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EXPLORING THE PHYTOCHEMICAL PROFILE AND POTENT HEALING PROPERTIES OF *PTERIS VITTATA* L.

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Abstract

Cancer and multi-drug resistant bacterial strains are major causes of global mortality. Natural products offer a safe and cost-effective alternative to combat these issues. This study aimed to investigate the antibacterial and anticancer properties of *P. vittata*. Fronds of *P. vittata* were extracted using different solvents, and their bioactivities (anti-bacterial and anti-cancer) and phytochemical composition were analyzed. The data analysis revealed that *P. vittata* contains a wide range of phytochemicals, including alkaloids, terpenoids, flavonoids, tannins, carotenoids, carbohydrates, saponins, quinines, coumarins, phlobatanins, and phenols. Quantitative analysis showed significantly higher levels of phenolics (948.78 ± 14.78), reducing sugar (687.55 ± 37.97), and ascorbic acid (419.64 ± 5.69), followed by flavonoids (292.67 ± 6.32). Furthermore, the organic solvent extracts demonstrated notable activity against specific bacterial strains. The chloroform extract exhibited a significantly larger zone of inhibition against *S. aureus* (17±0.57), followed by *E. coli* (15±0.57) and *K. pneumoniae* (14±0.57). The ethyl acetate extract significantly inhibited the growth of *S. aureus* (16.66±0.33) and *K. pneumoniae* (15.33±1.20). Similarly, the methyl alcohol extract showed higher zones of inhibition against *S. aureus* (17.33±1.20), followed by *E. coli* (12.66±1.45). The N-hexane extract also displayed remarkable activity against *S. aureus* (16.33±0.88), *P. aeruginosa* (11.33±1.85), and *K. pneumoniae* (11±1.00). Moreover, higher doses of the plant's ethanolic extract (1000ug cell viability assay, 2000ug colony formation assay) significantly inhibited the growth of both AGS and SGC cancer cell lines. However, the plant extract did not exhibit any anticancer potential at lower doses. Based on these findings, it can be concluded that *P. vittata* is rich in phytochemicals and its extracts possess remarkable antibacterial and anticancer properties. The active constituents of this plant could be utilized in the management of gastric cancer and diseases caused by multidrug-resistant bacterial strains.

Key words: *Pteris vittata* L., Fronds, Phytochemistry, Antibacterial Activity, Anticancer Activity.

1. Introduction

Antimicrobial resistance (AMR) is recognized as one of the top10 global threats to health in feature [1]. The misuse, overuse, and self-prescription of antibiotics worldwide are key factors contributing to the emergence of antimicrobial resistant strains [2]. Research studies have shown that poor and developing countries are more prone to the production of these strains as compared to developed countries [3]. The presence of drug resistant microbial strains leads to severe infections, resulting in high mortality rates and increased financial losses due to longer hospital stay [4]. The development of microbial resistance involves major phenomena such as tolerance, persistence, and resistance [5]. Studies have indicated that resistance is associated with an increase in minimal inhibitory concentration (MIC), while tolerance and persistence do not show such an increase in MIC [6].

Similarly, cancer is a pressing issue that is escalating globally and stands as a primary contributor to mortality, accounting for one in every six deaths [7]. The year 2018 witnessed a staggering 9.6 million fatalities and 18.1 million fresh instances of cancer reported worldwide [8]. It is projected that these numbers will continue to rise in the coming decades [9]. Globally, gastric cancer is the most prevalent among various types of cancers [10]. It ranks fifth in terms of frequency and is the fourth leading cause of death worldwide in 2020 [11]. Despite advancements in treatment and diagnosis, gastric cancer still has a bleak outlook with a high risk of recurrence and metastasis [12]. Peritoneum, lungs, lymph nodes, liver and bones are frequently affected by metastatic spread in cases of gastric cancer [13].

According to WHO 80% of the global population relies on herbal remedies for their primary healthcare needs [14]. Various parts of plants such as stem, leaves, root, fruits and seeds contain power full potent bioactive compounds such as glycosides, tannins, alkaloids, flavonoids etc, which enhances their immunity against microorganisms [15]. The fact that natural products have antimicrobial potential against multidrug resistant bacteria has been established through an increasing number of investigations [16]. Out of the 247 newly approved anticancer drugs, 217 are derived from natural products, while the remaining 29 are synthetic [17]. Anticancer drugs derived from natural products, specifically plants, disrupt multiple signaling pathways involved in cancer development and have fewer side effects compared to synthetic anticancer drugs [18].

