress et al., 2003) (Figure 2). Recently, *S. corbularia* has been identified to contain various biological activities such as antioxidant, antibacterial, anti-inflammatory and anticancer (Itharat et al., 2004; Jeeno et al., 2022). According to the previous study, the ethanol and methanol extracts from *S. corbularia*'s root part has higher total phenolic content and total flavonoid content compared to its water extraction. Some compounds have been found as Longistylin A, (-)-neolinderatin, neolinderatone, and stigmatellin Y discovered in *S. corbularia* ethanol extract were also shown to have anti-inflammatory and antioxidant properties in the same study (Jeeno et al., 2022).

2.2 *Phellinus linteus*

Phellinus linteus (*P. linteus*) is known as a medicinal mushroom in traditional oriental medicine which distributed in China, Japan, Korea and Thailand (Lee et al., 2015; Qin et al., 2023). This Basidiomycota can be found growing on different trees including trunk of popular, oak and mulberry (Sliva, 2010). The morphology of *P. linteus* has some characteristics were ungulate, sessile, hard woody and in the upper surface of basidiocarps was concentrically zonate with the color of dark chestnut (Hong et al., 2002; Min & Kang, 2021) (Figure 3). Both fruiting bodies and cultured mycelia were usually used to isolate the substance from *P. linteus* (Qin et al., 2023).

Figure 3. The appearance of *Phellinus linteus* fruiting body (source: https://www.out-grow.com/phellinus-linteus)

Genus *Phellinus* revealed has main component polysaccharides and other minor components such as terpenoids, flavonoids, polyphenolics (He et al., 2021). Especially hot water extraction from fruiting bodies of *P. linteus* has major polysaccharides >60 g/100g dry weight with total glucan content around 24.5 g/100g [comprised of α-glucan and β-glucan] (Kozarski et al., 2011). Other previous study reported the polysaccharides of *P. linteus* comprised of (1,3)-β-glucan with (1,6)- β link branch point (Baker et al., 2008; Reis et al., 2014). Numerous yellow polyphenol pigments from the genus *Phellinus*, including hispidin and hipolon, have been found to have antioxidant and anti-inflammatory (Li et al., 2019; Sarfraz et al., 2020). Recently, many studies have shown and clarified its immuno-modulator effects, including the inhibition of cell proliferation and metastasis, as well as the activation of apoptotic events against cancer both in vitro and in vivo (He et al., 2021; Konno et al., 2015; Mei et al., 2015).

2.3 Antioxidants

The importance of different reactive species, including reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS) has long been recognized as their role to maintenance the biological homeostasis, in the transmission of information, signaling, immunity and development, when available in

appropriate low amounts (Sies & Jones, 2020; Vona et al., 2021). ROS is mainly discussed to cause the pathogenesis and progression in many diseases. The sources of ROS production can be found both endogenous and exogenous. The endogenous ROS mainly produced in mitochondria and membrane bound NADPH oxidases (NOXs). ROS used to represented the free radicals such as superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical (•OH), alkoxyl radical (RO•), peroxyl radical (ROO•), as well as non-radical oxidant including hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) (Sies & Jones, 2020). The exogenous sources of ROS can be produced from the exposure of radiation (UV or X-rays), smoke, bacteria, viruses and drugs (Pham-Huy et al., 2008).

Oxidative stress in living things results from an imbalance between the production of reactive oxygen species (ROS) and the capacity to neutralize them. A lack of natural defense and an overabundance of reactive chemicals damage the lipids, proteins, and DNA in cells, which eventually triggers the pathogenesis of a number of illnesses. When created in excess, as in inflammation, ROS can lead to the creation of additional highly reactive species (Vaziri, 2008). the oxidative alteration of crucial enzymes or regulatory sites, whose redox modification results in altered cell signaling and programmed cell death. Oxidative stress and inflammation are tightly connected. Oxidative stress can trigger inflammation, which in turn triggers oxidative stress, producing a vicious cycle that results in cell damage (Ishibashi, 2013; Petrie et al., 2018).

Cardiovascular disease, diabetes, rheumatoid arthritis, cancer, and neurological illnesses are among the pathologies where ROS have been recognized as fundamental variables (Valko et al., 2007). Recently, the use of exogenous antioxidants has been advocated as a treatment (Di Meo et al., 2016). Antioxidants operate as free radical scavengers, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors, and synergists to delay or decrease lipid oxidation. Lipid oxidation may play a role in coronary heart disease, atherosclerosis, cancer, and aging in vivo

(Rufino et al., 2011). Vitamins A, C, and E, as well as carotenoids, flavonoids, and other simple phenolic compounds found in natural foods, have been shown to have antioxidant properties (Arnao et al., 2001).

There has been a lot of interest in producing and using effective natural antioxidants of certain polysaccharides extracted from plants, herbs, and fungi have antioxidant properties but low cytotoxicity. Many polysaccharides and polysaccharide-protein complexes derived from fungi have been employed as medicinal treatments (Ge et al., 2013). This explains why the hunt for natural antioxidants has expanded dramatically in recent years (Morais et al., 2014).

2.4 Breast cancer

There will be about 2.1 million diagnosed breast cancer worldwide with around 1 in 4 cases of cancer among females in 2018 (Bray et al., 2018). Over 3.8 million women in the United States have a history of breast cancer at the start of 2019. This figure comprises about 150,000 women with metastatic breast cancer, of whom 75% had stage I, stage II, or stage III breast cancer at the time of their first diagnosis (Mariotto et al., 2017).

Moreover, the disease occurs globally, its incidence, mortality, and survival rates vary significantly around the world, which could be attributable to a variety of factors such as population structure, lifestyle, genetic factors, and environment (Hortobagyi et al., 2005). With rapid growth of the population and aging, the factor of cancer risk developed as a leading cause of premature death due to other diseases relative to cancer, such as stroke and coronary heart disease in many countries, which was a reflection of social and economic development (Bray et al., 2018).

A hundred quantitative and qualitative studies related to breast cancer have been published recently with focus on therapeutic and diagnostic aspects using the cell lines as object. The history reported BT-20 was the first breast cancer cell line to be created in 1958 (Lasfargues & Ozzello, 1958). MCF-7, created in 1973 at the

Michigan Cancer Foundation, is still the most extensively utilized breast cancer cell line in the world 20 years later (Soule et al., 1973). Subsequently, it was discovered that breast cancer was a heterogeneous and complex condition. Histological type, tumor grade, lymph node status, and the presence of specific profiles like ER/PR and human epidermal growth factor receptor 2 (HER2) were used to classify the cells. Due to the ER/PR-dependent breast cancer subtype MCF-7, its expression is frequently responsive to treatment processes and is representative as a model for hormonal inquiry. In a metastatic model where ER, PR, and HER2 markers are absent, MDA-MB-231 and MDA-MB-468 are overrepresented, which could facilitate growth and lead to therapeutic resistance. The aggressive form of metastatic cancer is also increased in MDA-MB-231 due to large quantities of a mutant p53 gene (Bajalovic et al., 2022; Garcia et al., 2021; Holliday & Speirs, 2011).

Breast cancer treatment for all subtypes eventually leads to resistance, which often leads to increasing disease and death. Drug resistance is a common symptom of cancer and a primary cause of treatment failure (Lukong, 2017). The rising number of cancer fatalities requires the rapid development of therapeutic techniques with fewer cytotoxic side effects and resistance (Hashem et al., 2022).

2.5 Anticancer

Conventional cancer treatments include radiotherapy, chemotherapy, and surgical removal; however, these therapies are less effectiveness due to the risk of cells' resistance (Zhang et al., 2017). Natural substances have a number of advantageous properties, including the ability to treat cancer and enhance health. Alkaloids, taxanes, and flavonoids are only a few examples of the natural substances that constitute the basis of many currently used medicinal medications (Hasanpourghadi et al., 2017). Natural products have shown selective effects against cancer cells. Their chemical structures can also be used as models to create new medications with similar benefits or superior to those of currently available natural products, with fewer side effects and resistance (Cavalli et al., 2008).

It is expected that between 50% and 60% of cancer patients in the United States use plant or nutritional substances as a supplementary or substitute medication. The application is typically used as a single therapy or in conjunction with chemotherapy and/or radiation therapy. The anticancer properties of natural plant-derived bioactive substances were validated by evidence-based study. Many studies have resulted in an increase the number of comprehensive compounds used as chemical and biological functional agents with beneficial effects on human health (Meybodi et al., 2017).

Signaling pathways control cellular development and cause various changes in different cell types. Cell survival, proliferation, and motility are all regulated by several signaling pathways. When a mutationally activated route is blocked by an inhibitor, cancer cells can proliferate via an alternative signaling pathway. Thus, it suggested leading the resistance of cancer cells (Sever & Brugge, 2015). Natural products tend to target multiple oncogenic signaling pathways simultaneously by modulating the activity or expression, or both, of their molecular targets, affecting apoptotic cell, cell proliferation, migration/invasion, angiogenesis, metastasis, and other pathways that are specific to certain natural products. Most natural products target intrinsic apoptotic signaling pathways, which result in a number of intracellular signals that set off mitochondrial-initiated processes that kill cancer cells. It's interesting that natural products can be used as adjuvants to help chemoresistant tumors become more responsive to medication (Hashem et al., 2022).

A study showed that resveratrol, a non-flavonoid phytoalexin substance from plant derivative has been reported to inhibit cell proliferation and induce mitochondrial-mediated and caspase-independent apoptosis in transgenic adenocarcinoma of mouse prostate (TRAMP) cells. It was shown that resveratrol exerted its anticancer effects through changes in the expression of Bax/Bcl-2 and disturbance of the mitochondrial membrane potential in TRAMP cells (Kumar et al., 2017). Other study about reported some dietary patterns that contain a high intake

of phenolics-rich foods, primarily phytoestrogens that can bind to ER, such as isoflavones in soy-based products in Asian countries, have been linked to strong protective effects against breast cancer (Chen et al., 2014). As a result of their excellent selectivity, low cost, and minimal toxicity, natural compounds and their derivatives may be better chemotherapeutic agents.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Sample materials

- *Smilax* sp. and *Phellinus linteus* powdered: Nature Herb International Holding Co., Ltd.
- MCF-7 cell line: ATCC, Virginia
- MDA-MB-231 cell line: ATCC, Virginia
- MDA-MB-468 cell line: kind gift from Center of Excellence in Molecular Genetics of Cancer and Human Disease, Chulalongkorn University, Thailand
- HEK-293 cell line: kind gift from Center of Excellence in Molecular Genetics of Cancer and Human Disease, Chulalongkorn University, Thailand

3.1.2 Equipment

- Autoclave: model Tomy Sx 700E, Bio-active co. ltd., Thailand
- Reflux extractor: SCI
- Soxhlet column: Cytiva Life Science, China
- Freeze dryer: model Heto PowerDry LL 3000, Thermo Fisher Scientific, UK
- Rotary evaporator: model Eyela N-1000, Japan
- Refrigerator -20°C: Sanden, Thailand
- Refrigerator -80°C: model TSX series, Thermo Fisher Scientific, UK
- Microscope inverted: Motic, US
- Incubator CO_2: Thermo Fisher Scientific, UK
- Centrifuge: Wise spin, Swiss
- Laminar Air Flow: Clean series 1000
- Spectrophotometer: Thermo Fisher Scientific, USA
- Flow cytometer: Beckman Coulter Dx Flex Flow Cytometer, US

- Water bath: M-LAB, MTECH, Thailand
- Hot plate: model IKA C-MAG HS-10, Thailand
- Vortex: Four E's scientific, China
- Weight balance: Shimadzu Corporation, Japan
- Hot air oven: BINDER GmbH, Germany
- Ultra-violet light plate: LIO LAB LTD., PART., Thailand
- Fourier transform infrared (FTIR): INVENIO FT-IR Spectrometer, Bruker, Swiss
- High performance liquid chromatography (HPLC): Shimadzu Corporation, Japan
- Gel permeation chromatography (GPC): Shimadzu Corporation, Japan
- 96-well plate: Thermo Fisher Scientific, USA
- 6-well plate: Thermo Fisher Scientific, USA
- Micropipette: Gilson, USA
- Micropipette tips: NEST, China
- Serological pipette: OniLAB, USA
- Serological tips: NEST, China
- Flasks: Thermo Fisher Scientific, USA
- Falcon tubes: NEST, China
- Microtubes: NEST, China
- Silica gel plates: Sigma-Aldrich, USA
- Cryoprotection tubes: Axygen, USA
- Syringe 1 ml: Terumo, China
- Syringe filter 13mm: National Scientific, USA

3.1.3 Chemicals

- Ethanol 95%
- Distilled water
- P-anisaldehide reagent

- Follin-Ciocalteu reagent: Loba Chemie PVT LTD., India
- Sodium carbonate: Scharlau, Spain
- Gallic acid: Fluka, Sigma-aldrich, Switzerland
- Anthrone reagent: Sigma-Aldrich, USA
- D-Glucose: Thermo Fisher Scientific, USA
- D-Mannose: Kemaus, Australia
- D-Galactose: Kemaus, Australia
- D-Fructose: Kemaus, Australia
- D-Xylose: Kemaus, Australia
- 1-Butanol: Thermo Fisher Scientific, USA
- Acetic acid: Qréc, New Zealand
- NaOH: Qréc, New Zealand
- Sulphuric acid: Thermo Fisher Scientific, USA
- 2,2-diphenyl-1-picrylhydrazyl (DPPH): Sigma-Aldrich, USA
- Methanol: RCI Labscan, Thailand
- 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS): Sigma-Aldrich, USA
- Potassium persulfate: Ajex Finechem, New Zealand
- Ascorbic acid: commercial brand HICEE, Japan
- Dulbecco's modified eagle medium (DMEM): Gibco, USA
- Fetal Bovine Serum (FBS): Gibco, USA
- Antibiotic: Gibco, USA
- Phosphate Buffered Saline (PBS): Gibco, USA
- 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT): Abcam, UK
- DMSO: Merck, Sigma-Aldrich, USA
- Cisplatin: Glentham Life Science, UK

- Trypsin-EDTA: Gibco, USA
- 5-bromo-2'-deoxyuridine (BrdU) reagent kit: Roche, Germany
- Apoptosis Annexin V-FITC reagent kit: Abcam, UK
- Dye crystal violet staining: Abcam, UK

3.2 Methods

3.2.1 Extraction of *Smilax* sp. and *Phellinus linteus*

The powdered of *Smilax* sp. rhizome was extracted by 95% ethanol solvent and *P. linteus* fruiting body used hot water extraction, with a solid-to-liquid ratio of 1:60 w/v and heated at 80℃ for 6 hours in reflux extractor with soxhlet filter (Fauzantoro et al., 2017; Moldovan et al., 2019). The crude extract was collected and evaporated in a rotary evaporator for *Smilax* sp. extract product, and evaporated by lyophilizing for *P. linteus* extract product. The dried extracts were weighted to calculate the yield and were stored in refrigerator at -20℃ for the further use. The preparation of extracts to determinate the total polysaccharide content, total phenolic content, antioxidant activities and anticancer activities was used five samples. The variation of the samples were individually crude extracts from *Smilax* sp. and *P. linteus*, and in combinations with different ratios such as combination 1 (contained 50%:50% of *Smilax* sp.:*P. linteus*), combination 2 (25%:75% of *Smilax* sp.:*P. linteus*), and combination 3 (75%:25% of *Smilax* sp.: *P. linteus*). Primarily, the dried crude extract of *Smilax* sp. was diluted in DMSO, while *P. linteus* was diluted in distilled water for the stock solutions. After that, the stock was diluted into the serial of concentrations with the solution as mention in each method. For anticancer activities, sample stocks were freshly diluted with complete medium in serial concentration as required in each method. The content of DMSO in final medium was around 0.1%. All assays were conducted in triplicate.

