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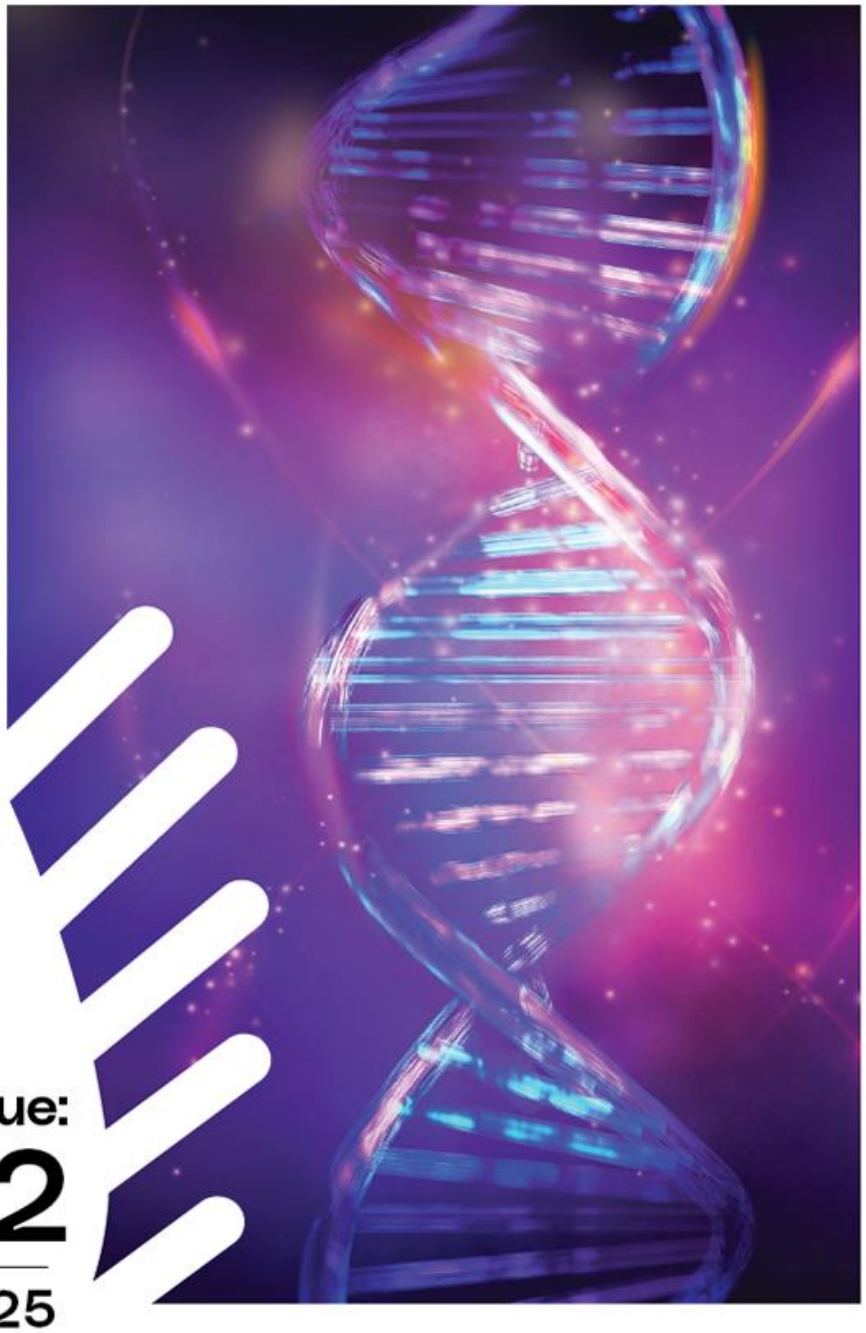

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REVIEW

Genetic polymorphism and current biotechnology approaches of therapeutic aspects within endometrial tumors

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Abstract

Among oncological diseases of women, gynecological diseases deserve special attention. Gynecological tumors contribute significantly to women's health throughout the world. Notably, gynecologic malignancies represent a prevalent category of cancers affecting women globally. Single nucleotide polymorphisms have emerged as a promising source of genetic information to better understand complex diseases such as cancer, in terms of etiology, interindividual differences and treatment response. In this review, we summarize some selected gene single nucleotide polymorphisms' implication in gynecological cancer susceptibility/predisposition, as well as the potential to use such genetic markers for improved diagnosis and individualized treatment of gynecological cancers. Furthermore, this review explores the advances in biotechnology that have contributed to the management of gynecological tumors, in particular endometrial tumors, with a focus on molecular diagnostics, therapeutic innovations, and personalized medicine. It is critical to investigate the single nucleotide gene polymorphisms associated with gynecologic cancer susceptibility/predisposition as some of them might be utilized as useful molecular markers for assessing gynecologic cancer predisposition and might be further used for diagnosis and treatment modalities in individuals with similar single nucleotide polymorphism profile. Moreover, recent advancements in biosensing technologies, particularly nano-biosensors

and microfluidic biosensors, have significantly enhanced the detection of gynecological tumor markers. Taken together, the revolution in cancer research, diagnosis, and treatment has been made possible by advances in biotechnology in recent decades.

Keywords: gynecological tumors, endometrial tumors, gene, single nucleotide polymorphism, biosensing

Introduction

Among oncological diseases of women, gynecological diseases deserve special attention. Notably, gynecologic malignancies represent a prevalent category of cancers affecting women globally, characterized by their diverse nature in terms of risk factors, management approaches, and outcomes (Bray et al., 2018). Ovarian cancer stands as the primary contributor to mortality linked to gynecologic malignancies and ranks as the fifth leading cause of death among women (Arora et al., 2023). Predominantly affecting postmenopausal women, the majority of ovarian cancer cases surface in individuals aged between 50 and 75 years (Momenimovahed et al., 2019). On a parallel note, endometrial cancer (EC) holds the position of the fourth most prevalent cancer in women. Its prevalence is notably heightened in postmenopausal females, with a peak incidence occurring between ages 50 and 70 (Makker et al., 2021). The risk factors associated with endometrial cancer encompass heightened exposure to estrogen, stemming from either endogenous sources (such as obesity) or exogenous influences (such as estrogen replacement therapy). In general, among the reasons for the development of woman's tumors (both .0benign, malignant), various factors are considered, among which hormonal imbalance (Nakashidze et al., 2014), the alteration of lipid profile (Kotrikadze, et al., 2019a), alteration of gene expression and alteration of the micrRNA (Petrovic et al., 2017; Shaikh et al., 2023; Kotrikadze, et al., 2019b) (Kotrikadze, et al., 2019c) (Kotrikadze et al., 2020). Moreover, women carrying the hereditary nonpolyposis colorectal cancer syndrome, commonly (Petrović et al., 2021) known as Lynch syndrome, confront a significantly elevated lifetime risk of developing uterine cancer (Endometrial Cancer Prevention (PDQ®) - NCI, 2023). This heightened risk underscores the need for regular screening and the implementation of risk reduction strategies in this specific population. Cervical cancer, with its alarming worldwide incidence and mortality rates, poses a substantial burden of disease globally. As the fourth leading cause of cancer-related deaths worldwide, with highest fatalities concentrated in developing countries (cervical cancer, n.d.). A clear and direct causal link exists between human papillomavirus (HPV) (Fernandes et al. 2022), the precursors to cancer, and the onset of cervical cancer. Notably, the high-risk HPV serotypes 16 and 18 contribute to more than 70% of cervical cancer cases among the approximately 100 identified HPV strains (Ahmed et al., 2017). Besides of mentioned, the another factors, including age (Liu et al., 2023a, Hitchins et. al. 2023), cigarette smoking, immunosuppression, and nutritional considerations, serve as predictive indicators for the advancement of HPV infection to cervical intraepithelial neoplasia (CIN) and subsequent invasive stages (Hewavisenti et al., 2023). According to the literature, the SNP gene is associated with numerous diseases (Castellanos-Rubio & Ghosh, 2019; Leitão et al., 2023; Nakashidze & Ahmad, 2019) including oncological disorders (Nakashidze et al., 2020).

In this review article, we summarize some selected Gene SNP's implications in gynecological cancers to understand disease genetic predisposition. In particular, this review focuses on the genetic polymorphism significance in the diagnosis (including early diagnosis) and treatment of gynecological cancers, which could further introduce single nucleotide polymorphisms (SNPs) as

tools to develop early intervention strategies. As gynecological tumors contribute significantly to women's health throughout the world, therefore it is important to develop new approaches. Notably, the revolution in cancer research, diagnosis, and treatment has been made possible by advances in biotechnology in recent decades. This review also explores the advances in biotechnology that have contributed to the management of gynecological tumors, with a focus on molecular diagnostics, therapeutic innovations, and personalized medicine.

Genetics and epigenetics aspects of the gynecological tumors

As is already widely recognized, the carcinogenesis of endometrial cancer is presently thought to be primarily caused by estrogen exposure (Rodriguez et al., 2019), aberrant mismatch repair (MMR) system (Ouh et al., 2024) genetic abnormalities (Marković et al., 2024), and incorrect DNA and microRNA methylation (Zmarzły et al., 2023) Based on clinicopathological features, endometrial cancer is categorized as type I or type II. Atypical endometrial hyperplasia, the mechanism by which type I endometrial cancer operates in an estrogen-dependent way, is usually more common in premenopausal or perimenopausal women.These tumors have minimal muscle invasion, well-differentiated endometrioid adenocarcinoma, reduced rates of lymph node metastases, and a generally good prognosis. They also show positive for estrogen and progesterone receptors. On the other hand, type II endometrial cancer operates independently of estrogen and typically presents in postmenopausal women. De novo carcinogenesis, or the direct development of cancer from a normal endometrium, is thought to be the cause, as opposed to endometrial hyperplasia or undetected precancerous lesions (Banno et al., 2014; Fan et al., 2021; Arciuolo et al., 2022; Galant et al., 2024). Research demonstrating variations in molecular markers based on histology supports the existence of at least two overarching classes of endometrial carcinoma (Arafa et al., 2010; Lim & Oliva, 2010; Galant et al., 2024). Numerous pathologists and gynecologists have adopted the perspective that there are at least two primary biological types of endometrial cancer, and potentially more. However, most epidemiological studies have evaluated risk factors for endometrial cancer as a whole, predominantly reflecting the risks associated with the prevalent type I tumors, particularly in predominantly Caucasian populations (Felix et al., 2010). Consistently, registry data indicates that type II cancers tend to occur more frequently in older women and those from non-white ethnic backgrounds (Lm et al., 2011; Katagiri et al., 2023; Wakkerman et al., 2024). Furthermore, certain epidemiological studies have revealed that type II cancers are less strongly associated with traditional type I risk factors, such as obesity (Wang et al., 2023), and hormonal factors (Sherman et al., 1997; Yang et al., 2013; Nees et al., 2022; Yang et al., 2024).

A steroid hormone essential to the development of the female reproductive organs (Deli et al., 2020), estrogen binds to cytoplasmic estrogen receptors (ER) to form dimers and control nuclear gene expression. In addition to ligand-independent AF-1 and ligand-dependent AF-2 transcriptional activation domains, ER has DNA-binding and ligand-binding domains. The balance of these domains varies across tissues (Metzger et al., 1995). Miyamoto et al. highlighted the significance of MMR deficiency in early-stage endometrial cancer. They found a positive correlation between blood estrogen levels and the expression of important MMR proteins, hMLH1 and hMSH2. Higher estrogen levels corresponded to increased MMR activity, suggesting a lower likelihood of cancer in a high estrogen-exposed environment. Conversely, low estrogen levels were associated with reduced repair activity, potentially increasing the risk of genetic errors and carcinogenesis, particularly during perimenopausal

conditions with sufficient estrogen but low MMR activity (McInerney & Katzenellenbogen, 1996; Miyamoto et al., 2006; Pierre et al., 2024). Various genes contribute to the development of the two types of endometrial cancer. Type I mutations involve PTEN, ß-catenin, and K-ras (Kanaya et al., 2005; Koo et al., 2022). PTEN, located on chromosome 10, serves as a tumor suppressor in several disorders and cancers, inducing apoptosis when functioning properly. Mutations in PTEN occur in 20-50% of endometrial cases, often associated with early-stage carcinogenesis (Saegusa et al., 2001; Khatami et al., 2023). B-catenin mutations (20-40% of type I cases) disrupt cell adhesion and contribute to early carcinogenesis, while K-ras mutations (10-31% of cases) play a role in both transition to cancer and invasive growth. In type II endometrial cancer, mutations in the HER-2/neu oncogene and the p53 tumor suppressor gene are prevalent. HER-2/neu mutations (present in 14-63% cases) inversely correlate with cellular differentiation. P53 mutations are found in 90% of type II cases but are less common in type I and hyperplasia. They contribute significantly to early-stage development and are associated with poorly differentiated cancer regions. Additionally, RB and c, with RB mutations associated with disease advancement and cyclin overexpression influencing cell cycle regulation and prognosis (Moreno-Bueno et al., 2003). The study analyzing 125,748 exomes from various databases confirms that MUTYH-associated genetic variants are linked to East Asian populations (Park et al., 2024). The investigation of HDR and MMR germline variants in cervical cancer has shown that MMR-related gene variants are linked to adenocarcinomas. This study suggests that targeting MMR genetics could enhance future therapeutic management (Kokemüller et al., 2025). According to another study, 308 somatic mutations were detected (56 mutations based on the analysis of 24 genes). Notably, functional mutations increase with age (Pandya et al., 2024).

Epigenetic control of gene expression involves DNA methylation, histone modification, and the activity of Polycomb group proteins. DNA methylation, crucial for genomic stability, occurs at CpG sequences during cell division (Goldberg et al., 2007). Common epigenetic changes involve DNA methylation and histone acetylation. MMR gene methylation is vital, impacting genes such as PTEN, TGF-BR2, IGF2R, and BAX, leading to microsatellite instability (MSI) and cancer development (The Epigenomics of Cancer - PMC, n.d.). In endometrial cancer, genes affected by DNA methylation include hMLH1, SPRY2, RASSF1A, RSK4, APC, CHFR, p73, CASP8, GPR54, CD1, HOXA11, and COMT. Aberrant methylation of these genes influences various cellular processes, such as cell adhesion, apoptosis, and DNA repair, contributing to cancer progression (Gu et al., 2022) . The CpG island methylator phenotype (CIMP) classification system identifies genome-wide methylation patterns, with CIMP-positive endometrial cancer associated with early carcinogenesis (Weisenberger et al., 2006; Yi et al., 2011). Understanding these epigenetic changes offers potential for targeted therapeutic interventions and prognostic markers (Zhang et al., 2011). Epimutation refers to alterations in germ cells' epigenetic patterns that either suppress the transcription of typically active genes, or activate genes which are usually suppressed (Ruiz De La Cruz et al., 2021). Epimutation can serve as the initial stage or a direct trigger for carcinogenesis, with endometrial cancer development possibly linked to epimutation in MMR genes like hMLH1 and hMSH2 (Banno et al., 2014).

Based on the above, the involvement of genetic/epigenetic factors highlights the need for tailored screening and therapeutic approaches that will enable the correct detection of important risk factors (genetic, hormonal, etc.) in the population. Ultimately, the treatment strategy for patients with endometrial cancer will be significantly improved.

Selected single nucleotide gene polymorphisms in gynecological tumors

The clinical implications of eNOS within cervical cancer (CA) carcinogenesis, clinicopathological features, and patient survival were the main topics of a Taiwanese investigation. Patients with cervical cancer who had genotypes TC/CC in eNOS SNP rs2070744 showed a decreased risk of parametrium invasion (OR = 0.16, 95% CI = 0.02-0.75, p = 0.009), pelvic lymph node metastasis (OR = 0.12, 95% CI = 0.01-0.89, p = 0.016), and advanced stage [odds ratios (OR) = 0.30, 95% confidence interval (CI) = 0.09-0.97, p = 0.036]. In summary, among cervical cancer patients, those with genotypes TC/CC in rs2070744, especially in Taiwan, showed a lower incidence of advanced stage, parametrium invasion, and pelvic lymph node metastases (Hung et al., 2019).

Significantly, patients with grade > 2 (p = 0.03) and those with positive cervical invasion (p = 0.042) had a higher distribution of A/A (rs4693608). Patients with T/C (rs4364254), on the other hand, showed a lower tumor grade. According to this study, a robust correlation between the HPSE SNP rs4693608 and susceptibility to endometrial cancer, indicating that HPSE SNPs could potentially serve as biomarkers for prognostic evaluation in endometrial cancer (Cao et al., 2020).

Examining the SNPs (rs389209) of CYP2D6 and (rs2031920, rs6413432, rs6413420) of CYP2E1, a noteworthy finding was made in a case control study evaluating the risk of genetic polymorphisms of CYP2D6 and CYP2E1 in cervical cancer: the variant allele A of CYP2E16 showed a significant increase in cervical cancer cases (OR=4.81; 95% CI: 1.57–14.77; p=0.005). The findings of this study support the hypothesis that among the Indian rural women under investigation, the rs6413432 SNP of CYP2E1*6 increases the risk of cervical cancer (Datkhile et al., 2022).

