Research Article **Phytopharmacology Research Journal (PRJ) Open Access**

Received: 17 Dec. 2025 | Revised: 22 January 2026 | Accepted: 25 January 2026 | Published online : 27 January 2026 | ISSN : 2959-9687

Anti-cancer Evaluations of solvent extracts and saponins of *Polygonatum verticillatum (L.)*

Fida Hussain[*1], Kainat Noor[1], Hina Gul[1], Zarafshan[1], Muska mahabat Khan[1], Muhammad Saeed Jan[*2]

[1]Department[t] of Pharmacy, University of Swabi, KP, Pakistan.

[2]Department of Pharmacy, Bacha Khan University Charsadda.

Correspondence:
Dr. Muhammad Saeed Jan &
Dr. Fida Hussain,
saeedjanpharmacist@gmail.com
E-mail:fida2k16@gmail.com

Funding Information
Not Applicable

Abstract

Cancer remains a leading cause of global mortality, and angiogenesis plays a pivotal role in tumor growth and progression. The present study aimed to evaluate the anti-cancer potential of solvent extracts and saponin fractions of *Polygonatum verticillatum* (L.) using phytochemical screening and an in vivo anti-angiogenic model. The whole plant was extracted with 80% methanol, followed by sequential fractionation using solvents of increasing polarity. Crude saponins were also isolated using a modified ethanol extraction method. Qualitative phytochemical analysis revealed the presence of alkaloids, glycosides, flavonoids, tannins, terpenoids, anthraquinones, and saponins in the crude extract.

The anti-angiogenic activity of the extracts and fractions was evaluated using the chorioallantoic membrane (CAM) assay. The chloroform fraction, saponin fraction, ethyl acetate fraction, and crude extract exhibited significant inhibition of angiogenesis in a concentration-dependent manner. Among all samples, the saponin fraction showed the highest potency with the lowest IC_{50} value, while dexamethasone was used as a positive control.

The findings of this study suggest that *Polygonatum verticillatum* possesses notable anti-angiogenic activity, which may be attributed to its rich phytochemical composition, particularly saponins. These results provide scientific evidence supporting the traditional use of the plant and highlight its potential as a promising source of natural anti-cancer agents. Further investigations are required to isolate active constituents and elucidate their underlying mechanisms of action.

KEYWORDS
Anti-angiogenic activity; Cancer; CAM assay; Saponins; *Polygonatum verticillatum* (L.)

1.0 INTRODUCTION

Cancer is one of the most complex and life-threatening diseases worldwide and remains a major cause of mortality and morbidity [1]. It is characterized by uncontrolled cell proliferation, resistance to programmed cell death, genomic instability, invasion, metastasis, and altered cellular metabolism. According to global health estimates, cancer ranks among the leading causes of death in both developed and developing countries, placing a significant burden on healthcare systems [2]. Despite substantial progress in cancer diagnosis and treatment, the overall success of current therapeutic strategies is limited due to drug resistance, systemic toxicity, high treatment costs, and undesirable side effects. These limitations highlight the urgent need for safer, more effective, and affordable anticancer agents [3].

Angiogenesis, the process of new blood vessel formation from pre-existing vasculature, plays a critical role in tumor initiation and progression. Rapidly growing tumors require a continuous supply of oxygen and nutrients, which is facilitated by angiogenic signaling pathways involving vascular endothelial growth factor

(VEGF), angiopoietins, fibroblast growth factors, and inflammatory cytokines. Excessive or abnormal angiogenesis contributes to tumor growth and metastasis; therefore, targeting angiogenesis has emerged as a promising therapeutic approach in cancer management. Inhibition of angiogenesis not only restricts tumor expansion but also enhances the effectiveness of cytotoxic and immunotherapeutic agents related to inflammation [4].

Polygonatum verticillatum (L.), a perennial medicinal plant belonging to the family Asparagaceae, is widely distributed in mountainous regions and has been traditionally used for the treatment of various ailments. However, systematic evaluation of its anticancer potential, particularly focusing on solvent extracts and saponin fractions, remains limited.Preliminary screening assays such as plant-based tumor inhibition models and cytotoxicity bioassays provide valuable insight into the anticancer potential of natural products before proceeding to advanced in vitro and in vivo studies [5-6]. Such assays are cost-effective, reliable, and useful for identifying promising candidates with antitumor activity. Evaluation of crude extracts alongside enriched fractions helps in correlating biological activity with specific classes of phytochemicals, thereby facilitating future isolation and mechanistic studies.

