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Anticancer and Antimicrobial Properties of *Passiflora incarnata*

Passiflora incarnata'nın Antikanser ve Antimikrobiyal Özellikleri

***Ayşegül ÖZTÜRK*[1], *Rukiye ASLAN*[2]**

ÖZ

Amaç: *Passiflora incarnata*, flavonoid ve fenolik içeriğine bağlı olarak terapötik özellikleriyle bilinen önemli bir tıbbi bitkidir. Bu çalışmanın amacı, *P. incarnata*'nın sitotoksik, oksidatif stres indükleyici, apoptotik ve antimikrobiyal etkilerini araştırmaktır.
Araçlar ve Yöntem: Sitotoksisite, SNU-1 mide kanseri hücrelerinde XTT testi ile değerlendirilmiş ve IC_{50} değeri 88.95 µg/mL olarak belirlenmiştir. Oksidatif stres belirteçleri, Total Antioksidan Kapasitesi (TAC) ve Total Oksidan Seviyesi (TOS) kitleri ile ölçülmüştür. Apoptotik aktivite, BAD ve Kaspaz-3 ELISA kitleri kullanılarak analiz edilmiştir. Antimikrobiyal etkiler, üç Gram-negatif bakteri (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 ve *Pseudomonas aeruginosa* ATCC 27853), iki Gram-pozitif bakteri (*Staphylococcus aureus* ATCC 29213 ve *Enterococcus faecalis* ATCC 29212) ve bir maya (*Candida albicans* ATCC 10231) olmak üzere altı mikroorganizma üzerinde agar disk difüzyon yöntemi ile test edilmiştir. İstatistiksel anlamlılık p<0.05 olarak kabul edilmiştir.
Bulgular: *P. incarnata*, TAC seviyelerini anlamlı şekilde düşürmüş ($p < 0.01$) ve TOS seviyelerini artırmıştır ($p < 0.01$), bu da oksidatif stres geliştiğini göstermektedir. Ayrıca BAD ve Kaspaz-3 düzeylerini artırarak ($p < 0.01$) apoptozun uyarıldığını göstermiştir. İnhibisyon zonları şu şekilde ölçülmüştür: *E. coli* 12.27 mm, *S. aureus* 14.37 mm, *K. pneumoniae* 11.96 mm, *P. aeruginosa* 9.9 mm, *E. faecalis* 12.5 mm ve *C. albicans* 15.6 mm.
Sonuç: *P. incarnata*, mide kanseri hücrelerinde oksidatif stres yoluyla apoptozu teşvik etmekte ve özellikle antifungal olmak üzere belirgin bir antimikrobiyal aktivite sergilemektedir. Bu özellikleri, antimikrobiyal dirence karşı daha fazla araştırmayı gerekli kılmaktadır.

Anahtar Kelimeler: antimikrobiyal aktivite; apoptoz; oksidatif hasar; passiflora incarnate; sitotoksisite

ABSTRACT

Purpose: *Passiflora incarnata* is a notable medicinal plant recognized for its therapeutic properties, largely attributed to its flavonoid and phenolic content. This study aimed to investigate the cytotoxic, oxidative stress-inducing, apoptotic, and antimicrobial effects of *P. incarnata*.
Materials and Methods: Cytotoxicity was assessed using the XTT assay in SNU-1 gastric cancer cells, determining the IC_{50} value as 88.95 µg/mL. Oxidative stress markers were evaluated via Total Antioxidant Capacity (TAC) and Total Oxidative Status (TOS) kits. Apoptotic activity was analyzed using BAD and Caspase-3 ELISA kits. Antimicrobial effects were tested against six microorganisms—three Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 278538539), two Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212), and one yeast (*Candida albicans* ATCC 10231)—via the agar disk diffusion method. Statistical significance was defined as p<0.05.
Results: *P. incarnata* significantly decreased TAS TAC levels ($p < 0.01$) and increased TOS levels ($p < 0.01$), indicating oxidative stress. It also elevated BAD and Caspase-3 levels ($p < 0.01$), suggesting apoptosis induction. Inhibition zones were *E. coli* 12.27 mm, *S. aureus* 14.37 mm, *K. pneumoniae* 11.96 mm, *P. aeruginosa* 9.9 mm, *E. faecalis* 12.5 mm, and *C. albicans* 15.6 mm
Conclusion: *P. incarnata* promotes apoptosis through oxidative stress in gastric cancer cells and exhibits notable antimicrobial activity, particularly antifungal, warranting further investigation in combating antimicrobial resistance.

