TÜBİTAK

Turkish Journal of Agriculture and Forestry

Volume 48 | Number 1 Article 13

2-1-2024

Bioassay-guided purification and identification of antimicrobial compound from corn silk extract and postharvest application against Fusarium verticillioides on cherry tomato

Ummara Khan
khanummaraft@gmail.com

Rita-Cindy AYE-AYIRE SEDJOAH
ritacindy19@gmail.com

Yuting Shao
2018208019@njau.edu.cn

Dyaaaldin ABDALMEGEED
2018116158@njau.edu.cn

Zichao WU
2021121005@stu.njau.edu.cn

See next page for additional authors

Follow this and additional works at: https://journals.tubitak.gov.tr/agriculture

Part of the Agriculture Commons, and the Forest Sciences Commons

Recommended Citation

Khan, Ummara; AYE-AYIRE SEDJOAH, Rita-Cindy; Shao, Yuting; ABDALMEGEED, Dyaaaldin; WU, Zichao; and Xin, Zhihong (2024) "Bioassay-guided purification and identification of antimicrobial compound from corn silk extract and postharvest application against Fusarium verticillioides on cherry tomato," *Turkish Journal of Agriculture and Forestry*: Vol. 48: No. 1, Article 13. https://doi.org/10.55730/1300-011X.3169
Available at: https://journals.tubitak.gov.tr/agriculture/vol48/iss1/13

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Agriculture and Forestry by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

Bioassay-guided purification and identification of antimicrobial compound from corn silk extract and postharvest application against Fusarium verticillioides on cherry tomato

Authors

Ummara Khan, Rita-Cindy AYE-AYIRE SEDJOAH, Yuting Shao, Dyaaaldin ABDALMEGEED, Zichao WU, and Zhihong Xin

This article is available in Turkish Journal of Agriculture and Forestry: https://journals.tubitak.gov.tr/agriculture/vol48/iss1/13

TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2024) 48: 139-153
© TÜBİTAK
doi:10.55730/1300-011X.3169

Bioassay-guided purification and identification of antimicrobial compound from corn silk extract and postharvest application against Fusarium verticillioides on cherry tomato

Ummara KHAN, **Rita-Cindy AYE-AYIRE SEDJOAH**, **Yuting SHAO**, **Dyaaaldin ABDALMEGEED**, **Zichao WU**, **Zhihong XIN***

Key Laboratory of Food Processing and Quality Control, College of Food Science and Technology, Nanjing Agricultural University, Nanjing, PR China

Received: 04.09.2023 • **Accepted/Published Online:** 21.10.2022 • **Final Version:** 01.02.2024

Abstract: Corn silk (*Stigma maydis*), the silky hairs on maize, is commonly discarded as waste. Despite its traditional medicinal use in countries like China, the United States, France, and Türkiye, its phytochemical and pharmacological attributes have received limited attention. In this study, agar well diffusion was used to evaluate the antimicrobial activity of free and bound crude extracts from corn silk obtained using different solvents. Compared to the free crude extract, the bound crude extract exhibited greater efficacy against the microorganisms tested. Consequently, sequential fractionation was performed on the bound crude extract, and it was found that the ethyl acetate extract showed the greatest activity against the microorganisms tested. Stigmasterol (STG) (compound 1) and stigmasteryl-3β-arachidate (compound 2) were isolated from the ethyl acetate active fraction, followed by their identification via mass spectrometry and nuclear magnetic resonance spectroscopy. STG showed favorable antimicrobial activity against *Staphylococcus aureus*, *Candida albicans*, *Mycobacterium smegmatis*, and *Escherichia coli*. In vivo the antimicrobial activity of STG was evaluated in artificially contaminated cherry tomatoes infected with *Fusarium verticillioides*. STG showed significant inhibitory activity compared to the control on cherry tomatoes when placed prior to inoculation at a concentration of 1.5 mg/mL STG and 2.5 mg/mL STG. This research paves the way for practical applications of STG isolated from corn silk in the food industry, where the demand for safe and natural antimicrobial agents is increasing.

Key words: Corn silk, bound phenolic, stigmasterol, antimicrobial activity, cherry tomatoes, *Fusarium verticillioides*

1. Introduction

In the search for antimicrobial agents, researchers have increasingly turned their attention to natural sources owing to their diverse chemical compositions and potential therapeutic properties (Anand et al., 2019). One such source is corn silk, a bundle of long, silky, yellowish hairs found on top of corn fruits, also known as *Zea mays* hairs (Abirami et al., 2021). Corn silk is a traditional medicinal herb used in various countries, including China, the United States, France, and Türkiye (Hasanudin et al., 2012). Utilized in traditional medicine, corn silk is known for its healing properties, particularly in the treatment of irritation and inflammation affecting the urinary bladder, prostate, and urinary system (Velazquez et al., 2005). It has been noted in various studies that corn silk has the ability to decrease hyperglycemia and inhibit the formation of immunoglobulin E by glycoproteins (El-Ghorab et al., 2007; Abirami et al., 2021; Singh et al., 2022a). Furthermore, corn silk has been recognized for its antioxidant (Nawaz et al., 2019) and antimicrobial properties (Emmanuel et al., 2016; Abinaya, 2021; Mohammadi et al., 2023). Corn silk contains a large number of bioactive chemicals, such as steroids, volatile oils, and other naturally occurring antioxidants like flavonoids and polyphenols (Singh et al., 2022b). In recent years, scientists have focused on exploring antimicrobial potential through a bioassay-guided extraction approach.

Bioassay-guided extraction is a powerful strategy for to identifying and isolating bioactive compounds from complex natural matrices (Najmi et al., 2022). This approach involves a series of steps combining biological testing and chemical analysis to pinpoint active compounds responsible for the bioactivity of interest (Xu et al., 2022). Upon identifying the bioactive fraction, researchers employed various chromatographic techniques, for example liquid chromatography (LC), high

* Correspondence: xzhfood@njau.edu.cn

This work is licensed under a Creative Commons Attribution 4.0 International License.

performance liquid chromatography (HPLC), and thin-layer chromatography (TLC), to further purify and isolate individual compounds. By progressively isolating and retesting the active fractions, researchers can narrow down the chemical constituents responsible for the antimicrobial activity observed (Afsar et al., 2022).

Corn silk contains more bound phenolic compounds than free phenolic compounds (Yang et al., 2019; Khan et al., 2023). A significant number of phenolic compounds exist in insoluble forms. Insoluble phenolic compounds covalently bind to cell wall components in plant cells (Zhang et al., 2020). In the present research, the utilization of bioassay-guided extraction demonstrated great potential for identifying antimicrobial compounds (such as stigmasterol (STG) and stigmasteryl-3β-arachidate) from bound crude extracts obtained from corn silk. These compounds have the ability to combat drug-resistant bacteria and various other microbial pathogens, offering promising prospects for further study and application.

The high moisture content and nutrient-rich nature of harvested fruits make them susceptible to attacks by plant pathogenic or opportunistic fungi (Rodrigues and Kakde, 2019). To mitigate the risk of mycotoxin contamination, it is essential to control fungal growth in fresh fruits, crops, and vegetables. Chemical fungicides are commonly used to inhibit fungal growth both before and after harvesting (Davies et al., 2021). Uncontrolled and extensive fungicide use in agricultural contexts has been identified as a major causative factor in the evolution of pathogen populations resistant to these agents. Consequently, higher concentrations of these antifungals are required, resulting in the elevated presence of toxic residues in food items (Poloni et al., 2021). Additionally, certain fungicides compounds are nonbiodegradable, resulting in their accumulation in the water, soil, and plants. This accumulation leads to environmental contamination and, when consumed through the food chain, poses potential risks to human health (Hashimi et al., 2020). As a result of these adverse impacts, there is growing demand for the development of new, environmentally friendly alternatives that are safe, biodegradable, and economically sustainable. Therefore, the aim of the present study was to investigate the antimicrobial attributes of free and bound crude extract through different solvents from corn silk. In preliminary screening, it was observed that the ethyl acetate bound extract of corn silk exhibited significant antimicrobial activity. Hence, the ethyl acetate extract was chosen for further antimicrobial activity. Through bioassay-guided methods, STG and stigmasteryl-3β-arachidate, with significant antimicrobial activity against phytopathogens, were isolated and identified through the application of nuclear magnetic resonance (NMR) spectroscopy and mass chromatography (Figure 1). Furthermore, STG was evaluated to control *Fusarium verticillioides* in cherry tomatoes, which could not only reduce losses caused by fungi, but also minimize the use of chemical fungicides.

