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Seyma Sagir
Lutfi Tutar
Yusuf Tutar

Green Nanotechnology Approach: Comparative Evaluation of Silver Nanoparticles from Two Plant Species and Three Solvent Systems

Dilek Bilgic Alkaya
Ceren Sivritepe
Kayra Simal Celik
Pervin Rayaman
Serap Ayaz Seyhan

Bioactive Properties of Saffron-Fortified Glycolic Propolis Extracts

Zeynep Berin Celebi
Gulsum Merve Boyracı
Atiye Degirmenci
Oktay Yildiz

02

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CONTENTS

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Review

Heat Shock Proteins in Cancer

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Abstract

Heat shock proteins (HSPs) are vital in the progression of cancer, aiding in the survival, proliferation, and metastasis of tumor cells. The overexpression of particular HSPs, such as HSP70 and HSP27, is often found in various malignancies, including lung, breast, and prostate cancers, and correlates with poor prognosis and enhanced resistance to chemotherapy. These proteins stabilize oncogenic proteins, inhibit apoptosis, and modulate the tumor microenvironment, contributing to cancer aggressiveness. Recent studies highlight the potential of HSPs as biomarkers for predicting cancer prognosis and treatment response. Targeting HSPs with specific inhibitors, notably HSP90 inhibitors, has come forth as a viable therapeutic approach to disrupt cancer-related processes and enhance the effectiveness of chemotherapy treatments. Targeting HSPs offers a multi-targeted approach, as these chaperones stabilize multiple oncogenic proteins simultaneously. Overall, this review aims to provide a comprehensive overview of HSPs in cancer, focusing on their role in tumor progression, their clinical implications as biomarkers and therapeutic targets, and the latest developments in HSP-targeted therapies.

Keywords: HSPs, molecular chaperones, cancer progression, therapeutic targets, cancer biomarkers

1. Introduction

Heat shock proteins (HSPs) represent a well-conserved group within the molecular chaperone family, which is essential for maintaining the cellular homeostasis, especially during stressful conditions. Their significance in cancer research has attracted considerable interest, as they serve both in safeguarding cellular integrity and promoting tumor development. HSPs are classified into several families based on their molecular weight, such as HSP27, HSP40, HSP60, HSP70, and HSP90. Each of these families demonstrates distinct roles and implications in relation to cancer (1). Various stress factors, including increased temperatures, oxidative stress, and interaction with detrimental substances, stimulate the synthesis of HSPs (2). The production of HSPs is crucial for cellular survival under stressful conditions, as they are instrumental in facilitating proper protein folding, preventing aggregation, and helping the degradation of improperly folded proteins. In the context of cancer, increased concentrations of HSPs are often associated with poor prognoses and treatment resistance (3). For example, HSP70 and HSP90 are often elevated in cancers, supporting cell survival by inhibiting apoptosis and promoting proliferation (4).

Recent findings have brought to light the numerous functions of HSPs in cancer development. Specifically, HSPs are vital in fundamental processes such as metastasis, angiogenesis, and the evasion of the immune system (5). The connection between HSPs and the tumor microenvironment is significantly important, underscoring the essential function of HSPs in promoting communication between cancer cells and their neighboring environments (6).

Additionally, HSPs have been recognized as promising biomarkers for the diagnosis and prognosis of cancer. A research has investigated their presence in bodily fluids, including urine and serum, as a non-invasive approach to cancer detection. The rare expression of HSPs in liquid biopsies has resulted in new possibilities for discovering cancer-specific biomarkers; however, the validation of these diagnostic markers is not yet complete. The ability of HSPs to serve as indicators of tumor burden and the effectiveness of treatment underscores their importance in clinical settings (7).

The capacity of HSPs to act as markers for tumor load and treatment efficacy highlights their significance in clinical practice. This method seeks to take advantage of the dependence of cancer cells on survival of heat shock proteins, particularly as traditional therapies frequently encounter challenges due to the protective functions these chaperones provide. The advancement of drugs aimed at targeting HSPs is a dynamic field of research, with numerous compounds presently in the clinical trial phase (8).

Apart from their involvement in the survival mechanisms of cancer cells, HSPs significantly influence the regulation of immune responses. Extracellular HSPs can boost anti-tumor immunity by serving as transporters for peptides derived from tumors, which aids in their presentation to immune cells (9). The immunogenic nature of HSPs has inspired studies into vaccines and immunotherapies based on HSPs, which are designed to exploit the ability of the immune system to determine and exclude malignant cells (10). The association of HSP-peptide complexes with antigen-presenting cells is fundamental for the activation of specific immune responses, illustrating the dual role of HSPs both guardians of tumor cells and catalysts for immune recognition (11).

Moreover, the regulation of HSP expression via epigenetic mechanisms is recognized as a critical component in the field of cancer biology. Epigenetic modifications, such as alterations in histones and DNA methylation, can influence HSP expression levels, which may subsequently affect the characteristics of tumors and the prognostic outcomes for patients. Gaining a deeper understanding of these regulatory pathways could offer valuable insights into the creation of targeted therapies designed to adjust HSP expression in cancerous cells (12).

2. HSPs and Cancer Hallmarks

HSPs are crucial in cancer development because they help stabilize and maintain the function of proteins involved in key cancer traits such as uncontrolled cell growth, avoiding cell death, and spreading to other tissues. When overproduced in tumors, HSPs contribute to cancer progression, making them attractive targets for treatment. Blocking HSP activity can interfere with several cancer-driving pathways, offering new possibilities for cancer therapies and improving the effectiveness of current treatments.

In the case of cancer, HSPs are primarily recognized for their functions in supporting folding, preventing aggregation, and facilitating the removal of incorrectly folded proteins. This role is especially vital in cancer, as cells frequently endure heightened stress levels resulting from rapid growth and adverse microenvironments. Zuo et al. (2024) point out that HSPs are significantly upregulated in cancerous tissues and are intricately linked to the processes of tumor formation and advancement. It is noted that HSPs are pivotal in determining the key characteristics of cancer, as they can either activate or inhibit specific signaling pathways, thus promoting the survival and growth of cancer cells (13).

The levels of HSPs are frequently elevated in response to various stressors, including heat shock, low oxygen conditions, and oxidative stress, which are often found in the tumor microenvironment. In their research, Li et al. (2012) elucidate the role of glucose-regulated protein 78 (GRP78), a HSP family member, in several essential characteristics of cancer. These characteristics include the proliferation of tumor cells, resistance to programmed cell death, evasion of the

immune response, metastasis, and angiogenesis. The authors highlight that GRP78 functions as a stress sensor, adapting to the dynamic conditions of the tumor microenvironment and thus supporting the development of cancerous features (14).

HSPs are crucial to assist in the proper folding of proteins, preventing their aggregation, and enhancing the removal of misfolded proteins. These functions are essential for maintaining cellular integrity under stress such as heat shock, oxidative stress, and nutrient deprivation. In the context of cancer, HSPs are often overexpressed, contributing to the survival and proliferation of malignant cells. This overexpression frequently occurs as a reaction to the tumor microenvironment, which is defined by a range of stressors that cancer cells must manage to survive and grow.

The possibility of employing HSPs as therapeutic targets in the fight against cancer is becoming more widely accepted. Ban et al. (2019) investigated the epigenetic modifications of HSPs within the realm of cancer, proposing that these proteins could serve as potential therapeutic targets as well as diagnostic markers. They highlight that the modulation of HSP expression via epigenetic processes can profoundly influence the behavior of cancer cells, indicating that adjusting HSP levels could modify the characteristics associated with cancer (15).

The concept of HSPs extends into the tumor microenvironment, where they can affect immune responses and facilitate immune evasion, a key characteristic of cancer. In their study, Secli et al. (2021) indicated that extracellular HSPs (eHSPs) can foster the growth and malignancy of cancer cells by promoting processes including angiogenesis and epithelial-to-mesenchymal transition (EMT) (16). These mechanisms are essential for metastasis, as they allow cancer cells to migrate and infiltrate into adjacent tissues. The authors point out that acquiring knowledge about the roles of eHSPs within the tumor microenvironment could facilitate the creation of advanced diagnostic and treatment options for cancer.

Beyond their roles in apoptosis and the tumor microenvironment, HSPs are also vital in metabolic reprogramming, a hallmark of cancer. Avolio et al. (2020) underscore the significance of these metabolic adaptations, which are crucial for cancer cell survival and growth under stress. Specifically, members of the mitochondrial HSP90 family are key regulators of these metabolic pathways, which frequently undergo changes in cancerous cells. The interplay between HSPs and metabolic reprogramming indicates that targeting these proteins could obstruct the metabolic versatility that cancer cells depend on for their development and survival (17).

The investigation into the function of HSPs in immune evasion is an emerging area of research. The tumor microenvironment acts as both a physical barrier for immune cells and a dynamic system that can modify immune responses. According to Becker (2014), solid tumors can foster an immune-permissive environment by utilizing non-transformed host cells (18). HSPs may contribute to this phenomenon by affecting the expression of immune-modulatory factors, thus facilitating the evasion of immune surveillance.

In conclusion, HSPs are crucial in cancer biology, greatly influencing fundamental tumor characteristics like cell survival, proliferation, metastasis, and the evasion of immune responses. Acting as molecular chaperones, HSPs are essential for preserving cellular homeostasis and ensuring that proteins fold correctly, which is critical for the survival of cells under conditions of stress. Their involvement in supporting various hallmarks of cancer, as defined by Hanahan and Weinberg (2011), underscores their importance in tumor development and progression (19). Due to their multifaceted functions, HSPs are promising targets for therapeutic intervention. A deeper understanding of their mechanisms and interactions within the tumor microenvironment is expected to yield valuable insights, potentially leading to more effective cancer treatment strategies. Ongoing research continues to highlight the critical contributions of HSPs to the complex biology of cancer and their potential to improve diagnosis, prognosis, and therapy.

3. Small Heat Shock Proteins (sHSPs)

Small heat shock proteins (sHSPs) constitute a varied group of molecular chaperones essential for preserving cellular proteostasis during different stress conditions. These proteins, generally between 12 to 43 kDa in size, are found throughout all life forms, underscoring their evolutionary importance and functional adaptability (20). The primary function of sHSPs is to prevent the aggregation of

incorrectly folded proteins, which helps to shield cells from the adverse effects of different stressors, including increased temperatures, oxidative stress, and mechanical pressure (21).