Pteridophytes, a category of plants, are recognized for their medicinal properties and have become increasingly important in the development of novel drug therapies [19]. Many species within this plant division contain a diverse range of secondary metabolites with medicinal properties that can be utilized in the treatment of various diseases. However, these plants are often overlooked [20]. Based on facts, pteridophytes have been identified as sources of phytochemicals with a diverse range of medicinal properties, including antimicrobial, antioxidant, anti-inflammatory, and anticancer effects [21].

The study aimed to investigate the phytochemicals, antibacterial properties, and potential anticancer effects of *Pteris vittata* from the Pteridaceae family, also known as the bark family. Specifically, the research focused on its ability to combat multi-drug resistant bacteria and gastric cancer cell lines (AGS and SGC7901).

2. Materials and methods

2.1 Plant collection and processing

***Pteris vittata* was collected from District Mansehra and processed at Hazara University Mansehra, KPK, Pakistan. Initially, the plant's fronds were dried in the shade and then ground into a fine powder using an electric grinder. To form the extracts, 400ml of five different solvents (methyl alcohol, N-hexane, chloroform, ethyl acetate, and distilled water) were mixed with 50g of the fronds powder. These solutions were placed in a shaker at room temperature (25±27◦C) for seven days. After this period, each sample was filtered using F1001 grade filter paper and then completely dried using a rotary evaporator. The dry extracts from**

each solvent were stored at 4◦C until further analysis. All experimental procedures were approved by the Advance Studies and Research Board (ASRB) at Hazara University, Mansehra, Pakistan.

2.2 Phytochemical analyses

Qualitative detection of various phytochemicals was conducted by observing changes in solvent colours. Flavonoids, terpenoids, tannins, saponins, phenols, glycosides and carbohydrates were identified using standard protocols used by [22], while phlobatanins, alkaloids, carotenoids and coumarins were determined following protocols [23]. Quantitative phytochemical analysis of *P. vittata* was performed using a spectrophotometer (UV 1900) such as prolines [24], anthocyanin [25], flavonoids and phenols [26], ascorbic acid [27] and sugar content [28].

2.3 Antibacterial activity

Antibacterial potential of *P. vittata* was assessed against four multi-drug resistant bacterial strains *Staphylococcus aureus (*ATCC-6538), *Escherichia coli (*ATCC-8739), *Klebsiella pneumonia* (ATCC-10031) and *Pseudomonas aeruginosa* (ATCC-1238). To conduct the experiment, 01ml of DMSO (Dimethyl Sulphoxide) and 120mg of dry extracts were taken in eppendrof tube and mixed well using gyro-mixture. These diluted samples were then used to evaluate the antibacterial activity of *P. vittata* by disc diffusion method (DDM) [29]. The levofloxacin discs with the concentration of 5ug were utilized as a positive control, while DMSO was employed as the negative control.

2.4 Anticancer activity

The anticancer potential of *P. vittata* was evaluated against human gastric cancer cell lines SGC (Scirrhous gastric cancer) with ATCC=7901 and AGS (Adenocarcinoma gastric cancer) with ATCC= CRL-1739, using an ethanolic extract. Both cell lines were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum and incubated in a humidified incubator with a constant supply of CO2 at 37◦C. The anticancer activity of *P. vittata* was determined using the colony formation assay [30] and cell viability assay [31]. This part of the study was conducted by the Fifth Affiliated Hospital of Zhengzhou University, School of Medicines, China.

2.5 Statistics

The statistical analyses were performed by using software (statistix 8.1). All experiments were performed in triplicate, and the average of the replicates was recorded and mean of replicates was noted. The ANOVA test was performed to observe significant differences among different treatments. A P-value ≤ 0.05 was considered significant.

3. Results

3.1 Phytochemical analyses

3.1.1 Qualitative phytochemical analysis

Table.1. Phytochemical constituents of *P. vitata* in different extracts

Tests	Chloroform	Ethyl acetate	Methyl alcohol	N-hexane	Distilled water
Flavonoids	-	-	+++	-	+++
Carotenoids	+++	+++	++	+++	-
Terpenoids	-	-	+++	++	+++
Phlobatanins	++	-	++	-	-
Alkaloids	++	+++	-	+++	++
Saponins	++	-	+++	-	-
Quinines	-	++	-	-	+++
Carbohydrates	++	-	++	-	-
Glycosides	++	-	-	-	++
Phenols	+++	++	+++	-	-
Tannins	+++	-	+++	-	++
Coumarins	+++	+++	++	+++	+++