Examining the Eastern UP population in India retrospectively, the study focused on genetic variations in the upstream region of the transcription start site of BRN3A, acknowledged for its involvement in promoting an anti-apoptotic cellular environment and facilitating epitheliotropic transformations in HPV-mediated cervical cancers. The allele frequency was 1.32 times higher in cancer cases than in control subjects ($\chi 2 = 6.315$, p = 0.012). After removing heterozygous conditions, the odds ratio (OR) analysis showed a significant correlation between cancer risk and the SNP in homozygous (GG) conditions (OR = 2.60, $p \leq 0.004$). The findings suggest the potential utility of this genetic variation in the non-coding region for predicting, diagnosing, or anticipating the progression of the disease (Prakash et al., 2022).

It is suggested that the FOKI and TaqI polymorphisms are associated with CIN2+ (cervical intraepithelial neoplasia (CIN)2+) risk (Li et al., 2022). The study, which concentrated on women from Bangladesh, found that the genotypes A/A and C/A + A/A together are linked to a higher risk of cervical cancer. The purpose of the study was to investigate the relationship between Bangladeshi females' risk of developing cervical cancer and the -160C/A genetic polymorphism in CDH1. The results of the comparative analysis showed that the variant A/A genotype and the combined (C/A + A/A) or 'any A' genotypes showed a 3.80-fold (95% CI=1.150-12.561, P=0.029) and 1.93-fold (95% CI=1.126-3.323, P=0.017) higher risk of developing cervical cancer, respectively, compared to the normal C/C genotype. Additionally, a positive correlation between the incidence of cervical cancer and the -160C allele was found, increasing the risk by 1.81 times (OR= 1.814, 95% CI=1.152-2.857, p=0.01). Interestingly, it was discovered that women with an early marital history (less than 18 years) and the homozygosity of the -160A/A variant were more likely to develop cervical cancer ($\chi 2 = 6.605$,

p=0.037) (Rahman et al., 2023). A meta-analysis study revealed the significance of 42 SNPs in cervical cancer development.

There was a universal observation of a wild-type phenotype in FGFR3 rs121913483 in the female population of southwest China. There was a significant rise in the rs25487 mutation found in the cervical cancer population. A 2-locus SNP-SNP interaction pattern involving rs25487 and rs1042522 showed a significant correlation with the risk of cervical cancer (OR = 4.63, 95% CI = 1.83-11.75; cases vs. elderly group: OR = 17.61, 95% CI = 4.34-71.50). In the female population of southwest China, this study represents the first time that a novel interaction between the XRCC1 and TP53 genes has been found. This interaction is strongly linked to susceptibility to cervical cancer risk (Liu et al., 2019).

The four SNPs for the EXOC1 (rs13117307), BCL2 (rs2279115), CCAT2 (rs6983267), and CARD8 (rs7248320) genes associated with CC. The same study suggests that the SNPs rs13117307, rs2279115, rs6983267 correlate also with some clinical characteristics in CC (Feng et al., 2022). Notably, the robust correlation between ANPGTL4 and the predisposition to CA was established, signifying its potential implication in cervical neoplasia. The ANPGTL4 polymorphism exhibited an association with an elevated risk of developing cervical neoplasia, as evidenced by the dominant model (OR = 12.48, CI = 4.9-31.82, $p < 0.0001$) and the additive model (OR = 30.54, CI = 7.35-126.89, $p < 0.0001$) (Rahmani et al., 2020). According to this study, there is an association between IL6 rs2069837, TGFB1 rs1800469, TLR9 rs187084, MMP7 rs11568818 and CCa ($p < 0.05$) (Wang et al., 2015). Also some immune and inflammatory genes, including the IL1B, IL6, IL10, IL18, TGFB1, CCR5, CD40, TLR9, and MMP7, are associated with CCa (Das et al., 2022).

The study suggests that CYP17A1 gene rs743572 SNP, (CC genotype ($p \leq 0.001$), C allele frequency ($p \leq 0.001$)) also CYP19A1 rs10046 SNP (CT genotype ($p = 0.023$), T allele frequency ($p = 0.015$)) are associated with ovarian cancer. Moreover, the rs743572 and rs10046 SNPs were associated with some clinical characteristics of ovarian cancer. In particular, CYP19A1 rs10046 associated to post-menopausal ovarian cancer. Authors suggest that genes CYP17A1 and CYP19A1 SNPs increase the risk for ovarian cancer development within South Indian Women (Kumar et al., 2022). Ovarian cancer risk is also substantially correlated with H19 rs2107425, miR-146a rs2910164, and miR-196a rs11614913 SNPs (H. Liu et al., 2023). The study found a relationship between the incidence of uterine fibroids (UF) in Caucasian women and the VDR polymorphisms rs731236, rs1544410, and rs2228570. (Uterine Fibroid Incidence and vitamin D Receptor Gene Polymorphisms in Caucasian Women, n.d.).

Thus, the importance of SNP in developing gynaecological tumours within several populations is confirmed. Notably, eNOS genotypes are associated with a reduced risk of advanced stages and metastasis. Additionally, SNPs HPSE, CYP2D6, and CYP2E1 are linked to increased cancer development risk and are suggesting their potential as biomarkers for early detection, etc. These findings suggest further exploration of genetic variations to enhance personalized approaches/ strategies for treating the gynaecological tumour.

Selected gene single nucleotide polymorphisms in endometrial tumors

Endometrial cancer (EC) ranks among the three prominent malignant tumors affecting female reproductive organs, predominantly affecting postmenopausal women (Mahdy et al., 2022). Recent clinical data indicate a global rise in EC incidence, with a noticeable uptick in diagnoses among younger women (Williams et al., 2024).

An elevated risk of EC has been linked to various reproductive factors in women, including late age at menopause, younger age at menarche, nulliparity, infertility, age of birth of the first child, and prolonged use of unopposed estrogens in hormone replacement therapy (Ignatov & Ortmann, 2020). Identifying oncogenes or tumor suppressor genes capable of predicting the malignant potential of EC, holds promise for advancing early clinical detection, treatment strategies, and prognostic assessments.

A study was done to investigate the relationship between the single nucleotide polymorphism (SNP) DACH1 gene and the susceptibility of people who have EC. Compared to T allele carriers, carriers of the C allele at the DACH1 gene rs9529895 locus showed a significantly lower risk for EC (odds ratio = 0.56, 95% confidence interval: 0.38–0.84, P<0.01). The impact of the DACH1 gene rs9529895 locus SNP on the risk for EC was found to be influenced by factors such as age, body mass index, smoking, alcohol consumption, and diabetes. In conclusion, there is a substantial association between the risk for EC and EC patients' progression-free survival (PFS). The underlying mechanism is presumed to involve the modulation of DACH1 expression levels by the DACH1 gene rs9529895 locus SNP (Xu et al., 2020).

A study focused on the impact of resistin gene polymorphisms, specifically 420C > G and 62G > A, on the susceptibility to endometrial cancer (EC), revealed that within the EC group, 420 GC (47.9%) emerged as the predominant gene polymorphism among Resistin 420 profiles. Notably, the investigation into Resistin 62 gene polymorphisms indicated a significantly higher prevalence of the 62GC polymorphism in the EC group ($p < 0.01$). In contrast, the control group exhibited a higher frequency of 62 AG (52.9%), associated with a diminished risk of EC ($p < 0.01$, Odds Ratio: 0.37). Moreover, the alleles 420G+ and 62A+ were notably more prevalent in the EC and control groups, respectively (p: 0.02 and p< 0.01). The presence of the 420G+ allele increased the risk of EC by 1.99 times, while the presence of the 62A+ allele was linked to a reduced risk of EC (p< 0.01, Odds Ratio: 0.038). This study, for the first time, provides evidence that Resistin 420G > C and 62G > A gene polymorphisms play a role in the development of endometrial cancer (Ozgor et al., 2019).

A study examining both codominant and recessive models revealed that HPSE SNP rs4693608 exhibiting GG genotype demonstrated a protective effect against endometrial cancer (adjusted OR = 0.41, 95%CI = 0.21-0.81, p = .026 and adjusted OR = 0.43, 95%CI = 0.22-0.82, p =0.0076, respectively) (Cao et al., 2020).

A significant correlation was found in a Norwegian population study between women who carry the APOBEC3A/B deletion variant and a lower risk of endometrial cancer (OR = 0.75; 95% CI = 0.62-0.91; p = 0.003; dominant model). Within the subgroup of patients with endometrioid endometrial cancer, this correlation held true (OR = 0.64; 95% CI = 0.51-0.79; p = 3.6 × 10-5; dominant model). Those between the ages of 50 and 60 showed the greatest reduction in risk (OR = 0.51; 95% CI = 0.33-0.78; p = 0.002; dominant model). All of these results point to a potential association between the Norwegian population's APOBEC3A/B deletion polymorphism and a lower risk of endometrial cancer (Sofiyeva et al., 2023).

Especially in those with the SNP309T genotype, the MDM2 SNP55T-allele may be associated with a lower risk of endometrial cancer. The minor SNP55T-allele was associated with a lower risk of endometrial cancer in those with the SNP309TT genotype (dominant model: OR = 0.63; CI = 0.45-0.88; p = 0.01). Furthermore, the SNP55T-allele showed a correlation with a lower risk of endometrial cancer before the age of 50 (dominant model: OR = 0.56; CI = 0.34-0.90; p = 0.02), independent of the genotype in nearby SNPs (Helwa et al., 2021).

In Hainan Chinese Han women, the association between IL6 gene polymorphisms and an elevated susceptibility to endometrial cancer was highlighted in this study. Cai et al. (2019) found that rs1524107 (IL6) (T/C, OR = 1.61, 95% CI = 1.09-2.37, p = 1.55 × 10-2) and rs2066992 (IL6) (OR = 3.09, 95% CI = 2.11-4.53, p = 3.13 × 10-9) significantly increased the risk of endometrial cancer. Globally, the fourth most prevalent female cancer is uterine cervix cancer, representing a leading cause of mortality in women. Approximately 6.6% of gynecological cancer cases worldwide are attributed to it, with 85% of these instances occurring predominantly in developing countries (Bray et al., 2018).

Table 1. Some selected gene SNP in gynecological tumors.

Gene Abbreviation	Gene Full Name	Category	Cytogenetic location (Genomic coordinates (GRCh38)
CYP2D6	Cytochrome P450 Family 2 Subfamily D Member 6	Protein Coding	22q13.2 (22:42,126,499-42,130,810)
CYP2E1	Cytochrome P450 Family 2 Subfamily E Member 1	Protein Coding	10q26.3 (10:133,527,363-133,539,123)
DACH1	Dachshund Family Transcription Factor 1	Protein Coding	13q21.33 (13:71,437,966-71,867,204)
eNOS	Endothelial Nitric Oxide Synthase	Protein Coding	7q36.1 (7:150,991,017-151,014,588)
FGFR3	Fibroblast Growth Factor Receptor 3	Protein Coding	4p16.3 (4:1,793,293-1,808,867)
HPSE	Heparanase	Protein Coding	4q21.23 (4:83,292,461-83,335,153)
MDM2	MDM2 Proto-Oncogene	Protein Coding	12q15 (12:68,808,172-68,850,686)

VDR (Vitamin D Receptor Protein coding gene) ApaI SNP decreases the risk of UI by around 80% (p = 0.044 respectively). Notably, carriers of the VDR gene TaqI Tt, tt genotypes associated with the risk of UI by 4.26-fold (p = 0.001). Also, the carrier of the BB in BsmI increases the risk by 3.47-fold (p = 0.005) for unexplained infertility (UI) (Isbilen et al., 2020). Fok1 rs2228570 significantly increases the risk for OC development (Hu et al., 2023). Thus, numerous genes (Table 1) SNP affect the predisposition and progression of gynecological tumors. The SNP rs4938723, located in the promoter region of pri-miR-34b/c is associated with cervical cancer in the Chinese Han population. Specifically, the C allele revealed a risk factor for developing cervical cancer. Additionally, the CT genotype has been found to correlate with advanced clinical staging and poorer prognosis of patients (Xiong et al., 2014).

Endometrial cancer (EC) is a widespread tumor among the reproductive system's organ tumours, particularly affecting postmenopausal women. Among risk factors is the genetic predisposition regarding the specific single nucleotide gene potential for genetic markers to enhance early detection and inform treatment strategies for endometrial cancer, emphasizing the need for further research. Gene SNP's implication clarity improving early detection/treatment strategies/prognostic assessments, etc. Therefore, it opens new opportunities for the development of potential biomarkers for susceptibility/ disease progression within EC patients.

Molecular diagnostics/biomarkers, therapeutic advances and personalized medicine

Molecular biomarkers play a crucial role in the early detection and management of gynecological tumors, particularly in ovarian and uterine cancers. Commonly used biomarkers include cancer antigen 125 (CA125), which is widely recognized for ovarian cancer detection, although it has limitations in specificity (R. Zhang et al., 2022). Other significant biomarkers include human epididymis secretory protein 4 (HE4), P53, K-RAS, and estrogen and progesterone receptors, which are utilized for both early detection and therapeutic targeting in uterine cancer (Priya et al., 2024). Liquid biopsy is significantly transforming the detection and monitoring of gynecological tumors by providing a non-invasive method to analyze circulating tumor components in bodily fluids. By utilizing biomarkers like circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and extracellular vesicles, liquid biopsy enhances the ability to track tumor progression and response to therapies(C. Zhang et al., 2024; H. Wang et al., 2024). Moreover, advancements in genomic technologies, including next-generation sequencing, facilitate the identification of actionable mutations, thereby guiding treatment decisions (Ho et al., 2024).

The development of biotechnology has also contributed to the advancement of treatment for gynecological tumors. In recent years, targeted therapies have become one of the most important innovations. Drugs like PARP inhibitors have significantly transformed the therapeutic landscape for ovarian cancer, particularly for patients with BRCA mutations. These agents exploit the concept of synthetic lethality, targeting the DNA repair mechanisms that are compromised in BRCA-mutated tumors, thereby enhancing cancer cell death (Hirschl et al., 2024). The introduction of PARP inhibitors like olaparib, niraparib, and rucaparib has provided new treatment options that extend progression-free survival (PFS) and improve overall survival (OS) in these patients (Guduri et al., 2024). Immunotherapies, including immune checkpoint inhibitors like pembrolizumab, have also made significant advances in treating certain types of gynecological malignancies, like advanced or recurrent cervical cancer. As a result of inhibiting the PD-1/PD-L1 axis, these therapies enable the immune system to recognize and destroy cancer cells (Parvez et al., 2023).

Personalized medicine, leveraging genomics and proteomics, significantly enhances treatment outcomes for patients with gynecological tumors by tailoring therapies to individual molecular profiles. Genomic profiling, particularly through next-generation sequencing (NGS), allows for the identification of specific mutations and homologous recombination deficiency (HRD), which are crucial for determining the efficacy of platinum-based chemotherapy and PARP inhibitors (Hrytsay et al., 2024). Additionally, proteomics aids in discovering novel biomarkers that can improve diagnostic accuracy and therapeutic targeting, although challenges remain in translating these findings into clinical practice(Kaur Jawanda et al., 2024). Artificial intelligence (AI) and machine learning (ML) are revolutionizing personalized treatment strategies for gynecological tumors by integrating complex datasets to enhance diagnosis and treatment efficacy. AI-driven frameworks utilize multi-omics data, electronic health records, and advanced machine learning models, such as Convolutional Neural Networks (CNNs) and Generative Adversarial Networks (GANs), to tailor treatment plans based on individual genetic and clinical characteristics (Vegesna, 2024).

Biotechnology is poised to significantly enhance the diagnosis and treatment of gynecological cancers through various innovative approaches. Nanotechnology, particularly the use of nanoparticles, is being explored to improve the sensitivity and specificity of cancer detection, enabling earlier diagnosis and more effective imaging techniques (Keyvani et al., 2024). Additionally, advancements in next-generation sequencing (NGS) facilitate genome-directed precision medicine, allowing for the identification of molecular biomarkers that guide targeted therapies, thereby improving treatment efficacy and reducing overtreatment.

Thus, advancing molecular biomarkers/ technologies (liquid biopsies/ genomic profiling, etc.) has significantly improved the early detection/ treatment of gynecological tumors. New Innovations (PARP inhibitors, immunotherapies etc.) are changing the treatment approaches/ landscape. Biotechnology approaches continue to develop/improve. Their ongoing achievements ultimately lead to further enhanced diagnostic accuracy and, finally, improving patients' diagnostic/treatment outcomes.

Clinical implications of single nucleotide polymorphisms

There are currently several limitations to determine the translational role of SNPs in determining. Even though there have been well-validated SNPs' correlation to endometrial cancers, single SNPs alone are known not to have a high contribution in cancer risk. Reliable SNPs, when validated for their medical usage, could serve for accurate diagnosis and prognosis of endometrial cancer, as well as prediction of drug response (Baker-Ran & Kitson, 2024).

The NINJ2 SNP rs118050317 revealed increased risk for the development of the EC; Notably, the carrier of mutant allele C/CC genotype revealed the elevated leves of the CEA, CA125/AFP compared to the control group (Cheng et al., 2021). For SNPs which have been identified as causal of endometrial cancer, SNP-directed RNAi drugs are a promising option for treatment purposes, especially for untreatable cancers, by specifically targeting the transcripts of oncogenes, which provide the survival advantages for cancer cells (Gebert et al., 2020). SNPs can also serve in clinical settings for therapeutic decisions, as better tools compared to family history (Srinivasan et al., 2015). The identification of relevant genetic variants is crucial for proper disease management; early detection of sensitive variants increases the likelihood of successful treatment.