The ways in which sHSPs provide their protective mechanisms are complex and varied. For example, they can create large oligomeric complexes with misfolded proteins, thereby sequestering these proteins and inhibiting irreversible aggregation. The ability to bind and stabilize proteins during their unfolding process is crucial, as it helps them to refold later by ATP-dependent chaperone proteins (22). The dynamics of interaction between sHSPs and their substrates are affected by several factors, notably post-translational modifications, which can alter their chaperone function and stability (23).

Studies have shown that sHSPs are not only crucial for the cellular response to heat stress but also have important functions in neurodegenerative disorders. For instance, α-synuclein, a protein involved in Parkinson's disease, shares both structural and functional characteristics with sHSPs, indicating that these chaperones might engage with α-synuclein and influence its aggregation (24). The functions of sHSPs in safeguarding against neurodegeneration are reinforced by evidence demonstrating their increased expression in injured brain tissues, suggesting a possible neuroprotective function (25). In this context, sHSPs such as HSP27 have demonstrated a significant ability to mitigate toxicity induced by α-synuclein, underscoring their potential as therapeutic agents in neurodegenerative diseases (26).

The engagement of sHSPs with various cellular elements highlights their crucial role in sustaining cellular homeostasis. Specifically, in skeletal muscle, sHSPs have demonstrated their ability to safeguard against mechanical stress through interactions with mechanosensitive proteins, thus playing a key role in the regulation of physiological contraction and extension cycles (21). This key role of sHSPs in muscle tissue demonstrates their capacity to adjust to various cellular conditions and stress factors.

In conclusion, small heat shock proteins are crucial in mediating the cellular response to stress in a wide range of biological phenomenon. Their ability to prevent protein aggregation, modulate signaling pathways, and interact with various cellular components positions, making them as vital players in maintaining proteostasis and enhancing cell survival under adverse conditions. Current research related with various functions and regulatory mechanisms of sHSPs is consistently uncovering their importance in both health and disease, highlighting their potential as therapeutic targets for a variety of conditions, such as neurodegenerative diseases and disorders related to stress.

4. HSPs and Cancer

The role of HSPs in cancer is complex and varied. These proteins are recognized for their ability to aid in proper protein folding, inhibit aggregation, and support the breakdown of improperly folded proteins. This assistance is particularly vital for cancer cells, which frequently endure increased proteotoxic stress because of accelerated growth and metabolic imbalances (27). HSP90 has been thoroughly examined and is critical for maintaining the stability and operational integrity of several oncoproteins, especially those linked to cell signaling and proliferation (28).

Studies indicate that HSPs are frequently overexpressed among the different types of cancer, such as breast cancer, lung cancer, and hepatocellular carcinoma. This overexpression plays a significant role in promoting tumor survival, facilitating metastasis, and enhancing resistance to treatment (29). In the context of non-small cell lung cancer, the presence of HSPs has been correlated with drug resistance, attributed to their capacity to alter apoptotic pathways and facilitate cell survival when subjected to chemotherapy (30).

Additionally, HSPs are being recognized as promising diagnostic and prognostic markers for cancer assessment. Their abnormal expressions in tumor tissues and bodily fluids have been associated with the advancement of the disease and the outcomes for patients (31).

The therapeutic approach of targeting HSPs has seen increased interest in recent years. Inhibitors of HSP90, including geldanamycin, have exhibited potential in both preclinical and clinical environments by interfering with the chaperoning of various oncogenic proteins, ultimately resulting in the death of cancer cells. Furthermore, the suppression of HSF1, which is a transcription

factor controlling the expression of HSPs, has been suggested as an innovative approach for cancer therapy.

The extracellular roles of HSPs are receiving increasing focus. Recent research has shown that cancer cells can secrete HSPs, which may influence the tumor microenvironment, enhance angiogenesis, and accelerate metastasis. The extracellular activity of HSPs points to their potential as therapeutic targets and biomarkers, thus unlocking new frontiers for the treatment and monitoring of cancer.

4.1. HSPs in Breast Cancer

HSPs, particularly HSP70, HSP90, and HSP27, are often observed to be overexpressed across many types of cancer, including breast cancer. This overexpression is often correlated with more aggressive tumor characteristics and worse prognosis. For instance, while HSP70 is normally present in healthy cells, its expression becomes dysregulated in numerous tumor cells, contributing to their survival in stressful environments (32). The enhanced expression of these proteins is linked to the capacity of cancer cells to avoid apoptosis, a defining characteristic of cancer advancement. HSPs, as they help ensure proteins fold correctly, prevent their aggregation, and promote the elimination of misfolded proteins.

The role of HSPs in breast cancer is underscored by their engagement with critical signaling pathways. Significantly, HSP90 has been proven to interact with steroid receptors and multiple signaling proteins, which has directed research towards the formulation of therapies that specifically target HSP in the context of breast cancer (33). This interaction highlights the promise of HSPs as focal points for therapy, since blocking their activity may interfere with the signaling mechanisms that facilitate proliferation and persistence of tumors.

The regulation of HSPs is influenced by multiple factors, notably heat shock factor 1 (HSF1), which serves as a significant regulator to the heat shock response. Besides its function in regulating HSP expression, HSF1 has been linked to the enhancement of the cancer stem cell (CSC) phenotypic characteristics in breast cancer. Increased expression levels of HSF1 are associated with poor prognoses in breast cancer patients, suggesting that HSF1-driven mechanisms may contribute to heightened tumor aggressiveness and treatment resistance (34). This suggests that focusing on HSF1 may be an effective approach to disrupting the cancer stem cell phenotype and enhancing the efficacy of existing therapies.

The participation of HSPs in drug resistance represents a significant dimension of their function in breast cancer. Research indicates that HSPs can enable cancer cells to resist a range of chemotherapeutic drugs by shielding them from apoptosis triggered by these medications. Specifically, HSP27 and HSP70 have been linked to the emergence of resistance to cancer treatments, positioning them as promising targets for combination therapy approaches (35). The integration of HSP inhibitors with standard chemotherapy has demonstrated potential in preclinical research, indicating that these approaches may improve treatment effectiveness by addressing resistance mechanisms (36).

Beyond their functions within cells, HSPs have been identified as being released by cancer cells through exosomes. This secretion can affect the tumor microenvironment and modulate the immune responses. The presence of these extracellular HSPs may act as indicators of cancer advancement and treatment efficacy, underscoring their promising role in theranostics (37).

Recent transcriptomic research has revealed that specific HSP genes exhibit dysregulation across different molecular subtypes of breast cancer. This observation suggests that the expression patterns of HSPs could be utilized to classify breast cancer subtypes and predict patient outcomes. For instance, in a study discovered both shared and distinct HSP genes linked to overall survival, underscoring their potential as prognostic biomarkers (38). These results emphasize the significance of comprehension of the distinct roles of HSPs in various breast cancer subtypes, which may inform the creation of personalized treatment approaches.

In conclusion, HSPs have various important functions in breast cancer, affecting tumor development, resistance to treatment, and overall patient prognosis. Their participation in vital cellular mechanisms and signaling pathways put forward them as potential targets for therapeutic strategies.

4.2. HSPs in Lung Cancer

The expression of HSPs in lung cancer has attracted considerable interest because of their roles in tumor development, progression, and therapeutic response. This article seeks to examine the diverse functions of HSPs in lung cancer, referencing various studies that underscore their significance in cancer biology and treatment strategies.

The presence of high-molecular-weight (HMW) HSPs, particularly HSP-60 and HSP-70, has been identified in lung carcinoma through immunohistochemical methods. In a research study done by Michils (2001), a quantitative analysis was undertaken to evaluate the concentrations of (HMW and low-molecular-weight (LMW) HSPs in lung tissues, distinguishing between tumor and non-tumor samples. The results indicated that tumor tissues had markedly elevated levels of HSP-60 and HSP-70 when contrasted with healthy lung tissues, implying that these proteins may function in the malignant transformation and persistence of lung cancer cells (39). The elevated levels of HSPs in tumor tissues suggest their potential utility as biomarkers for diagnosing and predicting lung cancer outcomes.

The function of HSPs goes beyond simply their expression levels; they are also involved in the processes that contribute to drug resistance in lung cancer. Xia et al. (2021) conducted a review on the role of GRP78, a HSP family member, in lung cancer. GRP78 serves as a key stress sensor within the endoplasmic reticulum (ER), facilitating the ER stress responses that are vital for the survival of cancer cells in challenging environments. The findings highlight that GRP78 is frequently overexpressed in tumors and correlates with unfavorable outcomes for patients with lung cancer (40). This indicates that focusing on GRP78 may improve the effectiveness of current treatments by addressing resistance mechanisms.

Beyond their involvement in drug resistance, HSPs play key roles in multiple cellular processes that facilitate tumor progression. A study done by Ferreira et al. (2019) emphasizes the importance of immune-based prognostic biomarkers in lung cancer, indicating that the expression levels of specific HSPs may be associated with immune responses and the effectiveness of treatments (41). The connection between HSP expression and the immune microenvironment may offer valuable understanding of how lung cancer cells avoid detection by the immune system, thus promoting tumor development and spread.

Recent studies have investigated the connection between HSPs and alternative splicing in lung cancer. Awad and El-Hadidi (2021) examine how alternative splicing can produce different isoforms of HSPs, each potentially serving unique roles in the biology of cancer (42). This complexity introduces an additional dimension to our comprehension of HSPs in lung cancer, as various isoforms may have the potential to affect tumor characteristics and treatment responses.

The participation of HSPs in lung cancer is evident in the field of immunotherapy. Herbst (2019) highlights the progress made in cancer immunotherapy, especially regarding immune checkpoint inhibitors, and observes that the expression levels of HSPs could act as predictive biomarkers for assessing treatment efficacy (43). The capacity of HSPs to influence immune responses indicates that they may be utilized to improve the efficacy of immunotherapeutic approaches in lung cancer patients.

In conclusion, HSPs play a crucial role in the biology of lung cancer, affecting tumor development, resistance to treatment, and the capability to escape the immune system's response. Their increased presence in tumor tissues, correlation with unfavorable outcomes, and potential as targets for therapy emphasize the significance of HSPs in lung cancer studies. As investigations related with the functions of HSPs advances, they may provide new opportunities for therapeutic approaches and tailored treatment plans in the management of lung cancer.

4.3. HSPs in Colorectal Cancer

Colorectal cancer (CRC) represents a major health worldwide issue, ranking among the foremost causes of cancer-related illness and deaths. The pathogenesis of CRC is complex, involving multiple molecular mechanisms, with HSPs identified as key contributors. HSPs that support the folding, stabilization, and degradation of proteins, particularly in reaction to stressful situations. Their involvement in cancer biology, particularly

in CRC, has attracted growing interest due to their significant roles in tumor advancement, metastasis, and resistance to treatment.

