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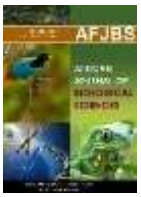

# INVESTIGATION OF ANTI CANCER ACTIVITY OF *PONGAMIA PINNATA* AND *STEVIA REBAUDIANA* IN BREAST CANCER USING ANIMAL MODEL

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**Abstract**

The prevalence of breast cancer is the highest among all types of cancer. The management of breast cancer involves various issues, including risk assessment and predictive diagnostics, focused prevention of metastatic disease, selection of appropriate treatment options, and ensuring cost-effectiveness of the treatments used. Extensive research has shown that phytochemicals have potential anti-cancer properties. They can keep cells from becoming cancerous, prevent the development of cancer, and hinder its spread to other parts of the body. Additionally, phytochemicals can boost the immune system and enhance the efficiency of traditional cancer treatments. The study has shown that the stevioside compound, extracted from *Stevia rebudiana rebaudiana* leaves, has an inhibitory effect on the growth of breast cancer cells (MDA-MB-231 and SKBR3). This compound acts by limiting cell proliferation and has potential as a targeted therapy for breast cancer. Furthermore, the findings also indicated that pure stevioside, when tested in a controlled environment, can enhance the susceptibility of breast cancer cells to 5-FU therapies. *Pongamia pinnata*, a native plant of the Indian subcontinent, has been extensively utilised in traditional medicine for various ailments due to its non-toxic nature and minimum or no adverse effects. This paper offers a thorough examination of the phytochemicals obtained from various components of *Pongamia pinnata* and the documented biological activity of the plant extracts and its constituents. Research was undertaken to discover the specific components responsible for the therapeutic function of the plant, based on traditional knowledge. This research involved extracting, isolating, and characterising the plant. *Pongamia pinnata* was found to possess important qualities, such as antimicrobial, antidiabetic, anti-inflammatory, and anticancer effects, when tested using in vitro and in vivo models for various disorders.

**Keywords:** Breast cancer, *Pongamia pinnata*, *Stevia rebaudiana*, Animal model

## Introduction

Breast cancer is a type of malignant neoplasm that is one of the most prevalent and widespread diseases that affect women. There are a number of factors, both internal and external, that can contribute to the development of breast cancer[1]. Poor lifestyle choices, environmental conditions, and social-psychological factors are all factors that have been linked to the incidence of this illness[2]. Hereditary mutations and family history are responsible for five to ten percent of breast cancer instances, according to research. Modifiable factors, on the other hand, are responsible for twenty to thirty percent of breast cancer cases[3-4]. The cells of the breast are the source of breast cancer. A tumour that is malignant, which is made up of a collection of cancer cells, has the capacity to penetrate and cause damage to the tissue that is surrounding it. In addition to being able to spread across the entirety of the body, it also possesses the capability to do so. There are times when breast cells undergo changes that make it more difficult for them to proliferate or function in a manner that is typical[5]. Nevertheless, breast cancer can develop on a random basis as a result of changes in breast cells. The development of breast cancer typically takes place in the cells that make up the lining of the ducts, which are the conduits that are accountable for transferring milk from the glands to the nipple. The condition known as ductal carcinoma is the name given to this particular form of breast cancer. It is also possible for cancer to emerge from the cells that are found in the lobules, which are clusters of glands that produce milk. The term "lobular carcinoma" is used to describe this particular form of cancer. Both ductal and lobular carcinomas have the potential to be in situ, which means that the cancer has not spread to other tissues and has remained confined in the place where it was initially discovered. In addition, they have the potential to be invasive, which indicates that they have the ability to spread into the tissues that are nearby[6]. There are many less common ways that breast cancer can manifest itself. This includes breast Paget disease, triple-negative breast cancer, and inflammatory breast cancer, among other types of breast cancer. There are a few uncommon forms of breast cancer, including non-Hodgkin lymphoma and soft tissue sarcoma[7]. Research reveals that the incidence of breast cancer has been steadily increasing in China, despite the fact that the country has a relatively low prevalence of the disease. By the year 2022, the disease would have a prevalence among Chinese women that would be greater than 100 instances per 100,000 individuals, with a total of 2.5 million women between the ages of 35 and 49 who would be affected. Therefore, in order to lessen the number of people who are affected by breast cancer,

it is absolutely necessary to do study on the elements that are related with the condition[8]. Breast cancer is the most common form of cancer among women around the world and the leading cause of death in women who have been diagnosed with cancer. There were around 2.09 million newly diagnosed instances of breast cancer in 2018, and the disease was responsible for the deaths of nearly 630,000 women. The incidence of breast cancer is on the rise, despite the fact that there are variations in the incidence of the disease across different regions[9]. In recent years, the incidence of breast cancer has been increasing by 17.6% and 15.6%, respectively, in China, which has the largest worldwide incidence of the disease across the globe. In spite of the fact that the incidence of breast cancer is relatively low across the globe (36.1/105) and mortality is 8.8/105, the large population of China is a contributing factor to these high numbers. The incidence of breast cancer is rising all over the world, which has resulted in a significant cause for concern for the health of the general population[10]. The disease known as breast cancer is a complicated condition that can be caused by a number of different variables, such as genetics, environment, and lifestyle decisions. For the purpose of gaining a better understanding of the disease's prevalence and making a contribution to the early identification of breast cancer, the purpose of this review was to investigate the epidemiology of breast cancer all over the world and to determine the risk factors that are connected with it. Genetic factors, particularly those that are passed down through families; diet and obesity, as the overall quality of life improves, leading to increased obesity rates among women and a high-fat diet; smoking and alcohol consumption; exposure to ionising radiation; and specific factors such as menstruation, childbirth, and lactation, which can also impact the likelihood of developing breast cancer. These individuals are considered to be the primary risk factors for breast cancer. To lessen the impact that exogenous hormones have on the body, it is recommended that we avoid using cosmetics that include oestrogen in our daily routines. This will help us to reduce the negative effects that these hormones have. The appeals in question have been the subject of a significant amount of discussion. Therefore, it is of the utmost importance to conduct a comprehensive analysis of the risk factors for breast cancer by employing meta-analytical approaches in order to provide direction for clinical prevention and therapy for the disease[11]. In this study, we conducted a meta-analysis on breast cancer risk factors in Chinese women by collecting relevant literature from 2001 to 2021. This was done despite the fact that before, Chinese researchers had already completed research that was comparable to the one we were conducting[12]. With the intention of providing Chinese women with vital information for the prevention of breast cancer, our goal