In this context, the present study was designed to investigate the anticancer potential of various solvent extracts and saponin fractions of *Polygonatum verticillatum* (L.). The study aims to provide scientific evidence supporting the traditional use of this plant and to explore its potential as a source of novel anticancer agents.

2.0 MATERIALS AND METHODS

2.1. Collection and Authentication of Plant Material

The whole plant of *Polygonatum verticillatum* (L.) was collected from the mountainous region of Lower Dir, Khyber Pakhtunkhwa (KPK), Pakistan, during the month of April, when the concentration of bioactive constituents is reported to be relatively high. The plant material was taxonomically authenticated by Prof. Muhammad Nisar, Chairman, Department of Botany, University of Malakand, Dir (L), KPK, Pakistan. A voucher specimen was deposited in the departmental herbarium for future reference.

2.2. Preparation and Extraction

The collected plant material was thoroughly washed with distilled water to remove extraneous matter and shade-dried at room temperature for approximately 21 days. The dried material was then chopped into small pieces and ground into coarse powder using a mechanical grinder. About 7.5 kg of the powdered material was soaked in 80% methanol (24 L) and kept at room temperature for 21 days with occasional shaking to ensure maximum extraction of phytochemicals.

After maceration, the mixture was filtered first through muslin cloth and subsequently through Whatman filter paper [7]. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40 °C to obtain a dark green crude methanolic extract. The dried crude extract was weighed and stored at 4 °C until further use.

2.3. Fractionation of Crude Extract

The crude methanolic extract was subjected to solvent–solvent partitioning to obtain fractions of varying polarity. The extract was suspended in distilled water and transferred to a separating funnel. Sequential fractionation was carried out using n-hexane, chloroform, ethyl acetate, and n-butanol. For each solvent, the mixture was vigorously shaken, allowed to stand for phase separation, and the organic layer was collected. Each extraction step was repeated three times to ensure complete separation.

The collected organic fractions were individually concentrated under reduced pressure using a rotary evaporator at 40 °C. The remaining aqueous layer was also concentrated to dryness. All solvent fractions were weighed, labeled, and stored in airtight containers for subsequent biological evaluation [8].

2.4. Extraction of Crude Saponins

Crude saponins were extracted using a modified ethanol extraction method. Briefly, 20 g of powdered plant material was extracted with 20% ethanol (100 mL) and heated in a water bath at 55 °C for 4 hours. The mixture was filtered, and the residue was re-extracted with additional 20% ethanol (200 mL). The combined filtrates were concentrated on a water bath to reduce the volume to approximately 40 mL.

2.5. Phytochemical Investigation

Qualitative phytochemical screening of the crude extract and solvent fractions of *Polygonatum verticillatum* (L.) was performed using standard procedures to detect major secondary metabolites, including glycosides, alkaloids, flavonoids, saponins, tannins, terpenoids, sterols, and anthraquinones.Glycosides were identified after acid hydrolysis followed by Fehling's test, indicated by a red precipitate. Alkaloids were detected using Dragendorff's reagent. Terpenoids and sterols were examined using the Liebermann–Burchard reaction, where color changes

confirmed their presence. Anthraquinones were detected by benzene extraction followed by ammonium hydroxide treatment, indicated by pink to violet coloration. Saponins were identified by the formation of persistent froth upon vigorous shaking [9].

2.6. Anti-angiogenic assay

The chorioallantoic membrane (CAM) experiment was used to determine the anti-angiogenic potential of plant extracts and saponins [1, 10]. The fertilized domestic chicken eggs were acquired from a hens trader in Chakdara, Pakistan, and incubated for 4-5 days at 37 °C in a humidified incubator (HYSC Korea (BI-81/150/250), touching them at least three times a day. The seven-day-old eggs were observed under a flash light after the incubation phase was completed in order to detect and encircle the embryo head. The yolk sacs were then detached from the shell membrane by puncturing a tiny hole at the narrow end of the eggs and aspirating 0.5-1 ml of albumin with an eighteen-gauge hypodermic needle. With forceps, the shell around the embryo air sac was separated, and the shell membrane around the air sac's base was peeled away. On the eighth day, a thermanox cover slip was carefully placed on the surface of CAM and incubated it with 10 different samples (31.25-1000 µg/ml). After that the 33 gauge needle was used to inject an optimum amount of methanol and acetone mixture (1:1) into the embryo chorioallantois after three days. The number of vessels in the CAM was counted after it was cut out of eggs. Under a microscope, vessels radially converging in the direction of the centre were counted. Each sample dose required at least twenty eggs.