HSPs are recognized for their elevated expression in multiple types of cancer, particularly CRC, where they play significant roles in tumor development and advancement. Javid et al. (2022) emphasized that various HSPs, such as HSP27, HSP40, HSP60, HSP70, HSP90, and HSP110, possess anti-apoptotic characteristics and are integral to mechanisms like tumor cell growth, invasion, and metastasis (44). This points to the potential role of HSPs as biomarkers to diagnose and prognose of CRC, since their expression levels are linked to the severity of the disease and the resulting patient outcomes.

The function of HSPs in CRC is complex and diverse. Buttacavoli et al. (2021) utilized a multi-omics strategy to explore the expression patterns of HSPs in breast cancer that may also be relevant to CRC due to the common pathways involved in tumor development (45). The involvement of HSPs in influencing the tumor microenvironment represents a significant aspect of their role in CRC. According to Lang et al. (2019), increased concentrations of HSPs within tumor cells correlate with unfavorable survival rates since they promote intrinsic characteristics of tumor cells, including unchecked growth and heightened metastatic capabilities (46).

Beyond their involvement in tumor biology, HSPs have also been linked to the emergence of resistance to cancer treatments. Zhang et al. (2020) examine the ways in which HSPs can affect tumor growth and metastasis, emphasizing their dual roles in cancer progression (47).

Additionally, the extracellular roles of heat shock proteins (HSPs) have attracted interest regarding their involvement in immune evasion and tumor advancement. Taha et al. (2019) investigated the capacity of extracellular HSPs to function as alarmins, affecting immune responses and possibly aiding tumor cells in resisting destruction by the immune system (48).

4.4. HSPs in Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the most frequent type of primary liver cancer. HSPs have been found to function in tumor development, metastasis, and resistance to treatment of HCC.

In HCC, the levels of several HSPs, including HSP27, HSP70, and HSP90, are frequently elevated, leading cancer cells to thrive under adverse conditions. For example, HSP70 has been associated with increased chemoresistance of HCC cells. Wang et al. (2021) indicated that incomplete radiofrequency ablation (iRFA) resulted in the upregulation of HSP70, which inhibited pyroptosis and allowed transformed cells to survive, thus enhancing chemoresistance (49).

The involvement of HSPs in the progression of HCC is further validated by their association with various oncogenic processes. As noted by Paul et al. (2024), HSPs are instrumental in regulating the cell cycle, apoptosis, and cellular proliferation, which are all critical components in cancer development (50). Specifically, HSP27 has been identified as a vital element in inhibiting apoptosis in HCC, thereby facilitating tumor growth and metastasis (51). This underscores the dual function of HSPs in enhancing cancer cell survival while also contributing to the aggressive characteristics of HCC.

5. Therapeutic strategies of HSPs

HSPs are key molecules for cellular stress response and are involved in numerous diseases, including cancer, neurodegenerative illnesses, and cardiovascular ailments. Functioning as molecular chaperones, they aid in the correct folding of proteins, prevent their aggregation, and promote the degradation of improperly folded proteins. Given their importance in sustaining cellular homeostasis, HSPs are receiving heightened attention as potential candidates for therapeutic targeting and as biomarkers.

In the context of cancer treatment, HSPs hold particular importance. HSP70 and HSP90 are frequently found to be overexpressed in cancer cells, which contributes to tumor progression and resistance to chemotherapy. The resistance encountered with ABL-tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia (CML) demonstrates the critical requirement for novel strategies to effectively overcome this obstacle. Research indicates that leukemia stem cells and the genetic variability in CML pathogenesis suggest

that targeting HSPs may improve the effectiveness of therapy (52).

HSPs play a role in ischemic heart disease and a range of cardiovascular conditions, alongside their link to cancer. Sueta et al. (2019) highlight the difficulties associated with antithrombotic treatment in cancer patients, pointing out that HSPs affect the management of therapy in individuals who have both malignancies and cardiovascular issues. This situation calls for a collaborative, multidisciplinary strategy to enhance patient care (53).

Digital health interventions broaden the scope of therapies related to health service providers. Kraft et al. (2021) examined the eHealth platform eSano, which offered internet and mobile-based solutions for mental and behavioral health issues. Incorporating HSPs-centered content into these platforms could improve patient engagement and compliance with treatment plans (54).

Additionally, healthcare safety professionals contribute to fostering a culture of safety within healthcare environments. Le et al. (2024) advocated for systematic reviews which aimed to identifying interventions that enhance safety culture, especially in the field of oncology. Gaining insights into how HSPs influence cellular stress responses may lead to the improvement of safety protocols and training initiatives, ultimately benefiting patient outcomes (55).

6. Conclusion

HSPs are essential components in the development of cancer, as they enhance the survival, growth, metastasis, and ability of tumor cells to evade the immune system. Their overexpression contributes to resistance against cell death and alters the tumor microenvironment, making them key factors in the advancement of cancer and the resistance to cancer treatment.

HSPs show promise as non-invasive biomarkers for cancer diagnosis, prognosis, and treatment monitoring, with their levels correlating to clinical outcomes across different cancers. Therapeutically, targeting HSPs -especially with HSP90 inhibitors- offers a multi-targeted approach by disrupting several oncogenic proteins simultaneously. Additionally, HSPs' role in immune regulation has spurred the development of HSP-based vaccines and immunotherapies.

Epigenetic control of HSP expression adds complexity but also presents new opportunities for targeted cancer treatments. Ongoing research about HSP functions within tumors is crucial for advancing cancer diagnosis and therapy.

In summary, HSPs are active drivers of cancer progression and valuable targets in diagnosis and treatment. Continued study on this subject is likely to improve patient outcomes.

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Original Article

Bioactive Properties of Saffron-Fortified Glycolic Propolis Extracts

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Abstract

Introduction: Propolis is a bee-derived natural product characterized by its complex chemical composition and pronounced antioxidant capacity. With the increasing consumer demand for functional foods, propolis has gained significant attention as a bioactive ingredient and is now widely incorporated into various formulations within the nutraceutical sector. Similarly, saffron, traditionally valued as a culinary spice, has recently attracted growing scientific interest due to its potential biological activities, particularly those associated with the modulation of central nervous system functions.

Methods: In this study, glycol-based propolis extracts containing 1% and 5% saffron were prepared. Their antioxidant properties were evaluated in terms of total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), and DPPH radical scavenging activity. The phenolic profiles of the extracts were also analyzed using HPLC-PDA with 26 phenolic standards.

Results: In the propolis extract, several phenolic and flavonoid compounds were identified, including p-hydroxybenzoic acid, caffeic acid, p-coumaric acid, ferulic acid, quercetin, trans-cinnamic acid, apigenin, rhamnetin, chrysin, pinocembrin, caffeic acid phenethyl ester (CAPE), and galangin. The incorporation of 1% saffron into the extract did not cause a notable alteration in the phenolic composition. However, supplementation with 5% saffron resulted in elevated concentrations of p-hydroxybenzoic acid, quercetin, apigenin, and rhamnetin.

Conclusions: This study suggests that the synergistic interaction between the bioactive constituents of propolis and saffron may enhance their overall biological efficacy. Nevertheless, the findings of the present study are limited to in vitro antioxidant assays. Therefore, further in vivo investigations are warranted to elucidate the underlying mechanisms of action and to optimize the formulation ratios for potential nutraceutical applications.

Keywords: Antioxidant, flavonoids, phenolic compounds, propolis, saffron

1. Introduction

Propolis is a natural bee product produced by honeybees using resins collected from plants (1). While it primarily consists of resins and beeswax, over 300 compounds have been identified in propolis, including vitamins B, C, and E, various minerals, phenolic acids, flavonoids, stilbene derivatives, terpenes, and amino acids. Among these, phenolic acids and flavonoids are particularly associated with the biological activities of propolis (2).

Historically, propolis has been used for its various therapeutic purposes such as embalming, wound care, and as an antiseptic (1). Today, due to its rich composition and broad spectrum of biological activities, propolis is being utilized in the development of nutraceutical products and in apitherapy. The World Health Organization (WHO) has stated that propolis can be safely used without interfering with medical treatments. Along with the growing global interest in functional foods, this has contributed to an increasing trend in the use of propolis as a food ingredient (3, 4).

However, several factors limit the use of propolis as a nutraceutical. Among these are its low water solubility, as well as its unpleasant taste and odor (3). Ethanolic extracts of propolis, which show better solubility than aqueous extracts, have been reported to exhibit higher antioxidant activity and a richer phenolic compound profile. Nevertheless, the potential toxicity of ethanol and its possible adverse health effects raise concerns among consumers, particularly regarding orally administered propolis drops. Therefore, the development of ethanol-free propolis extracts with high biological activity has become an important area of research (5, 6). In this context, glycol derivatives are being investigated as alternative solvents.

The phenolic content and antioxidant capacity of a water–polyethylene glycol (PEG) extract, prepared as an alternative to ethanolic propolis extracts, have been investigated. In one study, the antioxidant activities of ethanol and water–PEG extracts were found to be similar when assessed using ABTS and CUPRAC methods; however, ethanol extracts exhibited higher antioxidant activity based on DPPH and FRAP assays (6). Similarly, in another study comparing anhydrous PEG and ethanol extracts, no statistically significant difference was reported in terms of total polyphenol content (TPC). However, it was concluded that PEG was more effective in extracting polar compounds, whereas ethanol favored the extraction of more apolar phenolics. PEG is known to be a low-cost, non-toxic, and well-tolerated solvent. In fact, its use at certain concentrations is considered safe even in pediatric pharmaceutical formulations (5).

Saffron (*Crocus sativus* L.) is a spice commonly used to impart color, flavor, and aroma of foods. In addition to its culinary use, saffron has also been traditionally applied in cosmetic and therapeutic practices. Notably, it has been used as a tonic in Persian traditional medicine (7). Today, saffron is particularly studied for its effects on the central nervous system, and for its potential antidepressant, anti-inflammatory, and antioxidant properties (8).

The biological activities of saffron are mainly attributed to its active compounds: safranal, crocin, and crocetin. Some preclinical studies have demonstrated the antidepressant properties of crocin and crocetin. Clinical studies have also reported that saffron reduces anxiety scores in patients compared to placebo (9). In a six-week study conducted with patients diagnosed with moderate depression, saffron administered at a dose of 60 mg/day showed comparable effects to fluoxetine treatment at 40 mg/day (10). Due to its low toxicity, significant effects on oxidative stress and inflammation, anxiolytic properties, and its ability to modulate mitochondrial function, saffron extracts and their constituents are considered promising nutraceutical compounds in this field (7).