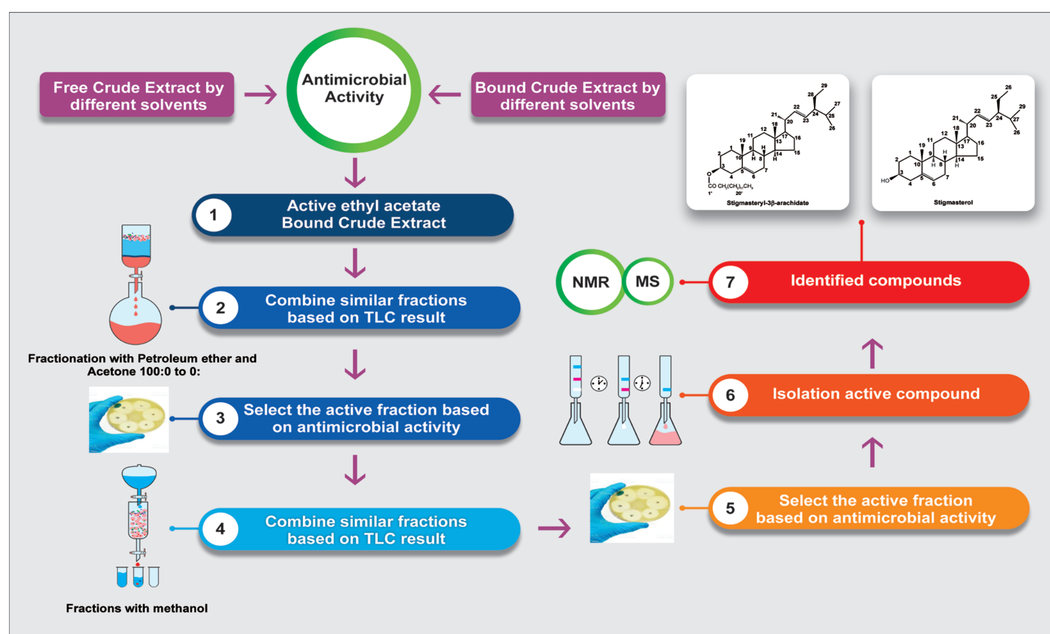

Figure 1. Bioassay-guided method for extraction and fractionation of the crude corn silk extract.

2. Materials and methods

2.1. Materials and reagents

Corn silk was purchased from the Xuanwu District's WeiGang Farmers Market (Nanjing, China). Chemicals (analytical grade), including HCl, NaOH, chloroform, ethyl alcohol, methanol, *n*-butanol, ethyl acetate, cyclohexane, acetone, DPPH, ABTS, and ascorbic acid, were purchased from Sinopharm Chemical Reagent Co., Ltd. (Wuxi, China).

2.2. Preparation of corn silk extraction

Distilled water was utilized to wash the corn silk, which was then left to dry naturally at room temperature for 3 days. The dried material was subsequently ground uniformly using an electric grinder. The powdered dried material was passed through a sieve with a mesh size of 60. Six solvents, i.e. methanol, ethanol, ethyl acetate, water, *n*-hexane, and *n*-butanol, were utilized to extract the free compounds' crude extract from 5 g of corn silk. To extract the bound compound crude extract, the leftover residue was digested with 2 mol/L NaOH for 150 min at room temperature using an electric plate with a magnetic stirrer and then neutralized with HCl. Six solvents, i.e. methanol, ethanol, ethyl acetate, water, *n*-hexane, and *n*-butanol, were utilized as extraction reagents. All extracts were screened for antimicrobial activity against a panel of microorganisms, and the extracts exhibiting significant activity were selected for further phytochemical separation, aiming to isolate bioactive compounds via bioassay-guided fractionation.

2.3. Isolation and purification of bioactive compound

Large-scale (900 g) digestion of corn silk was performed with 2 M NaOH for 1 day at room temperature after extracting the free crude extract for the purpose of isolating the active compound. The bound crude extract was extracted by ethyl acetate, dried, and separated into 75 fractions by flash chromatography on vacuum silica gel, using stepwise gradient elution with petroleum ether and acetone (0:100 to 100:0). The fractions were analyzed by thin-layer chromatography (TLC) plates coated with silica gel GF254 (10–40 μM), using chloroform and methanol, and the fractions were mixed. The active fractions were obtained using a bioassay-guided method for further purification and isolation. Furthermore, these fractions were subjected to bioactivity evaluation at 1 mg/mL against six pathogenic microbes: namely *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Mycobacterium smegmatis* (CMCC 93321), *Candida albicans* (CMCC 98001), *Fusarium verticillioides*, and *Botrytis cinerea*. The combined fraction was separated by column chromatography with chloroform and methanol (100:0 to 0:100, v/v) as the eluents to obtain the subfractions. Identical fractions based on the TLC results were combined and concentrated under reduced pressure and tested for antimicrobial activity. The highly active fraction was subjected to chromatography on a Sephadex LH-20 gel column using a chloroform and methanol elution system (1:1, v/v), which resulted in the isolation of compounds 1 and 2. The purity of the compounds was confirmed through analytical HPLC.

2.4. Microorganisms

The antimicrobial activity was tested on the free crude extract and bound crude extracts of the 20 strains. The selected pathogens were *Staphylococcus aureus* (ATCC 25923), *Mycobacterium smegmatis* (CMCC 93321), *Escherichia coli* (ATCC 25922), *Candida albicans* (CMCC 98001), *Fusarium verticillioides*, and *Botrytis cinerea*.

2.5. Antibacterial activity

Agar well diffusion was utilized to evaluate the antimicrobial activity of both the free crude extract and bound crude extracts (Asghar et al., 2020). The bacterial strains were cultured in LB broth and incubated at 37 °C for 24 h to assess their antibacterial activity. Corn silk extracts were dissolved in methanol at a concentration of 1 mg/mL. Sterile solution of LB agar was prepared and spread onto the plates. The plates were then swabbed with the test organisms. Using a sterile cork borer, four wells of 6 mm were punched into solidified media. Each well was filled with 25 μL of 1 mg/mL corn silk extract. Subsequently, different corn silk extracts were added to each well. Methanol was employed as a control. The plates were then incubated at 37 °C overnight and examined for the presence of inhibition zones. The diameters of the inhibition zones (mm ± standard deviation) were measured to evaluate the efficacy of activity. Two repetitions of the procedure were conducted and the average values for antagonistic activity were documented.

2.6. Antifungal activity

The microorganisms used in the study were obtained from the microbiology stock culture kept at the Key Laboratory of Food Processing and Quality Control at Nanjing Agricultural University in Nanjing, China. The fungal strains were cultivated in PDA medium at 25 °C for 7 days. The spore suspension method with modifications was employed to measure antifungal activity (Mota et al., 2012). Scraping was performed to collect spores from each fungal plate, which were then transferred to a 5-mL tube filled with sterile water. The tube was vigorously vortexed for 5 min and subsequently filtered through sterile cotton. This process allowed us to obtain a fungal suspension adjusted to a concentration of 1.0×10^6 CFU/mL. To evaluate the efficacy of the compound, a mycelial plug with a diameter of 6 mm was accurately placed at the center of the plates, previously inoculated with the fungus. Following an incubation period of 4 days at 28 °C, the experiments were performed in three repetitions. To determine the control efficacy, the measurements of the inhibition zones were recorded in millimeters. A secondary test to evaluate

antifungal activity was carried out using crude extracts, following the method described by Li et al. (2021) against the fungi. The mycelial growth inhibition rate (%) was estimated using the following formula:

Inhibition rate (%) = ($d1$- $d2$)/ x 100,

where $d1$ and $d2$ are the mean colony diameters of the control and treatment samples, respectively.

2.7. Determination of MIC, MBC, and MFC

A twofold broth microdilution technique was employed to determine the minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), and minimum bactericidal concentration (MBC) of the extracts, active fractions, and compounds. The methodology was based on the guidelines established by the Clinical and Laboratory Standards Institute (2010) with changes made for this study. In summary, a 100-µL volume of each extract was added to each well of a 96-well plate. The plate contained either 100 µL of sterile PDB medium and a fungal spore suspension at 10^6 spores/mL or 100 µL of sterile LB medium and a bacterial suspension at 10^8 CFU/mL. Consequently, the plant extracts exhibited concentration ranges of 5–900 µg/mL, while the isolated compounds and standard drugs displayed concentrations ranging from 0.5 to 70 µg/mL. The microplates were subjected to incubation for 24 h at 37 °C for bacteria and for 24 h at 30 °C for fungi. To determine the MIC, MBC, and MFC, a 10-µL volume from every well of the microplate was transferred onto suitable media (LA for bacteria; PDA for fungi) in a 16-cm petri dish. The dishes were then incubated at 37 °C for bacteria and at 30 °C for fungi for 24 h. After the incubation period, the MIC, MFC, and MBC values were assessed. Triplicate tests were conducted for each concentration of the extract.