3. RESULTS

3.1. Phytochemical investigation

Pv. Cr contains alkaloids, glycosides, tannins, terpenoids, anthraquinones, flavonoids, saponins, as well as oils but tested negative for sterols (Table 1).

Table 1: Phytochemical ingredients in crude fraction of *H. digitata*

S. No	Phytochemicals	Observations	Results
1.	alkaloids	Turbidity	+
2.	glycosides	Red color precipitate formation	+
3.	tannins	Bluish black color formation	+
4.	terpenoids	Reddish brown color appearance	+
5.	Anthraquinones	Red violet or pink color in aqueous layer	+
6.	Flavonoids	Yellow color formation and changed colorless when acid is added	+
7.	Saponins	Frothing bubbles formation	+

3.2. Anti-angiogenic assay

Angiogenesis is a process which is regulated by series of endogenous angiogenic and angiostatic factors under normal conditions [11].

In abnormal angiogenesis like atherosclerosis, malignancy and chronic inflammations [12], angiogenesis inhibitors are dominated by angiogenesis promoters leading to the abnormal proliferation and the migration of cells [13]. Researchers are looking to isolate and describe new anti-angiogenic drugs from the natural sources like plants since from last 15 years. In our current study, Pv.Chf, Pv.Sp, Pv.EA, and Pv.Cr shown maximum anti-angiogenic activity having 78.65 ± 1.66, 76.98 ± 1.03, 69.41 ± 1.13, and 65.35 ± 0.86% inhibitions at 1000 µg/ml with IC50of 28.63, 16.20, 86.73, and 460.51 µg/ml, correspondingly **(Table 2).** Dexamethasone was used as a standard having its IC50 value of 11.66 µg/ml. All other fractions showed concentration dependent but have less significant activity. In our observations, Ph.Sp showed highest anti-angiogenic activity with IC_{50} of 16.20 µg/ml. In all phytochemicals saponins have been shown anti-angiogenic potentials isolated previously from plants, like convallamaroside from *Convallaria majalis*, and Polyphyllin D from *Paris polyphylla*. Similarly, a large number of plants including crude extracts from *Viscumalbum, Populus nigra, Chrysobalanus icaco, Cassia garrettiana, Agaricus blazei* were defined for anti-angiogenic potentials. The isolated compounds including, shikonin from *Lithospermum erythrorhizon,*torilin from *Torilis japonica,* Deoxypodophyllo toxin from *Pulsatilla koreana,* resveratrol from grapes, epigallocatechin gallate from the green tea, genistein from ginseng and isoliquiritin from licorice have been described for anti-angiogenic activities both *in*-vitro and *in*-vivo studies.

Table 2: Anti-angiogenic activity of various samples of *H.digitata*

Sampl	**Percentanti-angiogenicactivityMean± SEM(*n* = 5)**	**IC50**

e	31.25 µg/ml	62.5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000µg/ml	**µg/ml**
Pv.Cr	29.24±0.22***	36.30±1.50***	40.52±0.60***	45.98±1.03***	52.37±0.35***	65.35±0.86***	460.51
Pv.Hx	21.92±0.51***	25.34±1.32***	26.68±0.91***	32.52±0.88***	39.85±1.38***	43.55±0.44***	1530.44
Pv.Cf	51.01±1.52***	55.10±1.80***	61.35±0.66***	60.95±1.23***	69.98±1.66***	78.65±1.66***	28.63
Pv.EA	44.50±0.56***	48.52±.0.88***	52.68±1.62***	56.05±0.84***	61.02±1.13***	69.41±1.13***	86.73
Pv.Aq	24.02±0.25***	27.10±1.17***	28.35±0.33***	39.35±0.90***	52.68±1.47***	61.44±1.43***	430.80
Pv.Sp	54.64±0.70***	58.22±0.72***	59.89±0.28***	64.20±1.17**	68.45±0.99*	76.98±1.03ns	16.20

Dexamethasone was employed as positive control, with IC50 value of 11.66 µg/ml. Values significantly various as compare to the standard drug *p <0.05, **p <0.01, and ***p <0.001 at 90% confidence interval. ns, Values not significantly different in contrast to standard drug.