Keywords: antimicrobial activity; apoptosis; cytotoxicity; oxidative damage; passiflora incarnate

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[1] Departments of Therapy and Rehabilitation, Vocational School of Health Services, Sivas Cumhuriyet University, Sivas, Türkiye.
[2] Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Sivas Cumhuriyet University, Sivas, Türkiye.

Corresponding Author: Ayşegül Öztürk, Departments of Therapy and Rehabilitation, Vocational School of Health Services, Sivas Cumhuriyet University, Sivas, Türkiye. e-mail: aysegulozturk@cumhuriyet.edu.tr

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INTRODUCTION

Medicinal plants have been utilized in traditional medicine for thousands of years to promote human health. Medicinal plants exhibit a diverse array of biological activities due to the bioactive compounds present in their biochemical structure.[1,2] Today, plants are regarded as the primary source for the discovery of new drugs and antimicrobial agents.[3]

Passiflora incarnata L. is a plant belonging to the Passifloraceae family, commonly known as passionflower. Species within the *Passiflora* genus are well-regarded for their sedative and analgesic properties. This plant exhibits a wide range of biological activities, attributed to its rich content of phenolic compounds and flavonoids.[4] *P. incarnata* is commonly used to treat insomnia, stress, gastrointestinal disorders, antioxidant, and anti-proliferative. In addition, *P. incarnata* has been shown to be effective against both Gram-positive and Gram-negative bacteria.[5–9] Gastric cancer (GC) is the fourth leading cause of cancer-related mortality globally and the fifth most diagnosed cancer worldwide.[10] This disease is frequently diagnosed at advanced stages with metastasis, primarily due to the absence of early and accurate diagnostic tools or specific clinical symptoms. Consequently, the five-year survival rate for GC remains approximately 32%.[11] Chemotherapy is a widely utilized cancer treatment approach that employs pharmaceutical agents to eliminate cancer cells. However, the efficacy of chemotherapy is often compromised due to the development of resistance by cancer cells over time and the significant side effects associated with these drugs. In this context, herbal compounds derived from natural sources have been recognized for their diverse therapeutic properties and hold considerable promise in cancer treatment, particularly due to their antiproliferative, oxidative damage and apoptotic potential.[12]

In recent years, the issue of antimicrobial resistance, stemming from the improper use of conventional antimicrobial drugs, has escalated into a global health crisis. The rise of multi-drug-resistant (MDR) bacteria complicates the treatment of infectious diseases and contributes to higher morbidity and mortality rates among patients.[13] Therefore, scientists have accelerated their search for alternative antimicrobials. The discovery of antimicrobial activity in plant-derived antimicrobials and their biochemical components is emerging as a promising strategy to combat antimicrobial resistance (AMR).[14] Specifically, the study sought to assess the effectiveness of *P. incarnata* against representative microorganisms. The aim of this study is to investigate the cytotoxic, apoptotic, oxidative damage-inducing, and antimicrobial properties of *P. incarnata*, exploring its potential as a therapeutic agent for gastric cancer and its efficacy in addressing antimicrobial resistance.

MATERIALS and METHODS

Experimental design

In this study, we investigated the antimicrobial effects of *P. incarnata* on standard bacteria, its antiproliferative effects on gastric cancer cells, as well as its impact on oxidative stress and apoptosis (Figure 1).