3.2.2 Preliminary compound screening

3.2.2.1 Evaluation of total phenolic content

The total phenolic content (TPC) was determined using Follin-Ciocalteu (FC) reagent method by Hong et al. (2021). The stock of extracts (*Smilax* sp. at concentration 400 µg/mL, *P. linteus* was 10000 µg/mL, combination 1 was 400 µg/mL, combination 2 was 800 µg/mL, and combination 3 was 400 µg/mL) and/or gallic acid (0-100 µg/mL) were dissolved in distilled water at appropriated concentrations and mixed with FC reagent (ratio of FC:water was 1:3) thoroughly for 5 minutes. After that, sodium carbonate (10% w/v) was added to the mixture and incubated for 60 minutes at room temperature in the dark condition. The absorbance of the mixture was detected at 765 nm using spectrophotometer. TPC was expressed as milligram of gallic acid equivalent (GAE) per gram defatted the crude extract (mg GAE/g crude dry extract) (Hong et al., 2021).

3.2.2.2 Evaluation of total carbohydrate content

The amount of total carbohydrates was determined as described by Chu et al. (2018). The stock of extracts was dissolved in distilled water at required concentrations (*Smilax* sp. at concentration 200 µg/mL, *P. linteus* was 100 µg/mL, combination 1 was 200 µg/mL, combination 2 was 200 µg/mL, and combination 3 was 200 µg/mL). The anthrone reagent (1 mg/ml) in concentrated sulfuric acid was added to sample solution and incubated at 4°C. After that, heated for 10 minutes in a water bath. After cooling to room temperature, the solution's absorbance at 630 nm was measured using a spectrophotometer. Calculations were made to compare the samples' total carbohydrate content to the reference solution, which included glucose (at concentration 0-300 µg/mL) (Chu et al., 2018).

3.2.2.3 Thin layer chromatogram

The qualitative analysis of crude extracts compound was conducted with thin layer chromatography (TLC) on silica gel plates with some solvent as a

mobile phase. The plate was visualized under the ultra-violet light and subsequently dipped with *p*-anisaldehyde reagent following by heating at 150°C for 2 minutes. The analysis for polysaccharide from *P. linteus* and phenolic compound from *Smilax* sp. were different. To know the characteristic of *P. linteus* polysaccharide with TLC, firstly crude extract was hydrolyzed using sulfuric acid at high temperature 121°C for 1 hour to generate the monosaccharide units as described by Tang et al. (2020) with modification (Tang et al., 2020). After that, the acid was neutralized with sodium carbonate. TLC for monosaccharide was performed using mixed solvents butanol:ethanol:water with ratio 5:3:2 as described by Reiffova & Nemcova (2006) (Reiffová & Nemcová, 2006). The crude extract of *Smilax* sp. was dissolved in ethanol and performed the TLC using mixed solvents butanol:acetic acid:water with ratio 4:1:5 as described by Martins et al. (2014) (Martins et al., 2014).

3.2.2.4 Structural characteristic of *Phellinus linteus*

Structural characteristics of *P. linteus* were carried out using three different methods such as high performance liquid chromatography (HPLC), fourier-transform infrared spectroscopy (FTIR), and gel permeation chromatography (GPC). HPLC has performed to investigate the monosaccharides composition which comprised the polysaccharide in *P. linteus*. Thus, before performance the HPLC sample extract was hydrolyzed as the same method as in TLC that described by Tang et al. (2020) with modification (Tang et al., 2020). The neutral product was filtered with syringe filter 13mm to HPLC tube and ready to analyze (Ramos-Andrés et al., 2021). FTIR was method to determine the functional group and preliminary configuration of *P. linteus* polysaccharide structure. For FTIR, the sample preparation was solid phase and data was analyzed in wavenumber ranging 500-4000 cm^{-1} using Origin 2023 software (Ghosh et al., 2021). GPC was conducted to identified the molecular weights (M_ws) of *P. linteus* polysaccharide.

Sample extract was dissolved the crude extract with distilled water become 5 mg/ml, filtered it and ready to perform the GPC (Wang et al., 2011).

3.2.3 Antioxidant activities

3.2.3.1 Determination with DPPH

The radical scavenging activity of extracts were assayed according to the DPPH method as described by Mariani et al. (2021) with modification (Mariani et al., 2021). The crude extract was serially diluted, mixed with 0.1 mM DPPH solution, and then incubated for 30 minutes at room temperature in the dark. Using a spectrophotometer, the extracts' lower absorbance was measured at 517 nm in comparison to the DPPH and methanol combination (control). The percentage of radical inhibition was calculated using the following formula:

Inhibition (%) = ((A control-A sample) x 100)/(A control) (1)

Where A control was the absorbance of DPPH solution without extract and A sample was the absorbance of sample with DPPH solution. The half-maximal inhibitory concentration (IC_{50}) was reported as the amount of antioxidant required to decrease the initial DPPH concentration in 50%. The blank was solvent solution of sample, negative control was solvent with DPPH, and positive control was ascorbic acid with DPPH.

3.2.3.2 Determination with ABTS

This assay was performed according to Gaber et al. (2021) (Gaber et al., 2021). The radical cation of 2,2′-azino-bis ethylbenzthiazoline-6-sulfonic acid ($ABTS^+$) was prepared by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate at the ratio of 1:1 (v/v) as a stock and leaving the mixture for 4 – 16 hours until the absorbance has stabled. The $ABTS^+$ stock solution was diluted with distilled water into an absorbance of 0.700 ± 0.05 at 734 nm. The photometric assay was conducted by mixing $ABTS^+$ solution with crude extract at different concentrations. After incubation for 10 minute in the dark, the absorbance mixture

was measured at 734 nm. The antioxidant activity of crude samples was calculated compared with the $ABTS^+$ solution at the stable absorbance according to the equation (1). The blank was solvent solution of sample, negative control was solvent with $ABTS^+$, and positive control was ascorbic acid with $ABTS^+$.

3.2.4 Anticancer activities

3.2.4.1 Cell lines preparation and culture

Three breast cancer cell lines (MCF-7, MDA-MB-231, and MDA-MB-468) and normal cells (HEK-293) were utilized in this study. According to Daddouissa et.al., (2019), all cell lines were cultured in complete medium which contained Dulbecco's modified eagle medium (DMEM), 10% FBS, and 1% antibiotic. All cells were cultured and incubated at 37°C with 5% CO_2, and after reached 70-80% confluence, they were used for further experimental treatment (Daddiouaissa et al., 2019).

3.2.4.2 Cytotoxicity assay

In this study used the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay to quantify the percentage of cell viability. Followed the method by Haryanti et al. (2019), $5x10^3$ cells/well were cultured in a 96-well plate for 24 h and added fresh medium mixed with various concentrations of the extract. Subsequent to 72 h, remove the cultured medium, and wash the cells with PBS. 0.5 mg/ml MTT in the medium were added into each well and incubated for 2.5–3 hours. After that, remove the solution and add DMSO for 10 minutes. The absorbance was measured using spectrophotometer at wavelength 595 nm. The absorbance data were provided as percent viability compared to untreated cells. The obtained data was used to calculate the percentage of viable/dead cells using equation as described by Daddiouaissa et al. (2019):

Cell viability (%) = (Abs of sample)/(Abs of control)x 100 (2)

Where Abs of the sample was the absorbance of treated cells and Abs of control was the absorbance of untreated cells. The half-maximal inhibitory concentration (IC_{50}) was reported as the ability of compound concentration that inhibit cell growth in 50%. IC_{50} has determined using curves constructed by plotting cell survival (%) versus the concentration of samples. Blank was DMSO with MTT solution, negative control was untreated cell with DMSO and MTT, and positive control was cisplatin treated cell with DMSO and MTT (Daddiouaissa et al., 2019; Haryanti et al., 2020).

3.2.4.3 Cell proliferative assay

Measuring proliferation rate with BrdU (5-bromo-2'-deoxyuridine) cell proliferation ELISA kit was used following the description by manufacturer protocol (Roche, Germany). Cells were planted at a density of $5x10^3$ cells/ml in a 96-well culture plate. The cells were cultivated for additional 24 hours with BrdU working solution after 72 hours of extract treatment incubation. The supernatant was then collected and the Fixative/Denaturing Solution was applied to fix and denature the cells for 30 minutes at room temperature. Anti-BrdU antibody solution was added and incubated at room temperature for 90 minutes. The unbound primary antibody was rinsed before the secondary antibody solution was added and incubated for 30 minutes. Finally, the substrate solution was added and incubated for 20 minutes in the dark. The amount of BrdU incorporated was determined using an ELISA reader at 370 nm.

3.2.4.4 Colony formation assay

As described by Sidorova & Petrikaite (2022) with modification, the cells were cultured into 6-well culture plate (1 $x10^5$ cells/well) in complete medium and allowed to attach overnight (Sidorova & Petrikaitė, 2022). After that, the cells were treated with the extracts for 72 hours. Afterward, the cells were trypsinized, added the fresh medium and suspended it with centrifuge at 3000 rpm for 5 minutes.

After that, the cell calculated into 2 x10^3 cells/well and cultured to the new 6-well plate for 10 days and changed the medium once in 3 days. After 10 days of incubation, cells were fixed and stained with solution which contained methanol, distilled water and 0.5% crystal violet solution at room temperature for 15 minutes. Plate was gently washed with PBS and distilled water. Photos of the cells were captured under a light microscope at 4x10 magnifications. Colonies were defined as cells growing (10-50 cells/colony for MCF-7 and 50-100 cells/colony for MDA-MB-231 and MDA-MB-468). The number of colonies were examined using manually counting in whole area of 6-well culture plate.

3.2.4.5 Apoptosis assay

The apoptosis assay was conducted among manufacturer protocol kit. The cell lines 2x10^5 cells/well were cultured and incubated in 6-well culture plate for 24 hours. Cells were treated with crude extract sample (the concentration of extracts were followed the value of IC_{50} of MTT test) for 72 hours. The cells were then trypsinized, washed in PBS once, and centrifuged at 3000 rpm for 3 minutes. To assess apoptosis induction, the collected cells were resuspended in Annexin V binding buffer. The cells were treated with annexin V DY 634 and propidium iodide reagents before being incubated in the dark for 15 minutes at room temperature. Flow cytometry was used to examine the apoptotic cells.

3.3 Statistical analysis

All experiments were performed in triplicate and the data were shown as their mean ± standard deviation (SD). The significance of differences between samples in the same group was examined by one-way analysis of variance (one-way ANOVA) with Tukey's HSD post-hoc test at P values less than 0.05 ($P<0.05$) using IBM SPSS 28.

CHAPTER IV

RESULTS

4.1 Physiochemical characterization of *Smilax* sp. and *Phellinus linteus*

The yield of *P. linteus* crude extract was higher by approximately 53% compared to *Smilax* sp. by about 18% (Table 1). The total phenolic content of all sample which are individually and in combination extracts were significantly different ($p<0.001$; all samples vs. *P. linteus*) (Figure 4A).

Table 1. Yield of *Smilax* sp. extract and *Phellinus linteus* extract

Yield (g/g)	*Smilax* sp.	*P. linteus*
	0.18 ± 0.02	0.53 ± 0.05

Note: All data was represented as the mean ± standard deviation (SD) of three independent experiments.

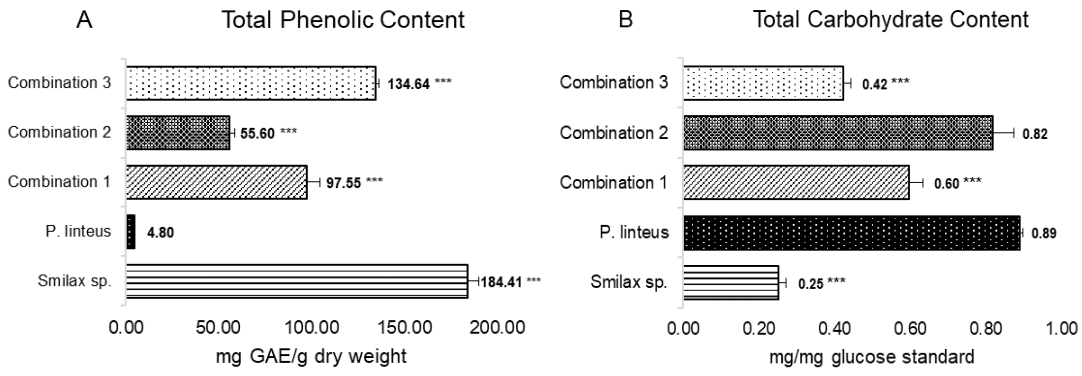


Figure 4. Chemical properties of *Smilax* sp., *P. linteus* and combinations. All data was represented as the mean ± standard deviation (SD) of three independent experiments; *** represented $P<0.001$ vs. *P. linteus* among the same group from statistical analysis using One-way ANOVA with Tukey's HSD post-hoc test. GAE, gallic acid equivalent.

