Analysis Of Antioxidant and Anticancer Properties in Methanolic Extract of Piper Sarmentosum Roxb. Against Mcf-7 Cells

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ABSTRACT

Cancer is by far one of the most worrying health issues that continue to be the major killing diseases worldwide, accounting for more than six million deaths. In the confrontation of advances in modern medicine such as surgery, chemotherapy, radiotherapy, and hormone therapy, cancer disease persists as a worldwide problem. In recent years, there has been a focused effort to explore new alternatives, with a particular emphasis on identifying medicinal herbs from local natural resources. Approximately, 60% of cancer drugs are derived from natural sources, due to their fewer side effects. *Piper sarmentosum* Roxb. which is locally known as 'kaduk', is a natural medicinal plant that has been used traditionally to treat headache, arthritis, menstrual pain, cough, and eczema. It is proven to have various biological properties such as anti-inflammatory, antioxidant, antimalarial, antiplasmodial, anti-diabetic, antifungal, and anti-carcinogenic. In this study, the phenolic content of *P. sarmentosum* was quantified using Folin-Ciocalteu method. Next, the antioxidant activity was determined using a DPPH scavenging assay. MTS assay was conducted to analyse the effect of the extract on human breast cancer cells, MCF-7. The results showed that the *P. sarmentosum* extract contained a high TPC which was 89.22 mg GAE/g. The extract also exhibited a good antioxidant activity with an EC50 value of 96.98 ± 2.29 μg/mL. The *in vitro* cytotoxicity test on MCF-7 cell proliferation showed the extract's IC50 at 24.63 ± 0.23 μg/mL. In conclusion, methanolic extract of *P. sarmentosum* contained a high phenolic content and is a potent antioxidant capacity as well as anticancer activity against the tested breast cancer cell.

Keywords: Breast cancer, medicinal plant, *Piper sarmentosum*, phenolic content, radical scavenging activity

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Kanser kekal sebagai kebimbangan kesihatan global, merugik lebih enam juta nyawa walaupun terdapat kemajuan dalam rawatan perubatan moden seperti pembedahan, kemoterapi, radioterapi dan terapi hormon. Usaha terbaru memberi tumpuan kepada alternatif penekanan pada herba ubatan daripada sumber semula jadi jadi tempatan. Sekitar 60% ubat kanser diperoleh daripada sumber semula jadi kerana kesan sampingan yang lebih rendah. *Piper sarmentosum* Roxb., dikenali sebagai 'kaduk,' ialah tumbuhan ubatan yang digunakan secara tradisional untuk menangani pelbagai masalah kesihatan seperti sakit kepala, sakit sendi, sakit senggugut, batuk dan ekzema. Secara saintifik, ia telah terbukti mempunyai sifat biologi seperti anti-radang, antioksidan, antimalaria, antiplasmodial, anti-diabetes, antikulat, dan anti-karsinogenik. Kajian ini dijalankan untuk mengukur kandungan fenolik *P. sarmentosum* menggunakan kaedah Folin-Ciocalteu. Selain itu, aktiviti antioksidan dinilai melalui ujian DPPH, manakala kesan ekstrak pada sel kanser payudara manusia (MCF-7) dianalisis menggunakan ujian MTS. Keputusan menunjukkan bahawa ekstrak P. sarmentosum mempermakern jumlah kandungan fenolik yang tinggi (89.22 mg GAE/g) dan menunjukkan aktiviti antioksidan yang baik (EC50 96.98 ± 2.29 μg/mL). Ujian sitotoksisisi in vitro pada percambahan sel MCF-7 menunjukkan IC50 ekstrak pada 24.63 ± 0.23 μg/mL. Kesimpulannya, ekstrak metanol P. sarmentosum mengandungi kandungan fenolik yang tinggi dan mempunyai kapasiti antioksidan yang kuat serta aktiviti antikanser terhadap sel kanser.