Key = -, absent; ++, moderately present; +++, strongly present

We performed a qualitative analysis of the phytochemicals present in different solvent extracts of *P. vittata* (figure 1, table 1). The results indicate that the chloroform and methyl alcohol extracts contained a significantly higher number of phytochemicals compared to the N-hexane, ethyl acetate, and distilled water extracts. In the chloroform extract, flavonoids, carotenoids, and quinines were absent, while all other compounds were present. This suggests that chloroform is not an effective solvent for extracting these specific phytochemicals from *P. vittata*. On the other hand, the methyl alcohol extract did not contain alkaloids, quinines, and glycosides, but all other phytochemicals were present. This indicates that methyl alcohol is not suitable for extracting these particular compounds. In contrast, the N-hexane, ethyl acetate, and distilled water extracts showed mild extraction of phytochemicals. These extracts contained a lower number of phytochemicals compared to the chloroform and methyl alcohol extracts. The majority of phytocompounds were absent in these extracts, suggesting that these solvents are less effective in extracting a wide range of phytochemicals from *P. vittata*. Overall, the results of the qualitative phytochemical analysis in Table 1 highlight the importance of solvent selection in phytochemical extraction. Different solvents have varying abilities to extract specific phytochemicals, and the choice of solvent can significantly impact the composition and quantity of phytochemicals obtained from a plant extract.

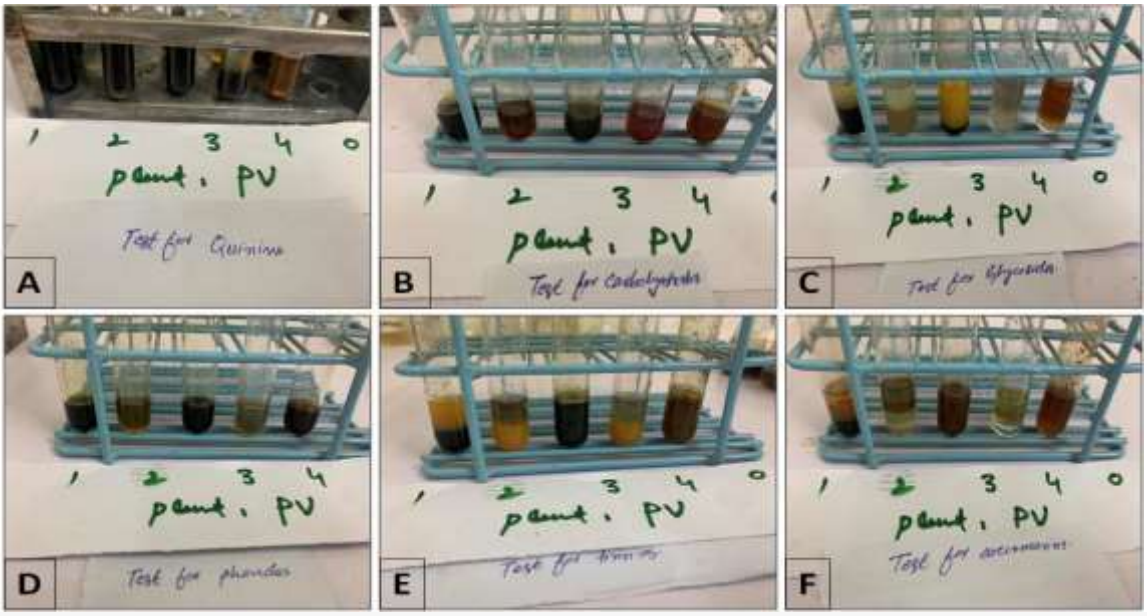

Figure.1. Qualitative phytochemical analysis of *P. vittata*, (A= test for quinines, B= test for carbohydrates, C= test for glycosides, D= test for phenols, E= test for tannins, F= test for coumarins) and (1, 2, 3, 4 and 0 represents chloroform, N-hexane , ethyl acetate, methyl alcohol and distill water extracts, respectively).

3.1.2 Quantitative phytochemical analysis

Table.2. Quantitative phytochemical analysis of *P. vittata*

S.No	Phytochemicals	Quantities (average ± standard error)	Units
1	Ascorbic Acid	419.64 ± 5.69	mM/g F.WT
2	Flavonoids	292.67 ± 6.32	Mg/g F.WT
3	Reducing Sugars	687.55 ± 37.97	uM/ml F.WT
4	Phenolics	948.78 ± 14.78	Mg/L F.WT
5	Prolines	1.96 ± 0.28	uM/g F.WT
6	Anthocyanins	25.36 ± 1.15	uM/g F.WT