Smilax sp. had the highest total phenolic concentration, at about 18.4% of dry weight, followed by combinations 3 (13.4%), 1 (9.76%), 2 (5.56%), and *P. linteus*,

which had the lowest total phenolic content, at about 0.48% of dry weight. Individual extract of *P. linteus* exhibited the highest total polysaccharide content of about 89% (w/w), followed by combination 2 (82%), combination 1 (60%), combination 3 (42%), and *Smilax* sp. (25%). The results were also statistically different ($p<0.001$; *Smilax* sp., combination 1 or combination 3 vs. *P. linteus*) (Figure 4B).

4.2 Preliminary structural analysis of crude polysaccharide from *Phellinus linteus*

The finding from HPLC assay of monosaccharide composition are listed in Table 2. The crude polysaccharide of *P. linteus* was shown to be a heteropolysaccharide consist of glucose, arabinose, and other monosaccharides based on the monosaccharide standards.

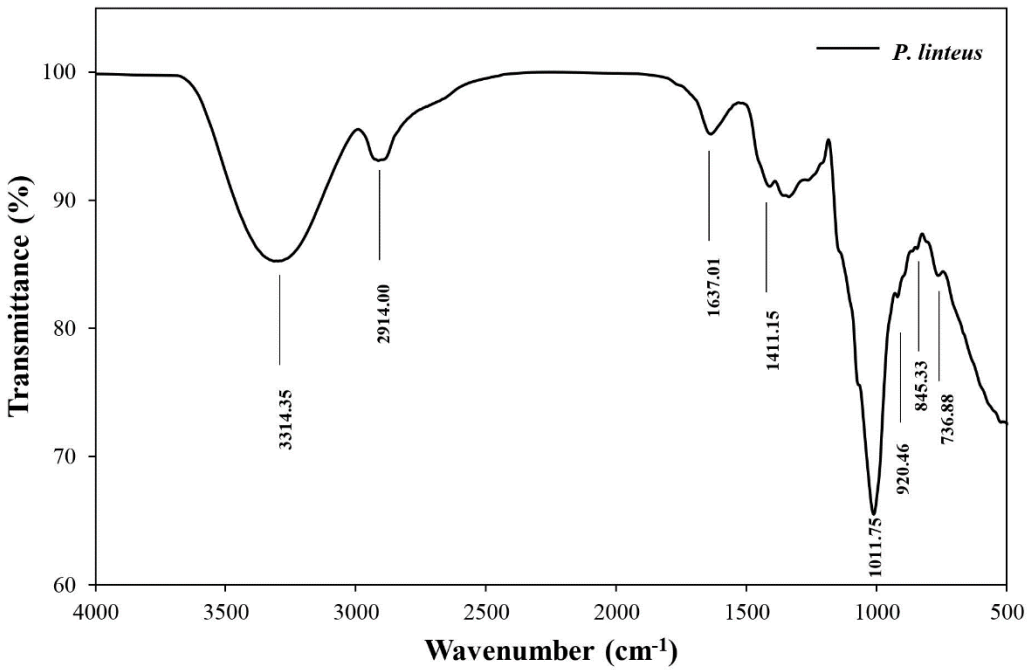


Figure 5. Fourier-transform infrared spectroscopy spectra of *Phellinus linteus* crude polysaccharide.

Table 2. Monosaccharide composition from *Phellinus linteus* crude polysaccharides

P. linteus	Monosaccharide composition (% w/v)		
	Glucose	Arabinose	Other monosaccharides
	96.42	1.68	1.89

w/v: mg/ml

FT-IR spectra of *P. linteus* crude polysaccharide was displayed in a range of wavenumbers between 500 and 4000 cm^{-1} (Figure 5). For the O-H stretching vibration, the profile of this crude polysaccharide showed a broad stretching peak at about 3314.35 cm^{-1}, and for the C-H stretching of CH2 groups, it had a weak stretching peak at around 2914.00 cm^{-1} (Ge et al., 2013; Pei et al., 2015). Uronic acid was present due to the COO-deprotonated carboxylic group inferred by absorption at 1637.01 cm^{-1}, and a peak at about 1411.15 cm^{-1} indicated the –OH group of phenol (Ghosh et al., 2021; Z. B. Wang et al., 2014). Strong banding at 1011.75 cm^{-1} suggested the vibration of the pyranose ring. Peak at 900 cm-1 (920.46 cm^{-1}) implied the β–configuration of sugar unit. The presence of β-D-glucosidic linkages was suggested by a faint band between 845.33 and 763.88 cm^{-1} (Ge et al., 2013; Ghosh et al., 2021; Z. B. Wang et al., 2014).

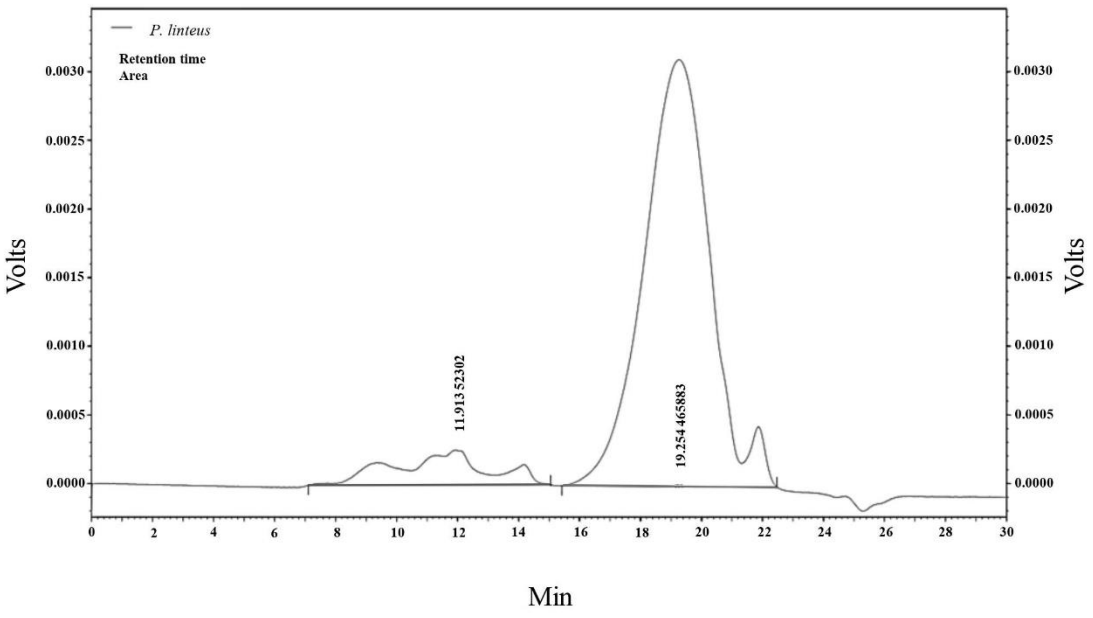


Figure 6. Gel permeation chromatography chromatogram of *Phellinus linteus* crude polysaccharide.

The molecular weight (M_ws) of the crude polysaccharide isolated from *P. linteus* was determined using GPC. The averages M_ws for the crude polysaccharide were 2,625.41 kDa and 2.416 kDa, respectively, with retention time of 11.913 and 19.254 for the two major peaks (Figure 6). According to the second peak's height, low molecular weight polysaccharide was the majority of *P. linteus* polysaccharide distribution.

4.3 Thin layer chromatogram

The major of monosaccharide found in the hydrolyzed *P. linteus* extract was glucose, due to the position of sample's band was nearest of glucose standard band visualized on the TLC with retention factor (Rf) around 0.46 (with green-blue shade) (Figure 7). The extracted *Smilax* sp. and all combinations had 3 bands on the TLC with the same Rf for all samples were 0.26 (blue-greenish), 0.72 (brown), and 0.80 (brown smear) using the p-anisaldehyde reagent and heat method. While, only band at Rf number 2 could be detected by ultraviolet (UV) light visualization at 254 nm (Figure 8).

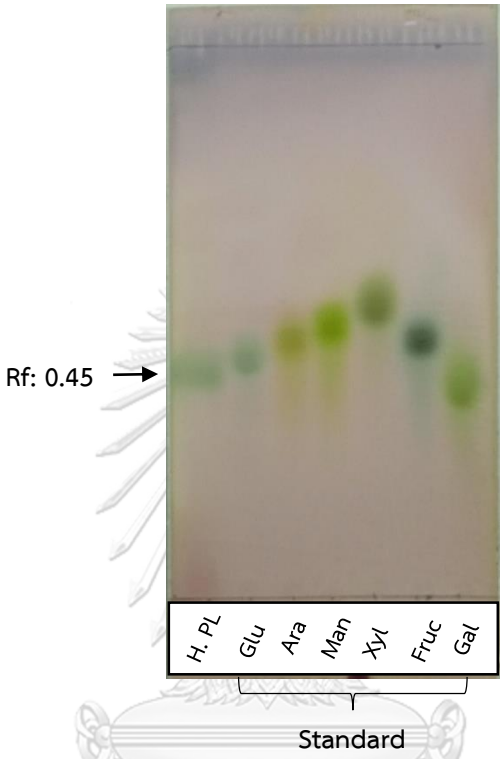


Figure 7. TLC visualization of hydrolyzed *Phellinus linteus* and standards using mixed solvents butanol:ethanol:water with ratio 5:3:2, which performed with p-anisaldehyde reagent following by heating at 150°C. H.PL (hydrolyzed *P. linteus*, Rf: 0.45); Glu (Glucose, Rf: 0.46); Ara (Arabinose, Rf: 0.48); Man (Mannose, Rf: 0.49); Xyl (Xylose, Rf: 0.54); Fruc (Fructose, Rf: 0.48); Gal (Galactose, Rf: 0.40).

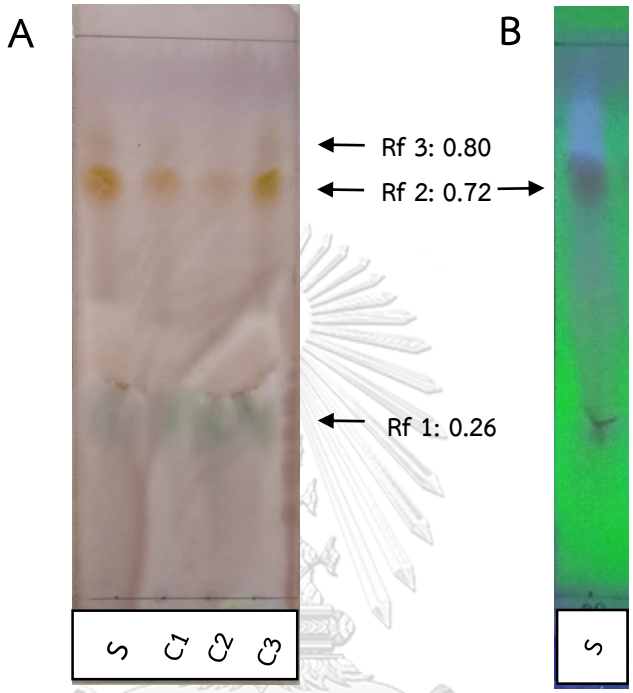


Figure 8. TLC visualization of *Smilax* sp. and combinations using mixed solvents butanol:acetic acid:water with ratio 4:1:5 as mobile phase. A: Performed with p-anisaldehyde reagent following by heating at 150°C, B: Performed under the UV-light. S (*Smilax* sp.), C1 (combination 1), C2 (combination 2), C3 (combination 3).

4.4 Antioxidant activities

The scavenging activity of all samples on DPPH and ABTS were displayed in Figure 9. *Smilax* sp., combination 3, combination 1, and ascorbic acid as positive control had no significant differences in the DPPH results, indicating that they had higher radical scavenging activity than combination 2 and *P. linteus* in a manner dependent on half-maximal inhibitory concentration (IC_{50}) ($P<0.001$; *P. linteus* or combination 2 vs. *Smilax* sp.).

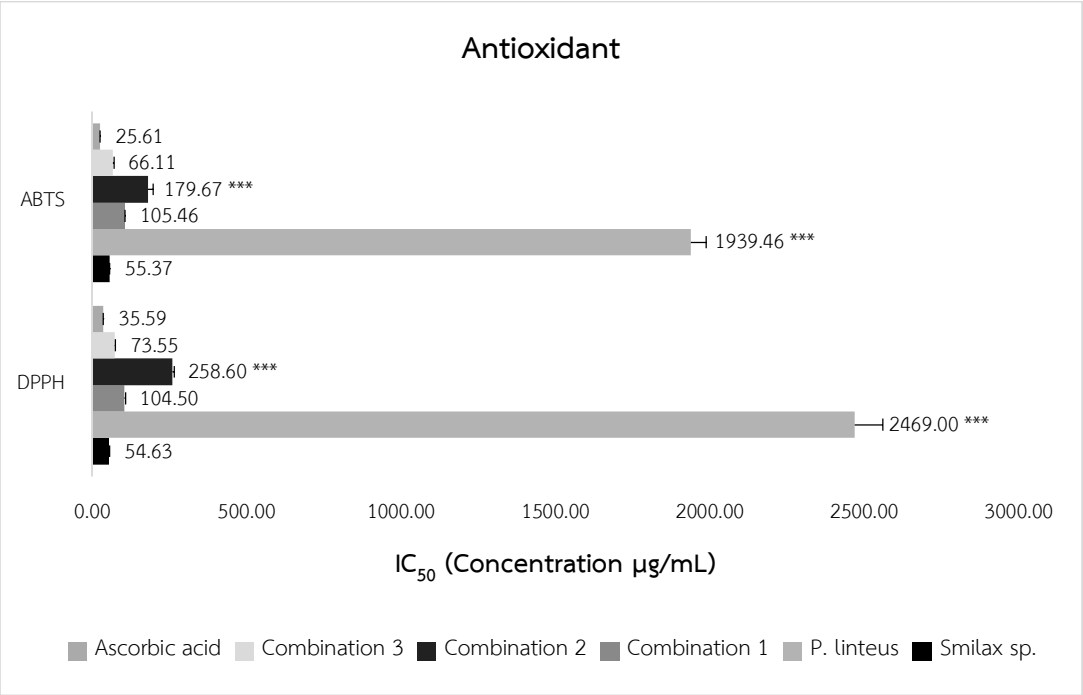


Figure 9. Antioxidant activities of *Smilax* sp. extract, *Phellinus linteus* extract, and combinations scavenging ability on DPPH and ABTS free radical reagent. All data was represented as the mean ± standard deviation (SD) of three independent experiments; *** represented P<0.001 vs. *Smilax* sp. among the same group (included the positive control) from statistical analysis using One-way ANOVA with Tukey's HSD post-hoc test.