Chemical materials

The dimethyl sulfoxide (DMSO) used in this study was purchased from Merck (Germany). The *Passiflora incarnata* L. extract was obtained from a commercial capsule formulation (300 mg/mL; Orzax İlaç, Türkiye). According to the manufacturer's certificate of analysis, the extract is standardized to contain defined amounts of total flavonoids, expressed as vitexin equivalents. As a commercially available product was used, no separate herbarium specimen was prepared. Distilled water was produced using the NS 104 distillation apparatus (Nuve, Türkiye).

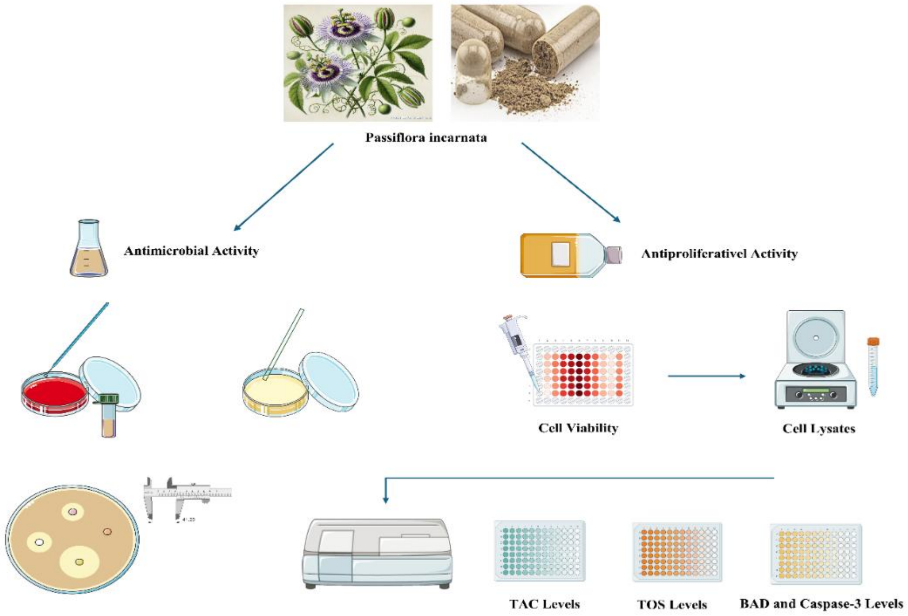

Figure 1. Cytotoxic, oxidative stress, apoptotic, and antimicrobial properties' experimental protocol of *P. incarnata* (The figure was created by Servier and is licensed under a Creative Commons Attribution 4.0 Unported License. *This figure was created by ChatGPT).

Cell culture

The SNU-1 cell line, a type of gastric signet ring cell carcinoma (CRL-5971), was obtained from the American Type Culture Collection (ATCC). The cells were grown in RPMI-1640 medium (Capricorn Scientific) with 10% fetal bovine serum (Sigma-Aldrich) and 1% penicillin-streptomycin antibiotic mix (Sigma-Aldrich). The cells were kept at 37°C in a humidified incubator with 5% CO2.12

Cell viability assessment

Cell viability was assessed using the XTT assay (Sartorius, Göttingen, Germany). SNU-1 cells were plated in 96-well plates at a density of 1×10^4 cells per well in 100 µl of DMEM and allowed to incubate overnight before the administration of *P. incarnata*. Concentrations were determined regarding prior study.[15] The next day, two cell groups were established to assess the antiproliferative effects. The control group received no treatment, while the *P. incarnata* group was exposed to different concentrations (400, 200, 100, 50, and 25 µg/mL) for 24 hours 16. Following the incubation period, the culture media were removed, and the wells were rinsed twice with phosphate-buffered saline (PBS). Subsequently, 100 µL of DMEM without phenol red and 50 µL of the XTT reagent mixture were added to each well. The plates were then incubated at 37°C for four hours. The plates were shaken, and absorbance was measured at 450 nm using an ELISA microplate reader (Thermo Fisher Scientific, Altrincham, UK). All experiments were conducted in triplicate, and cell viability was calculated as a percentage of viable cells relative to the untreated control group.[17,18]

Formation of cell lysates

Cells from each group were collected into sterile tubes and centrifuged at 2000 rpm for 10 minutes. After removing the supernatants, the pellets were resuspended in PBS (pH 7.4) at a density of ~1 million cells/mL. Intracellular components were extracted through freeze-thaw cycles, followed by centrifugation at 4000 rpm for 10 minutes at 4°C.19,20 The supernatants were collected for biochemical analysis, and total protein concentrations were determined using the Bradford assay kit (Merck Millipore, Darmstadt, Germany).