4. DISCUSSION

The present study was conducted to evaluate the anticancer potential of solvent extracts and saponin fractions of *Polygonatum verticillatum* (L.) through phytochemical screening and an in vivo anti-angiogenic assay using the chorioallantoic membrane (CAM) model. Angiogenesis is a fundamental process involved in tumor growth and metastasis, and its inhibition is considered a key therapeutic strategy in cancer management. Natural products, particularly medicinal plants, remain an important source of bioactive compounds capable of modulating angiogenic pathways [14].

Preliminary phytochemical analysis revealed that the crude extract of *P. verticillatum* contains a wide range of secondary metabolites, including alkaloids, glycosides, tannins, terpenoids, anthraquinones, flavonoids, saponins, and oils. The presence of these phytoconstituents supports the traditional medicinal use of this plant and suggests its potential for biological activity. Many of these classes of compounds have been previously reported to exhibit anticancer, cytotoxic, antioxidant, and anti-angiogenic properties. In particular, saponins and flavonoids are known to interfere with tumor-associated angiogenesis by modulating growth factor signaling and endothelial cell proliferation [15-16].

The anti-angiogenic activity observed in the CAM assay further substantiates the therapeutic potential of *P. verticillatum*. Among the tested samples, the chloroform fraction, saponin fraction, ethyl acetate fraction, and crude extract exhibited significant inhibition of blood vessel formation in a concentration-dependent manner. The chloroform fraction demonstrated the highest percentage inhibition at the maximum tested concentration, while the saponin fraction showed the lowest IC_{50} value, indicating strong potency. This suggests that moderately polar constituents and saponin-rich fractions are largely responsible for the observed activity.

The superior activity of the saponin fraction may be attributed to the known anti-angiogenic mechanisms of plant-derived saponins, which include suppression of VEGF expression, inhibition of endothelial cell migration, and induction of apoptosis in proliferating vascular cells. Several steroidal and triterpenoid saponins isolated from medicinal plants have previously been reported to inhibit angiogenesis both in vitro and in vivo, supporting the findings of the present study. Similarly, the notable activity of the chloroform and ethyl acetate fractions suggests the presence of bioactive terpenoids, flavonoids, and phenolic compounds that may act synergistically to inhibit neovascularization [17].

The CAM assay is a well-established, cost-effective, and reliable in vivo model for evaluating angiogenesis and anti-angiogenic agents. The significant reduction in blood vessel density observed in treated embryos indicates that the tested extracts can effectively interfere with angiogenic processes without causing overt toxicity at the tested concentrations. The comparison with dexamethasone, used as a standard anti-angiogenic agent, further validates the biological relevance of the observed effects [18-19].

Overall, the findings of this study demonstrate that *Polygonatum verticillatum* possesses promising anti-angiogenic activity, particularly in its saponin and chloroform fractions. These results provide scientific support for the traditional use of the plant and highlight its potential as a source of natural anti-cancer agents. Further

studies focusing on isolation, structural characterization, and mechanistic evaluation of the active constituents are warranted to explore their potential for anticancer drug development.

ACKNOWLEDGMENTS

The authors are thankful to the Department of Pharmacy University of Swabi, KP, Pakistan

CONFLICT OF INTEREST

The authors declare no conflict of interest.