2.8. Stability test

Comprehensive evaluation of the antifungal properties of STG involved subjecting it to a wide range of temperatures, varying intensities of ultraviolet (UV) light, and diverse pH conditions. The aim of this rigorous testing was to explore the effects of these factors on the ability of STG to inhibit fungal growth as described by Wang et al. (2020). Samples with identical concentrations of STG were exposed to various conditions including temperatures ranging from 4 to 121 °C for 25 min, UV light intensity of 100 µW/cm for between 10 and 60 min, and pH levels ranging from 1 to 11. To ensure accuracy, each treatment was performed three times and the inhibition rate (%) was determined using the formula

Inhibition rate (%) =($d1$- $d2$)/ $d1$ x 100,

where $d1$ and $d2$ are the mean colony diameters of the control sample and the treatment samples, respectively.

2.9. In vivo evaluation of STG on cherry tomatoes' fruit decay

The procurement of ripe and fresh tomatoes without any defects was carried out at a local market in Nanjing City, China. Double disinfection of the tomato fruit was performed using a combination of 75% v/v ethanol and 2% hydrogen peroxide. The fruit was then rinsed twice with water and left to air dry. Two concentrations of STG were selected: 1.5 mg/mL and 2.5 mg/mL. Using a sterile scalpel, a wound measuring 3 mm in diameter and 3 mm in depth was created on the cherry tomato fruit. After injection with 10 µL of STG, the wounds were allowed to air dry before being treated with 10 µL of suspension (2.0 × 10^6 CFU mL^{-1}) of *Fusarium verticillioides*. Subsequently, the samples were incubated for 9 days at 25 °C under 85%–90% humidity conditions. Three tomatoes were utilized in each treatment group and the experiment was conducted in triplicate. The lesion area (mm^2) was measured to evaluate the disease severity of the fruit during storage. The following formula was used to determine the control efficacy:

% Control efficacy = Σ ($L1$ - $L2$) / $L1$ x 100,

where $L1$ represents the mean lesion area observed in the control group and $L2$ represents the mean lesion area in the treatment group.

2.10. DPPH and ABTS antioxidant activity

The antioxidant activity of compounds 1 and 2 was calculated by measuring the decline in absorbance at 517 nm of the ethanolic DPPH solution in the presence of STG. A DPPH radical scavenging assay was performed as explained by Rivero-Pérez et al. (2007).

The experiment was performed three times. The antioxidant activity was expressed as

DPPH Inhibition %= $\left[1 - \frac{As}{Ao}\right] \times 100$,

where As is the absorbance of the DPPH radical solution blended with the compound/vitamin C, and A_0 is the absorbance of the DPPH radical solution mixed with ethanol.

The ABTS assay of both compounds was estimated according to Mechqoq et al. (2022) with some variations. In the dark, different concentrations of purified compounds or reference substances (ascorbic acid) were added to 3 mL of ABTS radical solution. The decrease in absorbance at 734 nm was measured. The standard curve was linear between 0 and 500 µM of ascorbic acid/mL. Each test was conducted in triplicate. The formula utilized to determine DPPH inhibition was also employed to compute ABTS percentage inhibition.

3. Results

3.1. Antimicrobial activity assay in vitro

3.1.1. Zones of inhibition exhibited by the free and bound crude extracts and active fraction

Multiple microbial strains were screened to assess the antimicrobial activities of corn silk free and bound crude extracts obtained from various solvents. After screening,

three bacterial strains and three fungal strains were selected for further analysis. The degree of growth inhibition exerted by the crude extracts on the microorganisms tested is visually represented in Figure 2, which summarizes the results in terms of the inhibition zones. The bound crude extract exhibited significant activity against all microorganisms, showing promising results comparable to those of free crude extracts. Among the different solvent extracts studied, ethyl acetate, methanol, and ethanol showed the highest degree of inhibition, followed by water, *n*-hexane, and *n*-butanol extracts. The ethyl acetate extract showed the highest level of antibacterial and antifungal activities among all the microorganisms tested. The bound crude extract exhibited the largest zone of inhibition against *M. smegmatis* (Figure 2a), *C. albicans* (Figure 2b), and *F. verticillioides* (Figure 2c) (15.3 mm, 13.6 mm, and 13.3 mm respectively), followed by *B. cinerea* (Figure 2d), *E. coli* (Figure 2e), and *S. aureus* (Figure 2f). In contrast, free crude extracts demonstrated a moderate level of antimicrobial activity against most of the microorganisms tested.

Among the nine fractions analyzed from the ethyl acetate bound crude extract of corn silk, fraction 4 demonstrated the most substantial inhibition zones (ranging from 17.5 to 20 mm) against both bacteria and fungi, as presented in Table 1. Furthermore, compounds 1 and 2 extracted from active fraction 4 showed good activity against both bacteria and fungi.

3.1.2. Minimum inhibitory concentrations

The MIC, MBC, and MFC values for the ethyl acetate free and bound crude extract, active fraction, and isolated compounds from the corn silk against bacterial and fungal strains are presented in Table 2. The study demonstrated that MIC values below 1000 µg/mL exhibited notable antimicrobial activity (York et al., 2012; Madikizela et al., 2014). The free and bound crude extracts, as well as the active fraction, demonstrated a broad spectrum of antibacterial activity against a diverse array of strains. MIC values, ranging from 200 to 800 µg/mL, provided evidence of varying degrees of antimicrobial effectiveness. Among the fractions tested, the ethyl acetate fraction displayed the most potent antibacterial activity against the microbes examined in the study. The MIC values for this fraction ranged from 200 to 300 µg/mL. The isolated compounds exhibited potent antibacterial activity, as evidenced by MIC values ranging from 12 to 25 µg/mL against all bacterial strains tested. Specifically, the STG compound demonstrated exceptional antibacterial potency among the three bacterial strains examined, as indicated by its highly inhibitory effect with an MIC value of 12 µg/mL against *M. smegmatis*.

The corn silk free and bound crude extracts, as well as the fractions, demonstrated varying levels of antifungal activity, with MIC values ranging from 250 to 800 µg/mL. Among the fungal strains tested, the partition fractions showed enhanced inhibitory activity against *C. albicans* compared with the crude extract, with MIC values ranging from 200 to 300 µg/mL. The ethyl acetate fraction displayed remarkable fungicidal activity against *C. albicans*, as evidenced by the most noteworthy MIC and MFC values of 200 µg/mL. The isolated compound STG exhibited significant fungistatic and fungicidal

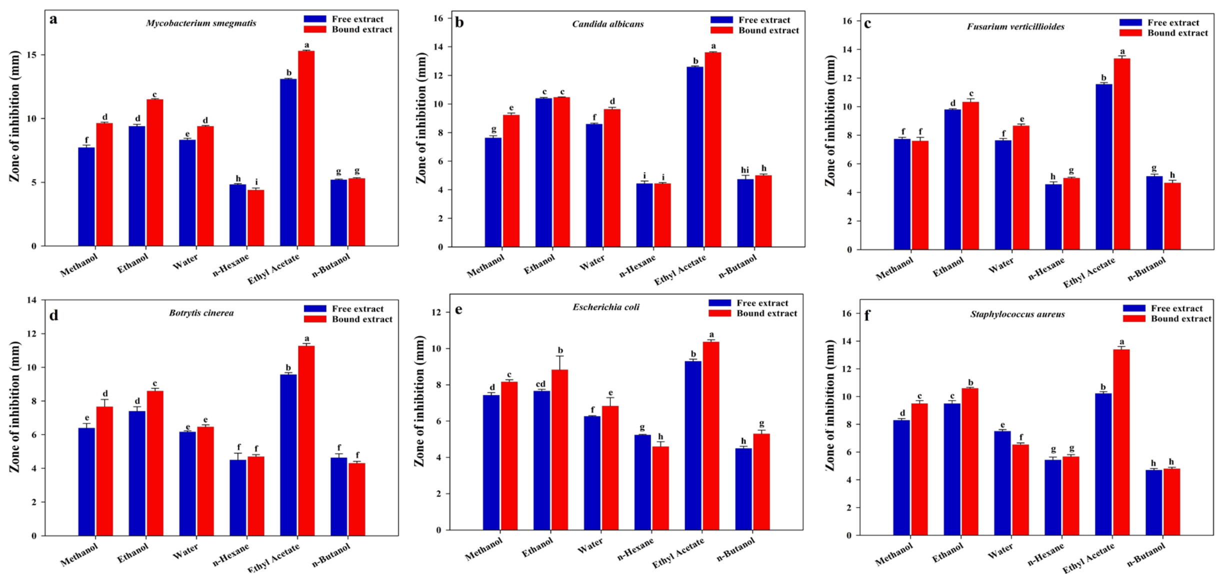

Figure 2. Antimicrobial activity detected by using different solvent extracts of corn silk (mean ± SE) against (a) *M. smegmatis*, (b) *C. albicans*, (c) *F. verticillioides*, (d) *B. cinerea*, (e) *E. coli*, and (f) *S. aureus*.