Kata kunci: Kanser payudara, tumbuhan ubatan, *Piper sarmentosum*, kandungan fenolik, aktiviti pemusnahan radikal
INTRODUCTION
Cancer is a complex genetic disorder which becomes a major public health burden in both developed and developing countries. It is predicted that by 2030, cancer incidence will ascend to 21.4 million, with the majority occurring in low and middle-income countries (De Silva et al., 2019). Breast cancer is more common in women than men, and it is the second leading cause of cancer deaths in women in the world. More than 50% of all breast cancer carcinoma with the estrogen receptor-positive type (Chia et al., 2015). Cancer is a multistep disease of genes a disorder that occurs in the normal processes of cell division by aberration of the genetic material of cells from many carcinogens such as radiation, viruses, and chemical carcinogens (Cullen et al., 2016).

According to Thavamani et al. (2013), about 3000 plants that possess medicinal properties of anticancer have been employed as potent anticancer drugs. Drug revelation from medicinal plants have crucial role in the cancer treatment. Plant secondary metabolites and their by-products over the last half century have been used in treating cancer (Vijayalakshmi et al., 2013). Plant’s bioactive compounds have the potential to inhibit, suppress, and delay the progression of carcinogenesis. The existence of vitamins and provitamins is well-established in plants, such as phenolic compounds, ascorbic acids, tocopherols, and carotenoids, and has been proven to be important in medicines. Antioxidant activity is commonly associated with phenolic, flavonols, and flavonoids (Chia et al., 2015). According to Do et al. (2014), the total phenolic content is equivalent to the plant’s antioxidant activity. Antioxidants could detain free radicals oxidation, and consequently inhibit or delay the carcinogenesis process (Ismail et al., 2018). Piperaceae family has contributed to various medicines and food grade spice for the past and present civilizations. This genus consists of over 1000 species worldwide with three main features: the cordate leaves, round berry-like fruits, and articulate stem. Asian tropics have 340 species of Piper in its warm, humid region and lowland of wet forests which take the form of herbs, lianas, shrubs, and woody climbers (Taher et al., 2020).

Piper sarmentosum Roxb. is known as kaduk among the locals contains phytochemical constituents such as amides, pyrones, sterols, flavonoids, neolignans, phenols, alkaloids, tannins, vitamin E, vitamin C, and xantophylls (Ugusman et al., 2012). The plant parts have been used traditionally as a remedy for various diseases such as cough, eczema, malaria fever, waist pain, headache, arthritis, and kidney stones by using the plant leaves (Ibrahim et al., 2022). Based on this literature, the current study was conducted to determine the total phenolic content and antioxidant activity of the methanolic extract of P. sarmentosum, as well as its cytotoxic activity against MCF-7 human breast cancer cells.

MATERIAL AND METHODS
Collection and extraction of plant material
P. sarmentosum plant was collected from Taman Pertanian Jubli Perak Sultan Haji Ahmad Shah, Kuantan, Pahang, Malaysia. The identification of the plant was carried out by Dr. Shamsul Khamis from Universiti Kebangsaan Malaysia. The leaves were harvested and rinsed under tap water before they were cut into small pieces and dried at room temperature overnight. Later, the leaves were dried at -80°C in a freezer for five days before being ground to form powder. Next, 10 g of the powdered samples were soaked in 80 mL methanol prior to adding 25 mL of 0.01 M hydrochloric acid (HCl) to the solution over 5 min as described by Rahman et al. (2014). The solution was mixed by using a shaker for 20 h at 100 rpm and 35°C. After
the cooling process, the sample was filtered through Whatman No. 1 filter paper. To dry the filtrate, 100 mL of the sample was evaporated for 4 h by using a rotary evaporator at 337 mbar with a water bath at 40°C. The crude extract was freeze-dried and stored at 4°C until further use.