Similar to DPPH results, *Smilax* sp., combination 3, combination 1 showed greater ABTS radical scavenging activity had no significant difference with ascorbic acid compared to combination 2 and *P. linteus*. The samples showed a trend from strong to weak scavenging activities were *Smilax* sp., combination 3, combination 1, combination 2, and *P. linteus* in accordance with DPPH and ABTS IC_{50} values.

4.5 Anticancer activities

4.5.1 Cytotoxicity effect

According to the findings, after 72 hours of treatment, *Smilax* sp., combination 3 and cisplatin as positive control exhibited the highest levels of cell growth inhibition and significantly different against MCF-7 compared to combination 1,

combination 2 and *P. linteus* (P<0.05; cisplatin vs. *Smilax* sp., P<0.01; combination 1 vs. *Smilax* sp., P<0.001; *P. linteus* or combination 2 vs. *Smilax* sp.) (Figure 10). *Smilax* sp. and combination 3 showed lower amounts than other samples to suppress growth on MDA-MB-468 (P<0.001; other extracted samples vs. *Smilax* sp. or combination 3).

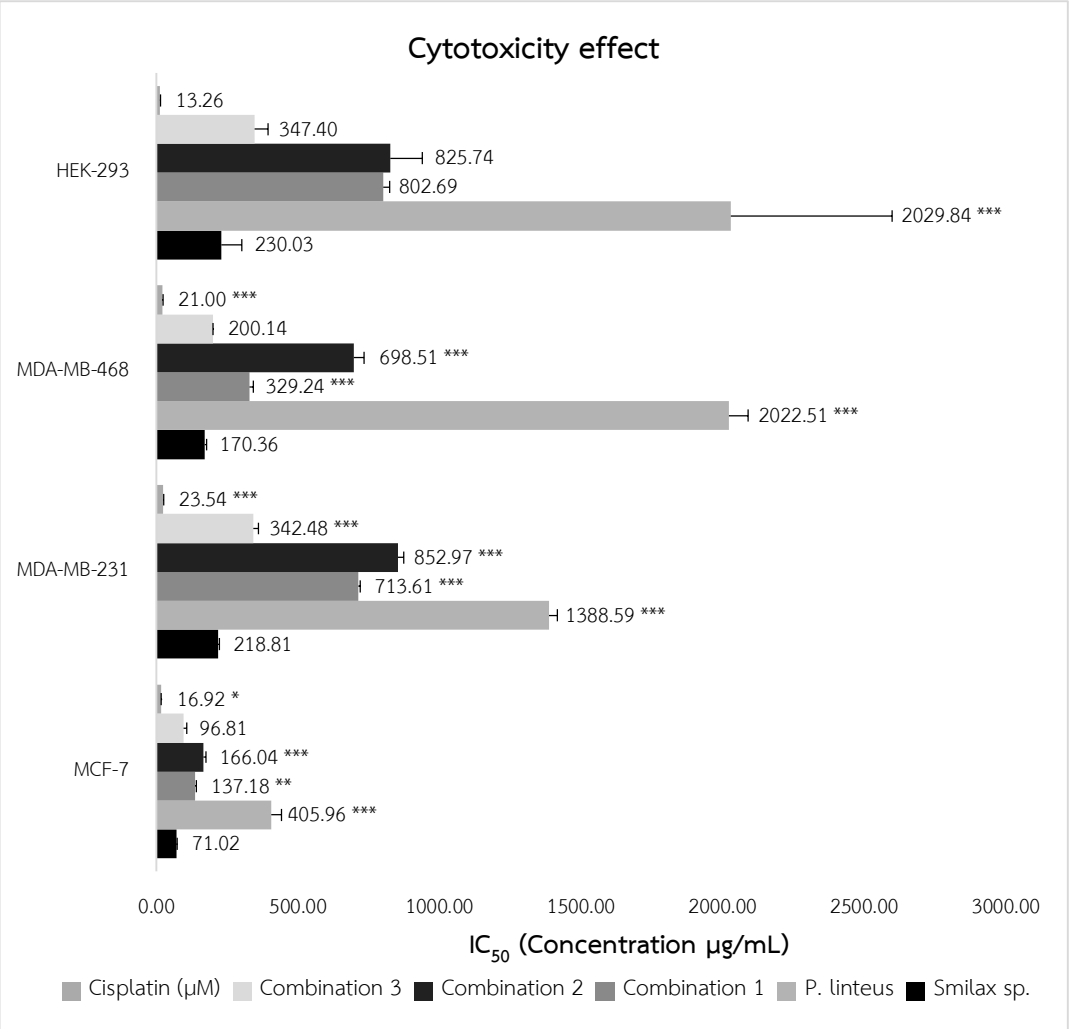


Figure 10. The Cytotoxic effects of *Smilax* sp. extract, *Phellinus linteus* extract, and combinations on the viability of breast cancer and normal cell lines were investigated. The mean standard deviation (SD) of three independent experiments was used to represent all data.; *, **, *** represented P<0.05, P<0.01 and P<0.001 vs. *Smilax* sp. among the same group (cell line's type, included the positive control) from statistical analysis using One-way ANOVA with Tukey's HSD post-hoc test.

On the MDA-MB-231 cell line, all extracted samples exhibited statistically different results ($P<0.001$; other extracted samples vs. *Smilax* sp.), and individually *Smilax* sp. extract shown a greater level of inhibition activity than the other extracts. The results demonstrated that the IC_{50} values against all cancer cell lines followed the same pattern, with *Smilax* sp. coming in first, followed by combinations 3, combination 1, combination 2, and *P. linteus*. Furthermore, the extracted materials were highly cytotoxic to MCF-7 but not to MDA-MB-231. According to an IC_{50}-dependent manner, the damage effect on HEK-293 cells was less than on MCF-7 cells. Beside *P. linteus* extract, most extracted samples were less cytotoxic to HEK-293 cells than to MDA-MB-468. However, the extracted samples become more toxic to HEK-293 as concentration increased, as did the results of *P. linteus* on MDA-MB-468 and all combinations on MDA-MB-231 cell lines.

4.5.2 Antiproliferative

Each cell line in this short term investigation of cell proliferation inhibition, received a 72-hour sample treatment before the addition of the BrdU incorporation reagent. The percentage of cell proliferation after 24 hours of BrdU incubation was measured and shown in Figure 11. Three different types of breast cancer cell lines were significantly inhibited in their ability to proliferate by *Smilax* sp. and *P. linteus* extracts individually and in combinations, compared to untreated cells.

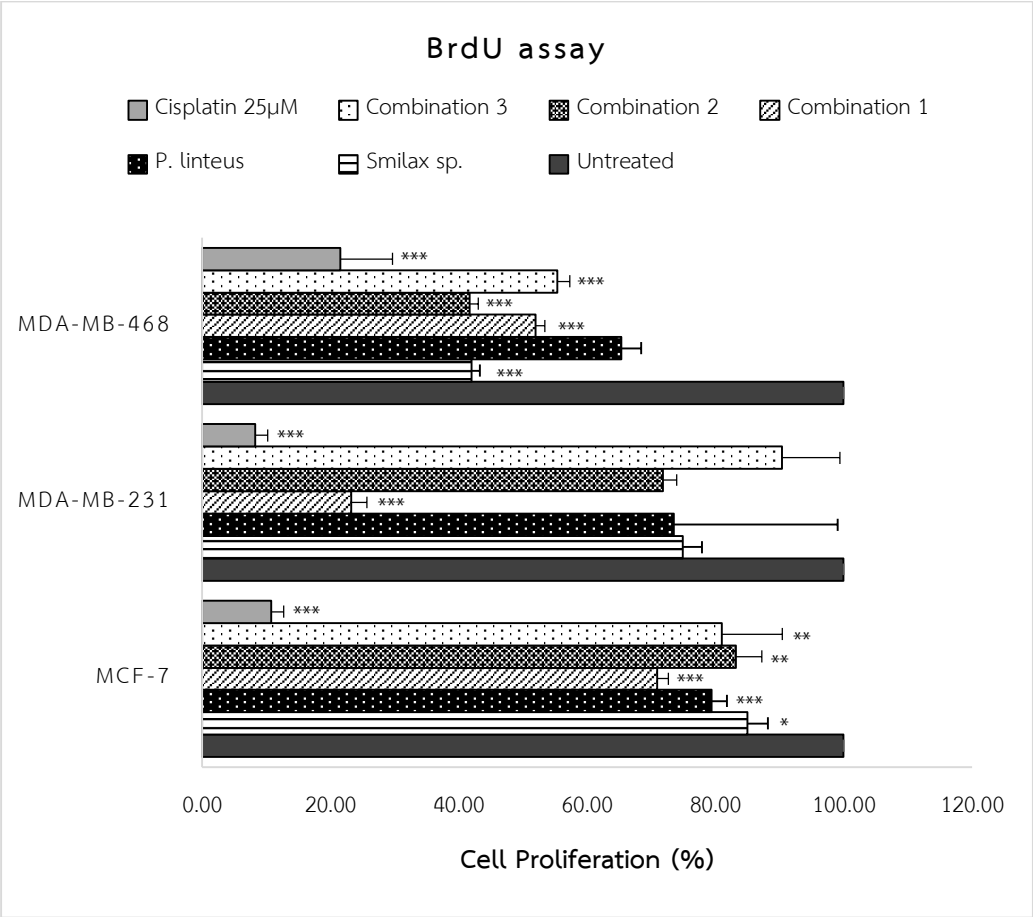


Figure 11. The antiproliferative effects of *Smilax* sp. extract, *Phellinus linteus* extract, and combinations were studied on three different breast cancer cell lines. For each sample of IC_{50}-treated cells, the percentage cell proliferation was expressed as the mean standard deviation of three replicates (One-way ANOVA with Tukey's HSD post-hoc test). *, **, *** represented P<0.05, P<0.01 and P<0.001 vs. the untreated among the same group (cell line's type).

Combination 1 exhibited the lowest amount of cell growth compared to the other samples, according to MCF-7 and MDA-MB-231 percentage data were 70.96±1.65% and 23.17±2.52%, respectively. Combination 2 nevertheless displayed the least amount of cell proliferation on MDA-MB-468. In addition, due to the value of each extract sample being below 70% of proliferation cells (*Smilax* sp. was 42.00±1.31%; *P. linteus* was 65.29±3.12%; combination 1 was 51.91±1.50%; combination 2 was 41.63±1.40%; combination 3 was 55.36 1.95%), all samples had

obvious antiproliferative activity on MDA-MB-468 compared to untreated cells. The positive control of 25μM cisplatin showed the lowest cell proliferative on all breast cancer cell lines.

4.5.3 Colony formation

A colony formation was investigated after 10 days' incubation post-treatment. The percentage of colonies on different breast cancer cell lines was shown in Figure 12.

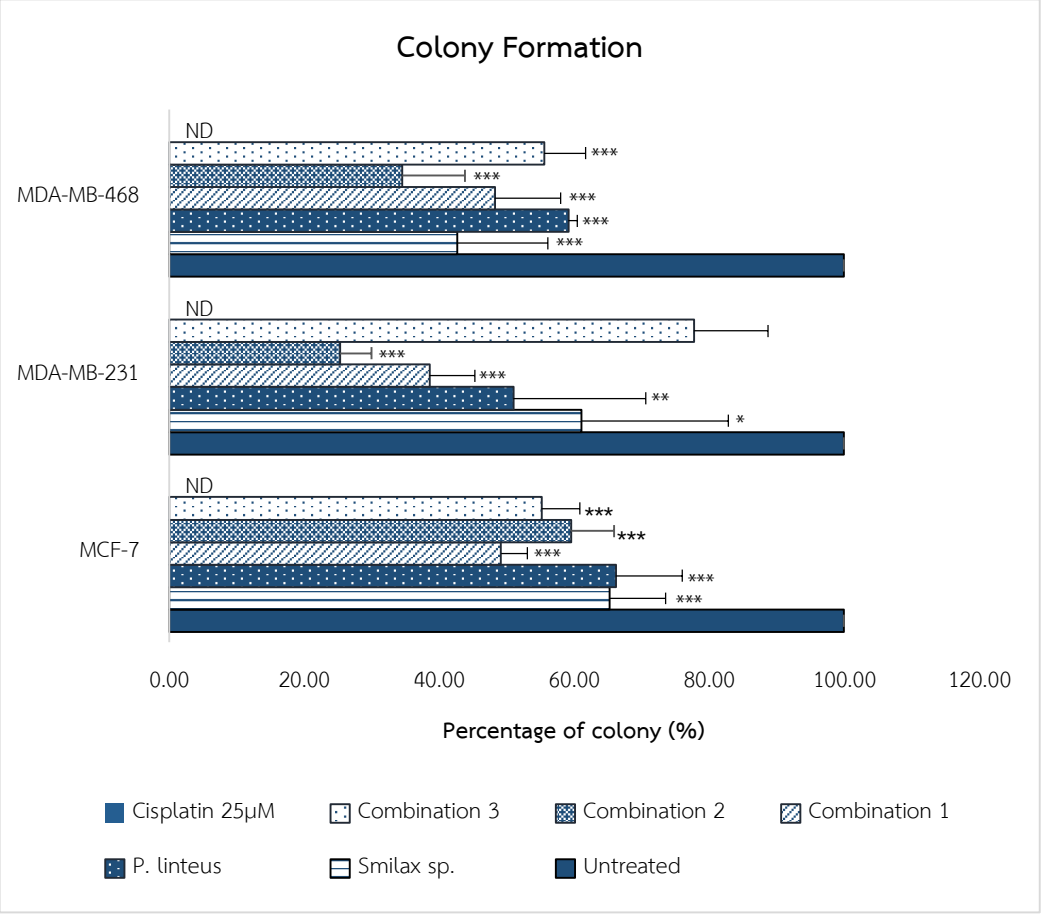


Figure 12. The colony formation test was used to assess the long-term inhibitory impact of *Smilax* sp. extract, *Phellinus linteus* extract, and combinations on three different breast cancer cell lines. For each sample of IC_{50}-treated cells, the percentage colony formation was expressed as the mean standard deviation of three replicates (One-way ANOVA with Tukey's HSD post-hoc test). *, **, ***

represented P<0.05, P<0.01 and P<0.001 vs. the untreated among the same group (cell line's type).