Table 1. The antimicrobial activity of corn silk fractions, obtained through ethyl acetate-bound crude extract, expressed in millimeters of mean values.

Pathogenic microbes	Fractions								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Staphylococcus aureus	0.00	0.00	0.80	18.5	15.6	10.5	14.7	9.0	13.8
Escherichia coli	0.00	0.00	0.85	18.0	14.8	9.0	9.0	0.00	10.5
Botrytis cinerea	0.00	0.00	0.75	17.5	13.3	0.00	13.5	13.7	0.00
Fusarium verticillioides	0.00	0.00	0.93	19.8	10.1	14.5	0.00	9.5	12
Candida albicans	0.00	0.00	0.86	20.0	9.0	0.00	12.5	8.5	0.00
Mycobacterium smegmatis	0.00	0.00	0.95	19.5	12.4	14.5	15.9	10.5	0.00

Table 2. Evaluation of antimicrobial concentrations (µg/mL) of CS extracts, active fraction, stigmasteryl-3β-arachidate, and stigmasterol.

Test organism	*S. aureus*		*E. coli*		*M. smegmatis*		*F. verticillioides*		*C. albicans*		*B. cinerea*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC	MIC	MFC
Free crude extract	800	850	700	800	650	700	800	900	600	650	700	750
Bound crude extract	600	650	500	550	300	350	700	750	250	300	500	500
Active fraction	300	300	250	300	250	300	300	500	200	250	250	300
Stigmasteryl-3β-arachidate	25	30	20	25.5	25	30.5	40	50	20	25.5	≥50	≥50
Stigmasterol	20	20	15	20	12.5	15.5	25.5	30	12	14	≥50	≥50

effects against *C. albicans*, with an MIC value of 12 µg/mL. Additionally, STG displayed notable antibacterial and antifungal potential, making it the most active compound overall.

3.1.3. Identification of isolated active compounds from the EA fraction

The ^{1}H NMR and ^{13}C NMR peaks of compounds 1 and 2 are given in Figure 3. Compound 1 (STG) $C_{29}H_{48}O$, white crystals; EI-MS: m/z 413 $[M+H]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ 5.35 (dt, *J* = 5.4, 1.9 Hz, 1H), 5.16 (dd, *J* = 15.2, 8.5 Hz, 1H), 5.02 (dd, *J* = 15.1, 8.7 Hz, 1H), 3.58 (m, 1H), (Figure 3a) ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.77, 138.31, 129.30, 121.71, 77.32, 77.00, 76.69, 71.82, 56.88, 55.98, 51.25, 50.18, 42.33, 42.23, 40.48, 39.70, 38.16, 37.27, 36.53, 31.91, 31.88, 31.68, 31.24, 29.70, 29.66, 29.36, 28.91, 25.40, 24.37, 22.69, 21.21, 21.08, 19.40, 18.98, 14.10, 12.24, 12.05, –0.02 (Figure 3b).

Compound 2 (stigmasteryl-3β-arachidate) was isolated as yellow crystals. It was determined to have the chemical formula $C_{49}H_{87}O_2$, based on ^{1}H and ^{13}C NMR spectroscopy, and ESI-MS analysis was performed, which detected an $[M + H]^+$ ion with an m/z value of 707. ^{1}H NMR (600 MHz, chloroform-*d*): δ 5.33 (m, 1H, H-6), 5.13 (dd, 1H, *J* = 9.0, 9.0 Hz, H-22), 5.01 (dd, 1H, *J* = 9.0, 8.4 Hz, H-23), 3.51 (m, 1H, W1/2 = 15.6 Hz, H-3a), 2.32 (t, 2H, *J* = 7.2 Hz, H2-2), 1.02 (s, 3H, Me-19), 0.92 (d, 3H, *J* = 6.3 Hz, Me-21), 0.87 (d, 3H, *J* = 6.2 Hz, Me-26), 0.85 (d, 3H, *J* = 6.2 Hz, Me-27), 0.83 (t, 3H, *J* = 6.5 Hz, Me-20), 0.79 (t, 3H, 6.1 Hz, Me-29), 0.67 (s, 3H, Me-18), 2.80–1.04 (m, 59 H, 26 × CH_2, 7 × CH) (Figure 3c). ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.01, 140.77, 138.34, 129.29, 121.75, 71.83, 56.78, 55.97, 51.25, 50.14, 45.85, 43.13, 42.22, 40.50, 39.78, 39.69, 39.08, 37.26, 36.51, 36.16, 33.75, 31.89, 31.47, 29.66, 29.60, 29.45, 29.37, 29.32, 29.25, 29.16, 29.08, 29.05, 28.93, 28.67, 28.26, 26.08,

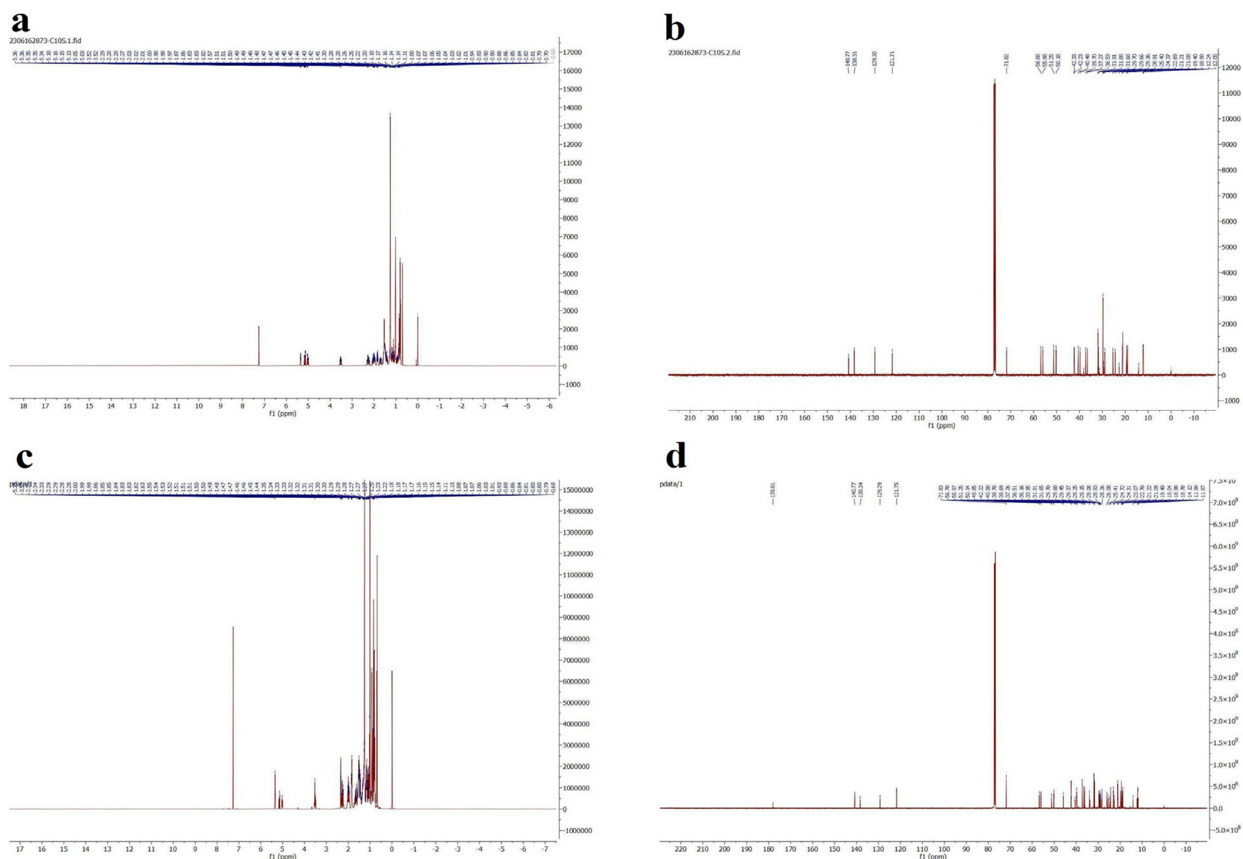

Figure 3. (a) ^{1}H NMR (b) ^{13}C NMR peaks of compound 1 (STG), (c) ^{1}H NMR (d) ^{13}C NMR peaks of compound 2 (stigmasteryl-3β-arachidate).