Determination of total phenolic content (TPC)
Folin-Ciocalteu method was adapted from Routray and Orsat (2012) to determine the total phenolic content (TPC) of *P. sarmentosum* methanolic extract. The extract (0.5 mL from 1 mg/mL stock) was mixed with Folin-Ciocalteu reagent of 2.5 mL which had been diluted with water (1:10 v: v) and 7.5 mL sodium carbonate (Na₂CO₃) solution. The mixture was allowed to stand for 120 min at room temperature. The absorbances were measured by a UV-vis spectrophotometer at 760 nm. The standard curve was plotted using 0 to 500 µg/mL solutions of gallic acids in methanol: water (50:50, v/v). The total phenolic content was expressed as milligram of gallic acid equivalents per gram of dry matter (mg GAE/g DM).

DPPH free radical scavenging activity assay
The antioxidant activity of the methanolic extract of *P. sarmentosum* was determined using the diphenylpicrylhydrazine (DPPH) free radical scavenging assay method, following the procedure outlined by Lee et al. (2011). Firstly, the stock solution of the methanolic extracts was diluted from 3.90 to 500 µg/mL and similar concentrations were applied for ascorbic acid as positive control. Approximately 5 µL of DPPH solution was then added to the methanolic extract of *P. sarmentosum* and ascorbic acid solution. The mixture was left for 30 min before measuring the absorbance at 517 nm by a microplate reader (Thermo, USA). The antioxidant activity was expressed in the percentage of scavenging activity on DPPH radical using the following formula:

\[ \text{DPPH activity} \% = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100\% \]

Then, the amount of extract needed to decrease free radicals’ concentration by 50% (EC₅₀), was determined from the interpolated graph.

MTS cell proliferation assay
To evaluate the effect of the *P. sarmentosum* extract against breast cancer, MCF-7 cells, MTS assay was employed as outlined by Ghazali et al. (2020). Briefly, 1x10⁴ cells/well of MCF-7 cells were seeded onto 96-well plates and grown for 24 h in the 5% CO₂ incubator at 37 °C. Subsequently, the cells were treated with serial concentrations of 180 µL/well extracts from 0.39 to 100 µg/mL, while vincristine sulfate at the concentration of 0.04 µg/mL was used for the positive control. After 72 h of treatment in a similar incubation condition, 20 µL/well of MTS (Promega, USA) solution was added to each well. The plates were incubated for 4 h in the incubator with the same parameters. The absorbance was then measured at 490 nm using a microplate reader. The percentage of inhibition on the MCF-7 cell proliferation was determined using the following formula:

\[ \% \text{ Cell inhibition} = 100 - \left[ \frac{(\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}})}{(\text{OD}_{\text{control}} - \text{OD}_{\text{blank}})} \right] \times 100\% \]

A dose-response curve for percentage of cell inhibition versus sample concentration was plotted and the sample concentrations that caused 50% inhibition of cell viability (IC₅₀) were determined from the graph. The test was done in triplicate.
Statistical analysis
The dataset was obtained in three replicates and expressed as mean ± standard error mean (S.E.M). T-test (Microsoft Excel, 2010) was applied to analyse the data. The probability of p<0.05 was considered as significant.

RESULT AND DISCUSSION

Total phenolic content (TPC) of *P. sarmentosum* methanolic extract
The findings regarding the methanolic extract of *P. sarmentosum* are intriguing and provide valuable insights into the plant's chemical composition. The TPC of the extract, at 89.22 mg GAE/g dry weight, indicated a substantial presence of phenolic compounds. This information is crucial, as phenolic compounds are known for their antioxidant properties and potential health benefits. This finding is supported by Rahman et al. (2014), emphasizing that the methanolic extract of *P. sarmentosum* contains naringenin, a type of flavonoid. Naringenin, recognized for its antioxidant and anti-inflammatory properties, contributes to the potential health benefits associated with *P. sarmentosum*. Furthermore, Rahman et al. (2014) identified additional flavonoids such as apigenin, myricetin, and quercetin in the aqueous-methanol extract, providing a more comprehensive understanding of the diverse chemical profile of *P. sarmentosum*. This collective evidence suggests that *P. sarmentosum* may offer a range of bioactive compounds with various potential health-promoting effects.