Evaluation of TAC and TOS levels

The total antioxidant capacity (TAS) (TAC) and total oxidative stress (TOS) (Rel Assay, Gaziantep, Türkiye) levels in cell supernatants were determined using an automated test method developed by Erel.21 TAS TAC was measured by the color change of the ABTS molecule resulting from its oxidation in the presence of hydrogen peroxide, where the color change accelerated in proportion to the antioxidant concentration and was measured at 660 nm. The results were expressed as µmoL Trolox Equivalent/mg protein. TOS was assessed based on the oxidation of the Fe^{2+}-o-dianisidine complex to Fe^{3+} ions by oxidizing agents in the samples. In an acidic environment, Fe^{3+} ions form a colored complex with xylanol, and this color change is proportional to the concentration of oxidizing molecules. The color change was measured at 530 nm, and the results were reported as µmoL H_2O_2 Equivalent/mg protein. The total protein levels were determined using the Bradford protein assay kit.[22]

Biochemical analysis

Human ELISA kits were utilized to evaluate the levels of BAD (cat. no: #E4102Hu; BT-Lab; China) and Caspase-3 (cat. no: #E4804Hu; BT Lab; China) in both *P. incarnata*-treated and control cell populations. The manufacturer's instructions performed the ELISA. Absorbance values, which indicate colorimetric changes in both control and treated samples, were measured at 450 nm using an ELISA reader (Thermo Fisher Scientific, Altrincham, UK). Following the manufacturer's guidelines, the concentrations of BAD and Caspase-3 in the cell lysates were determined.[23]

Antimicrobial screening

Test microorganisms

Gram-positive bacteria, Gram-negative bacteria, and a yeast fungus were examined in this study. Standard bacterial strains obtained from the ATCC were used in this study: *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 10231). Test bacteria were cultured overnight on 5% sheep blood agar in an incubator at 37°C. Yeast fungi were cultured on Sabouraud Dextrose Agar (SDA) (Aklab, Türkiye) at 30°C for 48 hours. The inoculum of both bacteria and yeast fungi was adjusted to the McFarland No. 0.5 standard (1 × 108 CFU/mL) using a sterile saline solution (0.9% NaCl).

Disk diffusion test

The *in vitro* antimicrobial activity of *P. incarnata* was assessed using the agar disk diffusion method for bacteria and yeast fungi, as recommended by the Clinical Laboratory Standards Institute (CLSI), with minor modifications to the procedure.[24–27] For this purpose, a sterile suspension of 200 µL of each prepared inoculum of bacteria and yeast fungi was evenly spread over the entire surface of Mueller Hinton Agar (MHA) (Aklab, Türkiye) using a cotton swab. The sample solution of the *P. incarnata* material to be tested was dissolved in DMSO, which exhibits no inhibitory activity. *P. incarnata* solution samples were then filtered through sterile cellulose acetate filters with a pore size of 0.45 µm (IsoLab, Germany). Blank white paper disks with a diameter of 6 mm (Bioanalyse, Türkiye), placed in a sterile petri dish, were impregnated with 10 µL of the prepared sample solutions (500 µg/mL per disk). The disks were kept at room temperature for 20 minutes and then in the refrigerator at +4°C for an additional 20 minutes to ensure saturation with the solution. Then, *P. incarnata* sample solutions impregnated disks were uniformly placed on MHA plates. The prepared plates were incubated overnight in an incubator at 37°C. Following incubation, the diameters of the inhibition zones around the disks were measured using a scale (mm).[28] The primary solvent (DMSO) was used as a negative control. All assays were repeated in three replicates, and the mean values were calculated. Antimicrobial activity was expressed as the mean zone of inhibition (mm) ± SEM.