was to do this. One definition of a risk factor is something that raises the probability of an individual developing cancer. It is possible that a habit, substance, or illness served as the source of the problem. The great majority of cancers are caused by a variety of risk factors that contribute to their development. This is a possibility. When compared to men, the likelihood of individuals having breast cancer is significantly higher in females. Women are more likely to develop breast cancer if their breast cells are exposed to oestrogen and progesterone throughout their lifetime[13]. Throughout its progression, breast cancer is characterised by a slow and unnoticeable progression. Regular screenings are the most common way for people to become aware of their illness. Nipple discharge, changes in breast shape or size, or the unexpected discovery of a breast lump are all possible medical complications that may be experienced by certain individuals. The manifestation of mastalgia is quite prevalent. A comprehensive physical examination, the utilisation of imaging tools, most notably mammography, and the collection of tissue samples for biopsy are all required in order to arrive at a diagnosis of breast cancer[14]. When detected in a timely manner, the likelihood of survival is increased. The ability of the tumour to spread through the circulatory and lymphatic systems results in a poor prognosis and the development of metastases that are located at a greater distance. Scientists are shifting their focus to alternative energy sources as the demand for fossil fuels continues to rise and the amount of fossil fuels that are being used continues to decrease. In recent years, M. pinnata has garnered recognition for its potential in the field of biofuels, which has emerged as one of the most promising choices in recent times[15]. In previous research, it was hypothesised that the leaves of *Stevia rebaudiana* has anticancer properties. As a result, the purpose of this study was to investigate the potentially damaging effects of pure stevioside on human breast cancer cell lines MDA-MB-231 and SKBR3. Based on the findings, it was revealed that pure stevioside was able to successfully inhibit the proliferation of several different cancer cell lines[16]. For MDA-MB-231 and SKBR3 cells, the IC50 values for the inhibition of cancer cell growth by stevioside were found to be 55 µM and 66 µM, respectively. Stevioside was found to be effective in inhibiting its growth. This provides evidence that purified stevioside possesses the capability to induce apoptosis, which suggests that it may have the potential to be an effective treatment for cancer cases. Nevertheless, past investigations have not offered a full explanation for the chemo sensitization effects of stevioside on breast cancer. In addition, the chemo sensitization potential of stevioside is evaluated in this study when it is considered in conjunction with 5-FU. The

findings of this research study highlight the significance of *Stevia rebaudiana* as a valuable reservoir of bioactive compounds that contain powerful anti-cancer capabilities.

**Material & Methods**

We collect information from a wide variety of reliable sources, including MDPI, SCI, WOS, and Scopus, in order to carry out an exhaustive search. For the purpose of this search, we are employing the following keywords: cancer, breast cancer, active substances *Pongamia pinnata*, and *Stevia rebaudiana*.

Ongoing research is being conducted with the objective of developing targeted therapies that particularly target those molecular markers or genetic abnormalities that are associated with breast cancer. These treatments are designed to improve the efficacy of treatment while simultaneously reducing the risk of undesirable effects. The application of immunotherapy, which is a method of treating breast cancer that involves employing the immune system to precisely attack cancer cells, is currently the subject of research that is currently being conducted. In order to attain a diagnosis that is both early and more accurate, the current research aims to develop screening techniques beyond mammography. This is being done by investigating molecular imaging and blood-based biomarkers. By conducting research into the genetic and epigenetic factors that influence the development of breast cancer, we are able to improve our understanding of individual susceptibility and contribute to the discovery of new therapeutic targets. Identifying additional lifestyle changes, medications, or treatments that can significantly reduce the risk of developing breast cancer is the primary focus of the research being conducted. The goal of collaborative research initiatives that are conducted by institutions and international organisations is to share information, resources, and knowledge in order to improve our understanding of breast cancer and the outcomes of therapy on a global scale. The goal is to reduce the incidence of breast cancer, as well as the number of deaths and illnesses associated with it, on a global scale. This will be accomplished by placing a constant emphasis on prevention through changes in lifestyle, early diagnosis through increased screening efficiency, and investment in cutting-edge research.

**Anti-tumor effects of *Stevia rebaudiana***

Because of the effect that stevia has on metabolism, which may result in reduced glucose levels in the bloodstream, consuming stevia can be a decision that is beneficial to personal health[17]. As was said before, this can have a number of positive effects on one's health, and it also allows tumour cells to be deprived of an essential supply of electricity. Plant derivatives have been shown to have cytotoxic and anti-proliferative effects in cancer cells and in vivo tumour models, according to information obtained from a recent study[18].