25.41, 24.72, 24.31, 22.70, 21.22, 21.09, 19.40, 19.04, 18.98, 18.90, 18.78, 14.12, 11.99, 11.87 (Figure 3d). The structures of both isolated compounds are given in Figure 4.

3.1.4. In vitro antimicrobial activity of STG

The antimicrobial activity of STG was highly promising against the microorganisms tested as shown in Figure 5. The results revealed that even at microgram levels of concentration, STG displayed excellent antimicrobial activity against both bacteria and fungi, demonstrating a clear dose-dependent response as shown in Table 3. The comprehensive results obtained from our study clearly indicate that STG exhibits a smaller zone of inhibition against both *S. aureus* (Figure 5a) and *C. albicans* (Figure 5b) when compared to the inhibition observed against *M. smegmatis* (Figure 5c) and *E. coli* (Figure 5d). STG exhibited remarkable effectiveness with the highest zones of inhibition observed against *M. smegmatis* and *E. coli*. Specifically, at a concentration of 1 mg/mL, the zones of inhibition measured 34.3 mm and 32.0 mm, respectively, indicating strong antimicrobial activity against these bacterial strains.

3.1.5. Stability of STG

The antifungal activity of STG against *F. verticillioides* was examined under different temperature, pH, UV, and concentrations (Figure 6). Interestingly, the results indicated that temperatures up to 65 °C did not significantly impact the antifungal activity of STG. However, a noticeable decrease in antifungal activity was observed when the temperature was raised to 75 °C or higher, although no significant difference was detected among the higher temperature ranges (Figure 6a). To summarize, the outcomes presented in Figure 6b indicate that STG maintains its antifungal activity regardless of pH variations within the range of 1 to 11. These findings highlighted the stability and efficacy of STG as an antifungal agent. The correlation between the duration of UV exposure and the gradual decrease in the inhibitory action of STG is represented in Figure 6c. The inhibitory effects of different concentrations of STG on the mycelial growth of *F. verticillioides* were examined and are depicted in Figure 6d. STG showed a strong inhibitory effect on *F. verticillioides* mycelial growth on PDA plates. Low levels of

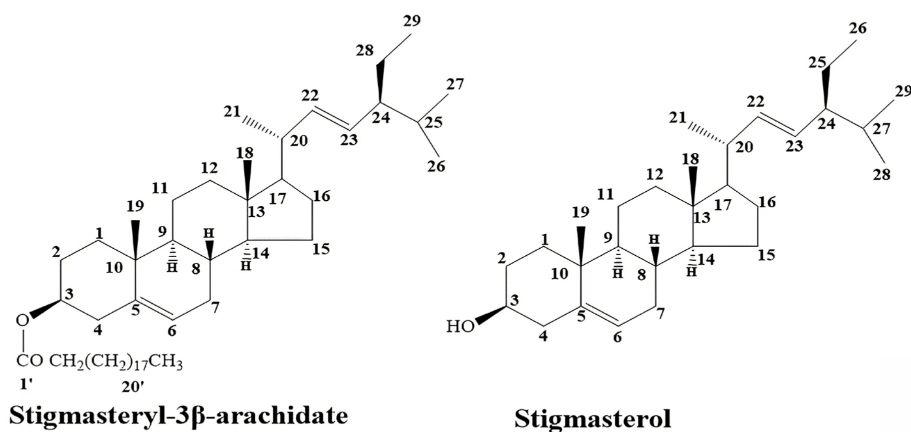

Figure 4. Structures of compounds isolated from corn silk.

Table 3. Antimicrobial activity of stigmasterol from corn silk against bacterial pathogens.

Concentration mg/mL	*S. aureus*	*E. coli*	*M. smegmatis*	*C. albicans*
0.2	10.5 ± 0.2	11.4 ± 0.1	11.4 ± 0.2	11.5 ± 0.3
0.4	15.8 ± 0.3	17.7 ± 0.2	18.3 ± 0.2	17.5 ± 0.2
0.6	20.9 ± 0.3	23.5 ± 0.3	24.4 ± 0.1	22.6 ± 0.2
0.8	25.1 ± 0.4	28.6 ± 0.1	30.6 ± 0.1	25.8 ± 0.1
1.0	27.8 ± 0.4	32.0 ± 0.1	34.3 ± 0.2	28.5 ± 0.1

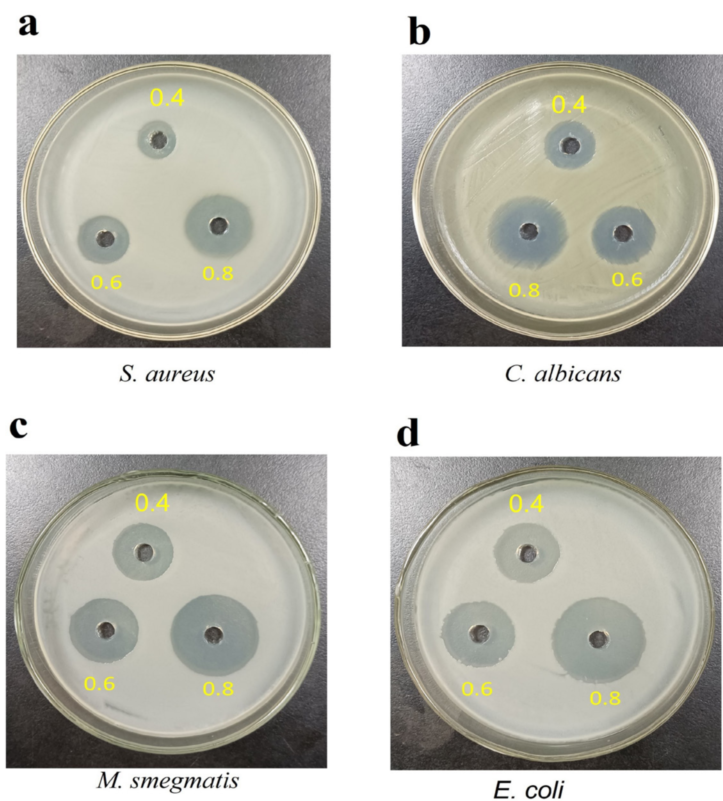

Figure 5. Zone of inhibition exhibited by STG at different concentrations (mg/mL) against (a) *S. aureus*, (b) *C. albicans*, (c) *M. smegmatis*, and (d) *E. coli*.

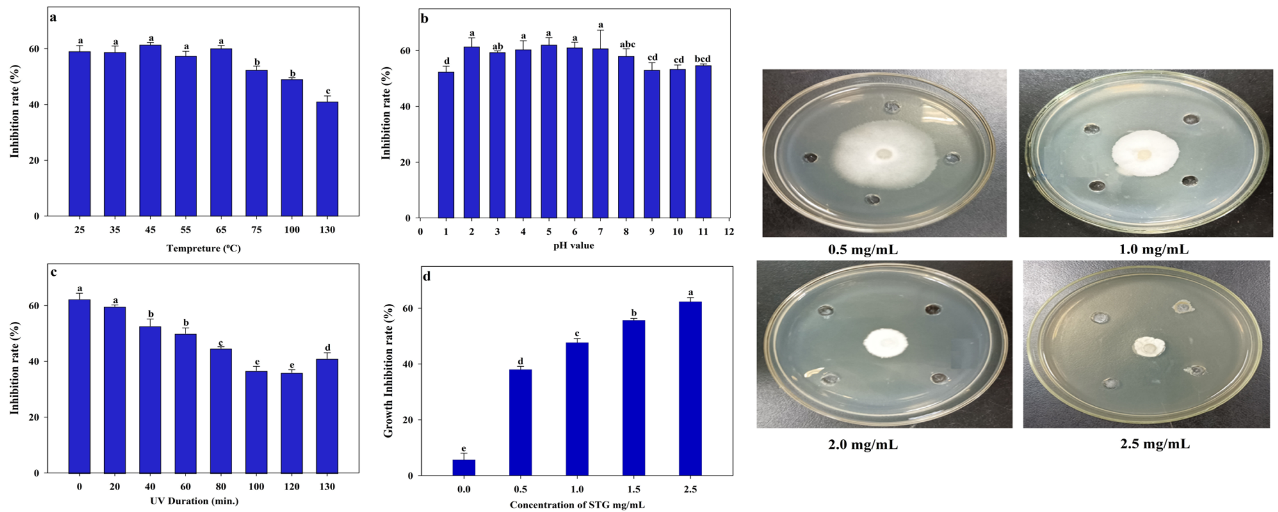

Figure 6. The antifungal activity of STG, and stability evaluation. Stability of STG under several conditions: (a) temperature, (b) pH, (c) UV radiation, and (d) mycelial growth inhibition of *Fusarium verticillioides* with varying concentrations of STG.