Statistical Analysis

The results are presented as the mean ± standard error of the mean (SEM). Data analysis was performed using GraphPad Prism software (version 10.0; Boston, USA). XTT assay data were assessed with a one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. TAC-TOS and ELISA results were analyzed using the student's t-test.

No ethical approval was required for this study, as it does not involve any human participants, animal subjects, or experimental procedures performed by the authors.

RESULTS

Effect of *P. incarnata* on cell viability in SNU-1 Cell Line

The effect of *P. incarnata* on cell viability was evaluated in SNU-1 cells. The treatment of SNU-1 cells with *P. incarnata* concentrations ranging from 50 to 400 µg/mL resulted in a significant decrease in cell viability compared to the control group ($p < 0.01$) (Figure 2). Furthermore, the IC50 value for *P. incarnata* in SNU-1 cells was determined to be 88.95 µg/mL after a 24-hour exposure.

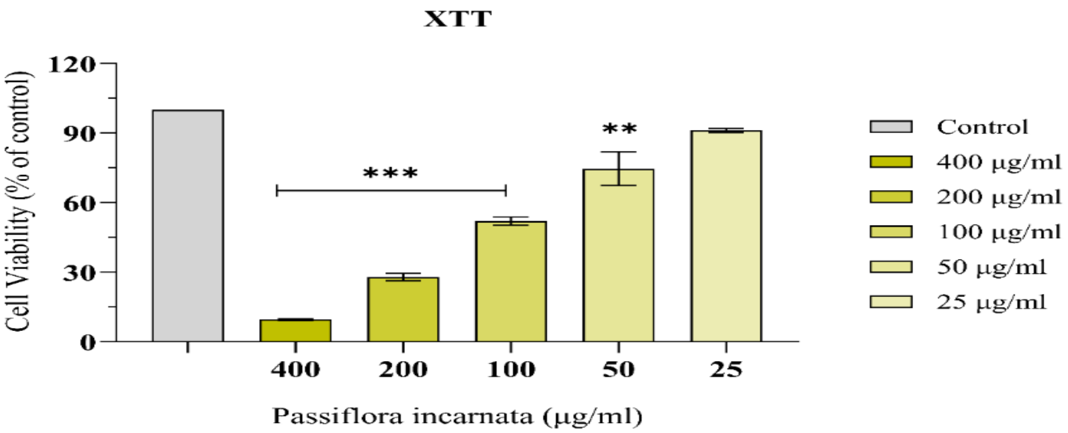

Figure 2. Effect of *P. incarnata* (24 h) on the viability of SNU-1 cells. Treatment with *P. incarnata* significantly reduced cell viability in a concentration-dependent manner compared with the control group. Values represent mean ± SEM. (*p < 0.05, **p < 0.01, ***p < 0.001 vs. control).

Effect of *P. incarnata* on TAC and TOS levels in SNU-1 Cell Line

SNU-1 cells were treated with a single concentration of *P. incarnata* (88.95 µg/mL) for 24 hours. As illustrated in Figure 3, *P. incarnata* significantly elevated decreased TAS TAC levels in SNU-1 cells compared to the control group ($p < 0.01$; Figure 3A). Conversely, *P. incarnata* also significantly increased TOS levels compared to the control group ($p < 0.01$; Figure 3B).