**Effect of *Stevia rebaudiana* Breast Cancer**

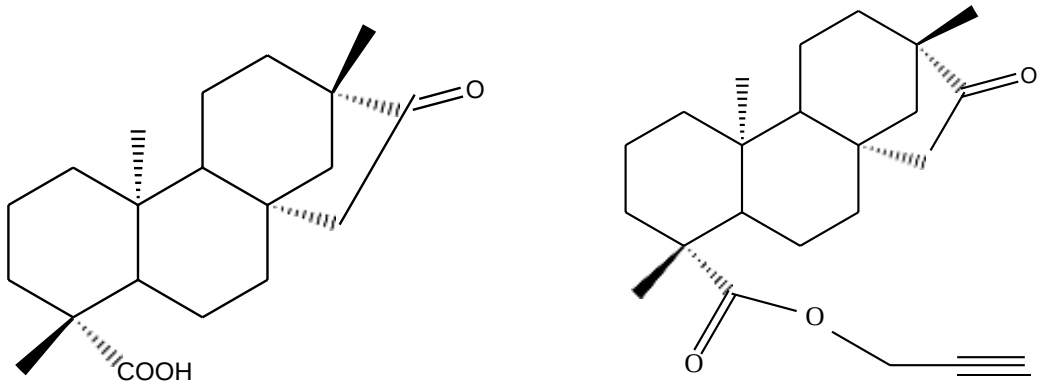
The number of pieces of evidence that point to the possibility that certain aspects of one's lifestyle, such as the food that one consumes, may be associated with an increased risk of breast cancer, which has emerged as the most prevalent form of cancer worldwide [19]. It is possible that the implementation of a dietary intervention that is based on plants, with the objective of preventing or treating the disease, has the potential to reduce the incidence of cancer and improve the general health of patients [20]. Based on the findings of the research that are discussed in this section, stevia products might be good additions to a diet following this pattern. As part of the preliminary investigation into *S. rebaudiana* metabolites, the effects of these chemicals were investigated on rodents using live animal models, and any adverse effects that may have been produced by these substances were investigated. Stevioside, which is the major steviol glycoside found in the stevia leaf, was the subject of a study that was carried out by Toyoda and colleagues on F344 rats in order to evaluate the potential cancer-causing effects of stevioside [21]. Two distinct amounts of stevioside, 2.5% and 5%, were administered to the rats over the course of 108 weeks as part of a modified diet that was administered to the rats. Following the completion of the investigation, the researchers came to the conclusion that there were no significant differences in the incidence of tumours between the groups that were being treated with stevioside and the groups that were serving as controls. This finding provides more evidence that the glycoside does not possess any characteristics that could potentially cause cancer. Particularly noteworthy is the fact that the incidence of spontaneous mammary adenomas in female rats was actually reduced in the groups that were treated with stevioside [22], underlining the potential of this compound as a cancer prevention agent.
The findings of the previous research were supported by subsequent investigations that investigated the inhibitory and toxic effects of stevia derivatives on different breast cancer cell

lines. Based on the findings of Gupta et al. [23], it was revealed that steviol, which is the primary aglycone of SGs, exhibited a significant cytotoxic effect on MCF-7 breast cancer cells. The amount of steviol that was administered resulted in a reduction in the viability of the cells. When the cell population was subjected to the highest concentration of Steviol (500 μM) for a period of 48 hours, the reduction was seen to be forty percent. Steviol was discovered to be responsible for putting a stop to the process of the cell cycle transitioning from the G2 phase to the M phase. In addition to this, it induced apoptosis by means of the mechanisms mediated by reactive oxygen species (ROS) [24]. It was discovered that the cytotoxic effects of stevioside on this cell line were comparable to those that were reported by a different research group. The cytotoxicity was shown to be dependent not only on the length of treatment but also on the dosage that was administered. Stevioside was found to have inhibitory effects on cell growth at lower concentrations (2.5–30 μM) when compared to steviol [25]. This finding suggests that the glycoside is a more potent anti-cancer drug than steviol. An increase in the cell population during the G0/G1 phase was seen in the cell cycle tests that were conducted in order to understand the mechanisms that are responsible for the cytotoxicity of stevioside. The fact that the cells were not replicating nor resting was something that the researchers noticed, which led them to believe that the cells might have died. After being exposed to a relatively low concentration of stevioside (10 μM), the cells were shown to be undergoing apoptosis, which was caused by reactive oxygen species (ROS). This was confirmed through the use of Annexin V staining. Apoptotic cells accounted for around 70 percent of the total population [26], and the impact became more obvious 72 hours after the therapy was administered. A study was conducted in which MCF-7 cells were used to investigate the cytotoxic effects of ammonium derivatives of steviol and isosteviol (19). Hydrolysis of stevioside results in the formation of isosteviol as a byproduct of the phenomenon. The findings of the study were quite surprising, as the scientists proved that two compounds that were synthesised, namely a mono-quaternized derivative of steviol and a bis-quaternized derivative of isosteviol (as illustrated in figure 1), exhibited a lethal impact (IC50 5 μM) on MCF-7 cells. This particular impact was just as powerful as the anti-cancer medication doxorubicin, with an IC50 value of 3 μM. Additionally, the two compounds demonstrated a distinct influence on cancer cells, as they demonstrated lesser toxicity in the Wi38 human embryonic lung cell line in comparison to the drug. This was proved by the fact that the medication was more toxic. The induction of apoptosis was the primary mechanism by which the two drugs triggered cell death in treated MCF-7 cells, as demonstrated by the staining of these

cells with Annexin V [24]. According to the findings of another research that looked at the effect that the chemicals had on the potential of the mitochondrial membrane and the production of early signs of cell death, the apoptotic process was launched through the internal pathway that was present within the mitochondria [27].