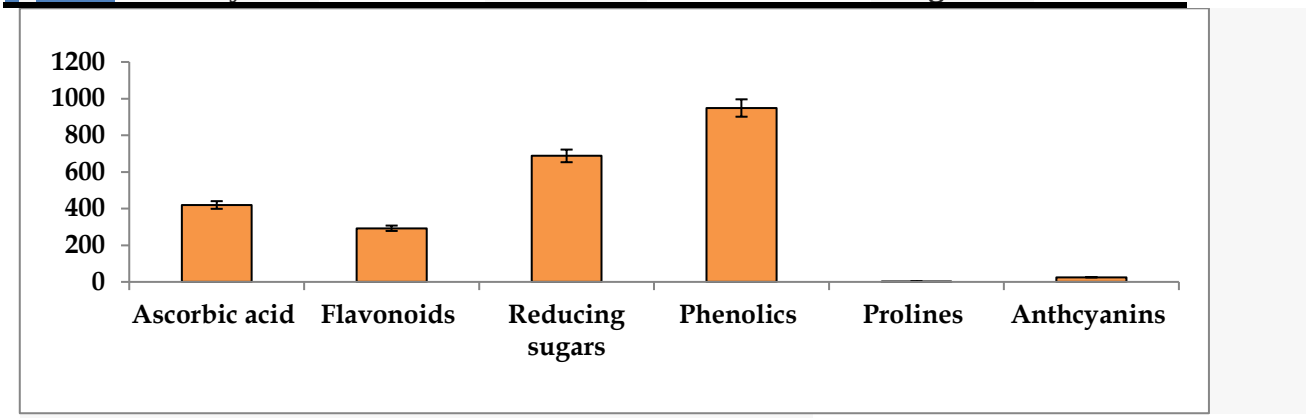

Figure.2. Quantitative phytochemical analyses of *P. vittata*

Results show the various phytoconstituents by quantitative analysis in *P. vittata*. The study measured the amounts of phenolics, reducing sugars, ascorbic acid, flavonoids, prolines, and anthocyanins using spectrophotometry (figure 2). According to the results, the highest quantity of phytoconstituents was observed for phenolics, with an average value of 948.78 ± 14.78. This indicates that *P. vittata* contains a significant amount of phenolic compounds, which are known for their antioxidant properties and potential health benefits. The second highest quantity was observed for reducing sugars, with an average value of 687.55 ± 37.97 (Table 2). Reducing sugars are a type of carbohydrate that can provide energy and contribute

to the overall sweetness of a plant. Ascorbic acid, also known as vitamin C, was found in relatively high amounts with an average value of 419.64 ± 5.69. Ascorbic acid is an essential nutrient that acts as an antioxidant and plays a crucial role in various physiological processes. Flavonoids, another group of plant compounds with antioxidant properties, were present in lower amounts compared to the other phytoconstituents. The average value for flavonoids was 292.67 ± 6.32. Although present in smaller quantities, flavonoids still contribute to the overall antioxidant capacity of *P. vittata*. On the other hand, prolines and anthocyanins were observed to be nearly absent during spectrophotometry. Prolines are amino acids that can act as osmoprotectants and play a role in stress tolerance in plants. Anthocyanins are pigments responsible for the red, purple, or blue colours in many fruits and flowers. Overall, the results from (Figure 2, table 2) provide valuable information about the phytoconstituents present in *P. vittata*, highlighting the abundance of phenolics, reducing sugars, and ascorbic acid, while indicating the lower presence of flavonoids and the absence of prolines and anthocyanins. These findings contribute to our understanding of the chemical composition and potential health benefits of *P. vittata*.

3.2 Antibacterial activity

Table.3. Antibacterial activity of *Pteris vittata*

Zone of inhibition (mm) ± Standard Error means

Key- CH= chloroform, EA= ethyl acetate, MA=methyl alcohol, NH= N-hexane, DW=distilled water, LEVO= Levofloxacin

S.NO	Bacterial strains	CH	EA	MA	NH	DW	LEVO
1	*Staphylococcus aureus*	17±0.57	16.66±0.33	17.33±1.20	16.33±0.88	00±00	24.66±0.88
2	*Klebsiella pneumoniae*	14±0.57	15.33±1.20	6.66±3.38	11±1.00	00±00	14±1.15
3	*Escherichia coli*	15±0.57	7±3.51	12.66±1.45	6.33±3.17	00±00	20.33±0.88
4	*Pseudomonas aeruginosa*	4.33±4.33	4±4.00	3±3.00	11.33±1.85	00±00	19±0.57