Biosensing technology and its role in early detection and diagnosis

Biosensing technology refers to sophisticated analytical devices that integrate biological components with electronic systems to detect and quantify biological or chemical reactions. These devices utilize biorecognition elements such as enzymes, antibodies, or nucleic acids to selectively bind to specific analytes, converting biological signals into measurable electrical signals (Abena, 2023). Biosensing technology has emerged as a powerful tool in the early detection, diagnosis, and monitoring of gynecological tumors, including ovarian, cervical, and endometrial cancers. As biosensing continues to evolve, it holds immense potential to enhance patient outcomes through more timely and accurate diagnostics in gynecological oncology.

Biosensors significantly enhance the early detection of gynecological tumors, including ovarian, cervical, and endometrial cancers, through their ability to identify specific biomarkers

in biological samples. Current research confirmed that the biosensors have revealed significant capability to detect breast cancer metastasis (Deng et al., 2023). Research is actively progressing in developing electrochemical biosensing techniques aimed at identifying precise and rapid biomarkers for the detection of breast cancer. Current studies indicate the need for affordable and efficient diagnostic biosensor technologies to enhance diagnostic capabilities and improve patient treatment options (Kiani et al., 2024). Biosensors have emerged as innovative tools for the detection of key biomarkers associated with gynecological cancers, significantly enhancing diagnostic capabilities. For ovarian cancer, biomarkers such as human epididymis protein 4 (HE4), alpha-fetoprotein (AFP), and cancer antigen 125 (CA-125) are commonly detected using advanced biosensor technologies, including electrochemical and photoelectrochemical immunosensors, which offer rapid and sensitive detection (Kovarova et al., 2023). In cervical cancer diagnostics, p16 is a notable biomarker identified through label-free electrochemical immunosensors (Kuntamung et al., 2024). Biosensors play a crucial role in detecting circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) in gynecological cancers through liquid biopsy techniques. These non-invasive methods allow for the collection of tumor-specific material from blood samples, facilitating real-time monitoring of cancer progression and treatment response. Recent advancements in electrochemical biosensing tools have enhanced the sensitivity and specificity of ctDNA detection, enabling the quantification of tumor-derived DNA in plasma samples (Sahin & Yıldırım Tirgil, 2024). The integration of biosensors with liquid biopsy techniques has shown promise in identifying genomic alterations and assessing cancer burden, which is vital for guiding therapy selection and predicting relapse (X. Wang et al., 2024).

Technological developments in biosensing

Recent advancements in biosensing technologies, particularly nano-biosensors and microfluidic biosensors, have significantly enhanced the detection of gynecological tumor markers. Nanomaterial-based biosensors have improved sensitivity and selectivity, enabling the detection of low concentrations of cancer biomarkers in complex biological samples, which is crucial for early diagnosis of gynecological cancers (Shahazi et al., 2024). Electrochemical immunosensors, leveraging nanotechnology, provide rapid and simplified detection of biomarkers, facilitating timely diagnosis and personalized healthcare (Kokilavani et al., 2024). Additionally, innovative methods such as nanopore sensing have been developed to identify specific ovarian cancer marker peptides in urine, showcasing the potential for non-invasive testing (Rockett et al., 2024). Electrochemical and optical biosensors are increasingly being employed in gynecological oncology to monitor specific biomarkers such as CA-125, HE4, and SCC-Ag, enhancing early detection and diagnosis. Electrochemical immunosensors, utilizing nanocomposites like CuCo-ONSs@AuNPs and molecularly imprinted polymers, have demonstrated high sensitivity and low limits of detection for CA-125, with ranges from 0.01 U/mL to 80 U/mL and LODs as low as 0.0089 U/mL (Wu et al., 2024). Additionally, multiplex biosensors have been developed for simultaneous detection of multiple biomarkers, including CA-125 and HE4, achieving linear detection ranges suitable for early-stage disease identification (Kumar et al., 2023).

Biosensors play a crucial role in ensuring complete tumor removal during gynecological

surgery by enhancing the detection of malignant tissues. For instance, intraoperative near-infrared fluorescence imaging using agents like pafolacianine and OTL38 allows surgeons to visualize cancerous lesions that are otherwise difficult to detect, significantly improving the completeness of cytoreductive surgery (Dindere et al., 2022). Additionally, techniques such as attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy provide rapid classification of fresh tissue samples, distinguishing between malignant and benign tumors with high accuracy, thus aiding in real-time decision-making (Malonek et al., 2020).

Biosensing technologies are poised to revolutionize gynecological tumor screening and diagnosis, particularly in low-resource settings, by providing cost-effective, portable, and efficient solutions. The development of lab-on-chip (LoC) technologies enables multiplex detection of various biomarkers, enhancing diagnostic reliability for gynecological cancers, including cervical and ovarian cancers (Nujhat et al., 2023).

Thus, the evolution of biosensing technologies has the potential to revolutionize diagnostics/ treatment in gynecological oncology. In particular, they have the ability to provide timely and accurate diagnostics that significantly improve the method for personalized therapy. It can be said that biosensing technology provides innovative/ effective methods for early detection/monitoring/ surgical assistance/enables personalized treatment strategies, etc. Taken together, the integration of biosensing technology into gynecological oncology may hold remarkable potential/new opportunities for transforming/improving the patient care aspects, including the following: early diagnostics/ ensuring timely intervention/facilitating personalized treatment approaches, etc.

Conclusions

It is critical to investigate the single nucleotide gene polymorphisms associated with gynecologic cancer susceptibility/predisposition as some of them might be utilized as useful molecular markers for assessing gynecologic cancer predisposition and might be further used for diagnosis and treatment modalities in individuals with a similar SNP profile. Moreover, recent advancements in biosensing technologies, particularly nano-biosensors and microfluidic biosensors, have significantly enhanced the detection of gynecological tumor markers.

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Conflict of interest

The authors must declare any conflict of interest.

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It is not required.

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Abbreviations

APC: Adenomatous Polyposis Coli; **BAX:** BCL-2 Associated X protein; **CIMP:** CpG Island Methylator Phenotype; **CHFR:** Checkpoint with Forkhead-Associated and Ring Finger Domains; **CASP8:** **Caspase 8; CD1**: Cluster of Differentiation 1; **COMT**: Catechol-O-Methyltransferase; **CIN**: Cervical Intraepithelial Neoplasia; **DACH1**: Dachshund family transcription factor; **GPR54**: G Protein-Coupled Receptor 54; **MLH1**: MutL Homolog 1; **HPSE**: Heparanase; **HOXA11**: Homeobox A 11; **IGF2R:** Insulin-like Growth Factor 2 Receptor; **EC**: Endometrial Cancer; **ER**: Estrogen Receptors; **HPV**: Human Papilloma Virus; **MMR**: Mismatch Repair; **MSI**: Micro-satellite Instability; **PTEN**: Phosphatase and tension homolog deleted on chromosome 10; **P73**: Tumor Protein P73; **RASSF1A**: Ras Association Domain Family Member 1A; **RSK4**: Ribosomal S6 Kinase 4; **SNP**: Single Nucleotide Polymorphism; **SPRY2**: Sprouty Homolog 2; **TGFBR2**: Transforming Growth Factor Beta Receptor 2

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REVIEW

Phytochemical and biological activities of *Eremurus spectabilis*: A review

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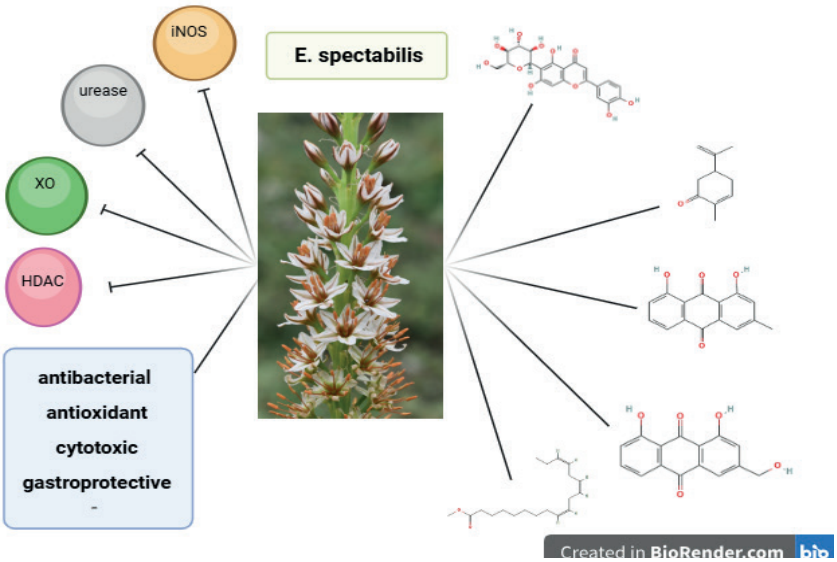
Abstract

Eremurus spectabilis M. Bieb., commonly known as the "foxtail lily," is a plant that is a member of the Xanthorrhoeaceae family. It is found in a variety of locations in Central and Western Asia, such as Afghanistan, Iran, Syria, and Turkey. Traditional medicine has long utilized *E. spectabilis* for its pharmacological properties, including antibacterial, antioxidant, cytotoxic, gastroprotective, and anti-inflammatory effects. Ethnobotanical research has indicated its use in treating dermatitis, diabetes, digestive issues, and inflammatory diseases. This review examines the therapeutic potential and phytochemical profile of *E. spectabilis*. The identification of thirty-five compounds that were isolated from the plant is given particular attention. Phenolic acids, terpenoids, flavonoids, and anthraquinones comprise these compounds. Furthermore, *E. spectabilis* was proven to be a source of essential nutrients, including polysaccharides, vitamins K, C, and D, as well as minerals like potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and copper (Cu). Despite its promising bioactivities, further research is needed to understand the underlying mechanisms, evaluate its full pharmacological potential, and assess the safety, efficacy, and appropriate dosing for clinical use.

Keywords: *Eremurus spectabilis*, foxtail lily, antibacterial activity, antioxidant effect, cytotoxicity, flavonoids



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Introduction

Eremurus (*Xanthorrhoeaceae*) is a genus of 62 species native to Central Asia and the Middle East, which has long been valued in traditional medicine. These plants have been used in ethnobotanical practices for various ailments, particularly in the regions where they grow, underscoring their importance in historical and ongoing medicinal applications (*The genus Eremurus.*, 2024; Salehi et al., 2017). One notable species, *Eremurus spectabilis M. Bieb*., known as the "foxtail lily," is also referred to by various local names, including "Çiriş ©," "çireş," "dağ pırasası," "yabani pırasa," "güllük," "kiriş," "sarı çiriş," and "sarı zambak." This species is native to South and Central Asia, encompassing Turkey, Iran, West Pakistan, Afghanistan, Iraq, Palestine, Lebanon, Syria, and the Caucasus. This perennial herbaceous plant can grow up to 1 meter tall and thrives in steppes, open scrublands, limestone rock formations, and scree at altitudes between 1000 and 2750 meters (Murathan et al., 2018).

E. spectabilis is historically utilized in traditional medicine due to its phytochemical composition. Phenolic acids, flavonoids, terpenoids, anthraquinones, and other secondary metabolite derivatives are naturally found in plants and fruits. These chemicals have a variety of medicinal properties (Murathan et al., 2018; Prakash & Sagar, 2021). As a medicinal plant, *E. spectabilis* is abundant in these compounds. Ethnobotanical studies indicate its use for treating various ailments, including scabies, diabetes, and intestinal issues (Arıtuluk & Ezer, 2012; Karaman & Kocabas, 2001; Korkmaz & Karakuş, 2015). Furthermore, the plant has demonstrated antibacterial, antioxidant, and cytotoxic activities in various studies (Aykutoğlu et al., 2023; Kanaani & Mohamadi Sani, 2015; Taskin et al., 2012; Tuzcu et al., 2017). Carvone, carvacrol, pentane, ©-caryophyllene, and valencene were discovered to be the primary ingredients of *E. spectabilis'* essential oil (Karaman et al., 2011). Moreover, compounds like inosine, methyl linolenate, chrysophanol, isoorientin, β-sitosterol, and sucrose have been extracted (Karakaya et al., 2017). Previous research also reveals the presence of polysaccharides, vitamins K, C, and D, along with essential minerals such as potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and copper (Cu), all contributing to its nutritional value (Bircan & Kırbağ, 2015; Cınar et al., 2017; Tosun et al., 2012).

Our literature review indicates a lack of detailed studies specifically focused on *E. spectabilis*. This review aims to compile data on the plant's phytochemical profile, nutritional value, pharmacological applications, and biological activities. Information on *Eremurus* species was gathered from electronic sources spanning the period from 2001 to early 2024.

Figure 1. The figure illustrates the different parts of *Eremurus spectabilis*: (a) stem and leaves, (b) root and rhizomes, (c) fruits, and (d) flowers (Tuncer, 2020).

Traditional uses

Historically, people have used the plant, in both its fresh and dried forms, as a wild edible vegetable, including leaves and roots, as well as a traditional medicinal or folk remedy. Ethnobotanical and ethnopharmacological research has shown its traditional use in alleviating gastrointestinal irritation, liver ailments, cutaneous infections like scabies and syphilis, rheumatism, and different inflammatory conditions (Karaman & Kocabas, 2001; Karaoğlan et al., 2018; Korkmaz & Karakuş, 2015). Traditional medicine extensively employs *E. spectabilis* to remedy jaundice, diabetes, and hyperlipidemia, in addition to addressing dysuria and hypertension (Amiri & Joharchi, 2013; Cınar et al., 2017).

Phytochemistry profile

E. spectabilis was revealed to have various phytochemical compounds, including flavonoids, terpenoids, anthraquinones, and sterols, which all contribute to its bioactivity profile (Karaman et al., 2011; Koldas, 2023; Tegin et al., 2024).

Carbohydrate composition

In several investigations, *E. spectabilis* revealed the presence of poly-, di-, and monosaccharides. Oligofructose, fructans, and inulin are among the polysaccharides that were identified during the evaluation of the plant's roots (Pourfarzad et al., 2015; Pourfarzad et al., 2014). Additionally, glucomannan was found in another assessment of the plant (Jahanbin & Beigi, 2015). As a disaccharide, sucrose was identified from an investigation conducted on the young leaves (Karakaya et al., 2017). Glucose, fructose, and arabinose are recognized as monosaccharides (Bircan & Kırbağ, 2015).

Phenolic compounds

E. spectabilis yielded several phenolic compounds, primarily from its aerial parts. In an investigation of the plant's aerial parts to analyze its phenolic compounds, LC-MS/MS results of

extracts obtained from Soxhlet extraction revealed the presence of various phenolic compounds, including hydroxycinnamic acids (a), hydroxybenzoic acids (b), phenolic acids (c), and flavonoids (d). Hydroxycinnamic acids (a), such as quinic acid (1) and malic acid (2), were the most abundant, highlighting their key roles in the plant's biochemical profile (Tegin et al., 2024). Another study on the aerial parts identified ferulic acid (3) as one of the prominent compounds (Di Simone et al., 2024). Other hydroxycinnamic acids, such as trans-caffeic acid (4), were also detected at relatively high levels. Hydroxybenzoic acids (b), including trans-aconitic acid (5) and gallic acid (6), were detected. Moreover, phenolic acids (c), like protocatechuic acid (7) and tannic acid (8), were found in moderate concentrations, further contributing to the plant's overall bioactivity. In addition to that, vanillin (9) was identified in smaller quantities, further contributing to the plant's potential role in flavor and bioactivity (Tegin et al., 2024). In another investigation, the methanolic extract of the plant yielded resveratrol (10), which is a polyphenolic compound (Bircan & Kırbağ, 2015).

Different extraction methods identified various flavonoids (d), primarily in the aerial parts of the plant. Isoorientin (11) is one of the flavonoids found in *E. spectabilis*. It has been found in many studies, mostly from the ethyl acetate and MeOH extracts of the leaves (Karakaya et al., 2017; Karaoğlan et al., 2018; Karaoglan et al., 2018). In other research, rutin (12), morin (13), and quercetin (14) were also among the flavonoids detected in the methanol extracts of the plant (Bircan & Kırbağ, 2015). Di Simone et al. (2024) conducted a recent study on the aerial parts, revealing the presence of quercitrin (15), delphinidin 3,5-diglucoside (16), isorhamnetin (17), and isoquercitrin (18) (Di Simone et al., 2024). The aerial parts also yielded the identification of hyperoside (19), a key flavonoid (Di Simone et al., 2024). In a different study done by Tegin et al. in 2024, the aerial parts were also found to contain kaempferol (20), apigenin (21), and luteolin (22) (Tegin et al., 2024).