The colony percentage of all extracted samples on MCF-7 (P<0.001; all samples vs. untreated), MDA-MB-231 (P<0.05; *Smilax* sp. vs. untreated, P<0.01; *P. linteus* vs. untreated, P<0.001 combination 1 or combination 2 vs. untreated), and MDA-MB-468 (P<0.001; all samples vs. untreated) showed a significant difference compared to untreated cells (expected as 100%). Combination 1 had the lowest value (49.17±3.85%) among samples against MCF-7 cell lines. On MDA-MB-231 cell lines, combination 2 (25.30±4.67%) and combination 1 (38.58±6.72%) had a strong effect on inhibiting the growth of colonies compared to other extracted samples and untreated cells. Combination 2 (34.47±9.38%) also significantly reduced the percentage number of colonies against MDA-MB-468 compared to other extracted samples and untreated cells. The positive control cisplatin treatment at 25 μM showed non-detectable colonies for all three different breast cancer cell lines.

4.5.4 Apoptosis

After 72 hours of incubation, MCF-7 cells treated with the IC_{50} values of *P. linteus* (3.64±0.21%), combination 1 (2.99±1.97%) and cisplatin (12.82±12.08%) had a higher percentage of late apoptotic cells than untreated cells (0.20±0.22%). However, there was no significant different between all samples compared to untreated cell. For early apoptosis, *P. linteus* (4.17±0.34%), combination 1 (1.28±0.44%) and cisplatin (21.69±4.56%) had higher percentages than other extracted sample and untreated cell (0.27±0.20%), however only cisplatin had substantially significantly different (P<0.001; cisplatin vs. untreated) compared to untreated cell (Figure 13A).

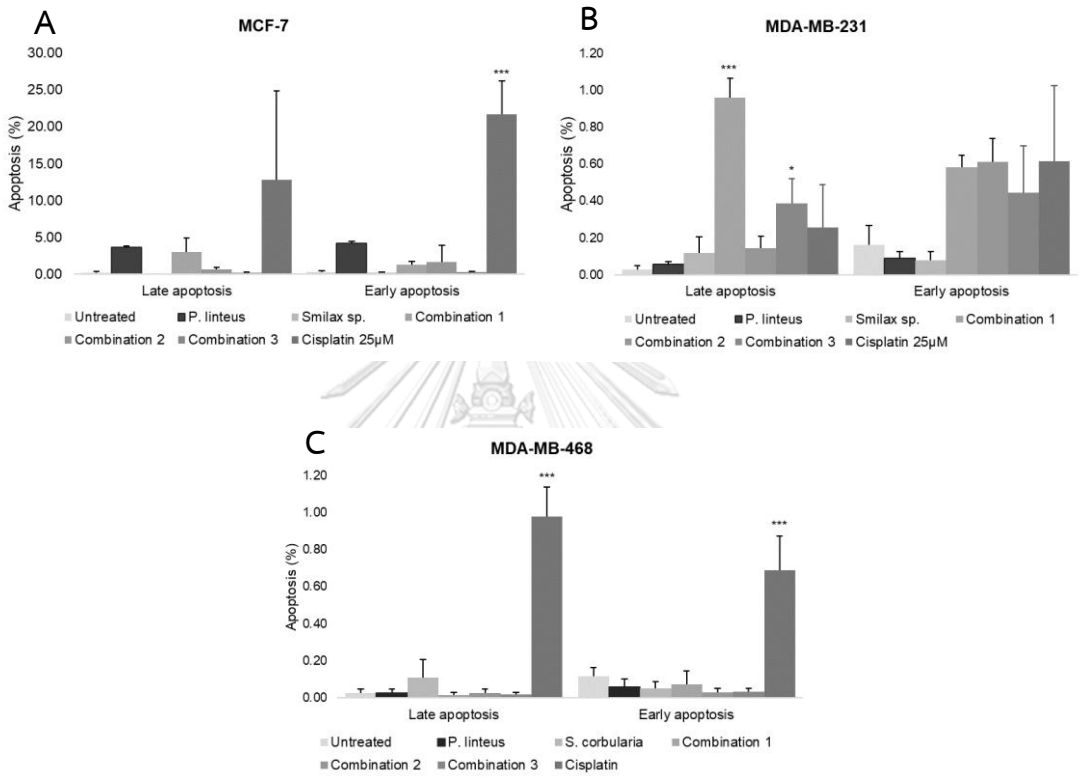


Figure 13. The apoptotic effect of Smilax sp. extract, Phellinus linteus extract, and combinations was studied on three different breast cancer cell lines. (A) MCF-7, (B) MDA-MB-231, (C) MDA-MB-468. For each sample of IC_{50}-treated cells, the percentage of cells in late apoptosis and early apoptosis was shown as the mean standard deviation of three replicates (One-way ANOVA with Tukey's HSD post-hoc test). *, *** represented $P<0.05$ and $P<0.001$ vs. the untreated among the same group (Late apoptosis or Early apoptosis in each cell line's type).

According to the MDA-MB-231 late apoptosis results, combinations 1 (0.96±0.10%) and combination 3 (0.39±0.13%) had greater values than other extracted samples and untreated cells (0.03±0.03%, Figure 13B). Combination 1 with values of 0.58±0.07%, combination 2 (0.61±0.13%), combination 3

(0.45±0.25%) and cisplatin (0.62±0.41%) had increased in early apoptotic rate compared to untreated (0.16±0.10%). Nevertheless, there was no significant different in early apoptosis of MDA-MB-231. All extracted samples showed the lowest apoptosis inductivity on MDA-MB-468 since there was no significant difference between extracted samples in late or early apoptosis when compared to untreated cells. In this result, only cisplatin that had the significant different compared to untreated cell both late and early apoptosis ($P<0.001$ cisplatin vs. untreated) (Figure 13C).

CHAPTER V

DISCUSSIONS

5.1 Preliminary compound screening

In this study, *P. linteus* was extracted from the powdered fruiting body by hot water, whereas crude extracts of *Smilax* sp. were obtained by ethanol extraction from the powdered root part. The findings showed that both samples had different yield extract percentages. It is common knowledge that factors such solvent type, extraction method, sample type, extraction process duration, and the obtained sample characteristics (specific culture, species and region growth distribution) may have an influence on the total yield of extraction, chemical properties, and bioactive substances (Kozarski et al., 2011; Moldovan et al., 2019; Zhang et al., 2018). According to this study, hot water extracted *P. linteus* extract had a lower total phenolic content than ethanol extracted from *Smilax* sp. Previous studies on the total phenolic content in *Smilax* sp. with ethanol extraction using the maceration process revealed a value of approximately 0.006 mg GAE/g (Jeeno et al., 2022), indicating that the modified extraction procedure using the reflux extractor in our recent study provides efficiency in the extraction of the total phenolic content. Furthermore, it is strongly advised to minimize the various factors that could affect the extract quality while continuously screening the active compounds of the extract using preliminary assays such as total phenolic content and total carbohydrate content.

On TLC analysis, *P. linteus* monosaccharides could not visualize under the UV due to its characteristic did not absorb the UV light (Ai et al., 2016). Meanwhile, for *Smilax* sp. there was one of three bands which had indicated to be a fluorescence substance in Rf number 2. Additionally, *Smilax* sp. extract in this study had 3 bands (0.26, 0.72, and 0.80) visualized with the p-anisaldehyde reagent which was different

from previous study of *Smilax* sp. root extract that had 5 bands of Rf (0.13, 0.23, 0.29, 0.39, and 0.67) (Martins et al., 2014). According to Caceres et al. (2012), the results showed some compounds that attributed to *S. domingensis* ethanolic extract were anthocyanin (Rf of 0.32-0.88), flavonoids (Rf of 0.27-0.81), and saponins with Rf values of 0.80 (Cáceres et al., 2012). However, TLC has poor reproducibility and poor color representation for microconstituents (Martins et al., 2014). Thus, after TLC the extracts still need advance analysis to know the exact compound inside.

This study showed that *P. linteus* crude extract has the greatest total polysaccharide content. Preliminary structural characterization implied polysaccharides from *P. linteus* extract were heteropolysaccrarides which the majority of sugar monomer unit was glucose as visualized by HPLC. Glucans with α-, β-, or both configuration forms are the most bioactive components of fungal polysaccharides (Kim et al., 2006; Kozarski et al., 2011). Antioxidant, anticancer, anti-inflammatory, and immunostimulating properties of these substances are well documented (He et al., 2021; Kozarski et al., 2011; Wong et al., 2020). In addition, the structure of *P. linteus* crude polysaccharide in line with FT-IR data suggests that it was made up of β -linked pyranose sugars, and spectroscopic data confirmed the presence of a small quantity of phenol. The average M_ws of the *P. linteus* crude polysaccharide in the GPC data was lower (3 kDa). A report of low molecular weight of the PL-N3 fraction polysaccharide considerably increased antioxidant activity, according to research on degraded polysaccharide using ultrasonic treatment without altering the basic structure of *P. linteus* polysaccharide (original PL-N). Additionally, polysaccharides with low molecular weights may be absorbed more readily and employed for medicinal purposes (Yan et al., 2016).

The biological activities of polysaccharides can be impacted by their extraction, molecular weight, monosaccharide compositions, and chemical structures (Qin et al., 2023; Z. B. Wang et al., 2014). In this study, crude extracts of *Smilax* sp. and *P. linteus* were combined to create three unique mixtures. Combination 3 had a greater total

phenolic content than the other combinations because it included 75% *Smilax* sp. extract. Combination 2 had a greater total polysaccharide content because *P. linteus* crude extract made up the majority of the mixture compared to other combinations. According to the results of the current investigation, the total phenolic and total polysaccharide contents of all combinations relied on the relative ratios of the constituents in each combination.

5.2 Antioxidants

This present study on individual *Smilax* sp. revealed high DPPH and ABTS radical scavenging activities than previous study on *S. corbularia* obtained by ethanol extraction (Jeeno et al., 2022). In addition, the antioxidant activity of individually *P. linteus* extract was stronger than earlier research on the fruiting body of *P. linteus* by water extraction scavenge the DPPH reagent (Kozarski et al., 2011). When compared to the other combinations, combination 3 had the greatest antioxidant. The antioxidant activities of both DPPH and ABTS from the high to low are represented as *Smilax* sp., combination 3, combination 1, combination 2, and *P. linteus*.

Although the results of combinations could not surpass those of individual *Smilax* sp. extract, combination 2 (which contained *P. linteus* extract at 75% of the total amount) might increase the scavenging activity by up to 8 times when compared to individual *P. linteus*. It is possible that the mixtures may have a compound interaction on biological activities between the two separate extracts. Moreover, earlier study has discovered a significant relationship between total phenolic content and antioxidant activity (Durgo et al., 2013). The sample with the highest total phenol content, such as individual *Smilax* sp. and combination 3, had the best ability to scavenge free radicals, which is consistent with this present finding.

5.3 Anticancers

The toxicity of a substance is indicated when cells are unable to transform tetrazolium salts into colorful formazan product (Ghasemi et al., 2021). When rated from strong to weak activity, the toxicity of all extracted materials on three different

breast cancer cell lines followed the same pattern, with individually *Smilax* sp. extract coming in first, followed by combinations 3, combinations 1, and combinations 2, and *P. linteus*. This indicates that the toxicity of extracted samples on cancer cells had a positive associated with antioxidant activity.

Reducing ROS-induced stressors by either scavenging free radicals or producing endogenous antioxidants is one of the many potential mechanisms of phytochemical antioxidants that may link their abilities as a cytotoxic agent to cancer cells. Targeting enzymatic antioxidants such SODs, GSH peroxidases (GPXs), peroxiredoxins (PRDXs), and catalase (CAT) as well as nonenzymatic antioxidant systems including NRF2 activators, vitamins, N-acetylcysteine (NAC), and GSH esters might increase endogenous antioxidant levels (Luo et al., 2022). Managing the H_2O_2-mediated oxidative stress can drastically affect the survival of MCF-7 cells with downregulated PRDX1 according to a prior work (Bajor et al., 2018). In addition, the antioxidant and cytotoxic properties of the medicinal herb *Medinilla speciosa* Blume were positively correlated in another study on the 4T1 breast cancer cell line (Sasikirana et al., 2021). It is in line with this present finding that individually *Smilax* sp. and combination 3, which had higher antioxidant activities compare to other extracts, also had a greater cytotoxic effect on breast cancer at lower concentrations.

The cytotoxic effect of the extracted samples in this present study was high on the MCF-7, moderate on the MDA-MB-468, and low cytotoxic effect on MDA-MB-231. It was indicated that every subtype of the cell, including breast cancer cell lines, would react to the extract differently. Excluding MCF-7 and MDA-MB-468, all extracted samples required a higher dose which can also impair the survival of normal cells HEK-293. For instance, when administered to MDA-MB-231 cells under the same conditions as IC_{50}-dependent treatments to HEK-293, the combinations were able to kill around 50% of the cells. In light of these results, we speculate that the crude extracts might selectively harm on breast cancer cells while having less of an impact on non-cancerous cells.

Cell proliferation was assessed in this study after treated with the extract samples for 72 hours. The cytotoxic agent might have an impact on cellular molecular systems and interfered the cell divisions (Sivapatham & Selvaraj, 2022). Therefore, not all cancer cells that survive a therapy can proliferate in those condition. This proliferation investigation discovered that combination 1 had the largest antiproliferative effect on MCF-7 and MDA-MB-231 cell lines, in contrast to the cytotoxic conclusion that combination 3 had the highest activity. Additionally, combination 2 exhibited the greatest inhibitory effect on the growth of MDA-MB-468 cells. It was proposed that distinct effects of chemical interactions on the cell mechanism exist.

Hispolon, a polyphenol found mostly in the genus *Phelinus*, has numerous anticancer actions, including suppressing cell proliferation (Sarfraz et al., 2020). Isolated hispolon from *P. lonicerinus* (Bond) ethanol extract had a statistically significant difference on estrogen-negative ER (-) MDA-MB-231 cells in high concentration, whereas it induced the proliferation of estrogen-sensitive ER (+) MCF-7 cells in lower concentration and reduced in high concentration. Moreover, hispolon significantly inhibited the cell proliferation on MCF-7 cells in the presence of E_2 (anti-estrogenic activity). This means hispolon had dual directional estrogenic and anti-estrogenic effects (J. Wang et al., 2014). Moreover, methanol extraction of *S. corbularia* also had estrogenic and anti-estrogenic against E_2-enhanced MCF-7 and co-treated E_2 on T47D in depended dose manner, due to its compound contained were flavonol rhamnoside, astilbin, cathecin, epicathecin, and resveratrol have the capacity to affect as antiproliferative agents (Wungsintaweekul et al., 2011). Thus, the combination of those bioactive chemicals from both separate extracts in certain ratios in this new study may have a synergistic effect on inhibiting breast cancer cell proliferation.