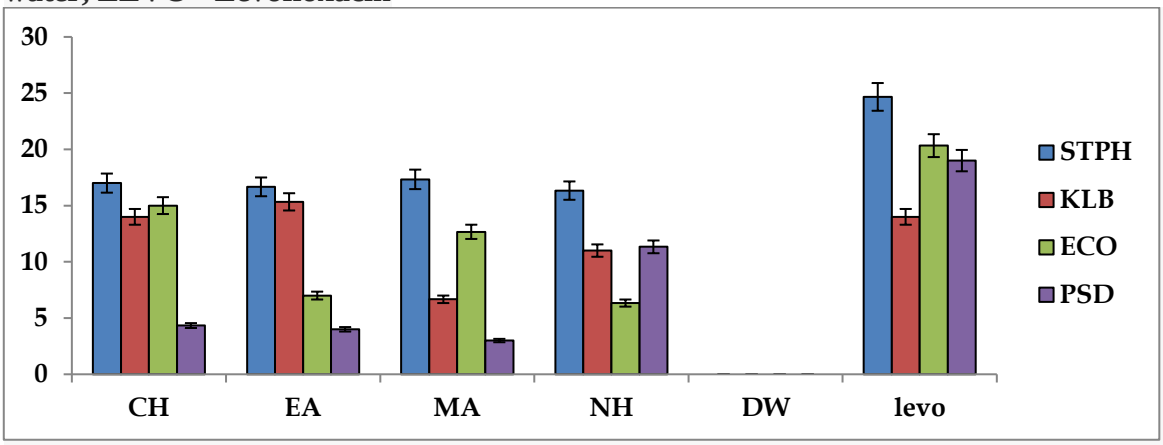

Figure.3. Graphical representation of antibacterial activity of *Pteris vitata*. (Key- CH= chloroform, EA= ethyl acetate, MA=methyl alcohol, NH= N-hexane, DW=distilled water, LEVO= Levofloxacin, STPH= *S. aureus*, KLB= *K. pneumoniae*, ECO= *E. coli*, PSD= *P. aeruginosa*)

We tested the antibacterial activity of different extracts of *P. vittata*. The extracts prepared in organic solvents demonstrated potential antibacterial properties, while the extract prepared

in distilled water did not show any inhibitory effect against the selected bacterial strains (Figure 3, Table 3). Among the organic solvent extracts, the chloroform extract exhibited the highest zone of inhibition against *S. aureus*, followed by *E. coli* and *K. pneumoniae*. However, this extract was not effective in inhibiting the growth of *P. aeruginosa* significantly. The ethyl acetate extract showed significant inhibition of *S. aureus* and *K. pneumoniae*, but only mild activity against *E. coli* and *P. aeruginosa* (figure 4). The methyl alcohol extract also showed higher zones of inhibition against *S. aureus* and *E. coli*, but mild activity against *K. pneumoniae* and P. *aeruginosa*. The N-hexane extract demonstrated remarkable activity against *S. aureus*, *P. aeruginosa*, and *K. pneumoniae*, but was less effective against *E. coli*. The levofloxacin disc, which served as the positive control, produced significantly higher zones of inhibition against all selected bacterial strains. On the other hand, the DMSO (negative control) did not produce any inhibitory zones against the selected bacteria (Figure 4). Overall, the results suggest that the organic solvent extracts of *P. vittata* have potential antibacterial activity, with the chloroform extract showing the highest efficacy against *S. aureus*, *E. coli*, and *K. pneumoniae*. However, further studies are needed to determine the specific antibacterial compounds present in these extracts and their mechanisms of action.