In this study, glycol extracts of high-activity propolis enriched with different concentrations of saffron were prepared, aiming to obtain antioxidant-rich extracts with high nutraceutical value.

2. Methods

2.1. Materials

Propolis was obtained from a local beekeeper in Turkey. Saffron was sourced from Iran. Food-grade glycol was used for extract preparation. All chemicals and phenolic standards used for the analyses were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of Extracts

All extracts were prepared in a solvent system containing 50% glycol. The first extract served as the control and contained only 30% (w/v) propolis. The second extract contained 30% propolis and 1% (w/v) saffron, while the third extract contained 30% propolis and 5% (w/v) saffron. The extracts were subjected to ultrasonic treatment for 40 minutes, followed by incubation at 50 °C with shaking at 100 rpm for 24 hours.

2.3. Total Phenolic Content (TPC)

The method described by Slinkard and Singleton (11) was used to determine TPC. In the analysis, 680 µL of distilled water, 400 µL of 0.2 N Folin–Ciocalteu reagent, 20 µL of the sample, and 400 µL of 10% sodium carbonate solution were used. After incubation for 2 hours, absorbance was measured at 760 nm using a spectrophotometer (Evolution™ 201, Thermo Scientific, USA). Gallic acid was used as the standard, and results were expressed as milligrams of gallic acid equivalents per milliliter (mg GAE/mL).

2.4. Total Flavonoid Content (TFC)

The method described by Mohammadzadeh et al. (12) was used for the analysis. For the assay, 0.5 mL of sample extract, 2.15 mL of methanol, 0.05 mL of 10% aluminum nitrate, and 0.05 mL of 1 M ammonium acetate were used. After incubation for 40 minutes, absorbance was measured at 415 nm using a spectrophotometer. Quercetin was used as the standard, and results were expressed as milligrams of quercetin equivalents per milliliter (mg QE/mL).

2.5. Antioxidant Activity

The ferric reducing antioxidant power (FRAP) assay was performed according to the modified method described by Benzie and Strain (13). The FRAP reagent was freshly prepared for the test. In each test tube, 3 mL of FRAP reagent and 0.1 mL of sample were added. After incubation for 4 minutes, absorbance was measured at 593 nm. Results were expressed as milligrams of Trolox equivalents per milliliter (mg Trolox/mL).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity assay was conducted following the method described by Molyneux (14). A fresh DPPH solution (0.04 mg/mL) was prepared for the test. In a test tube, 0.75 mL of DPPH solution and 0.75 mL of sample were mixed. After 50 minutes of incubation, absorbance was read at 517 nm. Results were expressed as SC_{50} (mg/mL) values.

2.6. Phenolic Profile

Equal volumes were taken from each extract to prepare the samples. First, the pH of the samples was adjusted to 2 with 1 N HCl. Subsequently, liquid-liquid extraction was performed twice using 15 mL each of ethyl acetate and diethyl ether (10 mL in the first extraction and 5 mL in the second extraction) at 200 rpm for 15 min. The organic phases were collected and evaporated using a rotary evaporator (IKA®-Werke RV 05 Basic). The residue was dissolved in 2 mL of methanol and then injected into the instrument for analysis. Twenty-six phenolic standards were analyzed using reversed-phase high-performance liquid chromatography (RP-HPLC, Shimadzu Corporation LC 20AT) equipped with a photodiode-array (PDA) detector, as described by Kara and Birinci (15). A C18 column (5 µm, 4.6 mm × 250 mm; GL Sciences) was used in the analyses. The injection volume was 20 µL, the column temperature was maintained at 30 °C, the flow rate was set at 1 mL/min, and measurements were performed at four different wavelengths (250, 280, 320, and 360 nm). In the method used, 70% acetonitrile (ACN)–ultrapure water (reservoir A) and 2% acetic acid (AcH)–ultrapure water (reservoir B) were used as the mobile phase. A gradient program with a total analysis time of 50 minutes was employed. Calibration curves were prepared for all 26 standards, with linear ranges of X–Y µg/mL and correlation coefficients (R^2) > 0.99. Within the analytical procedure, standard compounds were examined at four distinct wavelengths: 250, 280, 320, and 360 nm. The developed method for phenolic compound quantification was based on measuring each standard at its characteristic absorption wavelength. Specifically, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, rutin, ellagic acid, and daidzein were analyzed at 250 nm; gallic acid, catechin hydrate, epicatechin, syringic acid, trans-cinnamic acid, naringenin, hesperetin, chrysin, and pinocembrin at 280 nm; chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, apigenin, and caffeic acid phenethyl ester (CAPE) at 320 nm; while myricetin,

luteolin, quercetin, rhamnetin, and galangin were detected at 360 nm.

2.7. Statistical Analysis

Statistical analysis were performed using the SPSS Statistics 22.0 software. Results are presented as mean ± standard deviation. Data were analyzed using the ANOVA method and Tukey's test. A level of $p< 0.05$ was considered statistically significant. All analyses were performed in triplicate, and the results were expressed as mean ± standard deviation.

3. Results and Discussion

The antioxidant properties of the prepared extracts were evaluated in terms of TPC, TFC, FRAP, and DPPH radical scavenging activity (Table 1). The TPC value of the control extract containing 30% propolis was found 18.34±0.68 mg GAE/mL. In the Yildiz (16) study, the TPC value of 20% propolis-glycol (w/v) extract was determined as 49.78±2.55 mg GAE/mL. According to the TPC results, the addition of 1% saffron did not cause a statistically significant change in the antioxidant properties of the extract. However, a significant increase in TPC content was observed with the addition of 5% saffron. Previous studies comparing the antioxidant activities of various saffron extracts reported the highest TPC value (29.20 mg GAE/g) in an extract prepared with 80% ethanol and incubated for 24 hours at room temperature under shaking conditions (17). Another study demonstrated optimal antioxidant activity in extracts obtained through a combination of ultrasonic and microwave extraction using a 50% methanol/water solvent system, reporting a TPC value of 31.56 mg GAE/g (18).

Table 1. TPC, TFC and antioxidant properties of extracts.

	30% propolis	30% propolis+ 1% saffron	30% propolis+ 5% saffron
TPC (mg GAE/mL)	18.34±0.68^{a}	18.75±0.82^{ab}	19.74±0.14^{b}
TFC (mg QE/mL)	2.90±0.06^{a}	3.12±0.02^{b}	4.48±0.10^{c}
FRAP (mgTrolox/mL)	12.69±0.10^{a}	13.18±0.45^{a}	14.94±0.47^{b}
DPPH-SC_{50} (mg/mL)	0.066±0.002^{b}	0.049±0.0001^{a}	0.049±0.0001^{a}

Letters indicate statistical difference in the same row ($p < 0.05$).

In the present study, the control extract containing 30% propolis showed an increase approximate of 1.4 mg GAE/mL in TPC upon the addition of 5% saffron (0.05 g/mL). When normalized to the amount of saffron added, this corresponds to about 28 mg GAE/g. Moreover, in the extraction of whole saffron flowers, the total polyphenol content (TPC) was found to be 4.1 mg GAE/mL for the extract with an S:L ratio of 0.10 g/mL, whereas the extract with an S:L ratio of 0.30 g/mL exhibited a TPC of 9.6 mg GAE/mL (19). These results are in line with those reported in the literature.

Regarding TFC, the addition of both 1% and 5% saffron resulted in a statistically significant increase compared to the control extract. The observed enhancement in TFC with increasing saffron concentration may be attributed to the enrichment of the extract with the natural flavonoid components of saffron. Yıldız (16) reported the TFC of the propolis extract prepared with glycol as 6.81 mg QE/mL. In contrast, Hafshejani et al found that TFC of ethanolic propolis extracts (10% propolis (w/v), 72 hours, 40°C) ranged from 4.80–100.03 mg QE/mL (20). Moreover, Mahood et al. (21), the TFC of a 70% methanolic saffron extract was reported as 5.967 mg catechin equivalents per gram of dry weight and 241.797 mg QE/g (dry weight). Additionally, Belyagoubi et al. (22) reported a TFC value of 3.77 mg QE/g for a 70% ethanolic saffron extract.

The FRAP assay results of extracts were found between 12.69 to 14.94 mg Trolox/mL and presented in Table 1. The FRAP assay results showed that the addition of 1% saffron did not cause a significant increase in the ferric reducing antioxidant power of the extract, whereas the addition of 5% saffron led to a statistically significant enhancement. In a study investigating the antioxidant content of seven different propolis samples obtained from urban beekeeping, FRAP values were found to range from 10.93 to 29.55 mg Trolox/mL. In addition, the study also mentioned that factors such as geographical origin, harvest time, plant sources, season and climatic conditions affect the composition (23).

Changes in DPPH activity of the extracts are also shown in Table 1. As well seen, the SC_{50} of the propolis extract was found 0.066 mg/mL and both saffron enriched extracts were detected 0.049 mg/mL. Regarding DPPH radical scavenging activity, an increase in antioxidant activity was observed

with 1% saffron addition; however, higher saffron concentrations did not further enhance the activity.

The SC_{50} value of 11 different ethanolic propolis extracts was reported to vary between 4.62 and 1031.57 mg/mL (20). On the other hand, Rahaiee et al. (17) reported that the DPPH radical scavenging activities (SC_{50}) of ethanolic and methanolic saffron extracts ranged between 0.037 and 0.346 mg/mL. It should not be overlooked that the differences in bioactive properties of propolis and saffron, such as TPC, TFC and antioxidant activity could be affected by factors such as climate, harvest region, cultivation extraction method/ parameters and solvent (16, 24, 25).

Flavonoids such as quercetin, galangin, and apigenin, along with phenolic acids like caffeic and p-coumaric acids, represent the predominant phenolic constituents of propolis (26, 27). However, saffron and its floral by-products are rich in compounds such as apigenin, quercetin, kaempferol, rutin and *p*-hydroxybenzoic acid (28-30). These compositional similarities suggest that the incorporation of saffron into propolis formulations could enhance the extract's phenolic diversity and overall bioactivity. The increase in TPC, TFC and antioxidant activity observed in the 5% saffron-enriched extract may be attributed to phenolics sourced from saffron, including apigenin, quercetin, and kaempferol. From a biological standpoint, such compounds have been extensively reported for their potent antioxidant, antimicrobial, and anti-inflammatory properties, as well as neuroprotective effects, including the inhibition of amyloid aggregation associated with Alzheimer's disease (31, 32). Therefore, adding saffron to propolis not only improves the TPC, TFC, and antioxidant potential of the extract, but may also extend its spectrum of biological functions, supporting its potential application as a multifunctional nutraceutical ingredient.