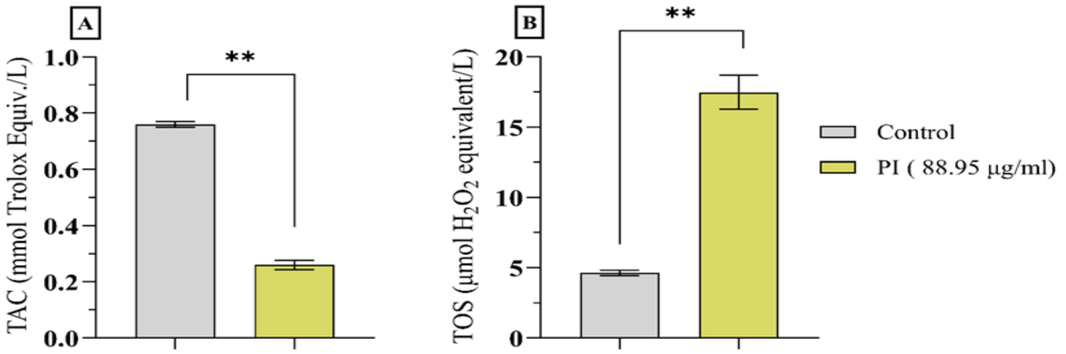

Figure 3. Effects of *P. incarnata* (88.95 µg/mL, 24 h) on (A) TAC and (B) TOS levels in SNU-1 cells. Treatment with *P. incarnata* significantly reduced TAC while increasing TOS levels compared with the control group. Values represent mean ± SEM. (**p < 0.01 vs. control).

Effect of *P. incarnata* on BAD and Caspase-3 Levels in SNU-1 Cell Line

To evaluate the effects of *P. incarnata* on apoptosis, levels of BAD and Caspase-3 were analyzed using ELISA. Exposure of cells to *P. incarnata* at a concentration of 88.95 mg/mL µg/mL for 24 hours resulted in a statistically significant increase in BAD and Caspase-3 levels compared to the control group (p < 0.01, Figure 4A, and 4B).

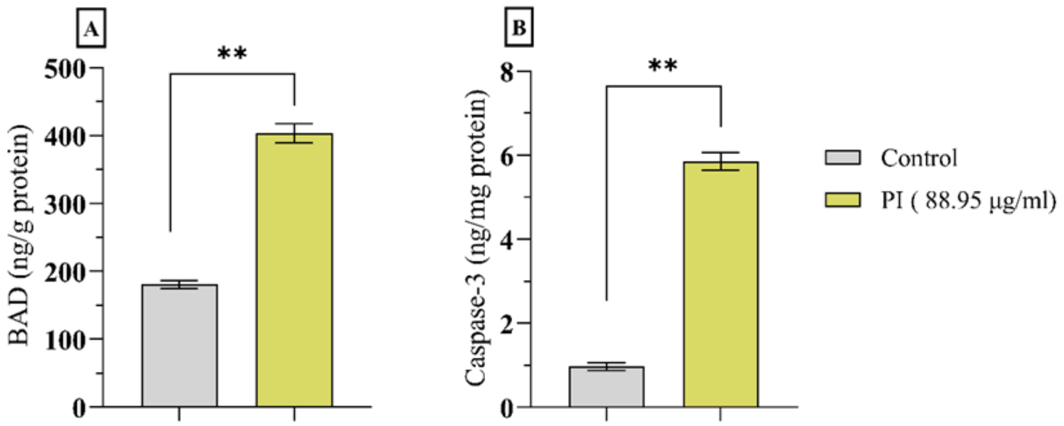

Figure 4. Effects of *P. incarnata* (88.95 µg/mL, 24 h) on **(A)** BAD and **(B)** Caspase-3 levels in SNU-1 cells. Treatment with *P. incarnata* significantly increased both BAD and Caspase-3 levels compared with the control group. Values represent mean ± SEM (**p < 0.01 vs. control).

Results of antimicrobial screening

In this study, we evaluated the antimicrobial activity of *P. incarnata* against three Gram-negative bacteria (*E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853), two Gram-positive bacteria (*S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212), and one yeast fungus (*C. albicans* ATCC 10231) using the agar disk diffusion method. The measured zones of inhibition against microorganisms were as follows: *E. coli* 12.27 mm, *S. aureus* 14.37 mm, *K. pneumoniae* 11.96 mm, *P. aeruginosa* 9.9 mm, *E. faecalis* 12.5 mm, and *C. albicans* 15.6 mm. Among the Gram-positive bacteria, *S. aureus* exhibited the most potent antibacterial activity (14.37 mm), whereas among the Gram-negative bacteria, *E. coli* demonstrated the strongest antibacterial activity (12.27 mm). . The results of the antimicrobial activity are presented in Table 1 and Figure 5.