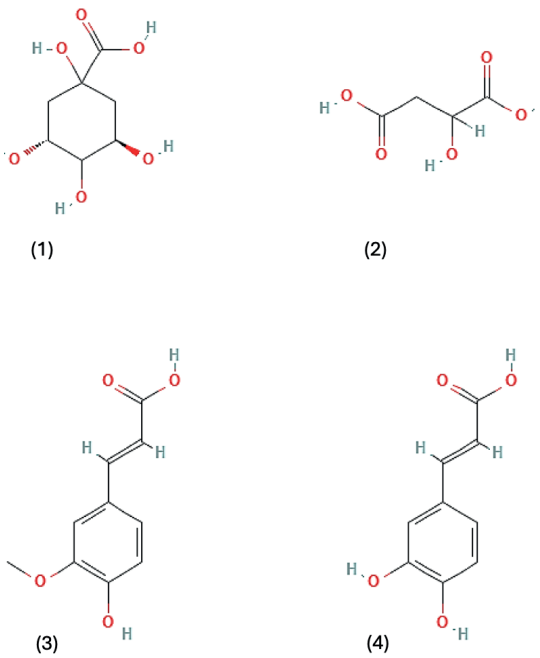


Figure 2. Chemical structures of hydroxycinnamic acids (a) isolated from E. spectabilis, including quinic acid (1), malic acid (2), ferulic acid (3), and trans-caffeic acid (4).

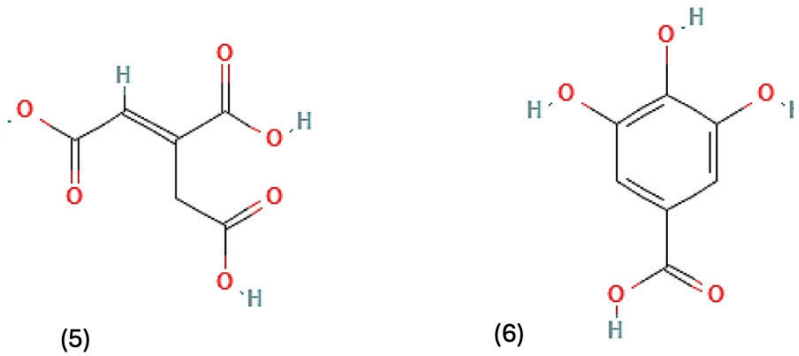


Figure 3. The structures of hydroxybenzoic acids (b) isolated from E. spectabilis, including trans-aconitic acid (5) and gallic acid (6).

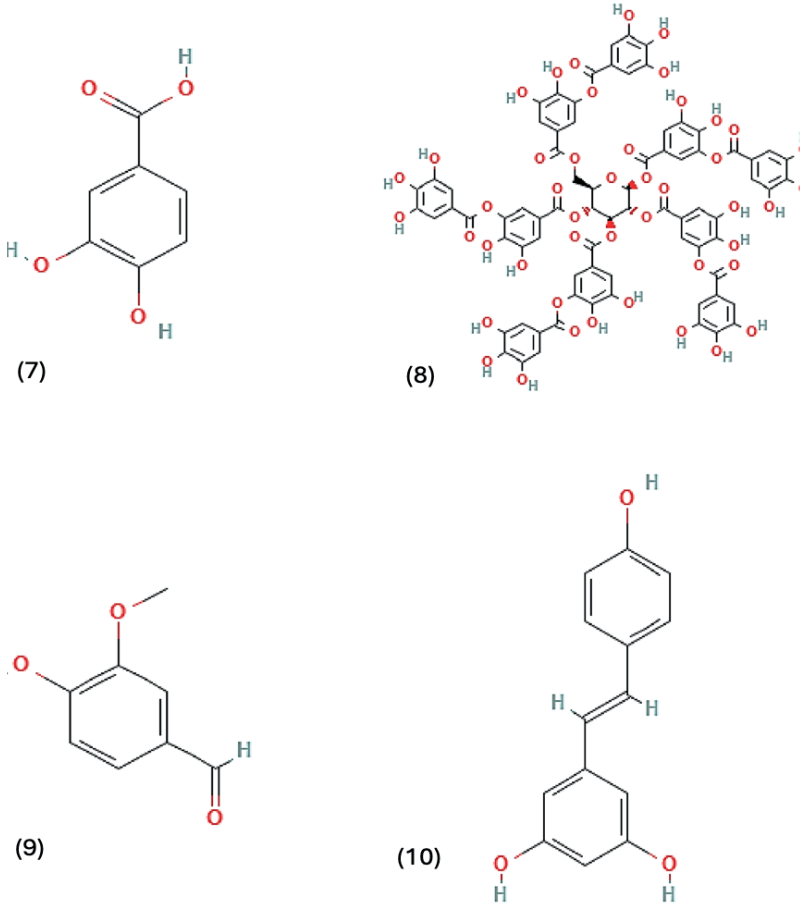


Figure 4. The structures of phenolic acids (c) isolated from E. spectabilis, including protocatechuic acid (7), tannic acid (8), vanillin (9), and resveratrol (10).

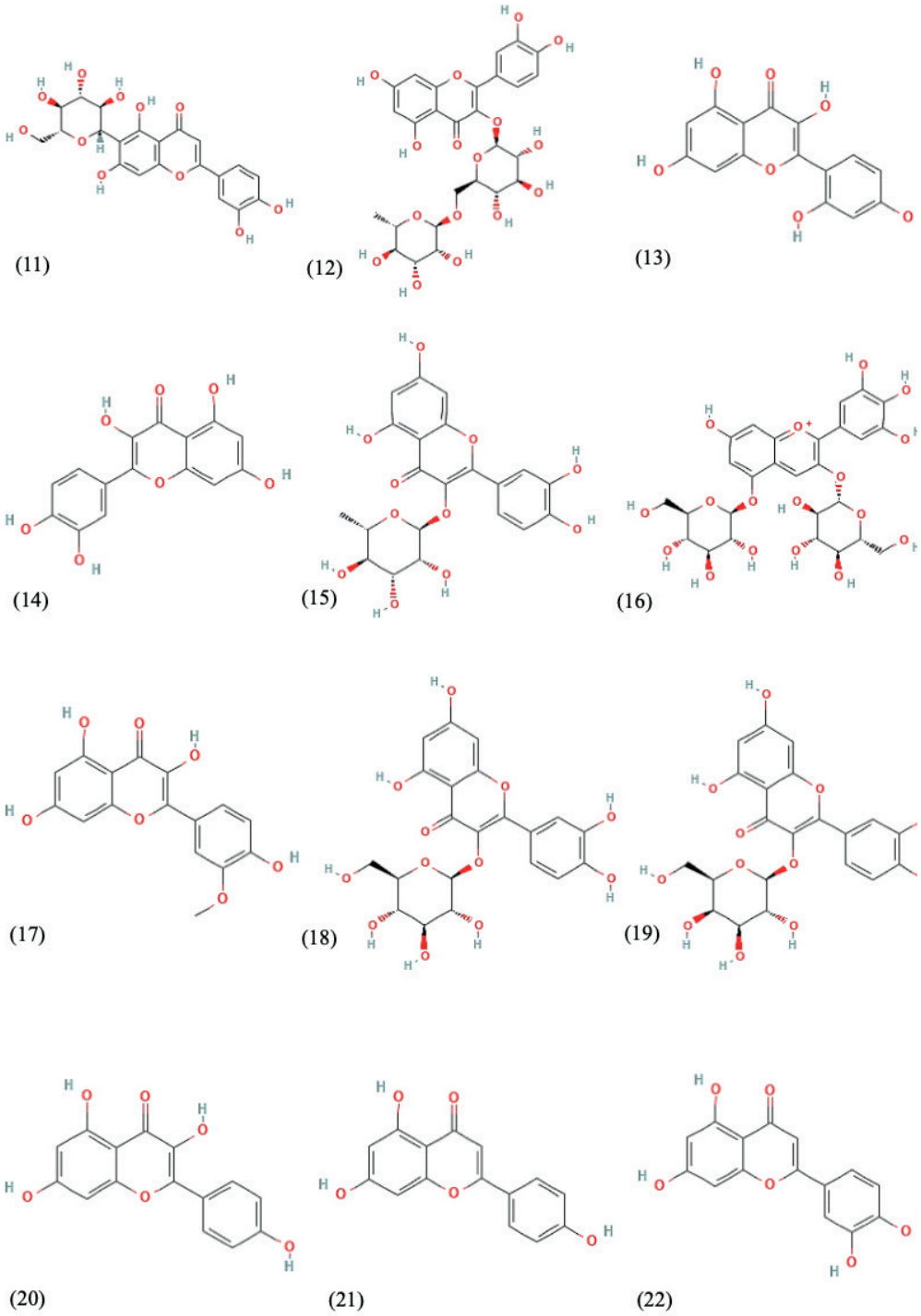


Figure 5. The structures of flavonoids (d) identified from the E. spectabilis, including isoorientin (11), rutin (12), morin (13), quercetin (14), quercitrin (15), delphinidin 3,5-diglucoside (16), isorhamnetin (17), isoquercitrin (18), hyperoside (19), kaempferol (20), apigenin (21), and luteolin (22).

Terpenoids

Carvone (23), carvacrol (24), (E)-caryophyllene (25), valencene (26), and cis-calamenene (27) were the major terpenoids detected from the aerial parts using hexane extraction (Karaman et al., 2011).

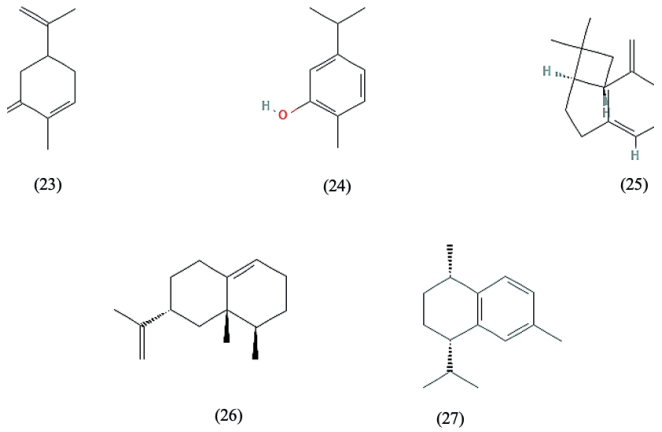


Figure 6. The structures of terpenoids identified from the E. spectabilis, including carvone (23), carvacrol (24), (E)-caryophyllene (25), valencene (26), and cis-calamenene (27).

Anthraquinones

A study by Koldas in 2023 on the aerial parts of *the plant* found that chrysophanol (28), aloe emodin (29), and chrysophanol-8-methyl ether (30) are all anthraquinones, while 7,10-bichrysophanol (31) and chrysalodin (32) are bianthraquinone compounds found in the chloroform extract (Koldas, 2023).

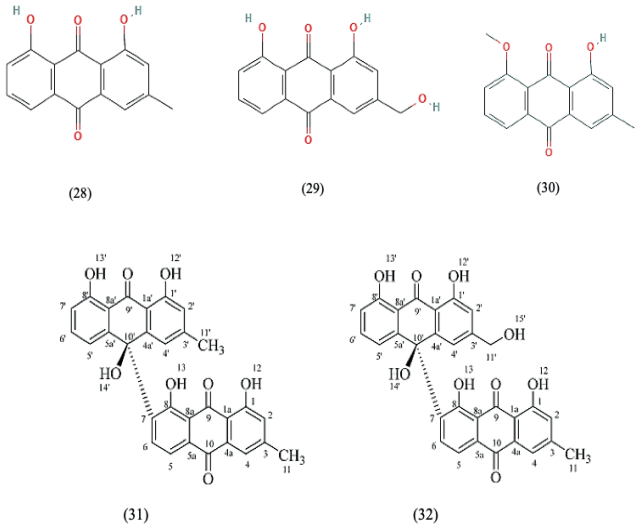
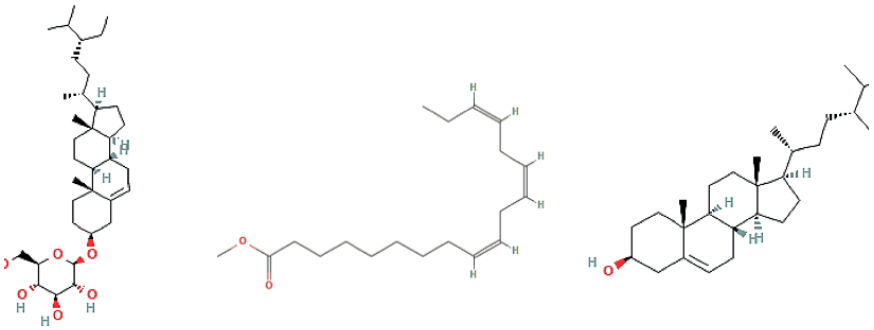


Figure 7. Anthraquinones: chrysophanol (28), aloe-emodin (29), and chrysophanol-8-methyl ether (30), and bianthraquinones: 7,10-bichrysophanol (31) and chrysalodin (32).

Sterols

The chloroform extracts from the aerial parts of *E. spectabilis* identified daucosterol (33) as a major sterol (Koldas, 2023). Methyl linolenate (34) and β-sitosterol (35) were also detected from the hexane extract of the above-ground parts, enhancing the plant's sterol composition (Karakaya et al., 2017).



(33) (34) (35)

Figure 8. The structures of sterols identified from the E. spectabilis, including daucosterol (33), methyl linolenate (34), and β-sitosterol (35).

Nutritional value of *E. spectabilis*

E. spectabilis is an exceptional dietary resource due to its remarkable nutritional profile. Its traditional use in local cuisines is substantiated by its minimal calorie and fat content, as well as its beneficial dietary fiber levels. Tosun et al. (2012) reported a protein and ash composition of 1.20% and 0.87%, respectively. However, a subsequent study determined that these levels were below these values (Cınar et al., 2017; Tosun et al., 2012).

Another notable aspect of its profile is its vitamin content; *E. spectabilis* offers a remarkably high amount of vitamin C (129.4 mg/100 g) and vitamin D, which aids in the mitigation of common dietary deficiencies (Bircan & Kırbağ, 2015; Cınar et al., 2017). Vitamin K, α-tocopherol, and ergosterol were also determined (Bircan & Kırbağ, 2015). The plant is also an essential source of macro- and microelement constitution, particularly its high potassium-to-sodium ratio (Cınar et al., 2017). Its high concentration of calcium and magnesium further suggests that it can meet the daily mineral requirements (Tosun et al., 2012). Nevertheless, the concentration of iron has been observed to fluctuate; Tosun et al. (2012) reported 7.1 mg/100 g, while other assessments determined 2.42 mg/100 g (Cınar et al., 2017; Tosun et al., 2012).

Pharmacological effects

Antioxidant activity

E. spectabilis has been the subject of extensive research regarding its antioxidant properties due to its extensive collection of phytochemicals, including flavonoids, hydroxycinnamic acid derivatives, hydroxybenzoic acid derivatives, and organic acids.

A study conducted by Karaman et al. (2011) evaluated the antioxidant and antiradical properties of ethanol, methanol, and aqueous extracts. According to the findings, the methanol extract exhibited the

highest antioxidant activity (81.72 mg ascorbic acid/g), and the ethanol extract exhibited the strongest antiradical activity (Karaman et al., 2011). In another study, Falahi et al. (2019) used 2,2-diphenyl-1-picrylhydrazyl (DPPH) and phosphomolybdate methods to examine the activity of five distinct plants from west Iran, showing that *E. spectabilis* had the greatest antioxidant capacity (2.41 µg/mL) of all the species they evaluated (Falahi et al., 2019). Dervişoğlu et al. (2013) also used water extracts from leaves and roots to measure the plant's antioxidant capacity by assessing DPPH and metal chelating activities. The results demonstrated that the antioxidant capacity of root extracts was higher than that of leaf extracts. The hydroxyl radical scavenging activity of both leaf and root extracts was higher than that of ascorbic acid and BHA (Butylated HydroxyAnisole) and BHT (Butylated HydroxyToluene) and comparable to that of α-tocopherol (Dervişoğlu et al.). Moreover, acetone extracts from leaves had the highest antioxidant activity (3703.25 µg ascorbic acid/g) when compared to BHA (Tuzcu et al., 2017). Different extraction techniques demonstrated strong radical scavenging activity (73.89% per mg/mL extract) in a separate study that utilized Density Functional Theory (DFT) methods to analyze the aerial portions of *E. spectabilis*, with a focus on the mechanisms of Hydrogen Atom Transfer (HAT), Single Electron Transfer followed by Proton Transfer (SET-PT), and Sequential Proton Loss Electron Transfer (SPLET) processes (Tegin et al., 2024). Bircan & Kirbağ (2015) revealed that DPPH free radical scavenging activity was effective even at 10 µL, and the reduction of elevated lipid peroxidation (LPO) levels, along with decreased levels of oleic and linoleic acids in the FeCl group, suggested that the flavonoids in methanolic extracts may protect unsaturated fatty acids from free radical attacks (Bircan & Kırbağ, 2015). In other research, the antioxidant effect of eight *E. spectabilis* species from different areas was examined using DPPH radical and β-carotene/linoleic acid assays. *E. spectabilis* showed antioxidant effects in both β-carotene–linoleic acid (inhibition: 94.56%) and DPPH assays (inhibition: 73.69%) (Tosun et al., 2012). Besides that, a significant increase in the expression of the cytochrome c gene was observed in cells exposed to the leaf water extract, with no increase in BAX and BCL-X. Additionally, the assays for superoxide dismutase (SOD), catalase (CAT), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) demonstrated enhanced antioxidant activity in the cells treated with the extracts (Aykutoğlu et al., 2023).