During the course of their research, Khare and colleagues conducted a trial in which they administered stevioside to triple-negative MDA-MB-231 and HER2+ SKBR-3 breast cancer cells at doses ranging from 5 to 100 μM for a period of 48 hours [28]. The viability of the cells in both cell lines was reduced by approximately sixty percent as a consequence of this. In addition to this, the glycoside demonstrated the capability of enhancing the chemosensitivity of these cells to the anti-cancer medicine 5-Fluorouracil (5-FU), which is frequently utilised. When compared to the individual treatment with 5-FU or stevioside, the investigation into the underlying mechanism revealed that the combination administration of the two drugs led to an increase in the Bax/Bcl-2 protein ratio as well as an enhancement of the apoptotic process by DNA fragmentation [25]. This was discovered as a result of the investigation. The members of the Bcl-2 protein family have a substantial influence on either enhancing or inhibiting the intrinsic apoptotic pathways that are activated as a result of mitochondrial dysfunction. Therefore, the fate of the cell is determined by the equilibrium that exists between these individuals [28]. While Bax is responsible for the induction of cell death, Bcl-2 is responsible for inhibiting apoptosis by inhibiting the function of Bax during the process. Indicative of an overexpression of Bax, which ultimately results in the death of cells through the process of apoptosis [29], an enhanced Bax/Bcl-2 protein ratio is present. On the other hand, it was shown that the MDA-MB-231 breast cancer cells exhibited a high level of sensitivity to other Stevia metabolites, such as steviolbioside [27] and isosteviol derivatives [30]. The anti-cancer medicine 5-FU was used as a reference for this study. After administering a dose of 250 μg/mL for a duration of 48 hours, the administration of 5-FU (1.92 mM), steviolbioside (0.39 mM), and stevioside (0.31 mM) led to a suppression of cell growth of 70%, 35%, and 20%, respectively, as reported in reference [31]. As a result, the determination was made that steviolbioside is a more powerful antitumor molecule in comparison to the substance that it was initially composed of. However, the authors also observed that steviolbioside exhibited a higher level of toxicity towards normal cells in comparison to stevioside, which raises issues about the safety of its application [32]. A different investigation was carried out by the authors, in which they developed and manufactured a wide variety of isosteviol derivatives that were connected to a triazole group. In

order to produce alkyne isosteviol derivatives, it was necessary to modify either the carboxylic (as shown in figure 2B) or the ketone group of the isosteviol molecule. The chemical molecule known as isosteviol triazole was subsequently constructed with the help of these modified components. The latter was made up of a 1,2,3-triazole group that was coupled to an aromatic ring. This aromatic ring was conjugated to the modified carboxylic group of isosteviol. The compound that was found to be the most potent among these chemicals, as depicted in figure 2C, had an IC50 value of 13.76 μM when tested on MDA-MB-231 breast cancer cells [33]. Other studies have also established the anti-cancer action of other 1,2,3-triazole hybrids and conjugates in a variety of human cancer cell lines, which has led to the identification of these compounds as potentially promising therapy possibilities [34].




**(A) isosteviol** **(B) isosteviol with a modified carboxyl group**

**Fig: 1 Using the information provided in the chemical structures of (A) isosteviol, (B) isosteviol with a modified carboxyl group are presented. It was ChemDraw Ultra that was used to recreate the structures that were found in the initial investigation.**

**Table 1: The biological effects of *Stevia rebaudiana* derivatives observed in breast cancer models are presented**

| Cell line model | Compound | Effects |
|---|---|---|
| F344 RATS | Stevioside | Decrease in mammary adenomas |
| MCF-7 | Steviol | G2M arrest, ros mediated apoptosis |
| | Stevioside | G1 arrest bax overexpression, apotosis |
| | Hydroalcholic extract | Increased cytotoxicity |
| | ZnS nanoparticles, aquous extract | Increased cytotoxicity |
| | Ammonium derivative of sterviol and isosterviol | Increased cytotoxicity |

**Effect of *Pongamia pinnata* for breast cancer**

Antimicrobials and pharmaceuticals have been used to an excessive degree, which has led to the development of resistance in a wide variety of diseases and parasites. This has created enormous problems for both human health and the environment. To add insult to injury, breast cancer is the second leading cause of death from cancer among women. In laboratory settings, the MCF-7 cell line is frequently utilised for the purpose of doing research on breast cancer[35]. Adenocarcinoma of the breast is the source of this substance, which exhibits a number of attractive characteristics that are unique to the mammary epithelium. Within the context of this circumstance, the development of medications that are both creative and beneficial to the environment is of utmost importance. The process of synthesising nanoparticles through the use of environmentally friendly methods is not only helpful to the environment but also cost-effective. In addition to this, it does not necessitate the use of hazardous chemicals or the addition of additional energy inputs. The purpose of this study was to investigate the anticancer effects of zinc oxide nanoparticles (ZnO NPs) that were manufactured from the seed extract of