Table 1. The diameter of the inhibition zone of *P. incarnata* against microorganisms.

Test Microorganisms	Strains	Diameter of Inhibition Zone (Mean±SEM, mm)
Escherichia coli	ATCC 25922	12.27±0.5
Staphylococcus aureus	ATCC 29213	14.37±0.2
Klebsiella pneumoniae	ATCC 700603	11.96±0.3
Pseudomonas aeruginosa	ATCC 27853	9.9±0.3
Enterococcus faecalis	ATCC 29212	12.5±0.8
Candida albicans	ATCC 10231	15.6±0.6

DISCUSSION

In recent years, *P. incarnata* has received increasing attention as a potential candidate for cancer therapy. Although its anti-cancer properties are increasingly demonstrated, the mechanisms underlying these effects are still under detailed investigation. This study is the first to demonstrate the antiproliferative, pro-oxidative, and pro-apoptotic activities of *P. incarnata* in SNU-1 gastric cancer cells and its antimicrobial effects against various pathogenic microorganisms. These findings highlight the diverse biological effects of *P. incarnata* as a natural therapeutic agent. Firstly, the XTT assay was employed to evaluate the dose-dependent cytotoxic effects of *P. incarnata* on the SNU-16 cell line. The experimental results revealed that *P. incarnata* significantly inhibited SNU-16 cell proliferation in a concentration-dependent manner. In addition, after 24 hours of treatment, the IC50 value was calculated to be 88.95 µg/mL. Deepika et al. nanoformulated the ethanolic extract derived from the leaves of *P. incarnata* using liposomes. They characterized this nanoformulation and investigated its anticancer effects across various cancer cell lines. The study determined that the nanoformulated ethanolic extract induced necrosis in cancer cells and inhibited cell growth in a dose-dependent manner. Additionally, it was found that the nanoformulation reduced toxicity to normal cells compared to the pure ethanolic extract.[5] In a recent study, the effects of extracts from the leaves of three *Passiflora* species on two acute lymphoblastic leukemia cell lines were investigated. Extracts from *Passiflora alata* and *P. incarnata* demonstrated potent activity against CCRF-CEM cells, whereas *Passiflora caerulea* exhibited weak activity. The pronounced effect of the *P. alata* extract was attributed to its high terpenoid content and the absence of flavone C-glycosides.[29] Amaral et al. investigated the antitumor activity of *P. alata* leaf extract (PaLE) in mice bearing S180 tumors. They found that PaLE inhibited cell proliferation by 75%, with an IC50 value of less than 30 µg/mL. Additionally, antitumor activities of 36.75% and 44.99% were observed following intraperitoneal administration. Toxicological assessments indicated minimal toxicity, with only minor changes recorded.[30] In our study, we found that *P. incarnata* induced oxidative damage in cancer cells. Additionally, consistent with previous research, *P. incarnata* induced apoptosis by increasing the levels of BAD and Caspase-3 in gastric cancer cells, thereby inhibiting their growth.

Inappropriate use of antibiotics contributes to the unchecked spread of antimicrobial resistance worldwide. The emergence of resistance among microorganisms to conventional antibiotics highlights the urgent need to discover alternative antimicrobial agents. The bioactive components present in the structure of *P. incarnata* indicate that it is a significant candidate for antimicrobial testing. Furthermore, the plant known as *Passiflora edulis* is regarded as an identical synonymous species of *P. incarnata*, as they exhibit similar morphological and microscopic characteristics.[31,32] The literature includes numerous studies investigating the antimicrobial activity of both *P. incarnata* and *P. edulis*.[8,33,34] This research has the potential to be one of the pioneering studies examining the antimicrobial activity of *P. incarnata*, particularly through the disk diffusion method.