Furthermore, to know the potential long-term inhibitory effects of the extract samples on exposure to three breast cancer cell lines, a colony formation assay was

performed with incubation for 10 days after treatment. Beside combination 3 treated on MDA-MB-231, most extracted samples showed significant inhibition of MCF-7, MDA-MB-231, and MDA-MB-468 colonies growth. In line with the short-term inhibitory effect, which was cell proliferation, the lowest percentage of colonies growth on MCF-7 was combination 1, and on MDA-MB-468, the lowest percentage of colonies was combination 2, compared to other extracts and untreated cells. Different results from cell proliferation occurred on MDA-MB-231, which was combination 2 had the lowest colony percentage value, followed by combination 1. Thus, in this study, certain combinations had a strong long-term inhibitory effect on breast cancer cell lines.

For the control positive, which was cisplatin in a small concentration of 25 μM, the results of colony formation were non-detectable on three distinct breast cancer cell lines. It suggested that the inhibition of the drug was almost 100% or that there was no colony growth under treatment with cisplatin. It can also be seen in the short term measurement of growth inhibition using the BrdU method, which showed the lowest result among all sample treatments on various breast cancer cell lines. However, cisplatin treatment could lead to adverse side effects for patients (Byrski et al., 2009; Smith & Talbot, 1992). To illustrate the cytotoxic effect of cisplatin on normal HEK-293 cells, this study showed the lowest value compared to three breast cancer cell lines (Figure 10). Moreover, a long period of cisplatin consumption contributes to drug resistance (Kashkoulinejad-Kouhi et al., 2021; Lee et al., 2019).

In accordance with apoptosis data, some samples including *P. linteus* and combination 1, displayed greater rates of late and/or early apoptosis. However, the result of apoptosis induction level of the extracted samples on breast cancer cell lines was low and have demonstrated by the values not exceeding 5%. It has been previously stated that all cells will perish if the mechanism necessary for their continuous existence is interrupted. The capacity of a cell to maintain cellular physiological homeostasis is influenced by a number of factors. Any substance or

agent may have an effect on the cells under stress and result in physiological changes when administered in a sub-lethal doses in a period time (Gerl & Vaux, 2005; Kroemer et al., 2022).

It has been reported that several biochemical substances induced programmed cell death. It was previously believed that the only type of programmed cell death that significantly improved cancer treatment was apoptosis. However, cancer cells create genetic mutations or epigenetic modifications of its key modulator pathways, such as mutation of the pro-apoptotic gene p53 and up-regulation of B-cell lymphoma-2 (BCL-2) protein, which is a protein inhibiting apoptosis. These modifications are prepared in order to avoid programmed cell death. As a result, cancer cells frequently lack the ability to induce apoptosis and may develop apoptotic resistance. Autophagy and necroptosis are two more non-apoptotic forms that have been added as a result of additional regulation of planned cell death (Alqudah et al., 2022; Su et al., 2015; Yang et al., 2023). This latest study may lead to other programmed cell deaths besides apoptosis due to the mitochondrial malfunction revealed by the MTT experiment and the lower growth of cancer cells.

This study revealed that among the combinations, combination 3 elicited higher antioxidant and cytotoxic effects due to the increase in total phenolic content, followed by combination 1 and combination 2. However, in further investigation of anticancer activities such as antiproliferative effect, long-term inhibition with colony formation, and apoptotic cells, it was shown that combination 1 had greater effectiveness against breast cancer cell lines and may lead to a synergistic effect. It is because the 50:50 combination may have each compound's best possible interaction against breast cancer cell lines. Moreover, the value of combination 1 in antioxidant and cytotoxic effects was closer to combination 3 than combination 2. In accordance with the result of this study with specific raw samples, extraction method, quality and quantity of the extracts compound, combination 1, which contained 50% *Smilax* sp. and 50% *P. linteus* extracts, has been recommended as an anticancer agent.

CHAPTER VI

CONCLUSION

In conclusion, ethanol extract of *Smilax* sp. and hot water extract from *P. linteus* together with their combinations for further investigation of the preliminary physiochemical screening, antioxidant activity and anticancer activity. The obtained *Smilax* sp. crude extract showed a high level of total phenolic content, while the crude *P. linteus* extract was enriched in total polysaccharide content. The combinations comprised total phenolic content and total polysaccharide content, which were dependent on the respective ratios of individual extract constituents. Furthermore, this study implied that the increase of antioxidant and cytotoxic activities is correlated with the increase of total phenolic content. Certain combinations had significantly reduced the cell proliferation and the total number of colony formation on three distinct breast cancer cell lines. However, the inductivity of apoptosis was not very potent and might be leading to other mechanisms of programmed cell death which deserves further exploration. Based on the results, the further investigation of anticancer activities such as antiproliferative effect, long-term inhibition with colony formation, and apoptotic cells, it was shown that combination 1 had greater effectiveness against breast cancer cell lines and may lead to a synergistic effect. Additionally, combination 1 had more effectiveness as anticancer agent.

REFERENCES

Ai, Y., Yu, Z., Chen, Y., Zhu, X., Ai, Z., Liu, S., & Ni, D. (2016). Rapid determination of the monosaccharide composition and contents in tea polysaccharides from Yingshuang green tea by pre-column derivatization HPLC. *Journal of chemistry*, *2016*.

Alqudah, M. A., Al‐Samman, R., Azaizeh, M., & Alzoubi, K. H. (2022). Amlodipine inhibits proliferation, invasion, and colony formation of breast cancer cells. *Biomedical Reports*, *16*(6), 1-7.

Arnao, M. B., Cano, A., & Acosta, M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. *Food chemistry*, *73*(2), 239-244.

Bajalovic, N., Or, Y. Z., Woo, A. R. E., Lee, S. H., & Lin, V. C. L. (2022). High Levels of Progesterone Receptor B in MCF-7 Cells Enable Radical Anti-Tumoral and Anti-Estrogenic Effect of Progestin. *Biomedicines*, *10*(8). https://doi.org/10.3390/biomedicines10081860

Bajor, M., Zych, A. O., Graczyk-Jarzynka, A., Muchowicz, A., Firczuk, M., Trzeciak, L., Gaj, P., Domagala, A., Siernicka, M., Zagozdzon, A., Siedlecki, P., Kniotek, M., O'Leary, P. C., Golab, J., & Zagozdzon, R. (2018). Targeting peroxiredoxin 1 impairs growth of breast cancer cells and potently sensitises these cells to prooxidant agents. *British Journal of Cancer*, *119*(7), 873-884. https://doi.org/10.1038/s41416-018-0263-y

Baker, J. R., Kim, J.-S., & Park, S.-Y. (2008). Composition and proposed structure of a water-soluble glycan from the Keumsa Sangwhang mushroom (Phellinus linteus). *Fitoterapia*, *79*(5), 345-350.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *68*(6), 394-424.

Byrski, T., Huzarski, T., Dent, R., Gronwald, J., Zuziak, D., Cybulski, C., Kladny, J., Gorski, B., Lubinski, J., & Narod, S. (2009). Response to neoadjuvant therapy with

cisplatin in BRCA1-positive breast cancer patients. *Breast Cancer Research and Treatment*, *115*, 359-363.

Cáceres, A., Cruz, S. M., Martínez, V., Gaitán, I., Santizo, A., Gattuso, S., & Gattuso, M. (2012). Ethnobotanical, pharmacognostical, pharmacological and phytochemical studies on Smilax domingensis in Guatemala. *Revista Brasileira de Farmacognosia*, *22*, 239-248.

Cavalli, A., Bolognesi, M. L., Minarini, A., Rosini, M., Tumiatti, V., Recanatini, M., & Melchiorre, C. (2008). Multi-target-directed ligands to combat neurodegenerative diseases. *Journal of Medicinal Chemistry*, *51*(3), 347-372.

Chen, M., Rao, Y., Zheng, Y., Wei, S., Li, Y., Guo, T., & Yin, P. (2014). Association between soy isoflavone intake and breast cancer risk for pre-and post-menopausal women: a meta-analysis of epidemiological studies. *PloS One*, *9*(2), e89288.

Chen, X., Li, H., Zhang, B., & Deng, Z. (2022). The synergistic and antagonistic antioxidant interactions of dietary phytochemical combinations. *Crit Rev Food Sci Nutr*, *62*(20), 5658-5677. https://doi.org/10.1080/10408398.2021.1888693

Chu, D., HUang, Z., & He, F. (2018). Comparison between sulfuric acid-phenol and sulfuric acid-anthrone methods used for determination of polysaccharides in shoots of Aralia elata (Miq.) Seem. *Agricultural Biotechnology*, *7*(3), 4.

Daddiouaissa, D., Amid, A., Kabbashi, N. A., Fuad, F. A., Elnour, A. M., & Epandy, M. A. (2019). Antiproliferative activity of ionic liquid-graviola fruit extract against human breast cancer (MCF-7) cell lines using flow cytometry techniques. *Journal of ethnopharmacology*, *236*, 466-473.

Dai, X., Cheng, H., Bai, Z., & Li, J. (2017). Breast cancer cell line classification and its relevance with breast tumor subtyping. *Journal of Cancer*, *8*(16), 3131.

Di Meo, S., Reed, T. T., Venditti, P., & Victor, V. M. (2016). Role of ROS and RNS sources in physiological and pathological conditions. *Oxidative Medicine and Cellular Longevity*, *2016*.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y.-H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of food and drug analysis*, *22*(3), 296-302.

Durgo, K., Koncar, M., Komes, D., Belscak-Cvitanovic, A., Franekic, J., Jakopovich, I., Jakopovich, N., & Jakopovic, B. (2013). Cytotoxicity of blended versus single medicinal mushroom extracts on human cancer cell lines: contribution of polyphenol and polysaccharide content. *International Journal of Medicinal Mushrooms*, *15*(5).

Fauzantoro, A., Muharam, Y., & Gozan, M. (2017). Improvement of nicotine yield by ethanolic heat reflux extraction of Nicotiana tabacum var. Virginia origin of ponorogo. *International Journal of Applied Engineering Research*, *12*(23), 13891-13897.

Gaber, N. B., El-Dahy, S. I., & Shalaby, E. A. (2021). Comparison of ABTS, DPPH, permanganate, and methylene blue assays for determining antioxidant potential of successive extracts from pomegranate and guava residues. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-021-01386-0

Garcia, E., Luna, I., Persad, K. L., Agopsowicz, K., Jay, D. A., West, F. G., Hitt, M. M., & Persad, S. (2021). Inhibition of triple negative breast cancer metastasis and invasiveness by novel drugs that target epithelial to mesenchymal transition. *Sci Rep*, *11*(1), 11757. https://doi.org/10.1038/s41598-021-91344-7

Ge, Q., Mao, J.-w., Zhang, A.-q., Wang, Y.-j., & Sun, P.-l. (2013). Purification, chemical characterization, and antioxidant activity of a polysaccharide from the fruiting bodies of sanghuang mushroom (Phellinus baumii Pilát). *Food Science and Biotechnology*, *22*(2), 301-307. https://doi.org/10.1007/s10068-013-0081-1

Gerl, R., & Vaux, D. L. (2005). Apoptosis in the development and treatment of cancer. *Carcinogenesis*, *26*(2), 263-270. https://doi.org/10.1093/carcin/bgh283

Ghasemi, M., Turnbull, T., Sebastian, S., & Kempson, I. (2021). The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis. *Int J Mol Sci*, *22*(23). https://doi.org/10.3390/ijms222312827

Ghosh, S., Khatua, S., Dasgupta, A., & Acharya, K. (2021). Crude polysaccharide from the milky mushroom, Calocybe indica, modulates innate immunity of macrophage cells by triggering MyD88-dependent TLR4/NF-kappaB pathway. *J Pharm Pharmacol*, *73*(1), 70-81. https://doi.org/10.1093/jpp/rgaa020

Habtemariam, S. (2017). Going back to the good old days: The merit of crude plant drug

mixtures in the 21st century. *International Journal of Complementary & Alternative Medicine*, *6*(2), 1-5.

Haryanti, S., Ratnawati, G., & Rahmawati, N. (2020). Curcuma zanthorrhiza Extracts Induce G2/M Cell Cycle Arrest and Apoptosis in 4T1 and MCF-7 Human Breast Cancer Cells. 4th International Symposium on Health Research (ISHR 2019),

Hasanpourghadi, M., Yeng Looi, C., Kumar Pandurangan, A., Sethi, G., Fen Wong, W., & Rais Mustafa, M. (2017). Phytometabolites targeting the Warburg effect in cancer cells: a mechanistic review. *Current Drug Targets*, *18*(9), 1086-1094.

Hashem, S., Ali, T. A., Akhtar, S., Nisar, S., Sageena, G., Ali, S., Al-Mannai, S., Therachiyil, L., Mir, R., & Elfaki, I. (2022). Targeting cancer signaling pathways by natural products: Exploring promising anti-cancer agents. *Biomedicine & Pharmacotherapy*, *150*, 113054.

He, P., Zhang, Y., & Li, N. (2021). The phytochemistry and pharmacology of medicinal fungi of the genus Phellinus: a review. *Food Funct*, *12*(5), 1856-1881. https://doi.org/10.1039/d0fo02342f

Holliday, D. L., & Speirs, V. (2011). Choosing the right cell line for breast cancer research. *Breast Cancer Research*, *13*(4), 7. http://breast-cancer-research.com/content/13/4/215

Hong, I.-P., Jung, I.-Y., Nam, S.-H., Park, J.-S., Cho, J. H., Lee, M.-W., & Guo, S.-X. (2002). Cultural characteristics of a medicinal mushroom, Phellinus linteus. *Mycobiology*, *30*(4), 208-212.

Hong, Y., Wang, Z., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. (2021). High-throughput screening and characterization of phenolic compounds in stone fruits waste by lc-esi-qtof-ms/ms and their potential antioxidant activities. *Antioxidants*, *10*(2), 234.