*Pongamia pinnata* on human MCF-7 breast cancer cells more specifically. In addition, the researchers investigated whether or not these nanoparticles have the capability of preventing the formation of biofilms by fungi and bacteria[36]. UV-Vis spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDX) were the techniques that were utilised in order to characterise the nanoparticles. At a dose of 25 µg ml-1, the growth of Gramme positive Bacillus licheniformis was significantly inhibited by Pp-ZnO nanoparticles, resulting in a zone of inhibition measuring 17.3 mm. After that came Gramme-negative Pseudomonas aeruginosa (14.2 mm) and Vibrio parahaemolyticus (12.2 mm), both of which were found in the next position. A concentration of 50 µg ml-1 of the Pp-ZnO nanoparticles was found to be effective in inhibiting the formation of biofilm by Candida albicans. A single treatment with Pp-ZnO nanoparticles resulted in a significant reduction in the cell survival of breast cancer MCF-7 cells. However, this reduction was only observed at concentrations over 50 µg, as demonstrated by cytotoxicity studies[37]. In order to evaluate the morphological changes that occurred in MCF-7 breast cancer cells after they were treated with Pp-ZnO NPs, phase contrast microscopy was utilised. As a result of the findings of this research, it appears that Pp-ZnO nanoparticles that were produced through environmentally friendly processes have the potential to be utilised as extremely effective agents in the fight against breast cancer and microbiological infections. The expression of cytochrome P450-1A1 (CYP1A1) is naturally regulated; nevertheless, it is triggered in two different ways: (a) by polycyclic aromatic hydrocarbons (PAHs), which can be transformed into carcinogens by CYP1A1, and (b) in the majority of breast cancers[38]. Therefore, if it is discovered that phytochemicals or flavonoids included in food are CYP1A1 inhibitors, then it is possible that they could be of assistance in the development of breast cancer and the prevention of carcinogenesis caused by PAH. *Pongamia pinnata* (L.) Pierre is an Indian medicinal plant that is abundant in flavonoids, and we have investigated the cancer chemopreventive characteristics of this plant in this study. The seeds methanolic extract has the ability to inhibit CYP1A1 in normal human HEK293 cells, which are cells that have an excessive amount of CYP1A1 expression. 0.6 µg/mL is the number that represents the IC50 for this inhibitor. An inhibitory impact on CYP1A1 is exhibited by the furanoflavonoids pongapin/lanceolatin B, which are classified as secondary metabolites. The value of the IC50 for this inhibitor is twenty nanometers. Pongamol, a furanochalcone, has the ability to inhibit CYP1A1 with an IC50 of only 4.4 µM. However, P5b, a semisynthetic pyrazole-derivative, has an IC50 of 0.49 µM, which

is about ten times more potent than pongamol[39]. When applied to HEK293 cells that have an overexpression of CYP1A1, the combination of pongapin/lanceolatin B and the methanolic extract of P. pinnata seeds provide protection against the toxicity caused by BHP. It is interesting to note that they also suppress the cell cycle of MCF-7 breast cancer cells that have an overexpression of CYP1A1, specifically during the G0-G1 phase. Additionally, they result in the suppression of cyclin D1 levels and the initiation of cellular senescence[40]. Since many years ago, a substantial amount of study has been carried out with the purpose of locating unique substances that possess medical qualities. There has been a resurgence in interest in the study of natural goods over the past twenty years. This is due to the fact that natural products account for 50% of the pharmaceuticals that are used in industrialised countries, with 25% coming from higher plants. Because of the usefulness that phytochemicals found in food have been shown to have in treating many types of cancer, researchers have lately begun looking into the phytochemicals found in food. Phytochemicals contained in medicinal plants have been proven to inhibit cell proliferation and activate apoptosis, which successfully inhibits the growth of a wide variety of cancer cells. This discovery was made through research that was carried out all over the world. Polyphenolic compounds are found in plants, and these molecules have a variety of pharmacological effects, including anti-carcinogenic and antioxidant properties[41]. The significant advantages of natural plant extracts, which include multiple bioactive chemicals, have been emphasised by a number of research that have been undertaken on cell lines as well as animal tissues. These studies have shown several advantages over single therapies[42]. It has been demonstrated through a number of studies that Stevioside had anticancer effects when applied to cell lines. When applied to colon carcinoma, it had anticancer properties. Additionally, the research demonstrated that it regulates reactive oxygen species (ROS), which in turn causes apoptosis to occur in the MCF 7 breast cancer cell line during the process. The investigation that was carried out after the current research was carried out utilising purified Stevioside that was derived from *Stevia rebaudiana* leaves. This inquiry validated the anticancer capabilities of Stevioside as well as its potential to promote sensitivity in breast cancer cell lines MDA-MB-231 and SKBR3[43]. For the purpose of determining the relative toxicity of the pure stevioside, studies on cell viability were carried out on the MDA-MB-231 and SKBR3 cancer cell lines. Stevioside demonstrated a high level of toxicity, notably against MDA-MB-231 cells, which were then given precedence over SKBR3 cells. There was a correlation between the amount of the drug and the decrease in the viability of these cells. Previous research has shown that there is

a correlation between experiments with high antioxidant activity and anti-cancer properties. This tendency is corroborated by several previously conducted investigations[44]. Due to the fact that caspase 3, caspase 9, and the Bax/Bcl-2 ratio are all crucial components of the apoptotic process, we decided to undertake additional research on these three parameters. The activation of caspase 3 and caspase 9, both of which are involved in the process of apoptosis, was brought about as a consequence of the administration of 5-FU in conjunction with stevioside. Additionally, the results of our inquiry utilising Western blot analysis showed that there was an increase in the expression of Bax and a decrease in the expression of Bcl-2. For the purpose of gaining a deeper comprehension of the process by which Stevioside induces cell death, researchers investigated the effect that it has on DNA fragmentation. DNA fragmentation is an essential component in the beginning stages of apoptosis. Laddering is a characteristic pattern of DNA fragmentation that was discovered through a study of cells that had been treated with pure stevioside and 5-FU. Within the context of apoptosis, this pattern is frequently observed, and it is indicative of the degradation of DNA between nucleosomes.