5.0 REFERENCES

1. Ejaz, I., et al., *Rational design, synthesis, antiproliferative activity against MCF-7, MDA-MB-231 cells, estrogen receptors binding affinity, and computational study of indenopyrimidine-2, 5-dione analogs for the treatment of breast cancer*. Bioorganic & Medicinal Chemistry Letters, 2022. **64**: p. 128668.
2. Khan, A., et al., *Phytochemical profiling, anti-inflammatory, anti-oxidant and in-silico approach of cornus macrophylla bioss (Bark).* Molecules, 2022. **27**(13): p. 4081.
3. Javed, M.A., et al., *Evaluation of pyrimidine/pyrrolidine-sertraline based hybrids as multitarget anti-Alzheimer agents: In-vitro, in-vivo, and computational studies.* Biomedicine & Pharmacotherapy, 2023. **159**: p. 114239.
4. Jan, M.S., et al., *Synthesis of pyrrolidine-2, 5-dione based anti-inflammatory drug: in vitro COX-2, 5-LOX inhibition and in vivo anti-inflammatory studies.* Latin Am J Pharm, 2019. **38**(11): p. 2287-2294.
5. Hussain, F., et al., *Exploitation of the multitarget role of new ferulic and gallic acid derivatives in oxidative stress-related Alzheimer's disease therapies: design, synthesis and bioevaluation.* RSC advances, 2024. **14**(15): p. 10304-10321.
6. Shi, N., et al., *Comparative analysis of the medicinal plant Polygonatum kingianum (Asparagaceae) with related verticillate leaf types of the Polygonatum species based on chloroplast genomes.* Frontiers in Plant Science, 2023. **14**: p. 1202634.
7. Mahmood, F., et al., *Ethyl 3-oxo-2-(2, 5-dioxopyrrolidin-3-yl) butanoate derivatives: anthelmintic and cytotoxic potentials, antimicrobial, and docking studies.* Frontiers in chemistry, 2017. **5**: p. 119.
8. Mahnashi, M.H., et al., *Neuroprotective potentials of selected natural edible oils using enzyme inhibitory, kinetic and simulation approaches.* BMC complementary medicine and therapies, 2021. **21**(1): p. 248.
9. Mahnashi, M.H., et al., *GC-MS Analysis and Various In Vitro and In Vivo Pharmacological Potential of Habenaria plantaginea Lindl.* Evidence-Based Complementary and Alternative Medicine, 2022. **2022**(1): p. 7921408.
10. Sadiq, A., et al., *Evaluation of crude saponins, methanolic extract and subsequent fractions from Isodon rugosus Wall. ex Benth: Potentials of anti-angiogenesis in egg and anti-tumorigenesis in potato.* Pakistan Journal of Pharmaceutical Sciences, 2019. **32**(5).
11. Mahnashi, M.H., et al., *Cytotoxicity, anti-angiogenic, anti-tumor and molecular docking studies on phytochemicals isolated from Polygonum hydropiper L.* BMC complementary medicine and therapies, 2021. **21**(1): p. 1-14.
12. Jan, M.S., et al., *Design, synthesis, in-vitro, in-vivo and in-silico studies of pyrrolidine-2, 5-dione derivatives as multitarget anti-inflammatory agents.* European journal of medicinal chemistry, 2020. **186**: p. 111863.
13. Iftikhar, F., et al., *Design, synthesis, in-vitro thymidine phosphorylase inhibition, in-vivo antiangiogenic and in-silico studies of C-6 substituted dihydropyrimidines.* Bioorganic Chemistry, 2018. **80**: p. 99-111.
14. Pervaiz, A., et al., *Comparative in-vitro anti-inflammatory, anticholinesterase and antidiabetic evaluation: Computational and kinetic assessment of succinimides cyano-acetate derivatives.* Journal of Biomolecular Structure and Dynamics, 2022: p. 1-14.
15. Ullah, I., et al., *Design, Synthesis, SwissADME Profile DFT Studies, in vitro and in silico Anti-diabetic Potential of Novel Amide Derivatives Based on Bis ((4-amino-4-oxobutanoyl) oxy) Zinc Scaffold.* Current medicinal chemistry, 2025.
16. Khan, A., et al., *Assessment of Preclinical Antioxidative and Anti-Inflammatory Activities of Cornus macrophylla Wall. Bark.* Food Science & Nutrition, 2025. **13**(7): p. e70620.
17. Alyami, B.A., et al., *Design, synthesis, antiproliferative activity, estrogen receptors binding affinity of C-3 pregnenolone-dihydropyrimidine derivatives for the treatment of breast cancer.* Steroids, 2022. **185**: p. 109059.
18. Zaman, M., et al., *Preclinical Evaluation of a Novel Tris Amine Derivative in Animal Models of Anxiety and Depression.* Phytopharmacology Research Journal, 2025. **4**(2): p. 142-146.
19. Gul, S., et al., *Phytochemical Profiling and Bioactivity of Celtis caucasica: Antioxidant, Antidiabetic, Anticholinesterase, and Anti-inflammatory Potential.* 2025.