The choice of solvent for extracting bioactive compounds can significantly influence the composition of the extract. The use of methanol in this study might have selectively extracted certain compounds, leading to the high TPC observed. However, it is worth noting that the efficacy of plant extracts can be influenced by other various factors, including geographical location, climate, and plant maturity (Ngo et al., 2017). Therefore, while these findings provide valuable insights, variations in *P. sarmentosum* extracts may exist based on different growth conditions and extraction methods. The relationship between absorbance (x) and the concentration of gallic acid solution (y) follows a linear equation, \( y = 0.0015x - 0.057 \), with a high coefficient of determination \( R^2 = 0.968 \). This suggests a strong correlation between absorbance values and gallic acid concentration, providing a reliable method for quantifying phenolic content in *P. sarmentosum* methanolic extract.

Antioxidant activity of *P. sarmentosum* methanolic extract
This study indicated that the methanolic extract of *P. sarmentosum* leaves demonstrated antioxidant activity, as evidenced by its effective concentration \( (EC_{50}) \) of 96.98 ± 2.29 μg/mL (Figure 1). The \( EC_{50} \) value represents the amount of extract required to reduce the concentration of free radicals by 50%, serving as a measure of the extract's antioxidant potency. Comparatively, the study also determined the \( EC_{50} \) value for ascorbic acid, a well-known antioxidant. Ascorbic acid’s \( EC_{50} \) was reported as 74.18 ± 5.34 μg/mL. This comparison establishes *P. sarmentosum* as a potent source of antioxidants, although slightly less potent than ascorbic acid in this assay. The findings suggest that *P. sarmentosum* could be a valuable natural source of antioxidants, which are known for their ability to counteract oxidative stress and protect cells from damage caused by free radicals. The fact that it is comparable to ascorbic acid, a widely recognized antioxidant, adds credibility to its potential health benefits.

According to Lee et al. (2011), the methanol extract obtained from the leaves of *P. sarmentosum* demonstrated superior antioxidant activity compared to extracts derived from hexane, chloroform, butanol,
aqueous, and ethyl acetate. Similarly, Yeo et al. (2018) conducted a study that confirmed the highest antioxidant activity in the methanol extract, with subsequent rankings of hexane, dichloromethane, and ethyl acetate in descending order of potency. This consistency in findings emphasizes the significance of methanol as an effective solvent for extracting antioxidant compounds from this plant, with superior outcomes compared to other extraction methods such as hexane, chloroform, butanol, aqueous, dichloromethane, and ethyl acetate.

![Figure 1: Antioxidant activity of methanolic extract of P. sarmentosum.](image)

**Cytotoxic activity of P. sarmentosum methanolic extract against MCF-7 cells**  
The study revealed that the methanolic extract of *P. sarmentosum* has demonstrated cytotoxic activity against the MCF-7 cell line. The 50% growth inhibition is represented by the IC₅₀ value, which, in this case, was determined to be 24.63 ± 0.23 μg/mL using the MTS assay (Figure 2). An IC₅₀ of 24.63 μg/mL indicated the concentration of the extract required to inhibit the proliferation of MCF-7 cells by 50%. This finding is noteworthy, particularly in the context of the criteria set by the American National Cancer Institute (NCI) (Campoccia et al., 2021). According to the NCI, active crude extracts are those that provide an IC₅₀ value of less than 20 μg/mL after 72 hours of exposure. Additionally, extracts with an IC₅₀ of less than 20 μg/mL are classified as highly cytotoxic. Given that the IC₅₀ value in the current study falls within the range of 20 to 30 μg/mL, it is categorized as active by NCI standards. This classification suggests that the *P. sarmentosum* methanolic extract holds promise as a potential anticancer candidate, warranting further investigation. The fact that the extract's activity is close to the threshold for high cytotoxicity is particularly encouraging. It implies a significant inhibitory effect on the growth of MCF-7 cells, and this characteristic aligns with the criteria that suggest the extract could be a potent candidate for further exploration in the development of anticancer therapies.