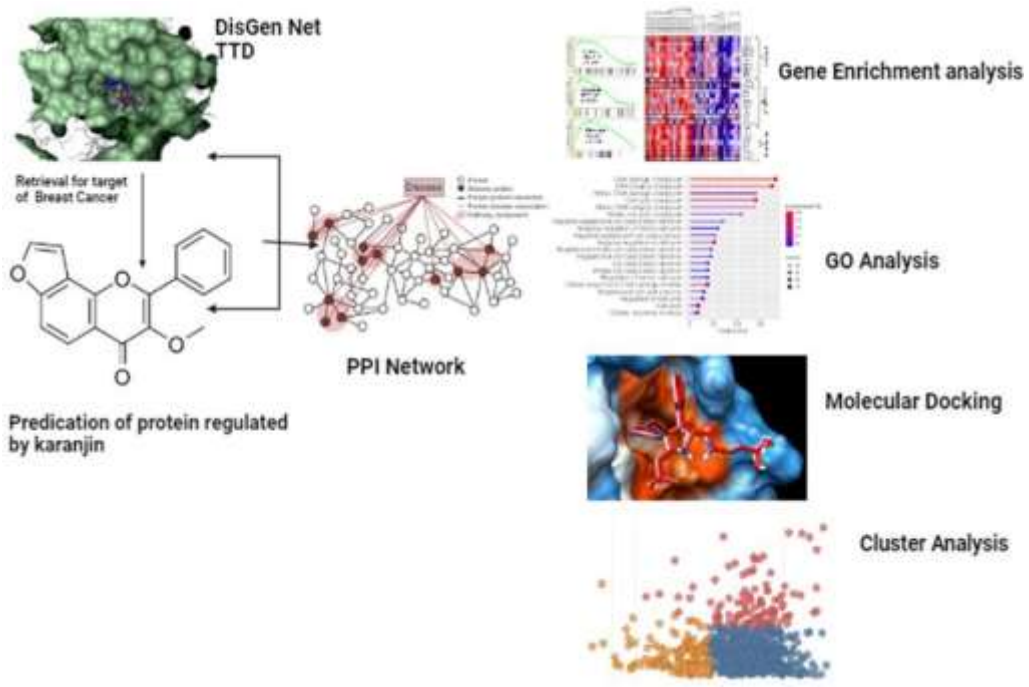



**Fig 2: Anti-tumor potential and mode of action of karanjin (*Pongamia pinnata*) against breast cancer; an *in-silico* approach**

The mucosal surfaces of the upper aerodigestive tract are the sites where Head and Neck Squamous Cell Carcinoma, also known as HNSCC, manifests after it has spread to the head and neck. This encompasses the mouth cavity, the pharynx, the larynx, and the sinuses of the paranasal region. Surgery, either on its own or in conjunction with radiotherapy and chemotherapy, is the most common treatment for head and neck squamous cell carcinoma (HNSCC)[45]. Therefore, it is of the utmost importance to give priority to the development of chemotherapeutic medicines that are biologically safe and demonstrate extraordinary anticancer effectiveness through the targeted activation of cancer cell-specific apoptosis while simultaneously minimising side effects. Recent studies in the field of chemotherapeutic agent development have specifically investigated the anti-cancer properties of natural compounds that are produced from herbal plants that are commonly used in traditional medicine or other natural substances that have been scientifically validated. The presence of phytoestrogens, which are naturally occurring compounds such as 17β-estradiol, can be discovered in a wide range of edible fruits, vegetables, and specific medicinal plants[46]. The oestrogen receptor is a receptor that phytoestrogens interact with Phytoestrogens have a role in a number of physiological and pathological processes, including reproduction, bone remodelling, cardiovascular function, immune system activity, and metabolic disorders. Furthermore, a recent study has shown that phytoestrogens have a major impact on the prevention and treatment of several forms of cancer, including liver, lung, colon, breast, prostate, and oral cancer. There is also evidence that phytoestrogens influence the therapy of cancer. In our most recent study, we discovered that biochanin-A, a phytoestrogen that was obtained from T. pretence, which is also referred to as red clover in traditional medicine, was responsible for inducing programmed cell death (apoptosis) in FaDu cells. This was accomplished through the death receptor-dependent extrinsic pathway as well as the mitochondria-dependent intrinsic pathway[47]. Formononetin is a phytoestrogen that is obtained from herbal plants. In this study, we demonstrated that formononetin causes cell death in FaDu cells by a process that involves death receptors and mitochondria. These are both intrinsic and extrinsic pathways of apoptosis.

During the course of this experiment, it was discovered that formononetin did not have any effect on the vitality of L929 fibroblasts, which were used as normal cells. In their study, Huh and colleagues discovered that the proliferation of human umbilical vein endothelial cells was augmented when they were subjected to a concentration range of 1–100 μM formononetin. On the other hand, this therapy did not have any effect whatsoever on the survival of normal

subchondral osteoblasts. As a result, these data indicate that the dose of formononetin that was utilised in this experiment, which ranged from 5 to 25 µM, is likely safe for normal cells. However, the survival rate of FaDu cells that were treated with 5 to 50 µM formononetin decreased in a manner that was directly proportionate to the dosage. According to our findings, formononetin was also observed to reduce the viability of a number of different cancer cell lines. These cell lines included human osteosarcoma U2OS cells (), human non-small-cell lung cancer A549 and NCL-H23 cells, human prostate cancer DU-145 cells,[48] human cervical cancer HeLa cells, and human breast cancer MCF-7 cells [49] The manner in which formononetin reduced the viability of these cancer cell lines was dependent on the duration of exposure as well as the concentration that was utilised. Although prior studies have demonstrated that formononetin is toxic to a wide variety of cancer cells, the work that we are currently conducting is the first to definitively establish that formononetin is responsible for the death of cells in human head and neck squamous cell carcinoma. In addition, the purpose of this research was to determine the precise concentration of formononetin that resulted in the death of FaDu cells while having no effect on normal fibroblasts[50]. An technique that is very effective in the treatment of cancer is one that involves directing the control of biological processes in order to hasten the death of cells. Throughout the course of development, apoptosis is an essential mechanism that plays a role in the elimination of cells that are not required in order to maintain equilibrium and stability in animals that live for a very long time. The initiation of the extrinsic apoptosis pathway, which is mediated by death receptors, occurs when adaptor proteins, such as Fas-associated death domain (FADD) and tumour necrosis factor (TNF) receptor-associated death domain (TRADD), are recruited through the binding of death ligands (such as FasL, TNF-related apoptosis-inducing ligand (TRAIL), and TNF) to TNF family death receptors. In the subsequent step, adaptor proteins cleave pro-caspase-8 in order to induce the activation of caspase-8. According the activation of caspase-8 leads to the subsequent activation of the executioner caspase-3, which ultimately results in the death of the cell. Both the activation of caspase-8 and the expression of FasL were increased in FaDu cells as a result of the administration of formononetin in this study. As a consequence of this, the administration of formononetin to FaDu cells resulted in an increase in the activation of caspase-3, which is a molecule that is targeted by caspase-8 and caspase-9. Additionally, as a consequence of this activation, there was an increase in the levels of PARP, which is a chemical that encourages the death of cells. According to the findings, the process of formononetin-induced apoptosis in FaDu