STG inhibited the growth of *F. verticillioides* mycelia, and the inhibition rate was 38% when the PDA plate contained 0.5 mg/mL STG. The inhibition rate increased with increasing STG concentration. When the STG content reached 2.5 mg/mL, the inhibition rate was 62.3%.

3.2. In vivo STG protective efficacy against *Fusarium verticillioides* on cherry tomatoes

The inhibitory effects of STG on the decay of cherry tomato fruits caused by *F. verticillioides* were investigated (Figure 7) by artificially inoculating a spore suspension of the fungus with varying concentrations of STG. It was observed that the control group had the highest level of fruit decay after 3 days of infection (Figure 7a). After 9 days of infection, clear indications of decay were observed on cherry tomatoes in all treatment groups. These signs were marked by the occurrence of white fungal lesions accompanied by indentations and the presence of fungal sporulation around the wound. At 9 days postinfection, all treatment groups exhibited a significant reduction in tomato fruit decay compared to the control group. However, 2.5 mg/mL STG only caused minor lesions at the point of application. The lesion areas of the control, 1.5 mg/mL STG, and 2.5 mg/mL STG groups were 37 mm^2, 14.8 mm^2, and 8.4 mm^2, respectively (Figure 7b). The control efficacy rose with an increase in the concentration of the STG. The 2.5 mg/mL STG treatment group showed the maximum control efficacy of 80.31% on day 9, as shown in Figure 7c. These results demonstrate that STG significantly inhibited *F. verticillioides* and decreased tomato fruit decay.

3.3. Antioxidant activities of the isolated compounds

In the present study, we examined the influence of the isolated compounds STG and stigmasteryl-3β-arachidate obtained from corn silk on the ability to scavenge 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay radicals and DPPH. The determination of the antiradical power of the compounds involved calculations based on a calibration curve with an R value of 0.9872. The corresponding results are illustrated in Figure 8.

Both compounds exhibited dose-dependent enhancement in their ABTS (Figure 8a) and DPPH (Figure 8b) radical scavenging activity. The isolated compounds, known for their functional properties, demonstrated remarkable DPPH scavenging capability. At a concentration of 150 µM, the STG and stigmasteryl-3β-arachidate displayed their highest antioxidant activity, exhibiting scavenging activity of 70.98% and 68.5%, respectively. In comparison, the standard ascorbic acid demonstrated activity of 88.7%. The reported values represent the mean of three replicate experiments, and the data are expressed as the mean ± SD. The ABTS assay is a widely utilized in vitro test for assessing the scavenging capacity of radicals. Nonetheless, in order to execute this method, it is essential to generate ABTS radicals, which can be readily achieved by conducting a reaction between ABTS salt and potassium persulfate. The ABTS radicals exhibit reactivity towards a wide range of antioxidant compounds. Among the compounds isolated, STG and stigmasteryl-3β-arachidate exhibited antioxidant activity at a concentration of 180 µM, with scavenging activities of 65.87% and 64.6%, respectively. In comparison, the standard ascorbic acid demonstrated activity of 85.02%.

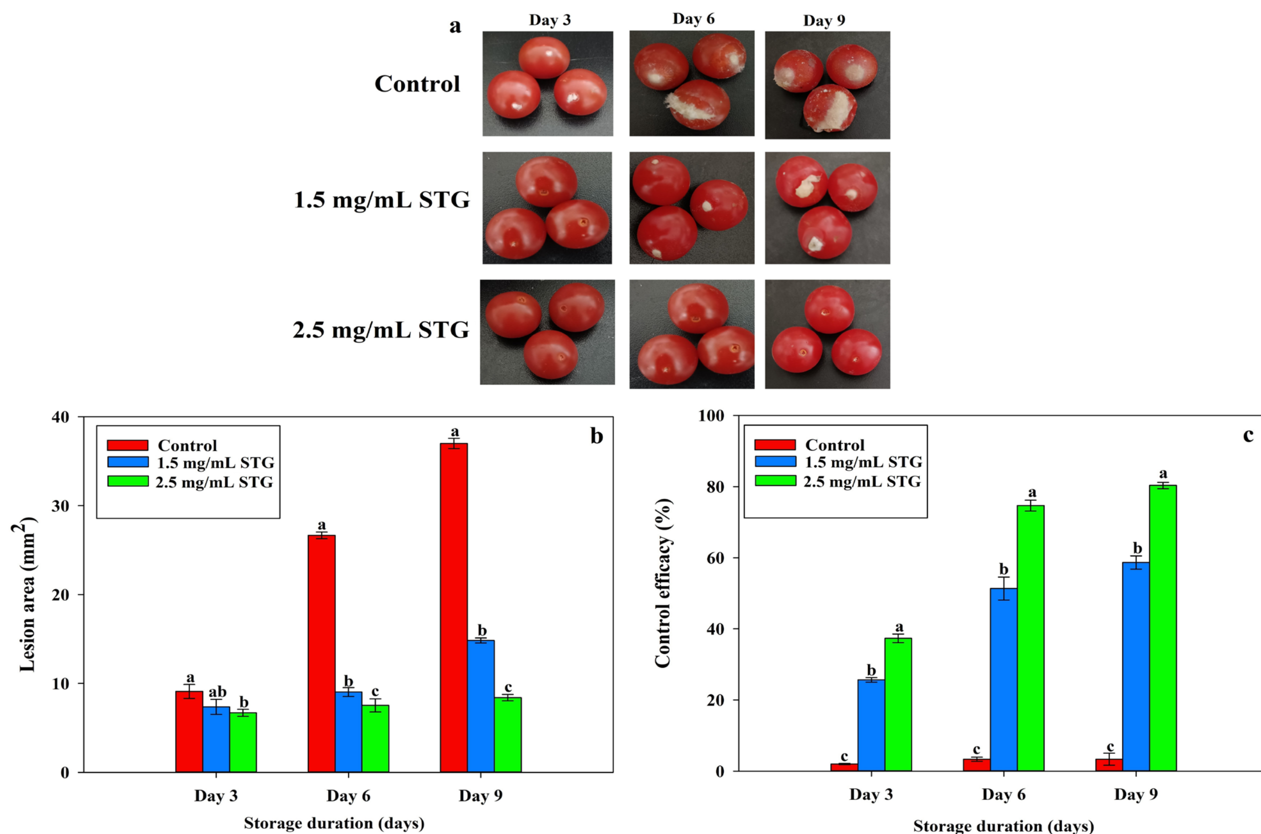

Figure 7. (a) Effect of different concentrations of STG and control (untreated) on postharvest tomatoes fruit disease induced by *F. verticillioides* infection, (b) lesion area of cherry tomato decay after treatment with different concentrations, (c) control efficacy of cherry tomato decay after treatment with different concentrations.

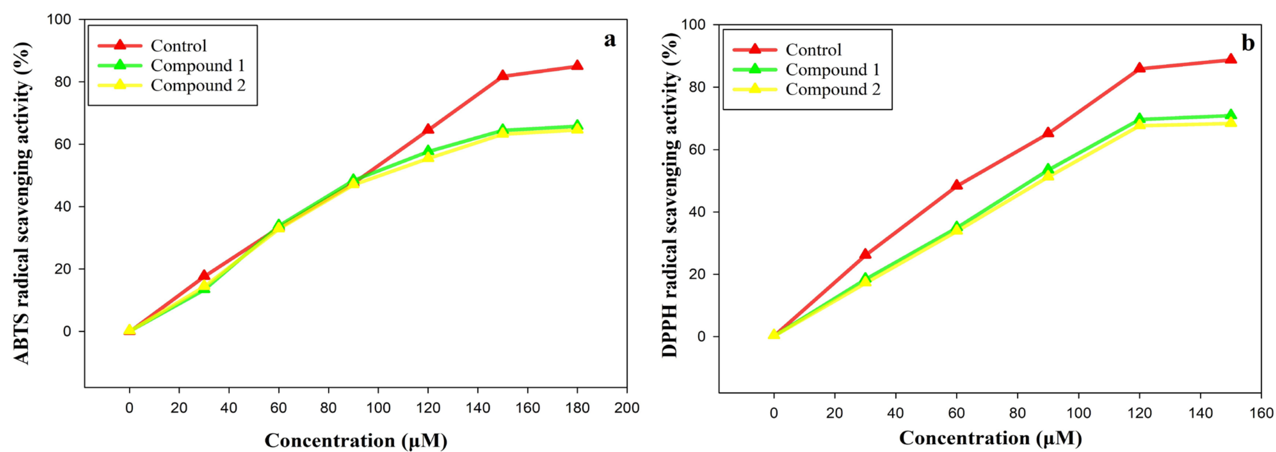

Figure 8. (a) ABTS and (b) DPPH radical scavenging activity of control (ascorbic acid), STG, and stigmasteryl-3β-arachidate.