The current study reported a lower IC₅₀ value (24.63 μg/mL) compared to the study by Amid et al. (2009), which used the Sulforhodamine B cytotoxicity assay (IC₅₀ of 70 μg/mL) on MCF-7 cells. In contrast, the study by Zainal Ariffin et al. (2009) reported a lower IC₅₀ value (12.5
µg/mL) for the ethanolic extract of *P. sarmentosum* against the HepG2 hepatoma cell line compared to both the current study and Amid et al. (2009). This indicated a potentially stronger cytotoxic effect on HepG2 cells with the ethanolic extract in the study by Zainal Ariffin et al. (2009). These variations in IC₅₀ values may be attributed to differences in the assay methods, extraction techniques, and cell lines used in each study. The findings provided by Azizi et al. (2010) regarding the genetic differences between the MCF-7 and T-47D breast cancer cell lines added a layer of complexity to the understanding of the responses of different cancer cell lines to treatments, including those involving natural extracts such as *P. sarmentosum*. T-47D cells exhibit higher estrogen receptor (ER) expression compared to MCF-7 cells. Conversely, T-47D cells show lower progesterone receptor (PR) expression compared to MCF-7 cells. They also suggest the presence of a mutation in the p53 gene in T-47D cells. T-47D cells are more sensitive to valproic acid (VPA), suberoylanilide hydroxamic acid (SAHA), and cisplatin (CDDP) treatments compared to MCF-7 cells.

The antioxidant and anticancer properties of the methanolic extract of *P. sarmentosum* can be attributed to the synergistic effects of its diverse array of compounds. Phenolic compounds, flavonoids, alkaloids, and terpenoids such as myricetin, quercetin, apigenin, naringenin, rutin and vitexin, collectively contribute to the observed effects, highlighting the potential of this natural extract as a source of bioactive compounds with therapeutic implications (Ismail et al., 2018; Zainol Abidin et al., 2023). The relationship between the total phenolic content, antioxidant activity, and anticancer effects underscores the holistic potential of *P. sarmentosum* as a natural therapeutic agent. Phenolic compounds, with their antioxidant properties, can neutralize oxidative stress, a factor implicated in cancer development (Ibrahim et al., 2022). The observed inhibitory effect on MCF-7 cells further supports the idea that the phenolic-rich extract may hold promise in cancer prevention or treatment.
Understanding the specific mechanisms through which *P. sarmentosum* exerts its cytotoxic effects on cancer cells could provide valuable insights for further research and potential therapeutic applications. It is important to note that while the cytotoxic activity against cancer cells is promising, further studies are needed to explore the selectivity of the extract—determining its impact on normal cells versus cancer cells. Additionally, identifying and isolating specific bioactive compounds responsible for the observed cytotoxicity would contribute to a more comprehensive understanding of *P. sarmentosum*’s potential as a source of anticancer agents.

**CONCLUSION**

In conclusion, the study on *P. sarmentosum* underscores its potential as a valuable natural resource in cancer research. The findings of this research revealed that the methanolic extract of *P. sarmentosum* exhibited a substantial phenolic content (89.22 mg GAE/g), indicating its rich biochemical composition. Moreover, the plant demonstrated noteworthy antioxidant activity, as evidenced by its ability to scavenge DPPH radicals. The study extended its investigation to assess the effect of the extract on human breast cancer cells (MCF-7), revealing a significant inhibitory effect on cell proliferation with an IC$_{50}$ value of 24.63 ± 0.23 μg/mL. These results not only validate the traditional uses of *P. sarmentosum* but also highlight its potential as a source of natural compounds with anticancer properties. This study provides valuable insights that may serve as a foundation for future research, including investigations into its mechanisms of action, the isolation of bioactive compounds, toxicological assessments, and testing in animal models.

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