Antimicrobial effects

The studies on *E. spectabilis* highlight the variability in antimicrobial activity due to differences in extraction methods, solvent types, bacterial strains, and geographical regions of the plant.

Methanolic extracts of the aerial parts of *E. spectabilis* from Bingöl, Kahramanmaras, and Nakhcevan exhibited significant antibacterial activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus megaterium*, *Escherichia coli*, and *Enterobacter aerogenes*, with the strongest inhibition observed against *Enterobacter aerogenes* (14.970 mm) from the Nakhcevan sample. Antifungal activity was observed only against *Saccharomyces cerevisiae*, with no effect on other yeast strains (Murathan et al., 2018). However, Kanaani and Sani (2015) found no activity in methanolic root extracts using the agar diffusion method but reported antibacterial effects against *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella enterica*, and *Escherichia coli* using the microdilution broth assay (Kanaani & Mohamadi Sani, 2015). Moreover, Taskin et al. (2012) documented antifungal activity from chloroform and aqueous extracts against *Candida albicans* and antibacterial effects from ethyl acetate and aqueous extracts against *Klebsiella pneumoniae* and *Staphylococcus aureus* (Taskin et al., 2012). In another study, Bircan and Kirbağ (2015) also observed inhibition zones of 12 mm against *Staphylococcus aureus*, 14 mm against *Escherichia coli*, 9 mm against *Candida albicans*, and 8 mm against *Epidermophyton spp.*, indicating broad

antimicrobial potential, particularly stronger against bacteria than fungi (Bircan & Kırbağ, 2015). Karaman et al. (2011) found that methanolic, ethanol, and aqueous extracts displayed antimicrobial activity, with the strongest effect from aqueous extracts (10%) against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Karaman et al., 2011). In a subsequent study, Tuzcu et al. (2017) revealed that aqueous, acetone, and ethanol extracts demonstrated antimicrobial potential against *Listeria monocytogenes*, *Saccharomyces cerevisiae*, *Staphylococcus aureus*, and *Escherichia coli* using the disk diffusion method (Tuzcu et al., 2017). Furthermore, in a distinct study, the plant exhibited higher antibacterial activity against seven bacteria compared to commercial antibiotics penicillin (10 µg/disc) and amoxicillin-clavulanic acid (30 µg/disc). The above-ground parts were more effective, showing moderate activity against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Escherichia coli*, but low activity against *Salmonella typhimurium*. The underground parts exhibited weaker effects, with the lowest inhibition against *Bacillus subtilis* (7.00 mm) and the highest against *Escherichia coli* (8.33 mm) (Tegin et al., 2024). However, it is observed that at a 1% concentration, no inhibition was found, but increased extract concentrations resulted in stronger inhibition zones, with *Pseudomonas aeruginosa* being more sensitive and *Yersinia enterocolitica* more resistant (Karaman et al., 2011).

Cytotoxic activity

The anticancer activity of *E. spectabilis* was investigated in prostate cancer (PC-3) cells using both organic and aqueous extracts from its leaves and roots. A 2017 study by Tuzcu et al. found that acetone, ethanol, and water extracts at concentrations of 250 and 500 µg/mL decreased cell proliferation by up-regulating Bax and caspase-3 mRNA while down-regulating Bcl-2 mRNA (Tuzcu et al., 2017). Additionally, another study showed that aqueous leaf extracts exhibited significant cytotoxic effects, with an $IC_{50\ of\ 250\ \mu g/mL}$ and maximal growth inhibition at 500 µg/mL. The root extract did not induce cell death, while the leaf extract did, as evidenced by increased expression of the cytochrome C gene. Furthermore, the anticancer potential of the leaf extract on PC-3 cells was confirmed by increased lipid peroxidation, indicated by elevated levels of malondialdehyde (MDA) (Aykutoğlu et al., 2023).

Another assessment evaluated the cytotoxic activity of aqueous and hexane-ethanolic extracts of *E. spectabilis* on rhabdomyosarcoma (RD) and Vero cells. The extracts demonstrated dose- and time-dependent inhibition of cell proliferation, with RD cells showing sensitivity to all tested concentrations (10–0.001 µg/ml over 24–72 hours), while Vero cells were resistant at higher concentrations (100 and 10 µg/ml) (Abubaker, 2015). The effects of *E. spectabilis* lyophilized and nanoparticle plant leaf extracts on the cellular and enzymatic immune systems of rats with hepatocellular cancer (HCC) were investigated by Genç and Çelik. In HCC rats, the plant extracts modulated T lymphocyte subsets (CD3+, CD4+, CD8+, and CD4+/CD8+ ratio). CD3+ and CD8+ T cells showed reductions in the groups treated with cancer along with lyophilized plant leaf extract (CLPLE), while CD4+ T cells exhibited a broader decrease across all experimental conditions compared to the normal control. Furthermore, following treatment with lyophilized plant leaf extract (LPLE) and nanoparticle plant leaf extract (NPLE), lung and spleen tissues showed increased levels of the enzymes myeloperoxidase (MPO) and adenosine deaminase (ADA), suggesting improved immunological responses (Genç & Çelik, 2024).

E. spectabilis-derived isoorientin exhibits encouraging anticancer efficacy against HT-29 colorectal cancer cells as well as SH-SY5Y neuroblastoma cells. By altering genes linked to the cell cycle and

apoptosis, isoorientin efficiently suppresses cell growth, with IC50 values of 250 µM and 125 µM, respectively. It triggers apoptotic pathways by upregulating p53, p21, caspase-3, caspase-8, caspase-9, and ATR and downregulating CCND1, CDK6, Bcl-2, Bax, CHEK1, CHEK2, and ERCC1 (Gundogdu et al., 2018; Karaoğlan et al., 2018). Moreover, various extracts of the plant, particularly the ethanolic and ethyl acetate extracts, exhibit inhibitory effects on histone deacetylase (HDAC), a key regulator of cancer cell proliferation, migration, angiogenesis, immune evasion, and treatment resistance, highlighting the plant's potential as an anti-cancer agent (Bertan & Refiye, 2021; Hai et al., 2022). However, in the study conducted by Karakaya et al., *E. spectabilis* extracts and isolated compounds did not show cytotoxic activity against various cancer cell lines, including HeLa, A-549, MCF-7, mPANC96, U87MG, PC3, and CaCo-2. Nevertheless, the hexane extract exhibited inhibition of inducible nitric oxide synthase (iNOS) in RAW 264.7 murine macrophage cell lines, with an IC50 value of 25 µg/ml, suggesting limited anticancer activity but potential anti-inflammatory properties via iNOS inhibition (Karakaya et al., 2017).

Overall, the cytotoxic activity in the mentioned studies is primarily observed in acetone, ethanol, and aqueous extracts of *E. spectabilis* leaves, which inhibited proliferation in PC-3 and RD cells. Additionally, isoorientin demonstrated cytotoxic effects against HT-29 and SH-SY5Y cells, while root and hexane extracts generally exhibited limited or no cytotoxicity.

Gastroprotective efficacy

The gastroprotective activities of the aerial parts of *E. spectabilis* and its major component, isoorientin, were investigated in rats using an indomethacin-induced gastric damage model on rats. Increasing glutathione (GSH) levels and superoxide dismutase (SOD) and lowering lipid peroxidation activity in rat stomach tissue were achieved by all doses of isoorientin and methanol extract. However, the 500 mg/kg dose of methanol extract worked best, similar to the standard drug famotidine, and it counteracted the oxidative stress caused by indomethacin. Researchers attribute the plant's effectiveness to a potential synergistic effect of its components, not just isoorientin, which aligns with its traditional use and highlights *E. spectabilis*'s promise as a natural antiulcer agent (Karaoğlan et al., 2018). In another study, both the ethanolic and ethyl acetate extracts had substantial urease-inhibitory activity, with the ethyl acetate extract exhibiting greater inhibition (Bertan & Refiye, 2021). Blocking urease, which significantly contributes to stomach cancer and peptic ulcers due to excessive ammonia production, aligns with the gastroprotective properties of the plant (Follmer, 2010).

Antidiabetic activity

The aqueous extract of fresh *E. spectabilis* leaves was tested for its potential anti-diabetic effects. Compared to acarbose, the extract had strong inhibitory effects on α-glucosidase and α-amylase and an intermediate inhibitory effect on elastase. On the other hand, it had weak inhibitory effects on hyaluronidase and tyrosinase activities compared to their standard inhibitors. This suggests that consuming *E*. spectabilis leaves could potentially help manage postprandial blood glucose levels (Bayrak & Yanardağ, 2021). The plant may also be beneficial in the management of diabetes due to its dominant carbohydrates, glucose, sucrose, and fructose, as well as polysaccharides such as galactomannans. Its traditional uses also support its role in diabetes management (Bircan & Kırbağ, 2015).

Anti-inflammatory effect

E. spectabilis demonstrated anti-inflammatory properties mostly via the methanol extract, which significantly inhibited lipopolysaccharide (LPS)-induced cyclooxygenase-2 (COX-2) gene expression.

The phytochemicals, such as vanillic and ferulic acids, mitigate oxidative stress and disorders induced by inflammation, especially in the colon (Di Simone et al., 2024). Moreover, the hexane extract exhibited iNOS inhibition with an $IC_{50\ of\ 25\ \mu g/ml}$, indicating potential anti-inflammatory properties (Karakaya et al., 2017). Nonetheless, the plant exhibited no reduction of hPBMC proliferation or TNF-α secretion in vitro (Gaggeri et al., 2015).

Skin benefits

Bayrak & Yanardağ (2021) conducted research on the aqueous *E. spectabilis* extract, revealing a moderate inhibitory effect on elastase, which may help with skin problems (Bayrak & Yanardağ, 2021).

Table 1. Summary of *E. spectabilis* bioactivity.

Bioactivity	Plant Part	Extract Type	MOA\ cell lines\ bacteria types	References
Antibacterial	Aerial parts and root	Methanol, aqueous, ethyl acetate, acetone, ethanol	*E. aerogenes, B. cereus, S. aureus, E. coli, K. pneumoniae, L. monocytogenes, S. cerevisiae, B. subtilis, E. faecalis, P. aeruginosa*	(Bircan & Kırbağ, 2015; Karaman et al., 2011; Murathan et al., 2018; Taskin et al., 2012; Tegin et al., 2024; Tuzcu et al., 2017)
Antioxidant	Root & Leaves	Methanol, water, acetone, ethanol	Methanol extract exhibited antioxidant activity (81.72 mg ascorbic acid/g); lowering LPO; XO inhibition	(Bertan & Refiye, 2021; Karaman et al., 2011; Tosun et al., 2012)
Cytotoxic	leaves and roots	acetone, ethanol, and water	Up-regulating Bax and caspase-3 mRNA; down-regulating Bcl-2 mRNA in PC-3 cells	(Tuzcu et al., 2017)
Gastroprotective	leaves	MeOH, ethanolic, and ethyl acetate	Increasing GSH & SOD; lowering lipid peroxidation; blocking urease	(Bertan & Refiye, 2021; Karaoğlan et al., 2018)
Antidiabetic	leaves	aqueous	α-glucosidase and α-amylase inhibition	(Bayrak & Yanardağ, 2021)

Conclusion

E. spectabilis is a medicinally and nutritionally significant plant with a diverse phytochemical composition, including phenolic acids, flavonoids, terpenoids, and anthraquinones, alongside essential vitamins and minerals. It exhibits antibacterial, antioxidant, cytotoxic, and gastroprotective activities and is traditionally used for scabies, diabetes, and intestinal disorders. Its ethnobotanical significance and potential as a source for novel therapeutic agents highlight the need for further research to fully understand its pharmacological potential.

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Funding

None.

Conflict of interest

The authors declare no conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics committee approval

Ethics committee approval is not required for this study.

Authors' contribution statement

Nour Alhalak and Turgut Şekerler: contributed significantly to the preparation of the manuscript. They guided the overall structure of the review, gathered and analyzed relevant data from various sources, and synthesized the information into a coherent and comprehensive narrative.

Use of Artificial Intelligence: No artificial intelligence-based tools or applications were used in the preparation of this study. The entire content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

AI tools were only used solely to assist with language editing and to improve readability.

Use of Artificial Intelligence: No artificial intelligence-based tools or applications were used in the preparation of this study. The entire content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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REVIEW

Advances in boron compounds: Author's perspectives on their role in biotechnology from antimicrobial agents to cancer therapy

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Abstract

Boron compounds, both organic and inorganic, have emerged as versatile and promising materials with wide-ranging applications in medicinal chemistry, catalysis, and materials science. Organic boron compounds, including heterocyclic aminoboron derivatives and boronic acids, have shown significant potential as antimicrobial and anticancer agents, with research highlighting their effectiveness in treating infections and inhibiting cancer cell proliferation. Ongoing research, including the author's own studies, demonstrates the considerable potential of inorganic boron compounds, which should notbe overlooked. Boron Neutron Capture Therapy (BNCT) has garnered attention for its targeted approach to cancer treatment, facilitated by the development of innovative boron-based drug delivery systems. Inorganic boron compounds, have also contributed to advancements in catalytic processes, material stability, and electronic properties, offering opportunities for applications in organic electronics, flame-retardant materials, and drug development. The unique chemical reactivity of boron compounds, including their ability to inhibit enzymes such as β-lactamases and histone deacetylases, positions them as valuable tools in combating antibiotic resistance and cancer. This review provides a comprehensive overview of the properties, applications, and therapeutic potential of boron compounds, emphasizing their role in drug delivery, enzyme inhibition, and antimicrobial development. Ongoing research into the structural modification and functionalization of boron-based compounds continues to expand their scope, positioning them as key candidates for the development of novel therapeutic agents in biotechnology and medicine.

Keywords: boron compounds, biotechnological applications, boron neutron capture therapy, enzyme inhibition, antimicrobial effects.

Introduction

Boron compounds, both organic and inorganic, have gained significant attention in recent years for their remarkable versatility and broad applications across various fields, particularly in medicinal chemistry, catalysis, and materials science. These compounds are distinguished by their unique chemical properties, including their ability to form stable covalent bonds with biomolecules, their Lewis acidity, and their capacity to engage in reversible interactions with biological targets, which allow them to interact effectively with biological systems and contribute to advancements in both therapeutic and industrial applications (Adamczyk-Woźniak et al., 2016).

Organic boron compounds, such as boronic acid derivatives and heterocyclic aminoboron, have demonstrated notable antimicrobial and anticancer activity, opening new avenues for the development of targeted therapies. Boronic acids, for example, are well known for their ability to inhibit proteasomes and serine hydrolases, making them valuable in the treatment of multiple myeloma and other cancers (Adams, 2004). These applications underscore the chemical diversity and adaptability of boron compounds in different scientific domains.

A particularly promising area of boron-based research is Boron Neutron Capture Therapy (BNCT), a targeted cancer treatment that exploits the ability of boron-10 isotopes to capture thermal neutrons and undergo nuclear reactions that selectively destroy cancer cells while minimizing damage to surrounding healthy tissues (Barth et al., 2018a).

This manuscript explores the potential of boron-based compounds in biomedical applications, focusing on their enzymatic inhibition properties, their role in drug resistance, and their contributions to innovative drug delivery systems. As researchers continue to explore and refine the synthesis and modification of these compounds, the scope of their use in combating a wide range of diseases, particularly infections and cancer, continues to expand. The continued development of boron-based therapeutics represents an exciting frontier in biotechnology and pharmaceutical sciences, offering new hope for the treatment of some of the most challenging medical conditions of our time.

Boron compounds

Boron compounds, both organic and inorganic (Figure 1), are a diverse and highly versatile class of materials that have garnered significant interest in a variety of scientific fields, including medicinal chemistry, catalysis, materials science, and drug development. The unique properties of both organic and inorganic boron compounds, such as their ability to modify solubility, reactivity, and electronic properties, make them highly valuable in diverse applications. Ongoing research into the synthesis and functionalization of these compounds continues to unlock new possibilities in drug development, materials science, and industrial applications, underscoring the importance of boron chemistry in advancing technological and therapeutic innovations.

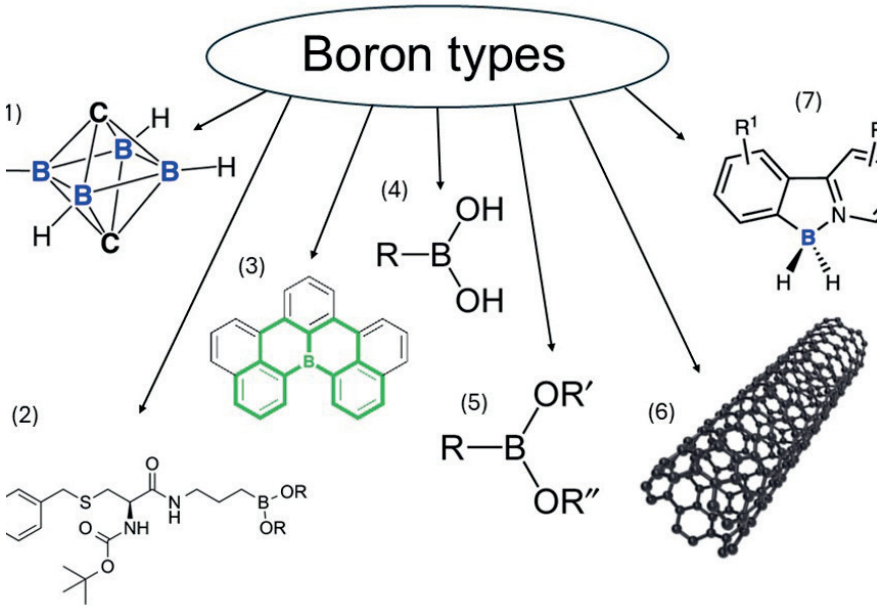

Figure 1. Commonly used boron compounds ((1) carboranes, (2) L-cysteine-based boron compounds, (3) cyclic boronates, (4) boronic acid, (5) boronate esters, (6) boron nitride nanotubes, (7) heterocyclic aminoboron) in biomedical applications.