4. Discussion

Microbial resistance to antibiotics has been established to occur as a result of the excessive and inappropriate use of commercial antimicrobial drugs. This circumstance encouraged researchers to investigate new antimicrobial sources, particularly those with novel modes of action. An appealing approach is to screen plant-based drugs with a history of traditional use, which offers benefits such as lower costs, fewer side effects, and widespread availability (Ahmed, 2017). Corn silk is a Chinese herbal medication that has been used for centuries (Jia et al., 2021). It has generally been used to treat edema (Jeong et al., 2017), gout, cystitis, nephritis (Sepehri et al., 2011), and kidney stones (Saad et al., 2021). Moreover, it displays pharmacological activities such as antitumor (Tian et al., 2013), hypoglycemic (Singh et al., 2022b), antioxidant (Wang and Zhao, 2019), and antimicrobial properties (Emmanuel et al., 2016). The objective of the present study was to assess the antimicrobial efficacy of corn silk extracts, specifically the free and bound crude extracts obtained through different solvent extractions. Furthermore, we explored the antimicrobial activity of the subfractions derived from the bound crude extract. The free crude extracts displayed moderate inhibitory activity against the microorganisms examined. Interestingly, the bound crude extract exhibited a greater antimicrobial activity against the test panel compared to the free extract. Notably, the ethyl acetate extract emerged as the most potent among the corn silk extracts, displaying the highest zone of inhibition when compared to the methanol and ethanol extracts. Abinaya (2021) described similar findings in their investigation on the leaf part of *Crescentia alata* Kunth, demonstrating similar effects against several microorganisms. Morshed and Islam (2015) found that corn silk extract exhibited sensitive responses in ethanol and methanol extracts to 11 bacteria out of the 12 tested. According to Nessa et al. (2012), petroleum ether and methanol extract of corn silk and flavonoids were active against 11 out of 12 bacteria. Similarly, Abirami et al. (2021) found that the ethanol corn silk extract exhibited the highest antibacterial activity at 900 µg and antifungal effectiveness was seen against *Aspergillus brasiliensis* and *Aspergillus niger* at a level of 2 mg/20 mL.

In the current study, the ethyl acetate bound crude extract was further fractionated with petroleum ether and acetone (100:0–0:100). In addition, fraction F4 exhibited the greatest activity against the microorganisms evaluated. Moreover, STG derived from active fraction F4 demonstrated varying levels of antimicrobial effectiveness against the microbes tested. To date, no standard criteria have been established to compare the MIC values of natural products for assessing their in vitro antimicrobial activity. In contrast, for medicinal plants a detailed classification scale has been proposed, which may be useful in demonstrating our samples' antimicrobial activity (Van and Holl, 2017). An MIC value of ≤160 µg/mL is regarded as potentially valuable for medicinal plant extracts, and a value <100 µg/mL indicates noteworthy or promising activity (Tang et al., 2003). The low MIC and MBC/MFC values of the STG (12–50 µg/mL, Table 2) showed that it has substantial antimicrobial activity against susceptible organisms. As compared to the extract, the isolated compound displayed a significantly lower MIC value and exhibited an improved inhibitory effect. In this way, STG can be isolated and purified from corn silk extract, which allows for an accurate evaluation of its activity, indicating that STG may be responsible for the antimicrobial effects observed. It is consistent with previous studies that STG exhibits broad-spectrum antimicrobial activity, for example, the in vitro antibacterial effectiveness of STG extracted from *Caylusea abyssinica* roots (Edilu et al., 2015). Moreover, the tested compound demonstrated effectiveness against *S. aureus*, *Pseudomonas aeruginosa*, *E. coli*, and *Salmonella typhimurium*, exhibiting an inhibition zone ranging from 11 mm to 18 mm. Likewise, Yinusa et al. (2014) reported that STG at 50 µg/mL demonstrated an inhibition zone of 20 mm to 24 mm against a variety of organisms, including *E. coli* (21 mm), *S. aureus* (21 mm), *B. subtilis* (24 mm), *Proteus vulgaris* (21 mm), *S. typhimurium* (21 mm), *S. pyogenes* (22 mm), *S. dysenteriae* (21 mm), *C. virusei* (20 mm), *C. albicans* (21 mm), and *C. tropicalis* (20 mm). In another study, Abinaya (2021) found that STG, a compound isolated from the leaf part of *Crescentia alata* Kunth, displayed the most potent antimicrobial effect against all tested microbial strains, with MIC values varying between 1.95 and 125 µg/mL. Kanokmedhakul et al. (2005) reported that steroids can act as inhibitors of bacterial transpeptidation by targeting the protein 'sortase' present on the surfaces of bacterial cells. Furthermore, the disruption of membranes is one of the mechanisms of sterol action in microbes (Tamokou et al., 2011).

Tomato (*Solanum lycopersicum*), belonging to the family Solanaceae, is widely recognized as one of the most significant and widely consumed vegetables globally (Aldubai et al., 2022). Multiple species of *Fusarium* contribute to significant crop losses in tomato production worldwide (Wang et al., 2011). Inhabiting the soil, the pathogen invades plants by infecting their roots and crown. Tomato wilt is commonly associated with various *Fusarium* species, such as *F. verticillioides*, *F. oxysporum*, and *F. equiseti* (Rozlianah and Sariah, 2010). At any stage

of growth, tomatoes can be infected by *Fusarium* species through both the roots and crown region. In the case of *Fusarium oxysporum*, for instance, the pathogen targets the vascular bundles, resulting in an early wilting syndrome as a response to stress (Adisa et al., 2018). In another study, Adss et al. (2017) found that H_2O_2 and salicylic acid were used as elicitors to induce the synthesis of defense-related proteins, resulting in less tomato rot caused by *Alternaria solani*. As evidenced by the decreased percentage of infected fruit and lesion diameter, STG displayed a longer-lasting effect. Possibly, STG prevented sporulation on the fruit's surface by inhibiting the pathogen's growth.

Phytosterols have gained considerable attention because of their thermal stability. The antifungal activity of STG was not affected significantly at different temperature or pH values. In addition, the inhibitory effect of STG decreased as UV exposure increased. These results were consistent with those of previous research. STG in soybean oil demonstrated outstanding thermal stability at 180 °C. This showed that STG has antioxidant activity and its addition to soybean oil could make it appropriate for home cooking or frying (Chang et al., 2020). STG provided significant protection when yeast cells were exposed to slow freezing. Additionally, it increases the survival rate of cultures stored at subzero temperatures (Tantratian et al., 2019).

Numerous studies have indicated that the effects of various commonly used antibiotics on bacterial cell wall synthesis result in deformation and lysis of both gram-negative and gram-positive bacteria (Kantele et al., 2015). The activity of transpeptidase, a crucial enzyme in the construction of peptidoglycan cell walls, can be disrupted by the binding of particular proteins within the bacterial cell wall (Cho et al., 2014). Because phytosterols are similar to endogenous sterols, they are thought to exhibit antibacterial effects because they substitute these substances within the cell membrane (Doğan et al., 2017). Moreover, it has been found that STG may affect multiple drug efflux pump proteins in multidrug-resistant *E. coli*; consequently, first-line antibiotic resistance is suppressed. Additionally, vinyl and peroxide bonds in steroid structures may contribute to their antibacterial effects (Vida et al., 2012). Future research should aim to understand the potential ways in which corn silk extracts and isolated compounds operate.