cells is regulated through the death receptor-mediated extrinsic apoptosis route. This mechanism involves the increased synthesis of the death ligand FasL. The mitochondria-dependent intrinsic apoptosis pathway is typically activated by the cleavage of Bid into tBid in response to apoptotic stressors, such as DNA damage, upregulation of oncogenes, deprivation of growth factors, elevated levels of Ca2+, exposure to DNA-damaging molecules, oxidants, and microtubule-targeting drugs. Moreover, the activation of caspase-8, which is an essential component of the death receptor-mediated extrinsic apoptosis pathway, can be a trigger for the beginning of the mitochondria-dependent intrinsic apoptosis route. This pathway is thought to be responsible for the death of cells. Through the process of cleavage, Bid is converted into tBid. This activation takes place. Through the process of oligomerization of activated Bax and Bcl-2-antagonist/killer (Bak), the release of intermembrane cytochrome c is brought about by the permeabilization of the outer membrane of the mitochondria. Certain anti-apoptotic factors, such as Bcl-2 and Bcl-xL, are responsible for regulating the oligomerization of activated Bax and Bak compounds. However, the activity of these anti-apoptotic proteins is controlled by pro-apoptotic factors like Bik, Bim, and Bad. These factors are responsible for regulating it. In this study, the activation of caspase-8 resulted in a decrease in the levels of Bid. This was accomplished by the breakdown of Bid into tBid in FaDu cells that had been treated with formononetin[51]. Furthermore, formononetin suppressed the production of anti-apoptotic proteins, such as Bcl-2 and Bcl-xL, which resulted in a reduction in the anti-apoptotic activity that was present in FaDu cells. Furthermore, formononetin was found to increase the synthesis of mitochondria-dependent pro-apoptotic proteins, specifically Bik and Bim. The sequential activation of caspase-9, caspase-3, and PARP occurred in FaDu cells that were treated with formononetin. This was caused by changes in the quantities of these compounds, which either promote or inhibit cell death through the mitochondria-dependent apoptosis pathway. The conclusion that can be drawn from these findings is that mitochondria-dependent intrinsic apoptotic pathways are responsible for the death of cells that are caused by formononetin. The activation of caspase-8, which is caused by increased expression of FasL in FaDu cells, is the first step in the process that is being described. Formononetin has been observed to trigger mitochondria-dependent apoptotic pathways in U2OS human osteosarcoma cells, A549 human non-small-cell lung cancer cells, and DU-145 and PC-3 human prostate cancer cells. This is in line with the findings of this study, which found that formononetin reduced the expression of Bcl-2 while simultaneously increasing the expression of Bax.

It is general knowledge that MAPKs are associated with a number of operations that take place in cancer cells. These operations include proliferation, differentiation, apoptosis, angiogenesis, invasion, and metastasis [46]. The current study discovered that the phosphorylation of ERK1/2 and p38 was suppressed in FaDu cells in a manner that was dependent on the dosage of formononetin that was administered to the cells. Recent research has shown that the MAPK signalling pathway can inhibit the activation of caspase-3 and caspase-9 by blocking the release of cytochrome c from mitochondria, either directly or indirectly [46–49]. This can be accomplished in a number of different ways. A cell signalling molecule known as NF-κB is responsible for controlling the activation of several genes that are involved in immunological responses, cell adhesion, cell differentiation, cell proliferation, angiogenesis, and apoptosis [50]. It is important to note that the activation of NF-κB is hindered by the binding of the NF-κB inhibitor, IκB, which is triggered through the phosphorylation of Akt [51]. It has been found by Alam and colleagues that there is a positive link between the expression of the anti-apoptotic factor Bcl-2 and the activity of NF-κB in oral squamous cancer. Furthermore, they discovered that this correlation is one that is directly connected to the progression of the disease as well as its resistance to therapy [52]. On account of the fact that MAPK and NF-κB signalling is frequently active in cancer cells, it is possible that the deactivation of this signalling pathway could potentially contribute to the anticarcinogenic effects. For the purpose of this investigation, the oral administration of formononetin was found to be effective in inhibiting the growth of tumours in comparison to the control group. Previous research has shown that formononetin effectively inhibits the growth of tumours, and our findings are consistent with those findings. These effects include the induction of apoptosis, which is the death of cells, as well as the blockage of the formation of tumours. In a number of different types of cancer cells, including human breast cancer, osteosarcoma, cervical cancer, and colon cancer cell lines, it has been demonstrated that formononetin possesses these effects. In addition, Tyagi et al. discovered that the consumption of resveratrol, which is a phytoestrogen that can be found in red wine, led to the programmed cell death of FaDu cells in animal models by raising the activity of cleaved caspase-3 [54]. According to the findings of the current inquiry, the histological examination revealed that the expression of cleaved caspase-3 was significantly elevated in the tumour tissues obtained from xenograft animals that were treated with formononetin. This was in contrast to the tumour tissues obtained from the control group[55].