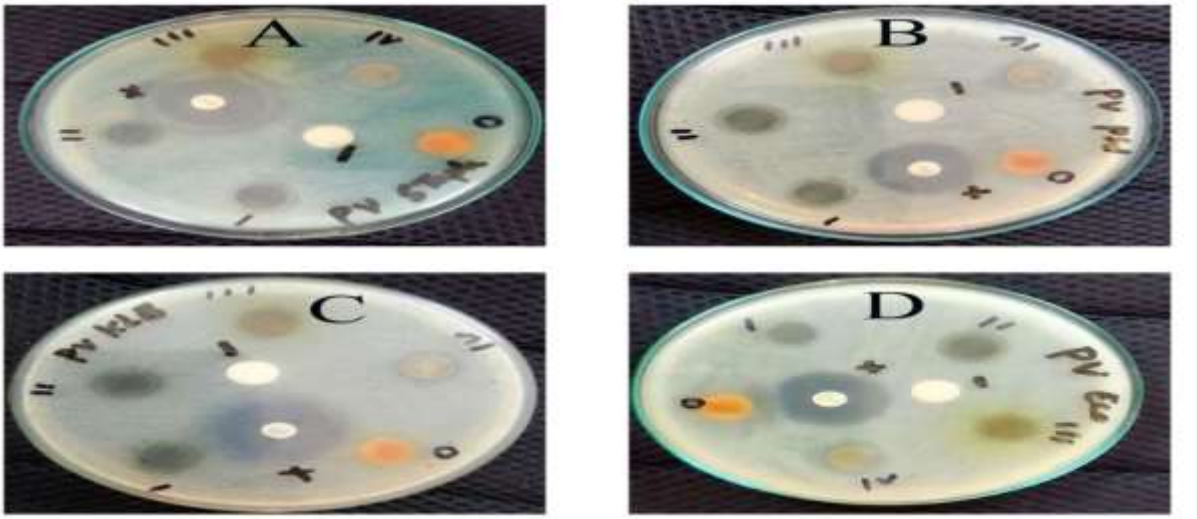

Figure. 4. Antibacterial activity of *P. vittata* (i= chloroform, ii= N-hexane, iii= ethyl acetate, IV= methyl alcohol, 0= distill water, positive control, negative control) A= *S. aureus*, B= *P. aeruginosa*, C= *K. pneumoniae*, D= *E. coli*.