In our study, we found that *P. incarnata* exhibited antimicrobial activity. Our results indicate that the antifungal activity of *P. incarnata*, which demonstrates moderate antimicrobial properties, is greater than its antibacterial activity. Furthermore, we established that it is more effective against Gram-positive bacteria than against Gram-negative bacteria. In a study conducted by Patil (2010), *P. incarnata* was evaluated as an antibacterial agent against *S. aureus* and *E. coli.* The study tested various concentrations, revealing an antibacterial effect >10 mm, which aligns with our findings. The observed antimicrobial activity was attributed to the flavonoids present in the plant's composition.[7] In a study examining leaf extracts of *P. incarnata*, antimicrobial activity was assessed against nine different bacterial species. The antibacterial activity against *S. aureus* observed in this study was consistent with our findings; however, antibacterial activity against *K. pneumoniae* was not reported.[35] Collectively, these findings distinguish the present study from earlier reports by demonstrating antimicrobial activities of *P. incarnata* within the same experimental framework. From a clinical standpoint, the moderate but consistent antimicrobial activity observed, particularly against *C. albicans* and Gram-positive bacteria, suggests that *P. incarnata* could serve as a complementary natural compound in managing microbial infections and combating antimicrobial resistance. While further *in vivo* and pharmacological studies are warranted, these results highlight both originality and the translational relevance of *P. incarnata* as a potential source of multi-target bioactive agents.

This study makes a distinctive contribution to existing body of knowledge by concurrently evaluating the anticancer and antimicrobial properties of *P. incarnata* within a single experimental design. In contrast to previous research that examined cytotoxic or antimicrobial effects separately, this work integrates oxidative stress and apoptotic biomarkers (BAD and Caspase-3) with antimicrobial assays, thereby providing a comprehensive assessment of the plant's dual bioactivity. Moreover, the use of a commercially standardized *P. incarnata* extract enhances the reproducibility and translational relevance of the findings. Collectively, these features highlight the originality of the present study and its potential to bridge the therapeutic interface between oncology and microbiology.

Although the antimicrobial findings of this study provide valuable preliminary insight into the potential of *P. incarnata,* standard reference antibiotics were nor included as positive controls in the disk diffusion assay. Therefore, the observed inhibition zones should be interpreted as indicative of intrinsic antimicrobial potential rather than direct clinical efficacy. Future comparative studies involving conventional antibiotics are warranted to further validate and contextualize these results.

Study Limitations

Although this study provides significant preliminary data, the lack of positive controls in the disk diffusion method and the focus on a single gastric cancer cell line limit the generalizability of the findings. Future research should include comparative analyses with conventional drugs and involve animal models to assess systemic effects.

Conclusion

In conclusion, this study demonstrates that *Passiflora incarnata* exerts significant antiproliferative, pro-oxidative, and pro-apoptotic effects on SNU-1 gastric cancer cells. The IC_{50} value of *P. incarnata* extract was calculated as 88.95 µg/mL, indicating a potent cytotoxic activity. Treatment with *P. incarnata* markedly decreased the TAC and increased the TOS compared with the control group, suggesting enhanced oxidative stress in cancer cells. Moreover, *P. incarnata* significantly elevated the expression levels of pro-apoptotic markers BAD and caspase-3, confirming apoptosis induction. In addition to its anticancer activity, *P. incarnata* exhibited notable antimicrobial effects against various Gram-positive and Gram-negative bacteria, as well as yeast species. These findings collectively highlight the therapeutic potential of *P. incarnata* as a natural anticancer and antimicrobial agent, warranting further mechanistic and in vivo studies to confirm its efficacy and safety.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethics Committee Permission

No ethical approval was required for this study, as it does not involve any human participants, animal subjects, or experimental procedures performed by the authors.

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Authors' Contributions

Concept/Design: AO, RA. Data Collection and/or Processing: AO, RA. Data analysis and interpretation: AO, RA. Literature Search: AO, RA. Drafting manuscript: AO,RA. Critical revision of manuscript: AO, RA. Supervisor: AO.

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