In this study, the phenolic contents of the extracts were analyzed using the HPLC-PDA method, targeting 26 phenolic compounds (Table 2). In the propolis extract, *p*-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ferulic acid, quercetin, *t*-cinnamic acid, apigenin, rhamnetin, chrysin, pinocembrin, CAPE, and galangin were detected. The addition of 1% saffron did not significantly alter the phenolic composition; however, enrichment with 5% saffron resulted in elevated levels of p-hydroxybenzoic acid, quercetin, apigenin, and rhamnetin. This outcome can be attributed to the naturally high abundance of these constituents in saffron (28-30).

Comparable results have been reported in previous studies. Analysis of the phenolic components of a 70% methanolic saffron extract revealed quercetin and epicatechin as the primary compounds (22) whereas ethanolic extracts were rich in gallic acid, kaempferol, quercetin, and pyrogallol (21). In another investigation, rutin, safranal, and picrocrocin were detected in all samples of 15 saffron samples from 11 different countries, while chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, luteolin, and apigenin were identified in varying amounts (29). These findings corroborate the present results, highlighting the role of saffron-derived phenolics in enriching the propolis matrix.

Although the composition of propolis varies with its botanical and geographical origin, Turkish propolis samples are known to contain abundant polyphenolic compounds such as pinocembrin, chrysin, CAPE, galangin, apigenin, quercetin, caffeic acid, p-coumaric acid, and trans-cinnamic acid. These compounds are thought to be responsible for the important biological activities of propolis, including antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory effects (15, 33, 34). The synergistic presence of both propolis – and saffron-derived phenolics in the enriched extracts may thus potentiate their overall biological efficacy.

Table 2. Phenolic profile of the extracts

	Phenolic Content	30% propolis	30% propolis+ 1% saffron	30% propolis+ 5% saffron
µg phenolic /mL sample	Gallic Acid	<LOD	<LOD	<LOD
	Protocatechuic Acid	<LOD	<LOD	<LOD
	Chlorogenic Acid	<LOD	<LOD	<LOD
	Catechin Hydrate	<LOD	<LOD	<LOD
	p-OH Benzoic Acid	10.12	10.38	17.74
	Epicatechin	<LOD	<LOD	<LOD
	Caffeic Acid	305.64	297.15	287.48
	Syringic Acid	<LOD	<LOD	<LOD
	Vanillic Acid	<LOD	<LOD	<LOD
	Rutin	<LOD	<LOD	<LOD
	Ellagic Acid	<LOD	<LOD	<LOD
	p-Coumaric Acid	177.20	178.70	169.07
	Ferulic Acid	212.17	207.30	201.01
	Myristin	<LOD	<LOD	<LOD
	Daidzein	<LOD	<LOD	<LOD
	Luteolin	<LOD	<LOD	<LOD
	Quercetin	62.22	65.08	80.44
	t-Cinnamic Acid	239.34	240.65	220.86
	Naringenin	<LOD	<LOD	<LOD
	Apigenin	108.71	109.53	120.78
	Hesperetin	<LOD	<LOD	<LOD
	Rhamnetin	260.67	266.12	294.03
	Chrysin	1423.67	1446.66	1469.59
	Pinocembrin	2168.90	2171.55	2214.25
	CAPE	798.52	819.69	821.27
	Galangin	1479.81	1483.87	1586.23

LOD: Limit of detection

4. Conclusion

In this study, the effect of saffron supplementation on the biological properties of propolis extract was investigated. From the results obtained in the current study, it was determined that increasing the proportion of added saffron has the potential to enhance both the phenolic composition and antioxidant capacity of the propolis extract. These findings suggest that the synergistic interactions between the bioactive constituents of propolis and saffron may augment their overall biological efficacy. However, the current study is limited to *in vitro* antioxidant evaluations; therefore, further in vivo investigations are necessary to elucidate the underlying mechanisms of action and to optimize formulation ratios. Overall, this research provides valuable insights into the potential health benefits and practical applications of saffron-enriched propolis extracts in the development of functional and nutraceutical products.

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Original Article

Green Nanotechnology Approach: Comparative Evaluation of Silver Nanoparticles from Two Plant Species and Three Solvent Systems

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Abstract

Introduction: Green synthesis using plant extracts provides an eco-friendly alternative to conventional nanoparticle production, reducing the use of toxic reagents while stabilizing particle surfaces. This study aimed to synthesize silver nanoparticles (AgNPs) using two plant species extracts and evaluate their antioxidant and antimicrobial potentials.

Methods: The plant was extracted with 70% methanol, 70% ethanol, and distilled water. Extracts were mixed with 1 mM $AgNO_3$ solution at 25 °C for 24 h to synthesize AgNPs. The total phenolic content was measured using the Folin–Ciocalteu method, expressed as gallic acid equivalents. Antimicrobial activity was assessed against seven bacterial and three yeast strains using agar well diffusion, and minimum inhibitory, bactericidal, and fungicidal concentrations were determined by microdilution following CLSI standards.

Results: The findings revealed that solvent type significantly influenced both the total phenolic content of the extracts and the physicochemical properties of the synthesized nanoparticles. Higher phenolic content was associated with enhanced stability and improved antimicrobial efficacy. Notably, AgNPs synthesized with ethanol extracts demonstrated stronger antibacterial activity, whereas those produced with aqueous extracts showed relatively lower bioactivity.

Conclusions: The plant extract can act as a natural reducing and capping agent for green synthesis of silver nanoparticles. Although free extracts lacked antimicrobial activity, AgNP-enriched extracts displayed broad-spectrum antibacterial and antifungal effects while retaining measurable antioxidant capacity. These findings suggest that plant-based AgNPs offer an environmentally friendly and biocompatible approach for enhancing the biological efficacy of phytochemicals and hold promise for biomedical applications.

Keywords: Silver nanoparticles, *Capparis spinosa* L., *Prunus laurocerasus* L., antioxidant activity, green synthesis

1. Introduction

Nanotechnology is a burgeoning discipline that has garnered global interest among researchers, with nanoparticles extensively utilised in scientific methodologies owing to their distinctive attributes, including diminutive size, extensive surface area, and targeted mechanisms of action. (1).

Oxidative stress, caused by an imbalance between free radicals and antioxidant defenses, plays a role in the pathogenesis of numerous chronic diseases, including aging and conditions such as cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions. Therefore, nanoparticles with strong radical scavenging abilities can function as protective agents by reducing oxidative damage (2).

Nanoparticles (NPs) are versatile molecules widely used in the biomedical field due to their biocompatibility, stability, and lack of toxicity (3). It has potential applications in medicine, such as drug delivery, antimicrobial, antioxidant, and other biological activities, as well as disease diagnosis (4). They can be easily functionalized for targeted drug delivery (3). The synthesis of metal nanoparticles (MNPs) is considered a progressive field attracting significant scientific research, holding importance in imaging and drug delivery. The small size of MNPs often allows them to leak through biological or physiological membranes that are generally impermeable to other macromolecules (5). Metal nanoparticles (platinum (Pt), copper (Cu), gold (Au), silver (Ag), zinc (Zn)) exhibit extensive antibacterial efficacy against many pathogens, including Gram-negative and Gram-positive bacteria as well as fungi, and are regarded as a viable alternative to antibiotics (6).

Extensive research has been conducted on the use of naturally occurring resources for synthesizing MNPs (7). The biological systems involved in the green synthesis of MNPs are microorganisms such as plants and their derivatives, bacteria, fungi, algae, and yeast (4). Plant extracts serve as reducing and stabilising agents in biosynthesis. Bioreduction entails the conversion of metal ions or metal oxides into zero-valent metal nanoparticles utilising phytochemicals, including tannins, polyphenolic compounds, amino acids, polysaccharides, and vitamins (7). Among metallic nanoparticles, silver nanoparticles (AgNPs) have become a focus of interest due to their special biological properties (8). The antibacterial efficacy of AgNPs against multidrug-resistant pathogens is significant and remarkable. The pursuit of novel antibacterial agents persists in response to the rising prevalence of infectious diseases caused by microorganisms and the scarcity of effective antimicrobial agents and antibiotics. Consequently, the pharmaceutical industry and research sectors are concentrating on the development of new drugs to address antimicrobial resistance effectively (9). When synthesized using plant-mediated methods, these antimicrobial effects can be further enhanced by the synergistic activity of bioactive plant metabolites. Traditional methods for producing AgNPs are expensive, toxic, and not environmentally friendly. To overcome these problems, researchers have found naturally occurring sources and their products that can be used for the synthesis of NPs.

The green synthesis of NPs is a newly emerging branch of nanotechnology. Green synthesis does not require high temperatures, energy, pressure, or harmful chemicals (10). Studies have not only identified the ability of natural extracts and microorganisms to form AgNPs but also their excellent antioxidant activities, which are higher compared to the substrates. It is believed that this activity stems from the preferential absorption of extract components on the surface of nanoparticles. Various studies have found that AgNPs obtained from plant extracts exhibit antitumor and antimicrobial effects (11). Csakvari et al. have demonstrated the usefulness of *Cannabis sativa* leaf extracts in mediating the green synthesis of AgNPs and their antibacterial activities against various human pathogens (12). Ali et al. elucidated the anti-candidal properties of AgNPs synthesised via the aqueous leaf extract of *Calotropis gigantea* (13)

Prunus laurocerasus L. (*P. laurocerasus*) , also known as cherry laurel, is an evergreen shrub from the Rosaceae family, native to the Black Sea region and widely found in Europe and Asia. Traditionally,

the fruits, leaves, and seeds have been used in folk medicine to treat ailments such as digestive disorders, coughs, and inflammation (14-16). The caper bush (*Capparis spinosa* L.) is a perennial plant belonging to the Capparidaceae family. The flower buds, fruits, and root bark of the plant are utilised in traditional medicine for their analgesic, cell-regenerative, wound-healing, diuretic, and tonic properties (17). Phytochemical studies on these plants have revealed high concentrations of phenolic compounds, flavonoids, anthocyanins, and other secondary metabolites with strong antioxidant and antimicrobial effects (14-16, 18).