Hortobagyi, G. N., de la Garza Salazar, J., Pritchard, K., Amadori, D., Haidinger, R., Hudis, C. A., Khaled, H., Liu, M.-C., Martin, M., & Namer, M. (2005). The global breast cancer burden: variations in epidemiology and survival. *Clinical Breast Cancer*, *6*(5), 391-401.

Ishibashi, T. (2013). Molecular hydrogen: new antioxidant and anti-inflammatory therapy

for rheumatoid arthritis and related diseases. *Current Pharmaceutical Design*, *19*(35), 6375-6381.

Itharat, A., Houghton, P. J., Eno-Amooquaye, E., Burke, P. J., Sampson, J. H., & Raman, A. (2004). In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. *J Ethnopharmacol*, *90*(1), 33-38. https://doi.org/10.1016/j.jep.2003.09.014

Jeeno, P., Tongban, S., Yana, P., Wongta, A., Sutan, K., Yadoung, S., & Hongsibsong, S. (2022). Tentative Identification of Phytochemicals from Smilax glabra and Smilax corbularia Extracts by LC-QTOF/MS and Their Bioactive Potential. *Plants (Basel)*, *11*(16). https://doi.org/10.3390/plants11162089

Kashkoulinejad-Kouhi, T., Safarian, S., Arnaiz, B., & Saa, L. (2021). Enhancement of cisplatin sensitivity in human breast cancer MCF-7 cell line through BiP and 14-3-3ζ co-knockdown. *Oncology Reports*, *45*(2), 665-679.

Khan, A. Q., Rashid, K., AlAmodi, A. A., Agha, M. V., Akhtar, S., Hakeem, I., Raza, S. S., & Uddin, S. (2021). Reactive oxygen species (ROS) in cancer pathogenesis and therapy: An update on the role of ROS in anticancer action of benzophenanthridine alkaloids. *Biomedicine & Pharmacotherapy*, *143*, 112142.

Kim, G.-Y., Lee, J.-Y., Lee, J.-O., Ryu, C.-H., Choi, B. T., Jeong, Y.-K., Lee, K.-W., Jeong, S.-C., & Choi, Y. H. (2006). Partial characterization and immunostimulatory effect of a novel polysaccharide–protein complex extracted from Phellinus linteus. *Bioscience, biotechnology, and biochemistry*, *70*(5), 1218-1226.

Konno, S., Chu, K., Feuer, N., Phillips, J., & Choudhury, M. (2015). Potent Anticancer Effects of Bioactive Mushroom Extracts (Phellinus linteus) on a Variety of Human Cancer Cells. *J Clin Med Res*, *7*(2), 76-82. https://doi.org/10.14740/jocmr1996w

Koyama, T. (1984). A taxonomic revision of the genus Heterosmilax (Smilacaceae). *Brittonia*, 184-205.

Kozarski, M., Klaus, A., Niksic, M., Jakovljevic, D., Helsper, J. P. F. G., & Van Griensven, L. J. L. D. (2011). Antioxidative and immunomodulating activities of polysaccharide extracts of the medicinal mushrooms Agaricus bisporus, Agaricus brasiliensis, Ganoderma lucidum and Phellinus linteus. *Food Chemistry*, *129*(4), 1667-1675.

https://doi.org/10.1016/j.foodchem.2011.06.029

Kress, W. J., DeFilipps, R. A., Farr, E., & Kyi, D. Y. Y. (2003). A checklist of the trees, shrubs, herbs, and climbers of Myanmar. *A checklist of the trees, shrubs, herbs, and climbers of Myanmar*.

Kroemer, G., Galassi, C., Zitvogel, L., & Galluzzi, L. (2022). Immunogenic cell stress and death. *Nat Immunol*, *23*(4), 487-500. https://doi.org/10.1038/s41590-022-01132-2

Kumar, S., Eroglu, E., Stokes III, J. A., Scissum-Gunn, K., Saldanha, S. N., Singh, U. P., Manne, U., Ponnazhagan, S., & Mishra, M. K. (2017). Resveratrol induces mitochondria-mediated, caspase-independent apoptosis in murine prostate cancer cells. *Oncotarget*, *8*(13), 20895.

Lasfargues, E. Y., & Ozzello, L. (1958). Cultivation of human breast carcinomas. *Journal of the National Cancer Institute*, *21*(6), 1131-1147.

Lee, J., Li, C., Surayot, U., Yelithao, K., Lee, S., Park, W., Tabarsa, M., & You, S. (2018). Molecular structures, chemical properties and biological activities of polysaccharide from Smilax glabra rhizome. *Int J Biol Macromol*, *120*(Pt B), 1726-1733. https://doi.org/10.1016/j.ijbiomac.2018.09.138

Lee, J. O., Kang, M. J., Byun, W. S., Kim, S., Seo, I. H., Han, J. A., Moon, J. W., Kim, J. H., Kim, S. J., & Lee, E. J. (2019). Metformin overcomes resistance to cisplatin in triple-negative breast cancer (TNBC) cells by targeting RAD51. *Breast Cancer Research*, *21*(1), 1-18.

Lee, W. Y., Hsu, K. F., Chiang, T. A., & Chen, C. J. (2015). Phellinus linteus extract induces autophagy and synergizes with 5-fluorouracil to inhibit breast cancer cell growth. *Nutr Cancer*, *67*(2), 275-284. https://doi.org/10.1080/01635581.2015.989374

Leone, J., Freedman, R. A., Lin, N. U., Tolaney, S. M., Vallejo, C. T., Leone, B. A., Winer, E. P., & Leone, J. P. (2021). Tumor subtypes and survival in male breast cancer. *Breast Cancer Research and Treatment*, *188*, 695-702.

Li, T., Wu, D., Yang, Y., & Chen, L. (2019). Review on structural characteristics and biological activities of Phellinus polysaccharides. AIP Conference Proceedings,

Lu, C. L., Zhu, W., Wang, M., Xu, X. J., & Lu, C. J. (2014). Antioxidant and Anti-Inflammatory Activities of Phenolic-Enriched Extracts of Smilax glabra. *Evid*

Based Complement Alternat Med, *2014*, 910438. https://doi.org/10.1155/2014/910438

Lukong, K. E. (2017). Understanding breast cancer–The long and winding road. *BBA clinical*, *7*, 64-77.

Luo, M., Zhou, L., Huang, Z., Li, B., Nice, E. C., Xu, J., & Huang, C. (2022). Antioxidant Therapy in Cancer: Rationale and Progress. *Antioxidants (Basel)*, *11*(6). https://doi.org/10.3390/antiox11061128

Machana, S., Weerapreeyakul, N., Barusrux, S., Thumanu, K., & Tanthanuch, W. (2012). Synergistic anticancer effect of the extracts from Polyalthia evecta caused apoptosis in human hepatoma (HepG2) cells. *Asian Pacific Journal of Tropical Biomedicine*, *2*(8), 589-596.

Mariani, F., Tammachote, R., Kusuma, I. W., Chavasiri, W., Punnapayak, H., & Prasongsuk, S. (2021). Phenolic contents and antioxidant activities of leaf extracts from Elaeocarpus submonoceras Miq. *Songklanakarin Journal of Science & Technology*, *43*(2).

Mariotto, A. B., Etzioni, R., Hurlbert, M., Penberthy, L., & Mayer, M. (2017). Estimation of the number of women living with metastatic breast cancer in the United States. *Cancer Epidemiology, Biomarkers and Prevention*, *26*(6), 809-815.

Martins, A. R., Soares, M. K., Redher, V. L., Bajay, M. M., Villela, P. M., Zucchi, M. I., & Appezzato-da-Glória, B. (2014). Use of anatomical, chemical, and molecular genetic characteristics in the quality control of medicinal species: A case study of sarsaparilla (Smilax spp.). *Economic botany*, *68*, 410-425.

Mei, Y., Zhu, H., Hu, Q., Liu, Y., Zhao, S., Peng, N., & Liang, Y. (2015). A novel polysaccharide from mycelia of cultured Phellinus linteus displays antitumor activity through apoptosis. *Carbohydr Polym*, *124*, 90-97. https://doi.org/10.1016/j.carbpol.2015.02.009

Meybodi, N. M., Mortazavian, A. M., Monfared, A. B., Sohrabvandi, S., & Meybodi, F. A. (2017). Phytochemicals in cancer prevention: a review of the evidence. *Iranian Journal of Cancer Prevention*, *10*(1).

Min, G.-J., & Kang, H.-W. (2021). Artificial Cultivation Characteristics and Bioactive Effects of Novel Tropicoporus linteus (Syn. Phellinus linteus) Strains HN00K9 and

HN6036 in Korea. *Mycobiology*, *49*(2), 161-172. https://doi.org/10.1080/12298093.2021.1892568

Moldovan, M. L., Iurian, S., Puscas, C., Silaghi-Dumitrescu, R., Hanganu, D., Bogdan, C., Vlase, L., Oniga, I., & Benedec, D. (2019). A Design of Experiments strategy to enhance the recovery of polyphenolic compounds from Vitis vinifera by-products through heat reflux extraction. *Biomolecules*, *9*(10), 529.

Morais, M. I., Pinto, M. E., Araujo, S. G., Castro, A. H., Duarte-Almeida, J. M., Rosa, L. H., Rosa, C. A., Johann, S., & Lima, L. A. (2014). Antioxidant and antifungal activities of Smilax campestris Griseb. (Smilacaceae). *Nat Prod Res*, *28*(16), 1275-1279. https://doi.org/10.1080/14786419.2014.895728

Nardin, S., Mora, E., Varughese, F. M., D'Avanzo, F., Vachanaram, A. R., Rossi, V., Saggia, C., Rubinelli, S., & Gennari, A. (2020). Breast cancer survivorship, quality of life, and late toxicities. *Frontiers in Oncology*, *10*, 864.

Nawab, A., Yunus, M., Mahdi, A. A., & Gupta, S. (2011). Evaluation of anticancer properties of medicinal plants from the Indian sub-continent. *Molecular and Cellular Pharmacology*, *3*(1), 21-29.

Pei, J. J., Wang, Z. B., Ma, H. L., & Yan, J. K. (2015). Structural features and antitumor activity of a novel polysaccharide from alkaline extract of Phellinus linteus mycelia. *Carbohydr Polym*, *115*, 472-477. https://doi.org/10.1016/j.carbpol.2014.09.017

Petrie, J. R., Guzik, T. J., & Touyz, R. M. (2018). Diabetes, hypertension, and cardiovascular disease: clinical insights and vascular mechanisms. *Canadian Journal of Cardiology*, *34*(5), 575-584.

Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International journal of biomedical science: IJBS*, *4*(2), 89.

Qin, D., Han, S., Liu, M., Guo, T., Hu, Z., Zhou, Y., & Luo, F. (2023). Polysaccharides from Phellinus linteus: A systematic review of their extractions, purifications, structures and functions. *Int J Biol Macromol*, *230*, 123163. https://doi.org/10.1016/j.ijbiomac.2023.123163

Ramos-Andrés, M., Aguilera-Torre, B., & García-Serna, J. (2021). Hydrothermal production of high-molecular weight hemicellulose-pectin, free sugars and residual

cellulose pulp from discarded carrots. *Journal of Cleaner Production*, *290*, 125179.

Raúl, S.-C., Beatriz, H.-C., Joseoziel, L.-G., & Francenia, S.-S. N. (2017). Phenolic compounds in genus Smilax (Sarsaparilla). *Phenolic Compounds*, 233-260.

Reiffová, K., & Nemcová, R. (2006). Thin-layer chromatography analysis of fructooligosaccharides in biological samples. *Journal of Chromatography A*, *1110*(1-2), 214-221.

Reis, F. S., Barreira, J. C., Calhelha, R. C., van Griensven, L. J., Ćirić, A., Glamočlija, J., Soković, M., & Ferreira, I. C. (2014). Chemical characterization of the medicinal mushroom Phellinus linteus (Berkeley & Curtis) Teng and contribution of different fractions to its bioactivity. *LWT-Food Science and Technology*, *58*(2), 478-485.

Rufino, M. S., Alves, R. E., Fernandes, F. A., & Brito, E. S. (2011). Free radical scavenging behavior of ten exotic tropical fruits extracts. *Food Research International*, *44*(7), 2072-2075.

Sarfraz, A., Rasul, A., Sarfraz, I., Shah, M. A., Hussain, G., Shafiq, N., Masood, M., Adem, S., Sarker, S. D., & Li, X. (2020). Hispolon: A natural polyphenol and emerging cancer killer by multiple cellular signaling pathways. *Environ Res*, *190*, 110017. https://doi.org/10.1016/j.envres.2020.110017

Sasikirana, W., Ekawati, N., & Dini, I. R. E. (2021). Correlation Between Antioxidant and Cytotoxic Activity of Parijoto (Medinilla speciosa Blume) Fractions in 4T1 Cell Line. *Indonesian Journal of Cancer Chemoprevention*, *12*(1), 21-27.

Sever, R., & Brugge, J. S. (2015). Signal transduction in cancer. *Cold Spring Harbor Perspectives in Medicine*, *5*(4).

She, T., Qu, L., Wang, L., Yang, X., Xu, S., Feng, J., Gao, Y., Zhao, C., Han, Y., & Cai, S. (2015). Sarsaparilla (Smilax Glabra Rhizome) Extract Inhibits Cancer Cell Growth by S Phase Arrest, Apoptosis, and Autophagy via Redox-Dependent ERK1/2 PathwayAnticancer Effect of Sarsaparilla Extract. *Cancer Prevention Research*, *8*(5), 464-474.

Sidorova, M., & Petrikaitė, V. (2022). The effect of beta adrenoreceptor blockers on

viability and cell colony formation of non-small cell lung cancer cell lines A549 and H1299. *Molecules*, *27*(6), 1938.

Sies, H., & Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature reviews Molecular cell biology*, *21*(7), 363-383.

Sivapatham, S., & Selvaraj, L. (2022). Currently available molecular analyses for personalized tumor therapy. *Biomedical Reports*, *17*(6), 1-6.

Sliva, D. (2010). Medicinal mushroom Phellinus linteus as an alternative cancer therapy. *Experimental and Therapeutic Medicine*, *1*(3), 407-411.

Smith, I., & Talbot, D. (1992). Cisplatin and its analogues in the treatment of advanced breast cancer: a review. *British journal of cancer*, *65*(6), 787.