Organic boron compounds

Organic boron compounds have gained significant attention in various fields of chemistry due to their unique properties and potential applications. These compounds, characterized by the presence of boron in their molecular structure, exhibit diverse functionalities that make them suitable for use in medicinal chemistry, materials science, and catalysis. One notable class of organic boron compounds is the heterocyclic aminoboron compounds, which have been investigated for their potential as antituberculosis agents. The research by Hall (2011) demonstrated that diamines with ethylene or propylene backbones yielded stable and active compounds, with derivatives from pinacol boronate esters showing enhanced antimycobacterial activity compared to their boronic acid counterparts. This highlights the importance of structural modifications in enhancing the biological activity of boron-containing compounds. Another significant area of research involves cyclic fluorodiamines containing boronate esters. Zhu et al. synthesized these compounds and evaluated their antimicrobial properties, noting that the protection of the boronic acid group via dehydration led to improved solubility in organic solvents, which is crucial for their application in biological systems (Zhu et al., 2017). The ability to manipulate the solubility and reactivity of boron compounds through synthetic strategies is a key aspect of their utility in medicinal chemistry. Boronic acids and their derivatives have also been explored for their role in catalysis and organic synthesis. For instance, Zu et al. reported on the catalytic enantioselective construction of chiroptical boron-stereogenic compounds, emphasizing the versatility of boron in facilitating complex organic transformations (Zu et al., 2021). This capability is particularly valuable in the synthesis of pharmaceuticals, where the stereochemical configuration of compounds can significantly influence their biological activity. In the realm of antimicrobial applications, boron compounds have shown promise as effective agents against various pathogens. Koldemir-Gündüz et al. highlighted the antimicrobial effects of boron compounds

against a range of bacteria and fungi, suggesting their potential as therapeutic agents in treating infections (Koldemir-Gündüz et al., 2021). The incorporation of boron into hybrid organic molecules has been proposed as a strategy to enhance antimicrobial efficacy while addressing issues related to drug resistance (Ganbar, 2019). Moreover, the development of boron clusters, such as carboranes, has opened new avenues for creating unique pharmacophores in biologically active compounds. These clusters exhibit distinct structural and electronic properties that can be tailored for specific interactions with biological targets, making them valuable in drug discovery (Issa et al., 2011). The modular nature of boron clusters allows for the incorporation of various functional groups, which can influence their antimicrobial activity and selectivity (Wang & Spokoyny, 2022). In summary, organic boron compounds represent a diverse and promising class of materials with significant potential in various applications, particularly in medicinal chemistry and antimicrobial development. Their unique properties, coupled with the ability to modify their structures, make them valuable candidates for further research and development in the quest for novel therapeutic agents.

Inorganic boron compounds

Inorganic boron compounds are a diverse class of materials that exhibit unique properties and functionalities, making them valuable in various applications, including catalysis, materials science, and medicinal chemistry. These compounds typically consist of boron in combination with other elements, often forming complex structures that enhance their chemical and physical properties. One significant area of research involves the synthesis of inorganic boron-based hybrid materials. For instance, Mergheş et al. explored the influence of boron on the structure and properties of hybrid compounds containing zirconium and phosphorus. Their findings indicated that the presence of boron significantly enhanced the thermal stability of these materials, attributed to boron's ability to form stable covalent networks (Mergheş et al.,2022). This characteristic is particularly beneficial in applications requiring high thermal resistance, such as in aerospace and automotive industries. Another interesting development in inorganic boron chemistry is the creation of boron-doped materials. Ando et al. synthesized boron-doped polycyclic π-electron systems, which demonstrated enhanced electron-accepting abilities due to the incorporation of antiaromatic borole substructures. These materials exhibited potential for use in photoresponsive applications, showcasing the versatility of boron in modifying electronic properties (Ando et al., 2021). Such modifications can lead to advancements in organic electronics, including organic light-emitting diodes (OLEDs). The coordination chemistry of boron compounds has also been a focal point of research. Zhi et al. reported on the construction of a series of three-dimensional inorganic-organic hybrid borates that link one-dimensional transition-metal coordination polymers with different inorganic boron oxides. This approach highlights the importance of coordination polymers in determining the final structures of borates, which can have implications for their use in catalysis and materials science (Zhi et al., 2018). The ability to manipulate the structural features of boron compounds opens avenues for designing materials with tailored properties for specific applications. In addition to their structural and electronic applications, inorganic boron compounds have shown promise in medicinal chemistry. For example, boron-containing compounds have been investigated for their potential as proteasome inhibitors, which are crucial in cancer therapy. Milani et al. synthesized L-cysteine-based boron compounds and evaluated their efficacy as proteasome inhibitors, indicating the therapeutic potential of these compounds in treating hematologic tumors (Milani et al., 2014). The ability of boron compounds to modulate biological processes underscores their significance in drug development. Furthermore, the flame-retardant properties of boron compounds have been extensively studied. Unlu et al.

compared the effectiveness of boron compounds and aluminum trihydroxide as flame retardant additives in epoxy resins. Their research demonstrated that boron compounds could enhance the flame retardancy of these materials by increasing char yield and suppressing smoke, making them suitable for applications in construction and manufacturing (Unlu et al., 2014). This aspect of boron chemistry is particularly relevant in the context of safety regulations and material performance standards. In summary, inorganic boron compounds exhibit a wide range of properties that make them suitable for various applications, from enhancing material stability to serving as therapeutic agents. Ongoing research into the synthesis and characterization of these compounds continues to reveal new functionalities and potential applications, highlighting the importance of boron chemistry in advancing technology and medicine.

Biotechnological applications

Cancer therapy

Boron compounds have garnered significant attention in cancer therapy, particularly through the mechanism of Boron Neutron Capture Therapy (BNCT) (Figure.2). This innovative approach leverages the unique properties of boron, especially the non-radioactive isotope boron-10. When boron-10 is irradiated with thermal neutrons, it undergoes a nuclear reaction that produces high-energy alpha particles and lithium nuclei, which effectively damage cancer cells while sparing surrounding healthy tissue (Barth et al., 2018b; Anufriev et al., 2020). The efficacy of BNCT depends on the selective accumulation of boron compounds within tumor cells, necessitating the development of advanced boron delivery systems that enhance tumor targeting and uptake (Luderer et al., 2016). One of the critical challenges in BNCT is achieving a sufficient boron concentration in tumor tissues. Studies indicate that an optimal tumor-to-normal tissue (T/N) boron concentration ratio of greater than 3:1 is necessary for effective treatment (Scorei & Popa, 2010; Wang et al., 2019). Various strategies have been proposed to improve boron delivery, including the use of receptor-targeted agents such as boron-rich peptides and liposomes, which can enhance the selectivity and uptake of boron compounds in cancer cells (Worm et al., 2019; Heber et al., 2012; Heber et al., 2014). For instance, the use of neuropeptide Y conjugates has shown promise in maximizing boron uptake and improving therapeutic efficacy in BNCT (Worm et al., 2019; Ahrens et al., 2014).

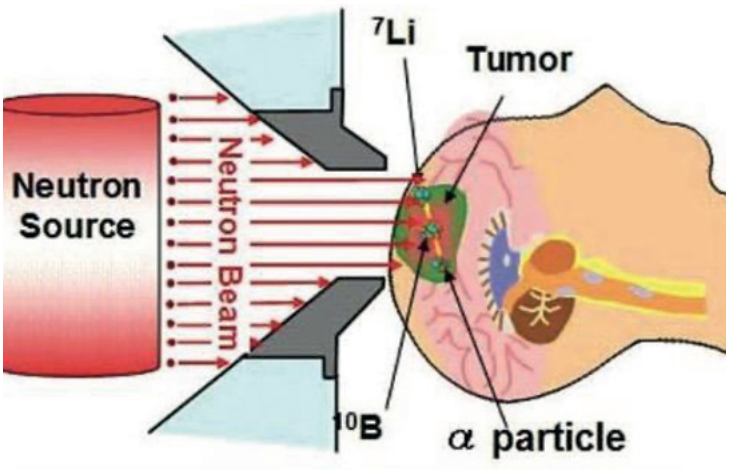

Figure 2. Boron Neutron Capture Therapy with the ^{10}B isotope.

Recent advancements have also explored the use of biodegradable nanoparticles and liposomes as carriers for boron compounds, enhancing the pharmacokinetics and biodistribution of boron in vivo (Li et al., 2017; Tamanoi et al., 2021). For example, hollow boron nitride nanospheres have been effectively utilized to deliver doxorubicin in prostate cancer models, highlighting the potential of boron compounds not only as neutron capture agents but also as carriers for chemotherapeutic drugs (Li et al., 2017). Furthermore, the development of carborane-based compounds, which possess high boron content and stability, has opened new avenues for improving the therapeutic ratio of BNCT (Yuan et al., 2019; Xiong et al., 2016). Research has indicated that certain boron-containing compounds can inhibit enzymatic activities associated with cancer cell proliferation and induce apoptosis (Scorei & Popa, 2010). This multifaceted approach, which combines the direct tumoricidal effects of BNCT with the chemotherapeutic properties of boron compounds, holds promise for improving treatment outcomes in various malignancies. In conclusion, the application of boron compounds in cancer therapy, particularly through BNCT, represents a promising frontier in oncology. Ongoing research into novel boron delivery systems and the optimization of boron accumulation in tumors are crucial for enhancing the efficacy of this therapeutic modality. Integrating boron compounds into existing treatment paradigms may offer synergistic effects, potentially leading to improved patient outcomes in cancer treatment.

Building on these advancements in boron-based cancer therapy, the development of innovative drug delivery systems plays a pivotal role in improving the targeted delivery and efficacy of therapeutic agents, including boron compounds, within tumor tissues.

Drug delivery systems

Recent studies have highlighted the potential of polyhedral boron compounds, such as carboranes and dodecaborates, as promising boron carriers due to their ability to deliver high concentrations of boron while maintaining relatively low toxicity (El-Zaria & Nakamura, 2009; Hattori et al., 2021).

Various strategies, including click chemistry and cycloaddition reactions, have been employed to create boron carriers that can improve the biodistribution and uptake of boron in cancerous tissues. For example, the synthesis of mercaptoundecahydrododecaborate derivatives via click chemistry has been proposed as a method to visualize boron localization in cells, thus facilitating more precise BNCT applications (El-Zaria & Nakamura, 2009; Zhu et al., 2010).

The integration of boron compounds into smart materials has also been explored, particularly in cancer radiation therapy. Boron crosslinked polymers have been designed to respond to specific stimuli, expanding their utility in targeted therapy applications (Vedelago et al., 2021) BNCT relies on the selective accumulation of boron-containing compounds in tumor cells, followed by irradiation with thermal neutrons, which induces a nuclear reaction that selectively destroys cancer cells while sparing surrounding healthy tissue (Barth et al., 2018a,b; Seneviratne et al., 2023). To achieve this, various innovative drug delivery systems have been explored, including nanoparticles, liposomes, and dendrimers, each designed to optimize boron accumulation in tumors and minimize systemic toxicity. Nanoparticle-based systems have emerged as promising candidates for boron delivery due to their ability to improve targeting efficiency and enhance the pharmacokinetics of boron compounds. For instance, carborane-conjugated polymeric nanoparticles have been developed to encapsulate doxorubicin, allowing for a dual therapeutic approach that combines chemotherapy with BNCT (Xiong et al., 2015). These nanoparticles are designed to remain stable in circulation while facilitating the selective release of boron compounds within the tumor microenvironment, thereby

enhancing therapeutic outcomes (Xiong et al., 2015; Heide et al., 2021). Additionally, the use of boron-rich nanotubes has shown potential in improving the delivery of boron compounds, as they can be engineered to possess high boron content while maintaining biocompatibility (Heide et al., 2021). Another effective strategy involves the use of liposomes as carriers for boron compounds. Liposomes can encapsulate boron-containing agents, such as boronophenylalanine (BPA), and enhance their delivery to tumor sites through the enhanced permeability and retention (EPR) effect (Koganei et al., 2012; Ailuno et al., 2022). Recent studies have demonstrated that liposomes with boron content can significantly improve tumor accumulation and therapeutic efficacy in BNCT (Koganei et al., 2012). Furthermore, modifications to liposomal formulations, such as the incorporation of targeting ligands, can further refine the specificity of boron delivery to cancer cells (Barth et al., 2018b). Dendrimers, which are highly branched macromolecules, have also been investigated for their potential as boron delivery systems. Their unique structure allows for the incorporation of multiple boron atoms, increasing the overall boron payload delivered to tumor cells (Dash et al., 2012). The leaky vasculature characteristic of tumors facilitates the preferential accumulation of dendrimer-based systems, thereby enhancing the likelihood of effective boron delivery (Dash et al., 2012). Moreover, the use of “click” chemistry to synthesize phenylene-cored carborane dendrimers has shown promise in enhancing cellular uptake and targeting specificity (Dash et al., 2012). In addition to these systems, antibody-based targeting approaches have been explored to improve the delivery of boron compounds to specific tumor types. By conjugating boron compounds to antibodies or peptides that bind to overexpressed receptors on cancer cells, researchers aim to achieve more precise targeting and enhanced therapeutic efficacy (Nakase et al., 2020; Worm et al., 2018). This method capitalizes on the biological targeting capabilities of antibodies to promote the selective accumulation of boron in tumor tissues. In conclusion, the development of advanced drug delivery systems for boron compounds is essential for optimizing BNCT and improving cancer treatment outcomes. Nanoparticles, liposomes, dendrimers, and antibody-based systems represent a diverse array of strategies that can facilitate the selective delivery of boron to tumor cells while minimizing exposure to healthy tissues. Continued research in this area is critical for translating these innovative delivery systems into clinical practice, ultimately enhancing the therapeutic efficacy of BNCT.

Alongside the advancements in drug delivery systems, the role of enzyme inhibitors in enhancing the therapeutic potential of boron compounds complements their efficacy, particularly in targeting key pathways involved in cancer cell proliferation and survival.

Enzyme inhibitors

Boron compounds have shown significant potential as enzyme inhibitors, particularly in the context of various therapeutic applications. Their inhibitory effects are primarily attributed to their ability to form covalent bonds with active site residues of enzymes, leading to the modulation of enzymatic activity. This mechanism has been extensively studied in relation to β-lactamases, proteases, and histone deacetylases, among others. One of the most notable applications of boron compounds lies in the inhibition of β-lactamases, which are enzymes produced by bacteria that confer resistance to β-lactam antibiotics. For instance, cyclic boronateshave been identified as potent inhibitors of various classes of β-lactamases, including metallo-β-lactamases (MBLs) and serine-β-lactamases (SBLs) (Brem et al., 2016). The cyclic boronates exhibit a broad spectrum of activity, effectively inhibiting enzymes such as VIM-1 and NDM-1, which are associated with multidrug resistance in clinical isolates (Cahill et al., 2017; Krajnc et al., 2019). The mechanism of inhibition involves the formation of a stable adduct with the active site serine residue, blocking the enzyme’s catalytic

activity (Krajnc et al., 2019). In addition to β-lactamases, boron compounds have been explored as inhibitors of histone deacetylases (HDACs), which play a crucial role in gene regulation and are implicated in cancer progression (Suzuki et al., 2009). Boronic acid-based HDAC inhibitors have been synthesized and shown to effectively inhibit HDAC activity in both enzyme assays and cellular models. The design of these inhibitors often involves adding aromatic groups that enhance binding affinity and selectivity for the target enzyme (Suzuki et al., 2009). Moreover, boron compounds have been reported to inhibit proteases, including serine proteases, through a competitive mechanism. Peptidyl boronates, for example, have been shown to inhibit the Lon protease of *Salmonella enterica* by forming a tetrahedral adduct with the enzyme's active site (Frase & Lee, 2007). This two-step inhibition mechanism involves an initial rapid binding, followed by a slower conformational change that stabilizes the inhibitor-enzyme complex, effectively rendering the enzyme inactive (Frase & Lee, 2007). The antioxidant properties of boron compounds also contribute to their enzyme inhibition effects. Studies have indicated that certain boron compounds can enhance the activities of antioxidant enzymes, potentially modulating oxidative stress responses in cells (Türkez et al., 2007; Akbari et al., 2022). This dual role as both an enzyme inhibitor and an antioxidant suggests that boron compounds may have therapeutic implications beyond traditional enzyme inhibition.