In this study, the biosynthesis of AgNPs was synthesized using three different extracts of *P. laurocerasus* L. fruit and *C. spinosa* L. seed. The phytochemical potential of these extracts was evaluated, particularly their total phenolic content (TPC), role in nanoparticle formation, and bioactivity. The antimicrobial activity of the obtained AgNPs was evaluated against selected pathogenic microorganisms, while their solubility in dimethyl sulfoxide (DMSO) was examined to assess their potential applicability in biomedical formulations.

2. Methods

2.1. Plant material

The *C. spinosa* L. samples were obtained from Caper Research and Development Food Production Center (Burdur). *P. laurocerasus* L. dried fruit was supplied by a local herbalist. The seeds of *C. spinosa* L. were dried at room temperature and then ground into powder using a grinder.

2.2. Preparation of plant extract

The extraction procedure was described by Alkaya et al.2019 (19). For the extraction process, 2 g of ground fruit sample was mixed with 25 mL of extraction solvent (70% methanol (Me), 70% ethanol (Et), and distilled water (w)). As part of optimization studies in biosynthesis, extraction conditions were carried out using 70% methanol, 70% ethanol, and water as solvents, at 60°C, and with a sequential extraction method lasting 1 hour and three consecutive extractions. The obtained extracts were filtered through Whatman blue band filter paper. Until the analysis stage, it was stored at – 20°C (19).

2.3. Synthesis of AgNPs

For the synthesis of silver nanoparticles, a 500 mL aqueous solution with a concentration of 1 mM was prepared from solid $AgNO_3$. 40 mL of this solution was taken and used in an incubator to mix with 40 mL of plant extract (70% methanol (AgNPMe), 70% ethanol (AgNPEt), and distilled water (AgNPw) in a 250 mL Erlenmeyer flask. It was left to react at room temperature for 24 hours in a thermal shaker at 37 °C (BIOSAN TS-100). Then, the samples were centrifuged at 5000 rpm for 20 minutes. The supernatant was discarded,and the pellet was washed once with distilled water. At the end of the process, the obtained AgNPs were transferred to beakers and dried in an oven at 80°C for 24 hours. The dried AgNPs were scraped and transferred to sterile tubes, and stored covered with aluminum foil to prevent light exposure (20).

2.4. UV-visible spectral analysis

The absorbance spectrum of the green synthesized AgNPs was analyzed in the 300−600 nm range using UV-vis spectroscopy (Shimadzu, UV-1601 spectrophotometer, Japan) (21).

2.5. Determination of TPC in extracts and AgNPs

TPC of extracts and AgNPs was determined with Folin-Ciocalteu modified method, using gallic acid (GA) as a standard phenolic compound (19). 100 µL of each sample was mixed with 4 mL of distilled water and 100 µL of Folin-Ciocalteu reagent. Then, 100 µL of 6% sodium carbonate solution was added. After the mixtures were incubated for 30 minutes, their absorbance values were measured using a spectrophotometer (Shimadzu UV-1601 spectrophotometer, Japan) within the 685–760 nm wavelength range (19). The calibration curve was prepared using a gallic acid standard in the concentration range of 62.5–1000 µM ($y = 0.0026x – 0.0563$, $R^2 = 0.9978$), and the results were expressed as gallic acid equivalents (GAE/mL) (Fig 1). All measurements were taken three times.

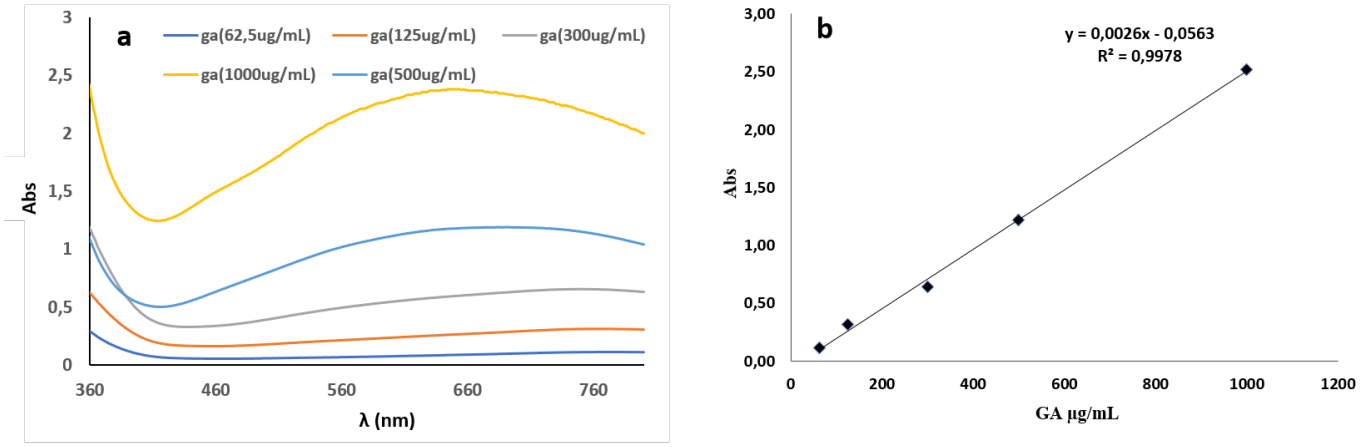

Figure 1. (a) Absorption spectra of GA at different concentrations and (b) GA calibration curve

2.6. Antimicrobial activity

The potential of plant extracts for antimicrobial activity was determined by using the agar well diffusion method. Additionally, minimum inhibitory concentrations (MICs) have been determined for the extracts. Subsequently, the extracts' minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) values were also determined.

2.6.1. Agar Well Diffusion Test

In our study, the following bacterial strains were used: *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606, and *Klebsiella pneumoniae* ATCC 4352; and the following yeast strains were: *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 90018, and *Candida tropicalis* KUEN 1021.

Bacteria were cultured on Mueller-Hinton agar (MHA) (Merck), and Yeasts on Sabouraud Dextrose Agar (SDA) (Merck), and incubated at 37°C for 24 hours. After incubation, microorganism suspensions were prepared from colonies formed in a 0.85% NaCl physiological saline (PSS) solution. Bacterial suspensions were adjusted to 108 CFU/mL, and yeast suspensions were adjusted to 10^6 CFU/mL based on a McFarland 0.5 standard turbidity value. Microorganism suspensions were spread onto the surface of MHA for bacteria and SDA for yeasts under aseptic conditions using sterile swab sticks, followed by the creation of 5 mm diameter wells on the surface of the medium using sterile punches. 50 µL (50 mg/mL) of extract dissolved in suitable solvents was added to the wells. Additionally, meropenem (10 µg/well) was used for bacteria, and amphotericin B (100 µg/well) as a positive control for yeast, DMSO as a solvent, and FTS as a negative control. The inoculated petri dishes were incubated at 37°C for 18-24 hours for bacteria and at 35°C for 24-48 hours for yeast, and the inhibition zones were measured at the end of the incubation period. The experiments were conducted in triplicate, and the average values were taken (22-24).

2.6.2. Determination of MIC for bacteria

MIC determination for bacteria was performed in accordance with Clinical and Laboratory Standards Institute (CLSI) standards. Cation-adjusted Mueller-Hinton Broth (CAMHB) was used as the culture medium. A bacterial suspension was prepared from colonies in an overnight bacterial culture according to the McFarland 0.5 turbidity standard and then diluted to a final inoculum concentration of 5×10^5 CFU/mL. 100 µL of CAMHB was distributed into sterile U-bottom microplates. Soluble extracts were placed in the first wells at 100 µL, and serial dilutions were made accordingly. Subsequently, 5 µL of bacterial suspension was added to the wells containing the extract, and the plates were incubated at 37°C for 24 hours (25).

2.6.3. Determination of the MIC for yeasts

The dilutions of the tested extracts were prepared in RPMI-1640 medium in U-bottom microdilution plates. The tested yeasts were inoculated onto SDA medium and incubated at 37°C for 24 hours. Subsequently, suspensions were prepared from the cultures in RPMI to a McFarland 0.5 turbidity. 100

µL of each suspension was taken and placed into the relevant wells. The prepared plates were incubated at 37°C for 24-48 hours. After a 24-hour incubation period, the plates were evaluated, re-incubated, and then re-evaluated at 48 hours.

At the end of the incubation period for bacteria and yeasts, the lowest extract concentrations at which no visible growth was observed were determined as MIC. Similarly, CAMHB, DMSO, and RPMI were used as negative controls, while meropenem and amphotericin B were used as positive controls (25-27).

2.6.4. Determination of minimum bactericidal and fungicidal concentrations

To determine the MBC and MFC values of plant extracts, 5 µL from each well of the microplate was seeded onto MHA for bacteria and SDA for yeast.. The Petri dishes were then incubated at 37°C for 24 hours for bacteria and 48 hours for yeasts. Finally, after incubation, the lowest dose of bacteria where no growth occurred was evaluated as MBC and in yeasts as MFC (28).

3. Results

3.1. TPC of extracts and AgNPs

The TPC of *P. laurocerasus* L. and *C. spinosa* L. extracts and their corresponding silver nanoparticles (AgNPs) was determined using the Folin–Ciocalteu method, and the results are presented in Table 1.

Table 1. TPC of plant extracts and AgNPs by the Folin-Ciocalteau method

Sample	Total phenolic content*	Sample	Total phenolic content*
P. laurocerasus-Me	181 ± 1.22	*C. spinosa* – Me	399 ± 2.01
P. laurocerasus-Et	113 ± 0.97	*C. spinosa* – Et	320 ± 1.29
P. laurocerasus-w	124 ± 1.11	*C. spinosa*-w	273 ±1.86
P. Me-AgNP	29 ± 0.88	*C.* Me-AgNP	340 ± 1.63
P. Et-AgNP	26 ± 0.93	*C.* Et-AgNP	123 ± 1.56
P. W-AgNP	24 ± 1.03	*C.* W-AgNP	77 ± 1.27

* mg gallic acid equivalent/ g dry plant±SD (n = 3); P. laurocerasus-Me and C. spinosa – Me: methanol extract; P. laurocerasus-Et and C. spinosa – Et: ethanol extract; P. laurocerasus-w and C. spinosa-w: water extract; P. Me-AgNP and C. Me-AgNP: AgNP obtained from methanol extract; P. Et-AgNP and C. Et-AgNP: AgNP obtained from ethanol extract; P. w-AgNP and C. w-AgNP: AgNP obtained from water extract.