Soule, H. D., Vazquez, J., Long, A., Albert, S., & Brennan, M. (1973). A human cell line from a pleural effusion derived from a breast carcinoma. *Journal of the National Cancer Institute*, *51*(5), 1409-1416.

Su, Z., Yang, Z., Xu, Y., Chen, Y., & Yu, Q. (2015). Apoptosis, autophagy, necroptosis, and cancer metastasis. *Mol Cancer*, *14*, 48. https://doi.org/10.1186/s12943-015-0321-5

Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, *14*(6), 2167-2180.

Tang, W., Liu, C., Liu, J., Hu, L., Huang, Y., Yuan, L., Liu, F., Pan, S., Chen, S., & Bian, S. (2020). Purification of polysaccharide from Lentinus edodes water extract by membrane separation and its chemical composition and structure characterization. *Food Hydrocolloids*, *105*, 105851.

Trachootham, D., Alexandre, J., & Huang, P. (2009). Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nature reviews Drug discovery*, *8*(7), 579-591.

Tufail, A. B., Ma, Y.-K., Kaabar, M. K., Martínez, F., Junejo, A., Ullah, I., & Khan, R. (2021). Deep learning in cancer diagnosis and prognosis prediction: a minireview on challenges, recent trends, and future directions. *Computational and Mathematical Methods in Medicine*, *2021*.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007). Free

radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology*, *39*(1), 44-84.

Vaziri, N. (2008). Causal link between oxidative stress, inflammation, and hypertension.

Vona, R., Pallotta, L., Cappelletti, M., Severi, C., & Matarrese, P. (2021). The impact of oxidative stress in human pathology: Focus on gastrointestinal disorders. *Antioxidants*, *10*(2), 201.

Wang, J., Hu, F., Luo, Y., Luo, H., Huang, N., Cheng, F., Deng, Z., Deng, W., & Zou, K. (2014). Estrogenic and anti-estrogenic activities of hispolon from Phellinus lonicerinus (Bond.) Bond. et sing. *Fitoterapia*, *95*, 93-101. https://doi.org/10.1016/j.fitote.2014.03.007

Wang, Y., Meng, X., Li, B., Sun, X., & Zhang, Q. (2011). Effect of ultrasonic-assisted extraction on antioxidant activity and molecular weight distribution of polysaccharides from Schisandra Chinensis (Turcz.) baill. 2011 4th International Conference on Biomedical Engineering and Informatics (BMEI),

Wang, Z. B., Pei, J. J., Ma, H. L., Cai, P. F., & Yan, J. K. (2014). Effect of extraction media on preliminary characterizations and antioxidant activities of Phellinus linteus polysaccharides. *Carbohydr Polym*, *109*, 49-55. https://doi.org/10.1016/j.carbpol.2014.03.057

Winterbottom, A. E. (2015). Of the China root: a case study of the early modern circulation of materia medica. *Social History of Medicine*, *28*(1), 22-44.

Wong, J. H., Ng, T. B., Chan, H. H. L., Liu, Q., Man, G. C. W., Zhang, C. Z., Guan, S., Ng, C. C. W., Fang, E. F., Wang, H., Liu, F., Ye, X., Rolka, K., Naude, R., Zhao, S., Sha, O., Li, C., & Xia, L. (2020). Mushroom extracts and compounds with suppressive action on breast cancer: evidence from studies using cultured cancer cells, tumor-bearing animals, and clinical trials. *Appl Microbiol Biotechnol*, *104*(11), 4675-4703. https://doi.org/10.1007/s00253-020-10476-4

Wu, Z., Gao, R., Li, H., Wang, Y., Luo, Y., Zou, J., Zhao, B., & Chen, S. (2021). New insight into the joint significance of dietary jujube polysaccharides and 6-gingerol in antioxidant and antitumor activities. *RSC Adv*, *11*(53), 33219-33234. https://doi.org/10.1039/d1ra03640h

Wungsintaweekul, B., Umehara, K., Miyase, T., & Noguchi, H. (2011). Estrogenic and anti-

estrogenic compounds from the Thai medicinal plant, Smilax corbularia (Smilacaceae). *Phytochemistry*, *72*(6), 495-502. https://doi.org/10.1016/j.phytochem.2010.12.018

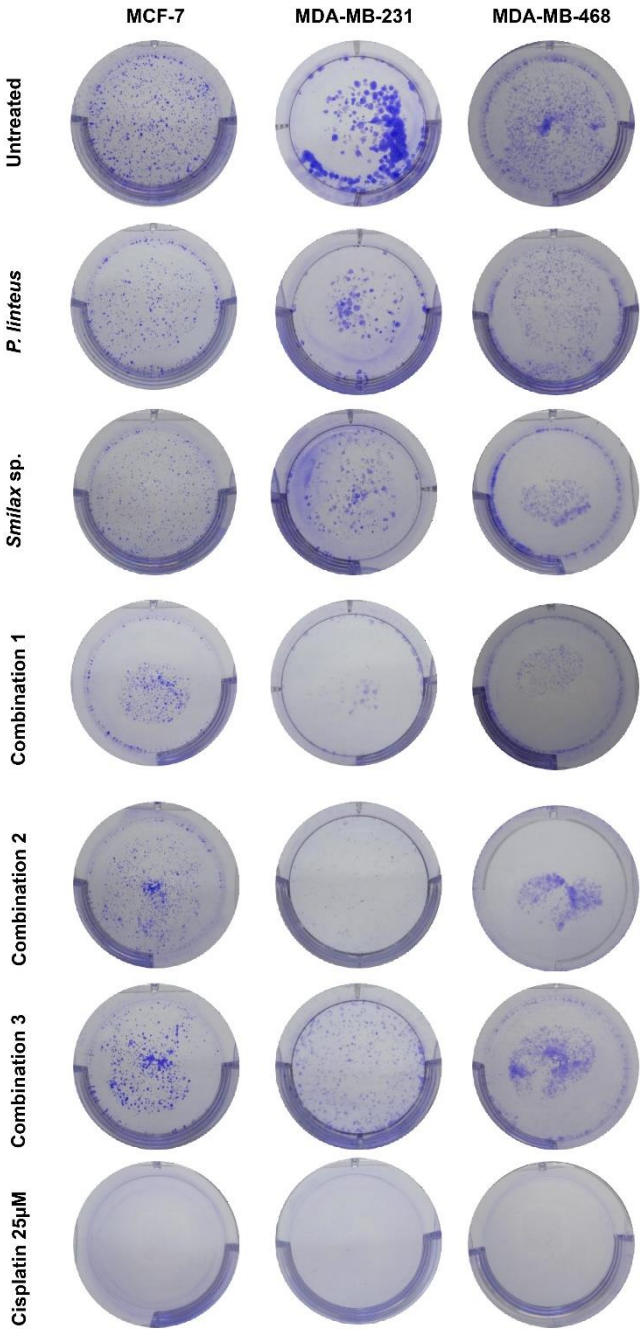
Yan, J. K., Wang, Y. Y., Ma, H. L., & Wang, Z. B. (2016). Ultrasonic effects on the degradation kinetics, preliminary characterization and antioxidant activities of polysaccharides from Phellinus linteus mycelia. *Ultrason Sonochem*, *29*, 251-257. https://doi.org/10.1016/j.ultsonch.2015.10.005

Yang, Y., Chen, Y., Wu, J. H., Ren, Y., Liu, B., Zhang, Y., & Yu, H. (2023). Targeting regulated cell death with plant natural compounds for cancer therapy: A revisited review of apoptosis, autophagy-dependent cell death, and necroptosis. *Phytother Res*. https://doi.org/10.1002/ptr.7738

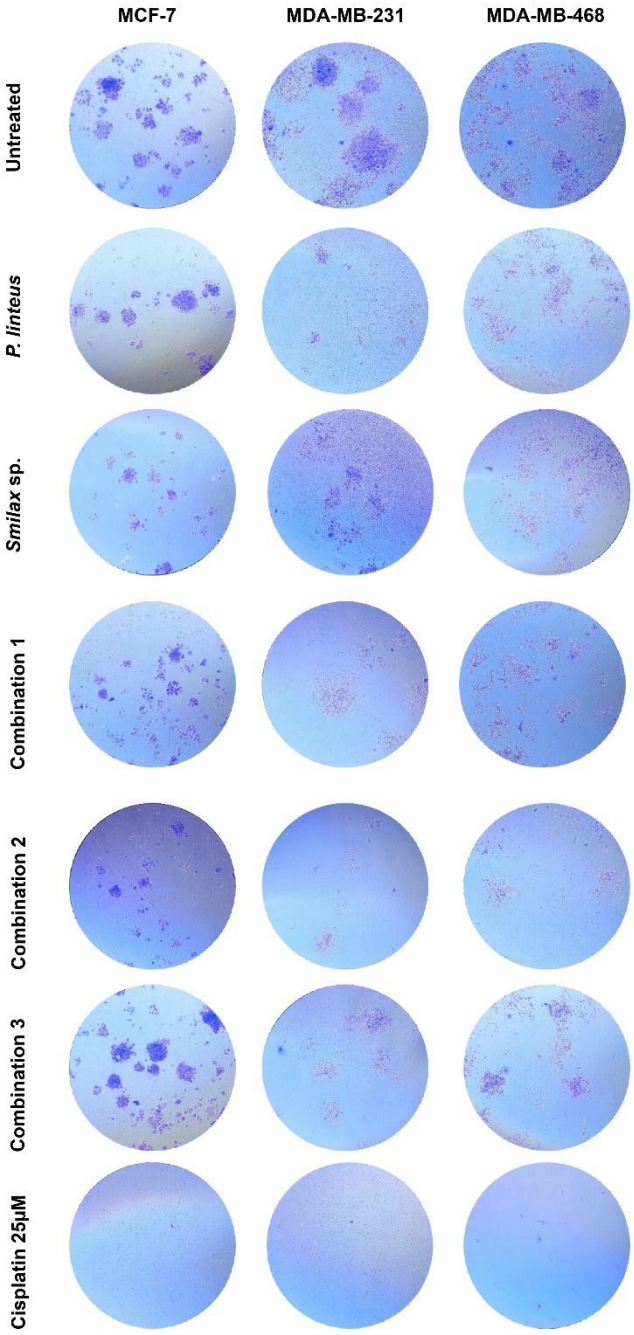
Zhang, C. L., Huang, T., Wu, B. L., He, W. X., & Liu, D. (2017). Stem cells in cancer therapy: opportunities and challenges. *Oncotarget*, *8*, 10.

Zhang, Q.-W., Lin, L.-G., & Ye, W.-C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese medicine*, *13*, 1-26.

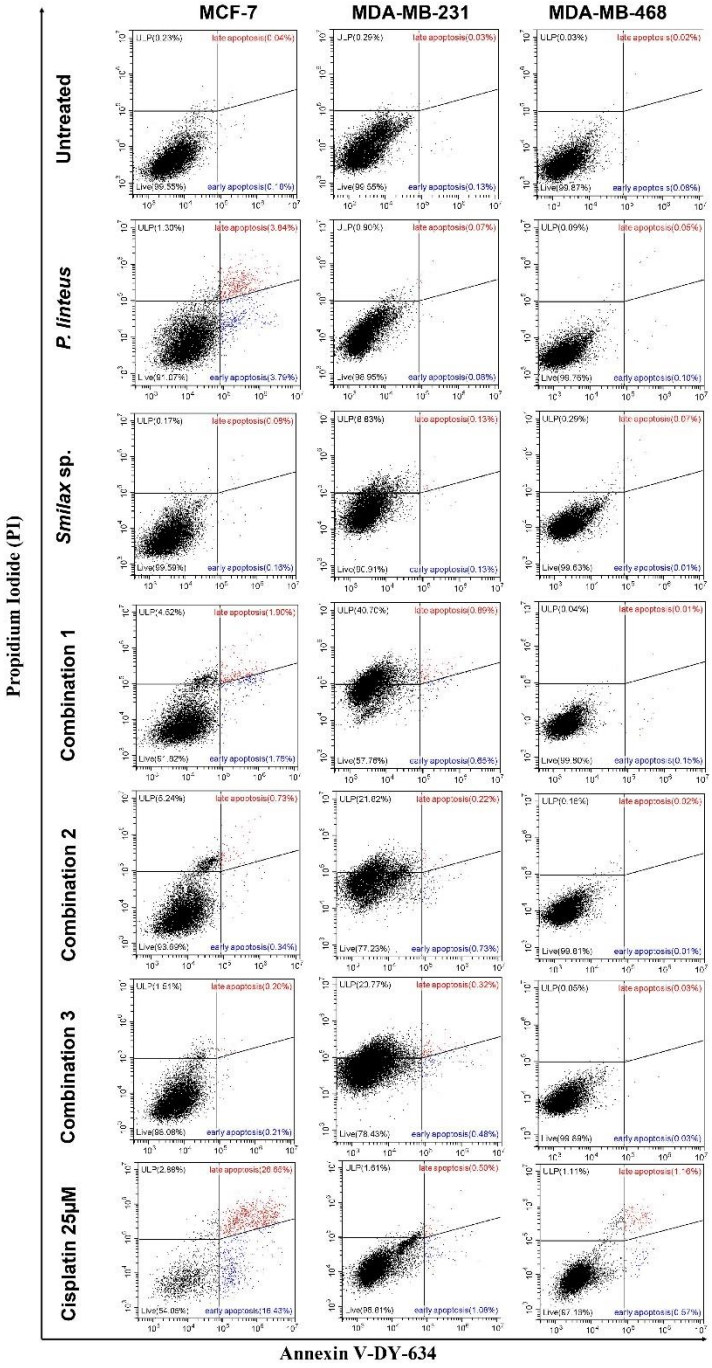
APPENDIX



Appendix Figure 1. The colony formation of three different breast cancer cell lines after 72 hours treatment of *S. corbularia*, *P. linteus* and combinations, with further incubation for 10 days (representative of whole well).



Appendix Figure 2. The colony formation of three different breast cancer cell lines after 72 hours treatment of *S. corbularia*, *P. linteus* and combinations, with further incubation for 10 days (representative under microscope at 40x magnification).



Appendix Figure 3. Representative flow cytometry dot plots of apoptotic cells using Annexin V-DY-634 and propidium iodide of the three replicates of 72 hours treatment on three distinct breast cancer cell lines with *S. corbularia*, *P. linteus* and combinations.

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