Boron compounds have also been investigated for their potential as enzyme inhibitors, particularly in proteasome inhibition. Boronic acids, a class of boron compounds, have been utilized to develop potent inhibitors of various enzymes, including those involved in cancer progression (Yang et al., 2003; Milani et al., 2014). The reversible nature of boronic acid interactions with target proteins allows for the design of selective inhibitors that can modulate biological pathways effectively. Moreover, the incorporation of boron into pharmaceutical agents has been shown to enhance their therapeutic profiles, as evidenced by the development of boron-containing retinoids and benzoxaboroles as treatment for diverse diseases (Das et al., 2022; Glynn et al., 2015).

In summary, boron compounds exhibit a diverse range of enzyme inhibition effects, making them valuable candidates for drug development in multiple therapeutic areas, particularly in combating antibiotic resistance and cancer. Their ability to form covalent interactions with enzyme active sites underpins their efficacy as inhibitors, and ongoing research continues to explore their potential in clinical applications.

In addition to their role as enzyme inhibitors, boron compounds also demonstrate significant antimicrobial effects, presenting promising avenues for combating bacterial infections and multidrug-resistant pathogens.

Antimicrobial effects

Boron compounds have been increasingly recognized for their antimicrobial properties, demonstrating effectiveness against a wide range of bacterial and fungal pathogens. The antimicrobial effects of boron are attributed to various mechanisms, including the disruption of microbial cell walls, inhibition of enzyme activity, and interference with metabolic processes. One notable finding is that boron compounds, such as boronic acid and its derivatives, exhibit significant antimicrobial activity against various strains of bacteria and fungi. For instance, Koldemir-Gündüz et al. (2021) reported that boron compounds effectively inhibited the growth of pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. The study highlighted that boron compounds have the potential to serve as potent agents against both bacterial and fungal infections, making them valuable in clinical settings. In addition to their broad-spectrum antimicrobial activity, boronic acids

have been identified as promising candidates for the development of novel antibacterial agents. These compounds can inhibit penicillin-binding proteins (PBPs), which are crucial for bacterial cell wall synthesis (Fontaine et al., 2014; Kollár, 2024). Kollár (2024) demonstrated that boronic acid derivatives effectively inhibit PBPs, blocking their function and leading to bacterial cell lysis (Kollár, 2024). This mechanism is particularly relevant to antibiotic resistance, as boronic acids can target resistant strains of bacteria. Furthermore, the potential of boron compounds as inhibitors of bacterial efflux pumps has been explored. The NorA efflux pump in *Staphylococcus aureus* plays a key role in multidrug resistance. Thamilselvan et al. found that boronic acid derivatives could inhibit the NorA efflux pump, thereby resensitizing drug-resistant strains to conventional antibiotics (Thamilselvan et al., 2021). This suggests that boron compounds may not only act as antimicrobial agents but also enhance the efficacy of existing antibiotics. The antimicrobial properties of boron compounds extend to their use in various formulations, including detergents and disinfectants. Boron compounds are commonly incorporated into cleaning products due to their ability to enhance stain removal and provide antimicrobial effects, which are crucial for maintaining hygiene in healthcare and industrial settings (Saraç et al., 2015). Their effectiveness in reducing microbial load on surfaces further supports their application in infection control. Moreover, the synthesis of boron-containing compounds has led to the discovery of new antimicrobial agents with enhanced activity. However, the claim regarding boromycin as a boron-based antibiotic was removed due to insufficient supporting references. Ongoing research into the structure-activity relationships of boron compounds continues to unveil new derivatives with improved antimicrobial properties. In conclusion, boron compounds exhibit significant antimicrobial effects against a variety of pathogens, making them valuable candidates for therapeutic applications. Their mechanisms of action, which include cell wall synthesis inhibition and efflux pump inhibition, position them as promising agents in the fight against antibiotic-resistant infections. Continued research into boron chemistry and its applications in antimicrobial formulations is essential for developing effective strategies to combat microbial resistance.

Suggestion about biomedical applications of boron compounds

To overcome the weaknesses of boron compounds in biomedical applications, several strategies can be employed. Enhancing tumor targeting and selectivity is crucial, with advanced delivery systems like nanoparticles, liposomes, and antibody-based targeting showing promise in improving boron accumulation in tumor cells while minimizing toxicity to healthy tissues. Incorporating receptor-targeted agents could further improve selectivity for cancer cells, maximizing therapeutic effects, especially in Boron Neutron Capture Therapy (BNCT). Additionally, boron compounds should be explored in combination with existing antibiotics or chemotherapeutics to address antimicrobial resistance and enhance the efficacy of conventional treatments. Optimizing pharmacokinetics and biodistribution is another key focus, with biodegradable nanoparticles and liposomes offering controlled release and improved stability in circulation. Expanding the range of applications for boron compounds is essential, particularly by exploring their potential as enzyme inhibitors in diseases like Alzheimer's or Parkinson's, in addition to cancer and infections. To reduce toxicity and side effects, more research is needed to refine the formulations of boron-based compounds and improve their safety profiles through targeted drug delivery systems. Furthermore, advancing research on boron clusters, such as carboranes, could lead to more stable and effective boron-rich compounds, improving therapeutic ratios in BNCT and other therapies. Finally, investigating new mechanisms of action, such as enzyme

inhibition and synergies with immunotherapy, could broaden the therapeutic potential of boron compounds, making them more versatile and effective in a variety of medical treatments. By focusing on these strategies, boron compounds can overcome their current limitations and become valuable tools in cancer, antimicrobial, and enzyme-targeted therapies.

Conclusions

Boron compounds, both organic and inorganic, have demonstrated significant potential across a wide range of applications in medicinal chemistry, catalysis, and material science. Additionally, AI approaches have been used to obtain comprehensive references from around the world.The ongoing research into boron compounds underscores their versatility and potential in various biomedical applications. Their unique chemical properties, such as the ability to form covalent bonds with enzymes and modify solubility, make them highly valuable in the development of therapeutic agents. In particular, organic boron compounds, such as heterocyclic aminoboron and boronic acid derivatives, have shown promise as antimicrobial and anticancer agents, while inorganic boron compounds contribute to advancements in catalysis and materials with enhanced stability and electronic properties. Furthermore, the integration of boron into drug delivery systems has opened new frontiers for targeted cancer therapies, such as Boron Neutron Capture Therapy (BNCT), with ongoing research focused on optimizing delivery mechanisms and enhancing therapeutic efficacy. The diverse enzymatic inhibition potential of boron compounds, particularly in combating antibiotic resistance and cancer progression, highlights their significance in drug development. As enzyme inhibitors, boron-containing molecules have exhibited broad-spectrum activity against bacterial pathogens, including drug-resistant strains, as well as promising effects on cancer cells. Moreover, their antimicrobial properties, combined with their role in enhancing the efficacy of conventional antibiotics, position boron compounds as valuable tools in the fight against infections. With continued research and advancements in the synthesis and modification of boron-based compounds, the scope of their applications will only expand, offering novel solutions for treating infections, cancer, and other diseases. The ability to modify and tailor the structure of boron compounds further strengthens their potential as versatile therapeutic agents, making them an exciting avenue for future research and development in biotechnology and pharmaceuticals.

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Conflict of interest

The author declares no conflict of interest.

Data availability statement

None.

Ethics Committee Approval

Ethics committee approval is not required for this study.

Authors' contribution statement

GCG; The author conceptualized and designed the study, conducted the literature review, and drafted the manuscript. The author was responsible for interpreting the data, writing the manuscript, and revising it for intellectual content. Additionally, the author supervised the research process and ensured the accuracy and integrity of the content throughout the study.

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REVIEW

The role of 3D printing in advancing biotechnology and bioengineering: A review

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Abstract

Three-dimensional (3D) printing, a subset of additive manufacturing technologies, has attracted significant attention from researchers for both laboratory-based and on-site prototyping since its widespread adoption. Its adaptability and versatility have made it an essential tool across various disciplines, particularly in biotechnology and bioengineering. While conventional manufacturing methods can offer precise material control and compatibility with biological fluids, they often pose significant challenges, such as high costs and the requirement for large, complex setups. These constraints limit their accessibility for the experimental needs of biotechnology and bioengineering. However, 3D printers, with their high adaptability and ability to process a wide range of materials, have proven to be remarkably effective in resolving these challenges. Their capability to create custom parts and structures while maintaining compatibility with biomaterials and fluids has opened new possibilities not only in tissue engineering, drug development, and biomedical device fabrication, but also across the broader fields of biotechnology, biochemistry, and related sciences. When examining the basic concept and development timeline of 3D printers, it becomes clear that emerging trends in artificial intelligence, robotics, and digitalization are expected to further accelerate their integration into real-world applications. These ongoing advancements are likely to benefit laboratories and production centers involved in biotechnology by speeding up experiments, paving the way for rapid production and testing, and making complex biofabrication processes more accessible and automated, including in areas like tissue engineering and personalized medicine.

Keywords: 3D printing, additive manufacturing, bioengineering, biofluids, instrumentation, rapid prototyping

Introduction

Additive manufacturing refers to the process of building a product by adding layers of homogeneous or different materials in a numerically controlled and stable manner, ensuring they are mechanically bonded and resistant to separation over time. Additive manufacturing, by definition, involves building an object by adding material layer by layer during the production process, in contrast to traditional (subtractive) manufacturing, which shapes the final product by removing material from larger blocks. As a result, one of the primary benefits of additive manufacturing is the significant reduction of waste material compared to traditional methods (Monfared et al., 2023; Narsimhachary & Kalyan Phani, 2024; Zhou et al., 2024).

The first patent application (stereolithography, SLA) in the field of additive manufacturing was filed in 1984 focused on computer-controlled stereolithography (United States Patent No. US4575330A, 1986). Four years later, the prototype was commercialized, marking a significant milestone in the history of AM with the process of solidifying liquid photopolymers using ultraviolet (UV) lasers guided by computer-controlled 3D motion mechanisms. Later, Fused Deposition Modeling (FDM or Fused Filament Fabrication, FFF) emerged. These techniques rely on combining thermoplastics or materials with similar thermal characteristics that are heated to their melting point, extruded into thin layers, and solidified through rapid cooling (Choi et al., 2011).

A pivotal development occurred with the RepRap project (initiated in 2005), which aimed to create self-replicating 3D printers capable of manufacturing their own components (Jones et al., 2011). The success of the RepRap project led to a rapid expansion of the open-source ecosystem surrounding 3D printing. The availability of easy-to-print and less hazardous materials like PLA after 2010 made printers more accessible, eventually reaching household use. Additionally, the expiration of FDM patents in 2009 and SLA patents in 2014 significantly contributed to the growth of makers developing 3D printers, further accelerating the widespread adoption of technology (United States Patent No. US5121329A, 1992; United States Patent No. US4575330A, 1986). Figure 1 presents simplified schematics illustrating the FDM and resin-based printing techniques.

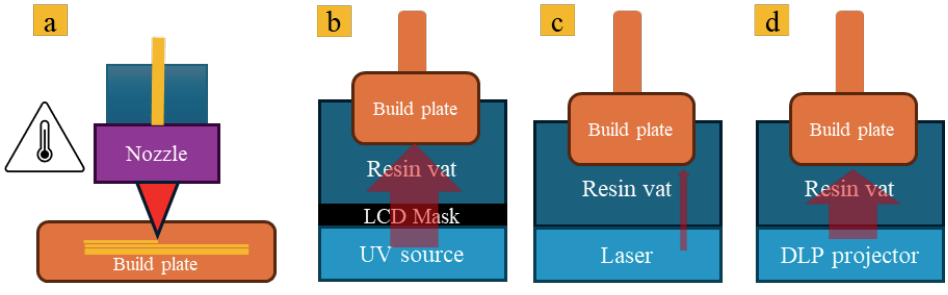


Figure 1. Simple schematics of basic 3d printing techniques, A: FDM, B: MSLA (Masked Stereolithography), C: SLA, D: DLP (Digital Light Processing)

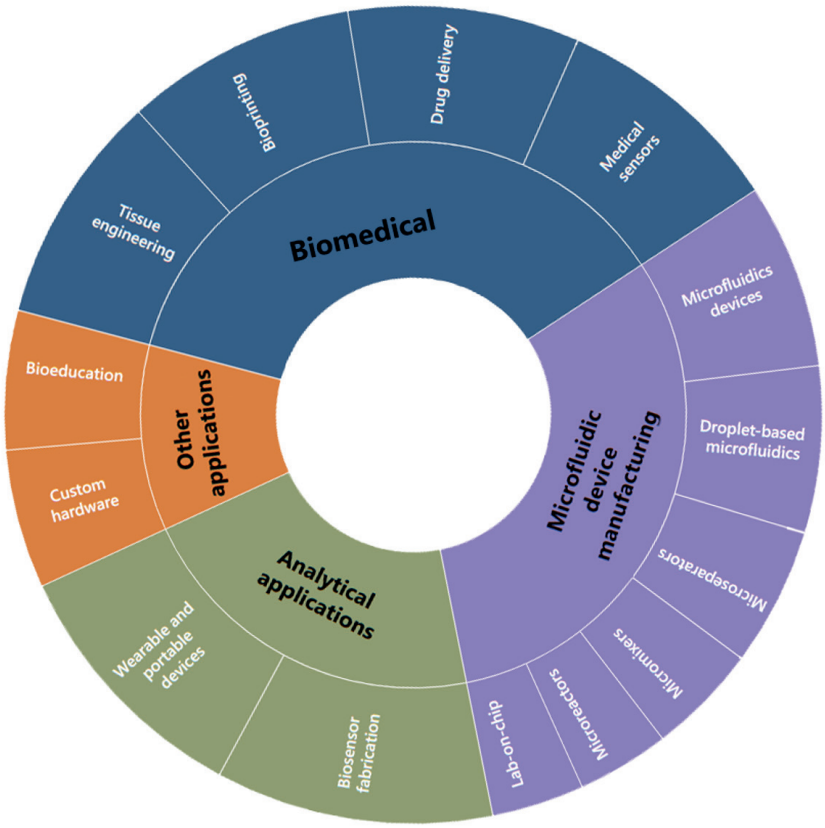
Table 1 compares various 3D printing methods (excluding bioprinting). FDM is typically favored for its affordability and wide range of material options, making it ideal for large prints. Resin-based techniques, though more sensitive and expensive, offer higher precision, with MSLA being a more

cost-effective alternative. Powder-based methods are faster and more sensitive but tend to be the most expensive, especially when combined with metal powder melting.

Table 1. Comparison of the main features of main 3D printing techniques.

Method	Printing principle and materials	Resolution	Cost
FDM	Extrudes various thermoplastic filaments layer by layer	Moderate	Low
SLA	Cures photopolymer liquid resin using UV light	Very High	Moderate
MSLA	Cures resin using a masked LCD screen to project light	Very High	Moderate-Low
SLS (Selective Laser Sintering)	Fuses powder material (polymer, metal) using a laser layer by layer	High	High
DLP	Cures resin layers using digital light projection	Very High	Moderate
MJF (Multi Jet Fusion)	Fuses powder layers using fusing and detailing agents (PA, TPU)	High	High
EBM (Electron Beam Melting)	Melts metal powder using an electron beam in vacuum	High	Very High

In this paper, three-dimensional printing technologies, which are becoming increasingly vital tools in bioscience due to the unique advantages they offer, are reviewed under the sorted headings outlined in Figure 2.



| **Figure 2.** Key applications of 3D printing technologies in bioscience.

3D printers in microfluidic device manufacturing

Microfluidic devices: The conventional method for producing microfluidic systems typically involved several steps, beginning with molding a poly(dimethylsiloxane) (PDMS) substrate using soft lithography. Although this method is effective, it requires multiple complex steps, specialized expertise, and a well-equipped infrastructure (Bhattacharjee et al., 2016). Compared to classical methods, 3D printing has streamlined the production process by reducing the number of steps and significantly lowering costs (Amin et al., 2016). In this field, as well as from a broader perspective, various 3D printing techniques can be applied. Resin and inkjet printers excel in precision, while MSLA printers offer a balance of accuracy and affordability. On the other hand, FDM printers are more cost-effective but are limited by their lower resolution, making them less suitable for applications requiring fine details (Waheed et al., 2016). 3D printing has enabled the rapid fabrication of microfluidic devices designed for specific applications, particularly in key areas such as biofluid mixing and separation, microreactors, and complex organ-on-chip assemblies. Thanks to the open-source nature of 3D printers, researchers can accelerate experiments by developing customized software and production workflows engineered to meet specific objectives (Y. Zhang et al., 2024).

Droplet-based microfluidics: 3D printers are successfully employed in droplet-based microfluidics, a technology that utilizes various physical actuation methods (e.g., magnetic, ultrasonic, pneumatic, thermal) to regulate droplet generation, making it possible to design custom components integrated into unified microfluidic devices (Aladese & Jeong, 2021; Moragues et al., 2023). Jiao et al. developed a 3D-printed droplet-based microfluidic chip and applied it for PCR detection of miRNA-21 in cellular samples, demonstrating its utility for sensitive and specific biomarker detection in cancer diagnostics (Jiao et al., 2019). Ji et al. developed a customizable microfluidic system using 3D-printed components capable of creating emulsion droplets with desired properties (Ji et al., 2018). Nguyen et al. demonstrated the production of cell-containing hydrogel microspheres by developing a 3D-printed system with an adjustable gap height (Nguyen & Seo, 2022). In one study, the desired surface hydrophobicity was adjusted by leveraging 3D printing's ability to work with various materials (Warr et al., 2021). In pharmaceutical research, 3D-printed droplet-based chips play a prominent role in drug synthesis, screening, and delivery applications (Trinh et al., 2023). Additionally, the droplet microfluidic method has been shown to produce sensors for a wide range of applications by utilizing inks with various functions (Zub et al., 2022).