3.3 Anticancer activity

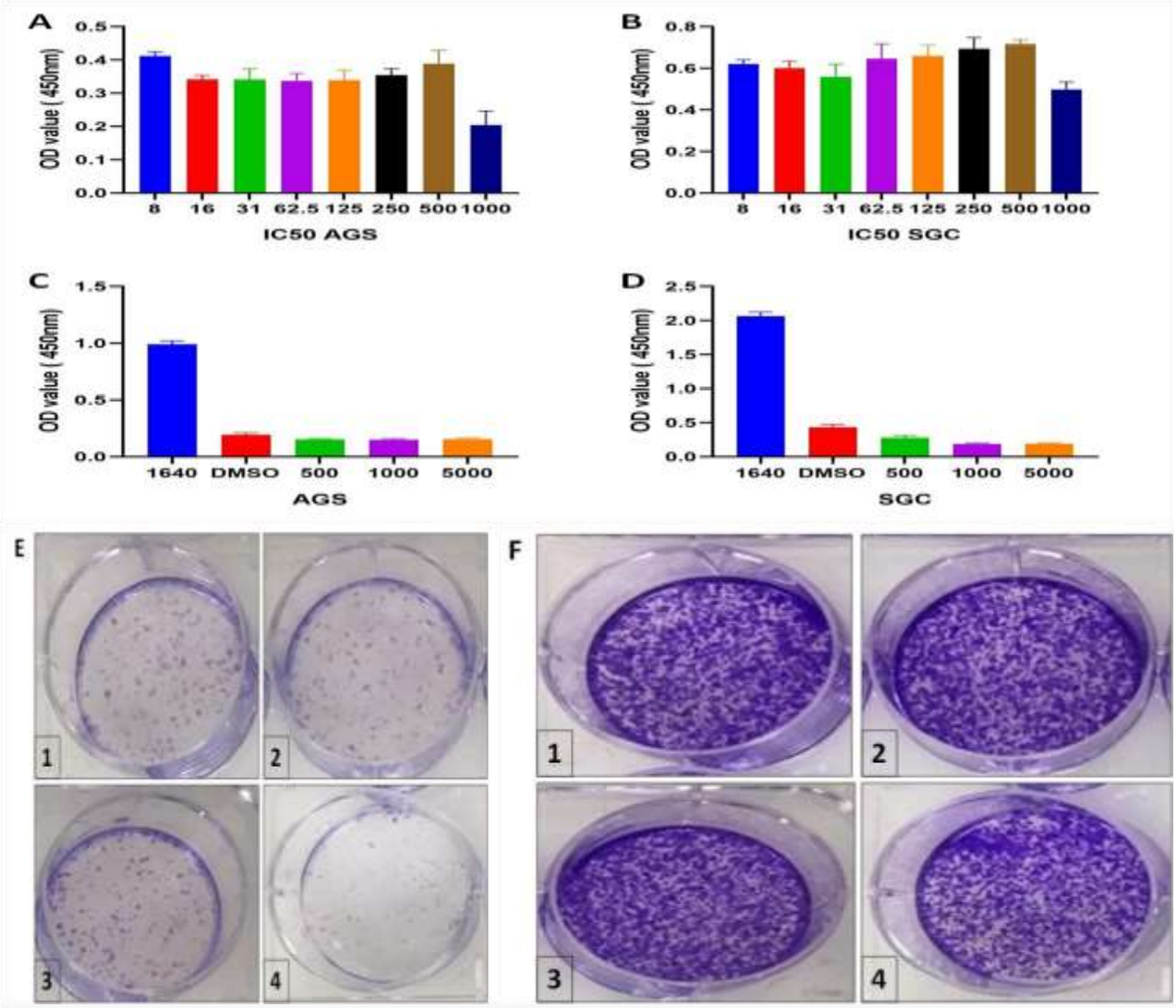

Figure.5. Graphical representation of the anticancer activity of *P. vittata*, A and C= Cell proliferation assay against AGS cell line, B and D= Cell proliferation assay against SGC cell line, E= colony formation assay against AGS cell line (1= cancer cell with normal media, 2= cancer cells with DMSO, 3= cancer cells with 500ug plant extract, 4= cancer cells with 2000ug plant extract), F= colony formation assay against SGC cell line (1= cancer cell with normal media, 2= cancer cells with DMSO, 3= cancer cells with 500ug plant extract, 4= cancer cells with 2000ug plant extract).

We determined the anticancer activity of *P. vittata*, a type of plant extract, against human gastric cancer cell lines AGS and SGC7901. The researchers conducted cell proliferation analysis on both cell lines initially. They found that at higher concentrations (1000ug) of the plant extract, the growth of AGS and SGC cell lines was significantly slowed down, as determined by IC50 analysis. However, at lower concentrations (125ug, 250ug, 500ug), the plant extract was unable to stop the growth of both cell lines (Figure5A and B).To further confirm the IC50 results, the researchers used 500ug and 5000ug of the plant extract. The analysis revealed a significant decrease in the viability of both cell lines compared to the negative control (Figure 5 C and D).
To provide additional evidence, the researchers conducted a colony formation assay. They found that at a concentration of 2000ug, the plant extract was able to retard the growth of the AGS cell line. However, at a concentration of 500ug, the extract failed to inhibit the growth of the AGS cell line. Similarly, the SGC cell line showed a decrease in viability at a concentration of 2000ug, while 500ug of the plant extract had no effect on its growth (Figure 5 E and F).

Overall, these findings suggest that *P. vittata* has anticancer activity against human gastric cancer cell lines, with higher concentrations of the plant extract being more effective in inhibiting cell growth. The results from the IC50 analysis and colony formation assay provide further support for the anticancer properties of *P. vittata*.

4. Discussion

In recent times, there has been a growing interest in the clinical use of natural products due to their numerous benefits and minimal side effects [32]. Medicinal plants are rich in bioactive phytochemicals, making them a potential source for discovery of new drug [33]. Plant-based food containing essential nutrients have shown promising results in preventing various respiratory diseases including asthma, pneumonia, bronchitis, flue and other respiratory infections [34]. Various phytochemicals present in plants like flavonoids, alkaloids, phenolics, terpenes, glucosinolates and anthocyanins having remarkable medicinal properties [35]. For instance, *Filipendula ulmaria* is a plant from which aspirin is derived which acts as a blood-thinning medication used in the treatment of cardiovascular diseases [36]. Terpenoids, major chemical constituents of plants have demonstrated hepatoprotective properties [37]. Proline, another natural product, exhibits potent antibacterial properties by interacting with bacterial proteins and rendering them inactive [38]. Phenolics, which are natural products derived from plants, have shown effectiveness in managing and preventing oxidative stresses, making them potential candidates for the treatment of cancer and other diseases [39].

In our current study, we conducted a comprehensive investigation into the phytochemicals, antibacterial and anticancer activity of *P. vittata*. We successfully screened a range of bioactive compounds including alkaloids, flavonoids, terpenoids, quinines, saponins, glycosides, carbohydrates, Phenols, tannins, coumarins and phlobatanins. The presence of these compounds confirms the medicinal properties of *P. vittata*. To identify these bioactive compounds, we utilized five solvents. Our results showed that chloroform and methyl alcohol extracts exhibited excellent efficacy of phytochemicals extraction, while N-hexane, ethyl acetate and distilled water shows poor extraction of phytoconstituents. The effectiveness of phytochemical extraction depends on polarity which determines their ability to dissolve the phytochemicals [40]. Our findings align with the studies conducted by [41] and [42] who recommend the chloroform and methyl alcohol as the most favorable solvent to obtain high yield of phytochemicals, resulting in extracts with superior biological activities. [43] Also support these findings, reporting limited extraction of phytochemicals with N-hexane. However, our results regarding distilled water extraction contradict the findings of [44] that successfully extracted various phytoconstituents of *S. oleraceus* and argue that water, due its polar nature, is the optimal solvent for phytochemical extraction. The absence of an inhibitory zone against the selected MDR bacteria suggests that the distilled water extract of *P. vittata* does not possess antibacterial properties. We speculate that the antibacterial components of the plant are non-polar in nature, which explains why they are not extracted by distilled water. Consequently, the extract does not exhibit any antibacterial potential.