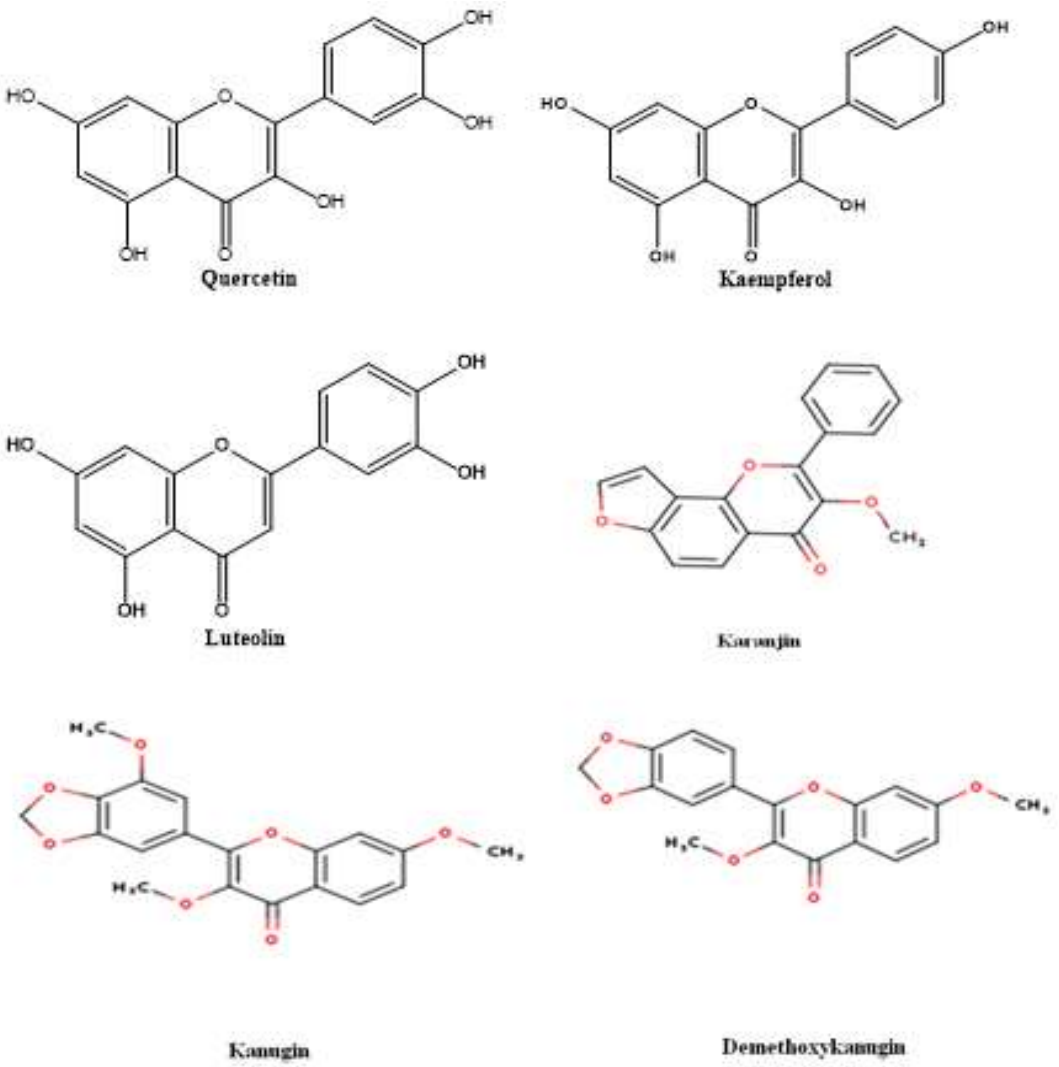



**Fig 3: Flavones of *Pangamic pinnata* and their derivatives**

## Conclusion

Overall, the findings of the study indicate that the stevioside molecule that is generated from the leaves of *Stevia rebaudiana* has the potential to act as an anti-tumor agent since it has a suppressive effect on the proliferation of breast cancer cells, specifically MDA-MB-231 and SKBR3. Furthermore, the data also suggested that pure stevioside, when evaluated in a controlled laboratory context, can increase the sensitivity of breast cancer cells to 5-FU therapy. This was demonstrated by the testing that was conducted. *Pongamia pinnata*, a plant that is indigenous to the Indian subcontinent, has been extensively used in traditional medicine for the treatment of a wide range of ailments due to the fact that it is non-toxic and has few or no bad effects. The purpose of this work is to provide a comprehensive analysis of the phytochemicals that were recovered from the various components of *Pongamia pinnata*, as well as the reported biological activities of the plant extracts and the constituents of the plant. Research was conducted in order to identify the specific components of the plant that are responsible for its curative abilities, using the knowledge that has been passed down through the generations. In the course of this investigation, the plant was extracted, isolated, and further characterised. It was discovered that *Pongamia pinnata* possessed notable properties, including as antibacterial, antidiabetic, anti-inflammatory, and anticancer activities, when it was evaluated utilising in vitro and in vivo models for a variety of illnesses. The phytochemicals that are responsible for these effects have been identified, they have been characterised, and they have been reported. Following the completion of both fundamental and advanced research on *Pongamia pinnata*, the subsequent step should be to place a high priority on gaining an understanding of the mechanism of action of bioactive components or extracts. This will allow for the accurate reporting of the complex pharmacological effects. In spite of this, additional investigations are necessary in order to conduct a comprehensive analysis of this phenomenon and the implications it has.

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