Micromixers: Micromixers are microfluidic devices used in biochemistry, both in the laboratory and in the field, for preparing homogeneous mixtures before they proceed, and the use of 3D printing for their fabrication has grown in significance (Razavi Bazaz et al., 2024). For example, Borro et al. successfully controlled the capacity and size of drug-loaded hydrogels using the micromixer they developed(Borro et al., 2019). Lavrentieva et al. utilized 3D-printed micromixers to achieve homogeneous mixing of precursors and crosslinkers, enabling the creation of stiffness gradients in photoactive hydrogels, an approach often applied in mechanobiology research (Lavrentieva et al., 2020). Bohr et al. produced nanocomplexes using micromixers they designed, enabling pilot-scale production speeds (Bohr et al., 2017). Researchers have designed micromixers that facilitate the rapid measurement of various biomarkers using portable biosensor systems **(Chan et al., 2016a; B. Liu et al., 2022; Plevniak et al., 2016)**. With rapid prototyping made possible by 3D printers, researchers can now conduct production tests of more efficient micromixers through computer simulation studies (Ammar et al., 2025; Liao et al., 2025; Z. Wang et al., 2023; Yin et al., 2021).

Microseparators: The use of 3D-printed microseparators is common in the separation of biological fluids and solids, serving purposes such as sample preparation, filtration, bioproduct purification, component separation, reaction setup, impurity removal, and diagnostics (Griffin & Pappas, 2023; Marković et al., 2024). In one study, 3D spiral separators were employed for the large-scale separation and extraction of stem cells (Ding et al., 2022). In another study, a different spiral design was developed to separate mammalian ovarian cells into a continuous flow system by incorporating 3D buffers (Enders et al., 2021). Amin et al. developed a portable 3D-printed device for the mass density-based separation of label-free heterogeneous cell mixtures in real-time continuous flow (Amin et al., 2017). Yang et al. harnessed the power of transferrin-receptor affinity to isolate cancer cells from biopsy fluids by modifying the surface of a 3D-printed device (Yang et al., 2023). Schellenberg et al. integrated a 3D-printed microseparator into a bioreactor outlet, eliminating the need for periodically replaced membranes and enabling continuous high-yield purification of monoclonal antibodies produced by cells (Schellenberg et al., 2023). Syed et al. designed a 3D-printed microcyclone separator for efficient and continuous harvesting of microalgae (Syed et al., 2017). With the rise of 3D printers, the development and testing of complex micromixers has become significantly easier (J. Clark et al., 2024; P. Li et al., 2021; Oldach et al., 2024).

Microreactors: Microreactors are all-in-one micro-bioprocessing solutions that garner attention due to their efficiency, scalability, and precise control over reaction conditions (Maier et al., 2020; Shrimal et al., 2020). Cingesar et al. designed a 3D microreactor and connected it to a microseparator to carry out methyl ester conversion under optimal conditions in the production of biodiesel from sunflower oil (Cingesar et al., 2025). One of the advantages of microreactors is the large surface area they offer, enabled by customizable and printable porosity options, which promotes homogeneous catalytic activity in continuous flow systems. Building on this advantage, Baena-Moreno et al. designed a system with internal surfaces in a gyroid geometry, which successfully increased the CO_2 conversion rate by 14% (Baena-Moreno et al., 2021). Alimi et al. similarly utilized a microreactor for flavonoid oxidation, significantly enhancing the reaction rate compared to a traditional batch reactor (Alimi et al., 2020). Ibáñez-de-Garayo et al. designed a microreactor specifically tailored for photocatalysts by creating a multichannel microarray, which evenly distributes light with high transmittance, effectively increasing the surface area (Ibáñez-de-Garayo et al., 2023). The advantages of 3D-printed microreactors are also leveraged in bioreactors, making them ideal for the cultivation of algae and other photosynthetic microorganisms (Castaldello et al., 2019; Podwin & Dziuban, 2017).

Lab-on-chip: Micro total analysis systems (μTAS), also known as lab-on-chips, are microfluidic devices typically used for analytical applications. They are created by integrating various functional units, such as the mixer, separator, and reactor designs previously mentioned, along with microvalves and microconcentrators (Patabadige et al., 2016). In one study, a low-cost flow analyzer for exposome determination from soil samples was developed using 3D printing technology (Cocovi-Solberg et al., 2019). Chiado et al. designed an optical analytical device that enables the detection of protein biomarkers, aiding in the early diagnosis of cancer (Chiadò et al., 2020). Adamski et al. developed a 3D-printed chip capable of performing DNA gel electrophoresis more cost-effectively and quickly (Adamski et al., 2016). 3D microchips are well-suited for artificial organ studies. For instance, in one study, an artificial nervous system chip was created to investigate viral infections in the nervous system (Johnson et al., 2016). Cardiovascular tissues and organs are also the focus of research and study using 3D microchips (Y. S. Zhang et al., 2016). Addario et al. designed a chip with the purpose of mimicking kidney tubule segments, which was used for in vitro tests related to chronic kidney disease (Addario et al., 2024).

3D printers in tissue engineering, bioprinting and biomedical

Bioprinting: Bioprinting refers to the process of combining biological materials to create structures such as tissues, organs, patches, or scaffolds, following principles similar to those of additive manufacturing (Mironov et al., 2006). The most notable difference from other 3D printing techniques is the use of bioinks as building materials (Decante et al., 2021). These bioinks, typically liquid, gel, or composite, are specifically developed to incorporate cells, microorganisms, macromolecules, and hormones (Daly et al., 2021). Additionally, bioprinting utilizes print heads such as various droplet-based microfluidic systems, pressurized syringe tips, pneumatic, or screw extruders, with the screws used to mix the materials for precise biomaterial deposition (Chen et al., 2023). These techniques typically require high-end printers, but with the adoption of photopolymer-based bioinks and high-resolution, low-cost resin printers, they have become more accessible and widely used in many laboratories (Tong et al., 2021). Unlike other 3D printing techniques, bioprinting can be performed directly on living tissues, organisms, or within a viscous medium to apply the bioink without disturbance and in a biocompatible manner (Singh et al., 2020). A viscous medium acts as a support structure in the technique known as submerged printing (H. Li et al., 2021). Additionally, several viscosity-lowering methods are employed to create stable 3D-printed structures while preserving biocompatibility (Colosi et al., 2016).

Tissue engineering and drug delivery: 3D printers are one of the basic tools in tissue engineering, as well as regenerative medicine (Bartolo et al., 2022). Whether using gel, solid polymer, or composite material, 3D printing is one of the most widely utilized techniques in the production of tissue scaffolds (Dutta et al., 2021; Radhakrishnan et al., 2021; Richards et al., 2013; Shao et al., 2019). The scaffold must have adjustable biodegradability and porosity, which is why 3D-printed fabrication is ideal, as it allows for precise control over these factors due to the wide availability of materials, ensuring the scaffold meets the specific requirements for tissue regeneration (An et al., 2015; Stratton et al., 2016; Wen et al., 2017). With 3D printing, scaffolds can be rapidly produced in geometries tailored for clinical applications (Blázquez-Carmona et al., 2021). 3D printers are widely used in cell seeding studies due to their ability to work with hydrogels. For instance, Xue et al. produced scaffolds with varying hardness by modifying 3D printing parameters, providing physical support for the seeded fibroblasts to grow **(Xue et al., 2019)**. Feng et al. achieved uniform and effective cell transplantation using a 3D-printed scaffold made from alginate and gelatin **(Feng et al., 2020)**. Similarly, drug-loaded tissue treatment patches, often created through 3D printing using biodegradable gels and materials, are also widely applied in biomedical treatments (Jang et al., 2021). This approach is also enabling personalized treatments (Manousi et al., 2024; Peng et al., 2017). 3D printers are also utilized in the production of microneedles, which play a crucial role in drug delivery and portable medical diagnostic devices (Detamornrat et al., 2022; Uddin et al., 2020).

Biomedical: 3D printing enables the creation of patient-specific biomedical devices that are ready for clinical use, particularly in fields such as orthopedics (Wong, 2016). 3D printers are being used to create metal alloy medical nails, wires, drug-loaded implants, and even tiny medical robots (Alam et al., 2020; Hari Raj et al., 2023; Honda et al., 2024; Wei et al., 2024; Ye et al., 2020). The use of 3D printing has expanded to larger models, such as customized arm and neck splints and braces, offering time and labor savings while improving efficiency compared to traditional plaster methods (Ambu et al., 2024; Boolos et al., 2022; J. Li & Tanaka, 2018). The use of 3D printers in dental applications

has shown successful clinical outcomes and is becoming increasingly widespread as both printer and material costs decrease, along with advancements in material research (Anadioti et al., 2020; Majeed et al., 2024; Tichá et al., 2024; van Noort, 2012). 3D printers are demonstrating significant potential in pharmaceutical drug research, facilitating the discovery of new drugs and enabling more personalized approaches to treatment development (Amekyeh et al., 2021; Michalski & Ross, 2014; Pugliese et al., 2021). There are also notable applications in otolaryngology, including the creation of eardrums and cartilage tissue replacements using 3D printing (Hu et al., 2023; Pugliese et al., 2021). 3D printers also serve an important role in advancing biomedical studies, particularly involving cell culture and cell line growth **(Bruno et al., 2019; Herreros-Pomares et al., 2021; Lerman et al., 2018)**.

Sensors: The role of 3D printers in biomedical sensor fabrication is also worth mentioning, such as a flexible wearable sensor that measures shoulder movement limitations, a piezoelectric insole that performs gait analysis, haptic devices that include a soft pressure sensor, and wearable biomechanical sensors made of a conductive transparent gel (Dimo et al., 2024; Latsch et al., 2024; Ntagios et al., 2020; Zeng et al., 2025).

Analytical applications of 3D printers

Biosensor fabrication: The production of most biosensors involves making functional modifications to a substrate material to enable specific biosensing capabilities, often incorporating multiple layers that work together to generate an electrically readable or optically visible signal (Katey et al., 2023). 3D printers are poised to play a significant role in this field due to their ability to work with materials that possess a wide range of functional biochemical and physical properties, such as bioinks, as well as those exhibiting conductive, magnetic, and optical activities (Byrne et al., 2024). For example, Hussaini et al. produced and modified electrodes for dopamine detection using a 3D printer, while Tiwari et al. fabricated microporous electrodes with a 3D printer to detect antibiotics in tissue scaffolds (Hussaini et al., 2024; Tiwari et al., 2024). Glasco et al. produced the electrodes of a new enzyme-free biosensor by 3D printing carbon material (Glasco et al., 2024). In another study, Wang et al. utilized a bioreceptor printed with bioink containing liver microtissue cells for the detection of deoxynivalenol (N. Wang et al., 2025).

Wearable and portable devices: 3D printers are also employed to create the necessary components that enable biosensors to function as portable or wearable devices (Ozer et al., 2022a). Examples include the production of microneedles for biomedical sampling, integration of sensor elements into compact structures such as specialized equipment like heaters and sampling chambers, and optical microfluidic devices that allow colorimetric measurements using a smartphone (Biswas et al., 2024; Chan et al., 2016b; Xu et al., 2024). 3D printers also facilitate the *in-situ* application of biosensors in various fields such as environmental monitoring, agriculture, and food safety (Ataei Kachouei et al., 2025; Ozer et al., 2024; Q. Zhang et al., 2021).

Custom laboratory equipment and educational tools produced using 3D printers

3D printed hardware: With the rise of digital manufacturing, also known as 3D printing, synchronized with the expansion of the open-source software and hardware ecosystem, researchers have increasingly started creating their own devices for both emergencies and regular use (Baden et al.,

2015; Ozer et al., 2022b). For instance, Behrens et al. fabricated a mini peristaltic pump; Holland et al. developed a custom syringe pump; Traciak et al. created a surface tension meter; Pechlivani et al. built a bioreactor; Sule et al. designed a centrifuge, Wilson et al. produced a micropipette, and Chagas et al. designed a fluorescence microscopy platform (Behrens et al., 2020; Chagas et al., 2017; Pechlivani et al., 2023; Sule et al., 2019; Traciak et al., 2021; Wilson & Mace, 2017).

3D printing in bioeducation: 3D printing has emerged as a standout tool in bio-related science education (bioeducation), offering innovative and interactive real-world objects that enhance students' understanding of the educational curriculum. For example, Gul et al. conducted a controlled educational experiment by creating 3D models of biomolecules in living organisms, revealing a clear difference in student comprehension (Gul & Yalinkilic, 2025). Lim et al. incorporated 3D printed models in anatomy lectures (Lim et al., 2016), while Augusto et al. used them in a cell biology course (Augusto et al., 2016). Boll et al. highlighted the usefulness of 3D models in synthetic biology within STEM education **(Oss Boll et al., 2023)**. Pinger et al. have highlighted the superior role of 3D printers in chemistry education (Pinger et al., 2020), and similarly, Renner et al. utilized 3D microfluidics in teaching continuous flow reactors and photoreactions (Renner & Griesbeck, 2020).

Challenges and limitations in using 3D printers

Despite the unique advantages, the use of 3D printers in biosciences also presents some limitations depending on the method applied. For example, in FDM printers, gaps may form between layers due to the way molten material is extruded and shaped. Although these gaps are typically smaller than 0.1 mm and not large enough to support biofilm formation, water molecules or chemical and biological contaminants can adhere to them (Aguado-Maestro et al., 2021). This makes it difficult to fully clean the printed object, especially between the layers. Post-printing surface finishing modifications, such as polishing, can help eliminate these gaps and improve the structure's suitability for bioscience applications. Notably, resin-based printers tend to exhibit fewer issues with interlayer gaps, offering a potential advantage in this regard. Additionally, high-temperature sterilization methods are often unsuitable for FDM-printed objects, necessitating the use of alternative sterilization techniques (Wiseman et al., 2022).

Another significant challenge is cost. While metal 3D printers used in biomedical and dental applications offer advantages over traditional manufacturing methods, they remain relatively expensive to acquire and operate. Additionally, although common materials and consumables used in 3D printing are generally affordable, specialized materials with advanced properties—such as high electrical conductivity, transparency, UV resistance, or a high Young's modulus or elasticity coefficient—come at a much higher cost. This can limit the accessibility of advanced applications and increase the overall expense of research or production on specific bio-applications (Lee et al., 2017; B. Li et al., 2023; Ma et al., 2023; Sachyani Keneth et al., 2021).

PLA, one of the most widely used materials in FDM printers, is typically derived from corn syrup and is biodegradable under controlled conditions. However, it does not break down as easily in natural environments as other biodegradable polymers like PCL (polycaprolactone) or PVA (polyvinyl alcohol). To address this, increasing attention is being given to materials enhanced with additives such as cellulose and lignin, which offer improved biodegradability and sustainability (Choe et al., 2022). In resin-based printers, the trend is shifting toward the use of bio-based photopolymers, which are gaining popularity for their environmentally friendly properties and compatibility with biological applications (Skliutas et al., 2020; Voet et al., 2021).

It is well-documented that mechanically formed microplastic particles can be dispersed into the air during the FDM printing process. However, when using PLA, this does not pose a significant health risk in spaces with normal ventilation. In contrast, materials like ABS release toxic fumes when subjected to high temperatures, making proper ventilation an absolute necessity (Gu et al., 2019; Salthammer, 2022). Similarly, resin printers emit harmful gases, including volatile organic compounds, during the printing process. To address this, many are equipped with HEPA and activated carbon filters to reduce emissions and improve safety during operation (Davis et al., 2019; Garcia-Gonzalez et al., 2024). Another consideration with resin printing is the need for post-processing, including additional curing and washing of the printed objects. The washing process typically involves ethyl alcohol, which can be hazardous if not handled properly. To address this, water-washable, bio-based resins are increasingly being used as a safer and more eco-friendly alternative (Y. Liu et al., 2024).

Conclusions

3D printers have attracted significant interest from the scientific community since they became widespread. The inherent precision of common 3D printing techniques and the variety of materials available, including biomaterials, have allowed them to quickly find a place in the field of bioengineering. In today's open-source digital era, biological products and related components within the biotechnology ecosystem—such as reactors, sensors, microfluidics, wearable devices, implants, and prostheses—are increasingly benefiting from the advantages of computerization thanks to 3D printing technology. With this unique output, researchers are exploring the vast potential of 3D printers across various fields, ranging from education to the synthesis of basic biochemical molecules. As they integrate new material handling techniques into their work, researchers are uncovering novel ways to enhance productivity, reduce research costs, and increase innovation and precision in bioengineering applications.

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Conflict of interest

The author declares no conflict of interest.

Data availability statement

Data sharing is not applicable to this review article as no datasets were generated or analyzed during the current study.

Ethics committee approval

Ethics committee approval is not required for this study.

Authors' contribution statement

Ismail Agir wrote and designed this article.

Use of Artificial Intelligence: No artificial intelligence-based tools or applications were used in the preparation of this study. The entire content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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