[45] Obtained similar findings when they assessed the antibacterial properties of *S. cumini*. They found that the distilled water extract did not exhibit any activity against the chosen bacteria when compared to the extracts obtained from organic solvents. Contrary to the findings of [46], our study revealed contrasting results. They reported that the distilled water extracts *D. Butyracea* leaves exhibited notable activity against *K. pneumoniae*. However, our study found that all organic solvent extracts (chloroform, ethyl acetate, methyl alcohol, N-hexane) of *P. vittata* demonstrated significant activity against the selected MDR bacterial strains. The organic solvent extracts of *P. vittata* exhibit notable antibacterial activity against certain MDR bacterial strains. We believe that antibacterial components in our plant are mostly non polar and extracted by organic solvents thus these extracts showed prominent antibacterial activity. Among the tested strains, *S. aureus* appears to be the most susceptible to the plant extracts. This could be attributed to the fact that *S. aureus* is a Gram-positive bacterium and easily absorbs the antibacterial components from the plant, thereby inhibiting

its growth. Similarly [47] determined that *S. aeurus* is most susceptible strain to methanolic bark extract of *Z. armatum*. In another study, [48], observed the same results in terms of *S. aureus* sensitivity to plants extracts. Likewise, the extracts of *P. vittata* also hindered the growth of *K. pneumonia* and *E. coli*, despite these bacteria being stronger due to an additional lipopolyscharide layer on their cell walls. However, the antibacterial components of our plants are more potent, as they penetrated the bacterial cells and killed them. Similarly, aqueous extracts of Aloe vera effectively inhibit the growth of gram negative bacteria (*E. coli* and *K. pneumoniae*), that are associated with urinary tract infections [49]. The *P. aeruginosa*, Gram negative bacteria was the most resistant strain to our plant extracts; we believe that these bacteria hinder the entry of antibacterial compounds from the plant extracts by having an extra lipopolyscharide membrane on their cell wall. Our findings align with a study by [50], who investigated the effects of different medicinal plants on bacterial strains and found that aqueous extracts of these plants did not produce any inhibitory zone against *P. aeruginosa.* However, our results contradict the findings of [51], they examined various extract of *L. inermis* and observed significant activity against *P. aeruginosa*.

The ethanolic extract of *P. vittata* shows promising anticancer effects against gastric cancer cell lines (AGS and SGC) at higher concentrations (1000ug for cell proliferation analysis and 2000ug for colony formation assay). This suggests that the extract contains bioactive compounds like flavonoids, lignins, phenolics, coumarins, etc., which are more abundant in higher concentrations. These compounds are believed to be responsible for inducing cell death in cancer cells. However, lower concentrations of the extract do not have enough of these compounds to effectively kill cancer cells. Similarly, the anticancer activity of horse tails ferns against liver cancer cell lines at higher concentrations showed remarkable anticancer activity [52]. On the same side, other plants also show potential activity against different cancer cell lines Km12, colo205, U031, A498, SKHEP, HEP3B, MG63.3 and MG63 [53]. Low concentration of *S. officinale* significantly inhibits the growth of the breast cancer cell line (MCF7) [54], while our plant has high activity at higher concentration.

5. Conclusion

In conclusion, the data analysis of *P. vittata*, revealed the presence of a wide range of phytochemicals, including alkaloids, terpenoids, flavonoids, tannins, carotenoids, carbohydrates, saponins, quinines, coumarins, phlobatanins, and phenols. The quantitative analysis showed higher levels of phenolics, reducing sugar, ascorbic acid, and flavonoids. The organic solvent extracts of *P. vittata* demonstrated notable activity against specific bacterial strains, with the chloroform extract showing the largest zone of inhibition against S. aureus. Additionally, higher doses of the plant's ethanolic extract showed significant inhibition of cancer cell growth in both AGS and SGC cell lines. These findings suggest that *P. vittata* has a potential source of bioactive compounds with antimicrobial and anticancer properties. Further research is needed to identify and isolate the specific compounds responsible for these activities and to explore their potential applications in medicine and pharmaceuticals.

6. Data availability,

The data produced in this study, including raw data and figures, can be obtained from the corresponding author without any hesitation.

7. Conflict of interest

All authors declare that they have no conflict of interest.

8. Funding statement

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10. Supplementary data

Not available

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