The analysis revealed that *C. spinosa* exhibited significantly higher phenolic content in all solvent extracts compared to *P. laurocerasus*. The methanol extract of *C. spinosa* showed the highest TPC, followed by the ethanol and water extracts. In contrast, *P. laurocerasus* displayed considerably lower values, with 181, 113, and 124 mg GAE/g for methanol, ethanol, and water extracts, respectively. The choice of solvent also influenced extraction efficiency. For both plants, methanol proved to be the most effective solvent for phenolic recovery, followed by ethanol, with water being the least efficient. This observation is consistent with previous reports that methanol, due to its polarity, facilitates the extraction of a broader spectrum of phenolic compounds compared to other solvents.

Following the biosynthesis of AgNPs using plant extracts, a marked reduction in phenolic content was observed. For *P. laurocerasus*-derived nanoparticles, the TPC values decreased sharply. This substantial decrease suggests that a majority of the phenolic compounds were consumed during nanoparticle synthesis, likely acting as reducing and stabilizing agents. Methanol, ethanol, and water mediated AgNPs of *C. spinosa* and *P. laurocerasus* showed lower but still phenolic content. These results indicate that while phenolics participate in nanoparticle formation, *C. spinosa* and *P. laurocerasus* possess a capacity to transfer and preserve phenolic compounds within the nanoparticle system.

3.2. UV-visible spectral analysis

UV-Vis absorption spectra of AgNPs synthesized with *C. spinosa* and *P. laurocerasus* extracts demonstrate their behavior in dimethyl sulfoxide (DMSO), a widely used polar aprotic solvent (Fig 2). The presence of a distinct surface plasmon resonance (SPR) band in the 400–450 nm range – typical for AgNPs – and the absence of broad secondary peaks suggest that the particles remain largely dispersed and stable in DMSO rather than precipitating or aggregating. In the figure, the main band appears only slightly shifted, which implies that the phenolic compounds from the *C. spinosa* extract are still attached and stabilizing the particles in DMSO (29).

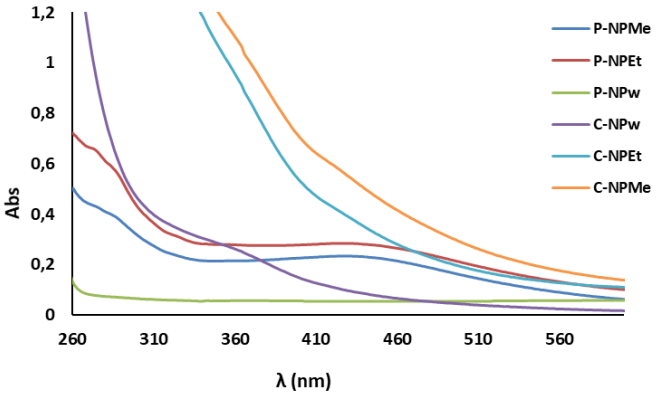

Figure 2. Spectra of AgNPs synthesized from *C. Spinosa* and *P. laurocerasus* extracts in DMSO

3.3. Antimicrobial activity

The results of the agar well diffusion assay to determine the antimicrobial effects of *C. spinosa* L. and *P. laurocerasus* extracts are presented in Tables 2 and 3. *C. spinosa* extracts and their AgNPs showed stronger antimicrobial effects across most tested microorganisms than those of *P. laurocerasus*. This trend is consistent with the higher total phenolic content previously observed in *C. spinosa*, which may contribute synergistically to antimicrobial activity. AgNPs obtained from both plant extracts exhibited inhibitory activity against the tested microorganisms, but the degree of activity varied depending on the plant species and the solvent used. In general, *C. spinosa* extracts exhibited broader and more potent antimicrobial activity than *P. laurocerasus* extracts. The AgNPs showed activity against both Gram-positive and Gram-negative bacteria, as well as fungi, although the degree varied. Generally, Gram-negative bacteria (with an additional outer membrane) were slightly more resistant than Gram-positive strains, but AgNPs still exhibited significant inhibitory effects.

Table 2. The agar well diffusion assay to determine the antimicrobial effects of *C. spinosa* L. extracts and AgNPs

Microorganisms	Inhibition Zone Diameter (mm)					
	C. spinosa-Me extract	*C. spinosa* Et-extract	*C. spinosa* Et-extract AgNPs-DMSO	*C. spinosa* Me-extract AgNPs-DMSO	Meropenem	Amphotericin B
***S. aureus* ATCC 29213**	0	0	12.61±0.43	11.81±0.28	34.68±0.29	-
***S. epidermidis* ATCC 12228**	0	0	10.48±0.05	10.34±0.05	51.25±0.27	-
***E. faecalis* ATCC 29212**	0	0	12.03±0.08	10.59±0.17	19.87±0.44	-
***E. coli* ATCC 25922**	0	0	11.74±0.07	11.79±0.24	35.06±0.15	-
***P. aeruginosa* ATCC 27853**	0	0	11.68±0.06	13.02±0.31	32.47±0.19	-
***A. baumannii* ATCC 19606**	0	0	11.00±0.09	12.05±0.17	32.50±0.24	-
***K. pneumoniae* ATCC 4352**	0	0	12.24±0.11	11.74±0.39	33.24±0.13	-
***C. albicans* ATCC 10231**	0	0	8.37±0.07	9.17±0.06	-	26.97±0.18
***C. parapsilosis* ATCC 90018**	0	0	10.33±0.11	12.48±0.31	-	24.26±0.13
***C. tropicalis* KUEN 1021**	0	0	8.15±0.07	8.94±0.47	-	19.58±0.21

Table 3. The agar well diffusion assay to determine the antimicrobial effects of *P. laurocerasus* extracts and AgNPs

Microorganisms	Inhibition Zone Diameter (mm)					
	***P. laurocerasus* – Me extract**	***P. laurocerasus-Et* extract**	***P. laurocerasus-Et* extract AgNPs-DMSO**	***P. laurocerasus* –Me extract AgNPs-DMSO**	**Meropenem**	**Amfoterisin B**
***S. aureus* ATCC 29213**	0	0	10.17±0.09	9.49±0.26	36.71±0.57	-
***S. epidermidis* ATCC 12228**	0	0	10.65±0.44	17.14±0.03	50.17±0.08	-
***E. faecalis* ATCC 29212**	0	0	13.85±0.58	9.96±0.48	18.97±0.63	-
***E. coli* ATCC 25922**	0	0	10.61±0.37	10.32±0.16	34.81±0.24	-
***P. aeruginosa* ATCC 27853**	0	0	13.02±0.04	13.06±0.02	33.29±0.18	-
***A. baumannii* ATCC 19606**	0	0	9.91±0.63	9.61±0.27	32.96±0.34	-
***K. pneumoniae* ATCC 4352**	0	0	10.84±0.15	9.76±0.38	33.07±0.03	-
***C. albicans* ATCC 10231**	9.66±0.18	8.17±0.06	11.93±0.39	11.01±0.03	-	27.43±0.32
***C. parapsilosis* ATCC 90018**	0	0	10.35±0.28	10.32±0.17	-	24.31±0.26
***C. tropicalis* KUEN 1021**	0	0	8.71±0.59	8.92±0.45	-	19.37±0.21

The antimicrobial potency of *C. spinosa* L. and *P. laurocerasus* L. extracts and their AgNPs was evaluated through the determination of MIC and Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC). The results are summarized in Tables 4 and 5.

Table 4. Minimum Bactericidal and Fungicidal Concentrations (MBC/MFC) and MIC of *C. spinosa* extracts and AgNPs against various control strains (mg/mL)

Microorganisms	***C.spinosa-Me* extract**		***C.spinosa* – Et-extract**		***C.spinosa*-Et extract AgNPs-DMSO**		***C.spinosa*-Me extract AgNPs-DMSO**		**Meropenem**		**Amphotericin B**	
	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC	MIC	MFC
***S. aureus* ATCC 29213**	0.11	> 6.5	0.11	> 6.5	0.05	> 3.4	0.05	> 3.4	2	4	-	-
***S. epidermidis* ATCC 12228**	0.23	> 6.5	0.43	> 6.5	0.10	> 3.4	0.10	> 3.4	0.25	0.50	-	-
***E. faecalis* ATCC 29212**	0.11	> 6.5	0.21	> 6.5	0.10	> 3.4	0.10	> 3.4	8	16	-	-
***E. coli* ATCC 25922**	0.45	> 6.5	0.85	> 6.5	0.20	> 3.4	0.20	> 3.4	0.06	0.12	-	-
***P. aeruginosa* ATCC 27853**	0.11	> 6.5	0.21	> 6.5	0.10	> 3.4	0.10	> 3.4	0.5	2	-	-
***A. baumannii* ATCC 19606**	0.23	> 6.5	0.43	> 6.5	0.10	> 3.4	0.05	> 3.4	2	4	-	-
***K. pneumoniae* ATCC 4352**	0.23	> 6.5	0.43	> 6.5	0.20	> 3.4	0.20	> 3.4	0.5	2	-	-
***C. albicans* ATCC 10231**	0.11	0.23	0.43	0.85	0.05	0.10	0.05	0.10	-	-	1	2
***C. parapsilosis* ATCC 90018**	0.23	> 6.5	0.43	> 6.5	0.03	0.10	0.05	0.10	-	-	1	2
***C. tropicalis* KUEN 1021**	0.11	0.23	0.21	0.21	0.20	0.20	0.42	0.84	-	-	1	2

MIC: Minimum Inhibitory Concentration, *MBC: Minimum Bactericidal Concentration, *MFC: Minimum Fungicidal Concentration

Table 5. Minimum Bactericidal and Fungicidal Concentrations (MBC/MFC) and MIC of *P. laurocerasus* extracts and AgNPs against various control strains (mg/mL)

Microorganisms	*P. laurocerasus*-Me extract		*P. laurocerasus* – Et extract		*P. laurocerasus-Et* extract AgNPs-DMSO		*P. laurocerasus-Me* extract AgNPs-DMSO		Meropenem		Amfoterisin B	
	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC	MIC	MFC
***S. aureus* ATCC 29213**	0.04	>1.2	0.09	> 1.4	0.063	> 2.016	0.063	> 2.016	2	4	-	-
***S. epidermidis* ATCC 12228**	0.15	>1.2	0.35	> 1.4	0.25	> 2.016	0.25	> 2.016	0.25	0.50	-	-
***E. faecalis* ATCC 29212**	0.15	> 1.2	0.18	> 1.4	0.13	> 2.016	0.13	> 2.016	8	16	-	-
***E. coli* ATCC 25922**	0.15	> 1.2	0.70	> 1.4	0.13	> 2.016	0.13	0.25	0.06	0.12	-	-
***P. aeruginosa* ATCC 27853**	0.15	> 1.2	0.18	> 1.4	0.13	> 2.016	0.13	> 2.016	0.5	2	-	-
***A. baumannii* ATCC 19606**	0.02	> 1.2	0.02	> 1.4	0.016	> 2.016	0.031	> 2.016	2	4	-	-
***K. pneumoniae* ATCC 4352**	0.15	> 1.2	0.18	> 1.4	0.13	> 2.016	0.13	> 2.016	0.5	2	-	-
***C. albicans* ATCC 10231**	> 1.4	> 1.2	> 1.4	> 1.4	0.13	0.25	0.13	0.25	-	-	1	2
***C. parapsilosis* ATCC 90018**	0.04	> 1.2	0.04	> 1.4	0.031	> 2.016	0.031	> 2.016	-	-	1	2
***C. tropicalis* KUEN 1021**	0.04	0.08	0.09	0.18	0.25	0.50	0.25	0.50	-	-	1	2

MIC: Minimum Inhibitory Concentration, *MBC: Minimum Bactericidal Concentration, *MFC: Minimum Fungicidal Concentration

Across all microorganisms tested, *C. spinosa*-derived AgNPs generally displayed lower MIC values compared to *P. laurocerasus*-derived AgNPs, in agreement with their higher phenolic content and stronger activity observed in agar diffusion assays. The methanol and ethanol extracts of *C. spinosa* exhibited inhibitory activity against all tested bacterial and fungal strains, with MIC values ranging from 0.11 to 0.85 mg/ml. However, their MBC/MFC values were generally greater than 6.5 mg/mL. *P. laurocerasus* extracts also showed inhibitory activity, with MIC values ranging from 0.02 to 0.70 mg/mL. Similar to *C. spinosa*, MBC/MFC values for the extracts were generally high (>1.2–1.4 mg/mL). The AgNPs synthesized from *C. spinosa* extracts demonstrated markedly improved antimicrobial activity. MIC values for AgNPs were significantly lower (0.03–0.20 mg/mL), and in several cases, fungicidal concentrations were achieved at similarly low levels. The antimicrobial performance of *P. laurocerasus*-derived AgNPs was significantly enhanced. MIC values ranged from 0.016 to 0.25 mg/mL, and several fungal strains demonstrated low MFC values (e.g., *C. albicans* 0.13/0.25 mg/mL; *C. tropicalis* 0.25/0.50 mg/mL). The results demonstrate that while plant extracts alone provide inhibitory activity, their conversion into AgNPs dramatically enhances antimicrobial potency, reducing MIC values and enabling bactericidal/fungicidal effects at lower concentrations.

4. Discussion

Plant extracts rich in bioactive compounds have recently been used in the green synthesis of NPs. The potential of biomolecules present in plant extracts to reduce metal ions to NPs is very important in the green synthesis process. Therefore, this study focused on the synthesis of AgNPs by reducing silver ions present in silver nitrate solution in the extractions of *C. spinosa* and *P. laurocerasus* with different solvents. The green method was developed for the synthesis of antioxidant and bactericidal AgNPs. For this, three solvents were used as reducing and capping agents for the synthesis of AgNPs.

AgNPs stabilized with *C. spinosa* and *P. laurocerasus* extract were synthesized with 1 mg/mL extract concentration and 1 mM $AgNO_3$ for 24 h at room temperature, and their spectra were taken. Based

on the knowledge that silver nanoparticles exhibit a yellowish-brown color in water, the reduction of silver ions to silver nanoparticles was confirmed by UV-Visible spectroscopy analysis. According to the obtained spectra, the faint SPR band in the AgNP water spectrum indicated that fewer phytochemicals could be synthesized from the NP during water extraction. These results showed that the antioxidant effect varies depending on the solvent, with the highest effect observed in ethanolic extracts, followed by methanol and aqueous extracts (ethanol>methanol>water). The enhanced biological performance of AgNPs synthesized using ethanol extracts can be attributed to the distinct phytochemical composition and extraction selectivity of ethanol as a solvent. Ethanol, with its intermediate polarity, effectively solubilizes a broad range of moderately polar phenolics, including flavonoids (e.g., quercetin, kaempferol, catechin), phenolic acids (e.g., gallic, caffeic, and ferulic acids), and tannins, which possess strong reducing and metal-chelating capacities. These compounds readily donate electrons to Ag^{+} ions, accelerating their reduction to metallic Ag^{o} nuclei, and simultaneously serve as natural capping and stabilizing agents. Consequently, the resulting nanoparticles exhibit improved surface stability, uniformity, and bioactivity. In contrast, methanol and water tend to extract either a wider range of highly polar compounds or fewer lipophilic phenolics, leading to less efficient nanoparticle nucleation and weaker biological activity (30). In this context, they indicate that the extraction solvent plays a critical role in both the yield of phenolic compounds and their binding potential to the AgNPs surface. Furthermore, it demonstrates that the phenolic compounds present in the extracts during biosynthesis not only act as reducing agents in the formation of nanoparticles but also directly affect the antioxidant properties of the synthesized particles. Beyond the contribution of residual phenolics, the antioxidant activity of the synthesized AgNPs can also be explained by intrinsic nanoparticle mechanisms. The high surface-area-to-volume ratio of AgNPs facilitates redox interactions with reactive oxygen species (ROS), enabling electron transfer and radical neutralization at the nanoparticle interface. Additionally, the adsorbed phytochemicals on the nanoparticle surface may enhance this catalytic process by forming a synergistic redox couple between the AgNP core and the organic layer. This dual mechanism — involving both phytochemical-derived surface functionalization and AgNP-mediated electron transfer — explains the persistent antioxidant effect observed even after the reduction in total phenolic content post-synthesis. Therefore, the superior performance of ethanol-derived AgNPs likely results from a combination of optimized phytochemical composition, effective surface stabilization, and enhanced catalytic redox activity (31,32).

Agar well diffusion experiments revealed that both methanol and ethanol extracts of *C. spinosa* L. and *P. laurocerasus* L. showed no inhibitory activity against the tested bacterial strains. In contrast, AgNP-extract combinations exhibited substantial inhibition zones aganist both Gram-positive and Gram-negative bacteria (Tables 2, 3). Accordingly, it was observed that *C. spinosa* L. AgNPs prepared with Et-DMSO and Me-DMSO extracts had inhibition zones ranging from 10.34 to 13.02 mm. The greatest inhibition was observed against *S. aureus* (12.61 ± 0.43 mm) and *K. pneumoniae* (12.24 ± 0.11). Similarly, *P. laurocerasus* L. AgNP conjugates exhibited broad-spectrum antibacterial activity with inhibition zones ranging from 9.49 to 17.14 mm. Specifically, *S. epidermidis* showed the highest sensitivity (17.14 ± 0.03 mm with Me-AgNPs-DMSO). Additionally, significant antifungal activity was observed between the two plants in our study. While *C. spinosa* extracts alone showed no inhibition zone against *Candida* species, both the methanol and ethanol extracts of *P. laurocerasus* exhibited limited activity against *C. albicans* (9.66 ± 0.18 mm and 8.17 ± 0.06 mm, respectively). When combined with AgNPs, both plants showed enhanced antifungal effects. *C. spinosa* AgNP extracts also showed antifungal activity against three *Candida* species, with inhibition zones ranging from 8.15 to 12.48 mm. The highest activity was recorded against *C. parapsilosis* (12.48 ± 0.31 mm with Me-AgNPs-DMSO). *P. laurocerasus*, AgNP combinations, showed antifungal activity against all three *Candida* species with inhibition zones ranging from 8.71 to 11.93 mm, and were most effective against *C. albicans* (11.93 ± 0.39 mm with Et-AgNPs-DMSO). Although the antifungal activity of both plant-based AgNPs was lower than that of amphotericin B (19.37-27.43 mm), the observed zones of inhibition clearly indicate that AgNP conjugation enhanced the extracts' weak antifungal potential. The inclusion of AgNPs significantly improved the antimicrobial properties of both extracts, providing consistent inhibition

against Gram-positive and Gram-negative bacteria, as well as *Candida* species. Although both plant-based AgNPs generally showed stronger and more balanced antibacterial activity against bacterial strains, *P. laurocerasus* AgNPs had the highest single inhibition value (17.14 ± 0.03 mm) against *S. epidermidis*. In terms of antifungal activity, *C. spinosa* AgNPs were more effective against *C. parapsilosis*, while *P. laurocerasus* AgNPs showed slightly better activity against *C. albicans*.

The MIC and MBC values determined by the microdilution method also support these findings (Table 4,5). The MIC and MBC values of plant extracts in their methanol and ethanol forms used alone were found to be significantly higher compared to the AgNP-supported forms. This situation reveals that the amount of extract required to completely stop microbial growth and achieve a bactericidal effect is significantly reduced by AgNPs. A similar trend has also been observed in the yeast species. Both the MIC and MFC values of AgNP-enriched extract against *C. albicans* and *C. parapsilosis* were significantly lower compared to the extracts alone. For example, the MIC value for *C. albicans* is only 0.43 mg/mL for the ethanol extract alone, while this value decreased to 0.05 mg/mL in the AgNP-ethanol combination. These findings support the synergistic effect of AgNPs against fungal pathogens as well. Overall, the data obtained from both diffusion and microdilution tests clearly show that *C. spinosa L*. and *P. laurocerasus* extracts are insufficient on their own in terms of antimicrobial effect, but their efficacy against both bacteria and yeasts is significantly increased when combined with AgNPs. These results suggest that AgNPs could be an essential strategy for enhancing the biological activity of plant compounds.

These findings clearly demonstrate that *C. spinosa* and *P. laurocerasus* extracts alone are largely ineffective against tested bacterial and yeast strains. However, their conjugation with AgNPs results in a significant improvement of antimicrobial activity. The observed enhancement can be attributed to the synergistic interaction between silver nanoparticles and phytochemicals adsorbed on their surface, which facilitates binding and improves antimicrobial efficacy.

5. Conclusion

This study demonstrated that the extract of *C. spinosa* and *P. laurocerasus* significantly enhances the antioxidant and antibacterial potential of AgNPs, making it a suitable and biocompatible medium for their synthesis. AgNPs produced with *C.spinosa* and *P. laurocerasus* extract exhibited strong antibacterial effects against all tested bacterial and yeast strains. Moreover, the biosynthesized AgNPs displayed notable antioxidant activity at concentrations effective against bacterial growth. These findings emphasize the promise of eco-friendly AgNPs synthesized in the presence of *C. spinosa* extract for diverse biomedical applications.

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