5. Conclusion

It is now common practice to utilize naturally active biomolecules from plant extracts for prolonging the shelf life of vegetables and fruits. It is evident from our study that the bound crude extract offers more potential as a source of natural antimicrobial agents when compared to the free crude extract. Through bioassay-guided fractionation, STG and stigmasteryl-3β-arachidate were isolated for the first time as antimicrobial compounds from the fraction derived from the ethyl acetate-bound crude extract of corn silk, highlighting their significance in this medicinal plant. Both compounds exhibited strong antioxidant and antimicrobial activity. STG showed significant in vitro and in vivo antifungal activity against *F. verticillioides* tomato fruit pathogens. Additional research is necessary to elucidate the fundamental mechanisms of action and an in-depth investigation should be conducted into their prospective utility within the domains of food preservation and pharmaceutical industries. The phytochemical and pharmacological analysis of corn silk represents an avenue of exploration that promises to pave the way for the creation of innovative therapeutic agents sourced from traditional herbal remedies.

Acknowledgments

This work was supported by the Key Research and Development Program of Jiangsu Province (BE2017374-2) and special funds of agro-product quality safety risk assessment of the Ministry of Agriculture of the People's Republic of China (GJFP201701505; GJFP20191101).

Author contribution: Conceptualization, Z.X.; Formal analysis, U.K., R.A.S., and D.A.; Methodology, U.K., R.A.S. Resources, Z.X.; Software, U.K Supervision, Z.X.; Writing—original draft, U.K. and U.K. Writing—review and editing, U.K., R.A.S., and Z.X. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: All authors declared no conflict of interest.

Ethical approval: This study does not require ethical approval from the Institutional Review Board

References

Abinaya R (2021). Isolation, characterization and biological activities of stigmasterol from leaf part of *Crescentia alata* Kunth (*Bignoniaceae*). European Journal of Medicinal Plants 32 (3): 9-21. https://doi.org/10.9734/ejmp/2021/v32i330377

Abirami S, Priyalakshmi M, Soundariya A, Samrot AV, Saigeetha S et al. (2021). Antimicrobial activity, antiproliferative activity, amylase inhibitory activity and phytochemical analysis of ethanol extract of corn (*Zea mays* L.) silk. Current Research in Green and Sustainable Chemistry 4: 100089. https://doi.org/10.1016/j.crgsc.2021.100089

Adisa IO, Reddy VL, Rawat S, Hernandez JA, Dimkpa CO et al. (2018). Role of cerium compounds in Fusarium wilt suppression and growth enhancement in tomato (*Solanum lycopersicum*). Journal of Agricultural and Food Chemistry 66 (24): 5959-5970. https://doi.org/10.1021/acs.jafc.8b01345

Adss I, Hamza H, Hafez E, Heikal H (2017). Enhancing tomato fruits post-harvest resistance by salicylic acid and hydrogen peroxide elicitors against rot caused by *Alternaria solani*. Journal of Agricultural Chemistry and Biotechnology 8 (1): 1-8. https://doi.org/10.21608/jacb.2017.38409

Afsar T, Razak S, Almajwal A, Shabbir M, Khan K et al. (2022). Bioassay-guided isolation and characterization of lead antimicrobial compounds from *Acacia hydaspica* plant extract. AMB Express 12: 156. https://doi.org/10.1186/s13568-022-01501-y

Ahmed IA, Mikail MA (2017). Paradigm shift: focusing on plant-based natural antimicrobials. Journal of Microbiology & Experimentation 5 (2): 00145. https://doi.org/10.15406/jmen.2017.05.00145

Aldubai AA, Alsadon AA, Migdadi HH, Alghamdi SS, Al-Faifi SA et al. (2022). Response of tomato (*Solanum lycopersicum* L.) genotypes to heat stress using morphological and expression study. Plants 11 (5): 615. https://doi.org/10.3390/plants11050615

Anand U, Jacobo HN, Altemimi A, Lakhssassi N (2019). A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery. Metabolites 9 (11): 258. https://doi.org/10.3390/metabo9110258

Asghar MA, Yousuf RI, Shoaib MH, Asghar MA (2020). Antibacterial, anticoagulant and cytotoxic evaluation of biocompatible nanocomposite of chitosan loaded green synthesized bioinspired silver nanoparticles. International Journal of Biological Macromolecules 160: 934-943. https://doi.org/10.1016/j.ijbiomac.2020.05.197

Chang M, Xu Y, Li X, Shi F, Liu R et al. (2020). Effects of stigmasterol on the thermal stability of soybean oil during heating. European Food Research and Technology 246 (9): 1755-1763. https://doi.org/10.1007/s00217-020-03528-6

Cho H, Uehara T, Bernhardt TG (2014). Beta-lactam antibiotics induce a lethal malfunctioning of the bacterial cell wall synthesis machinery. Cell 159 (6): 1300-1311. https://doi.org/10.1016/j.cell.2014.11.017

Clinical and Laboratory Standards Institute (2010). Performance Standards for Antimicrobial Susceptibility Testing: 20th Informational Supplement. Wayne, PA, USA: Clinical and Laboratory Standards Institute.

Davies CR, Wohlgemuth F, Young T, Violet J, Dickinson M et al. (2021). Evolving challenges and strategies for fungal control in the food supply chain. Fungal Biology Reviews 36: 15-26. https://doi.org/10.1016/j.fbr.2021.01.003

Doğan A, Otlu S, Çelebi Ö, Aksu P, Saglam AG et al. (2017). An investigation of antibacterial effects of steroids. Turkish Journal of Veterinary and Animal Sciences 41 (2): 302-305. https://doi.org/10.3906/vet-1510-24

Edilu A, Adane L, Woyessa D (2015). In vitro antibacterial activities of compounds isolated from roots of *Caylusea abyssinica*. Annals of Clinical Microbiology and Antimicrobials 14: 15. https://doi.org/10.1186/s12941-015-0072-6

El-Ghorab A, El-Massry KF, Shibamoto T (2007). Chemical composition of the volatile extract and antioxidant activities of the volatile and nonvolatile extracts of Egyptian corn silk (*Zea mays* L.). Journal of Agricultural and Food Chemistry 55 (22): 9124-9127. https://doi.org/10.1021/jf071646e

Emmanuel S, Olajide O, Abubakar S, Akiode S, Etuk-Udo G (2016). Chemical evaluation, free radical scavenging activities and antimicrobial evaluation of the methanolic extracts of corn silk (*Zea mays*). Journal of Advances in Medical and Pharmaceutical Sciences 9 (4): 1-8. https://doi.org/10.9734/jamps/2016/28530

Hasanudin K, Hashim P, Mustafa S (2012). Corn silk (*Stigma maydis*) in healthcare: a phytochemical and pharmacological review. Molecules 17 (8): 9697-9715. https://doi.org/10.3390/molecules17089697

Hashimi MH, Hashimi R, Ryan Q (2020). Toxic effects of pesticides on humans, plants, animals, pollinators and beneficial organisms. Asian Plant Research Journal 5 (4): 37-47. https://doi.org/10.9734/aprj/2020/v5i430114

Jeong YH, Oh YC, Cho WK, Yang HJ, Ma JY (2017). Maydis Stigma elicits analgesia and blocks edema in mice and inhibits inflammation in macrophages. American Journal of Chinese Medicine 45 (7): 1477-1496. https://doi.org/10.1142/S0192415X1750080X

Jia Y, Wang Y, Li R, Li S, Zhang M et al. (2021). The structural characteristic of acidic-hydrolyzed corn silk polysaccharides and its protection on the H_2O_2-injured intestinal epithelial cells. Food Chemistry 356: 129691. https://doi.org/10.1016/j.foodchem.2021.129691

Kanokmedhakul K, Kanokmedhakul S, Phatchana R (2005). Biological activity of anthraquinones and triterpenoids from *Prismatomeris fragrans*. Journal of Ethnopharmacology 100 (3): 284-288. https://doi.org/10.1016/j.jep.2005.03.018

Kantele A, Lääveri T, Mero S, Vilkman K, Pakkanen SH et al. (2015). Antimicrobials increase travelers' risk of colonization by extended-spectrum betalactamase-producing *Enterobacteriaceae*. Clinical Infectious Diseases 60 (6): 837-846. https://doi.org/10.1093/cid/ciu957

Khan U, Hayat F, Khanum F, Shao Y, Iqbal S et al. (2023). Optimizing extraction conditions and isolation of bound phenolic compounds from corn silk (*Stigma maydis*) and their antioxidant effects. Journal of Food Science 88 (8): 3341-3356. https://doi.org/10.1111/1750-3841.16682

Li X, Wu X, Yuan T, Zhu J, Yang Y (2021). Influence of the iodine content of nitrogen- and iodine-doped carbon dots as a peroxidase mimetic nanozyme exhibiting antifungal activity against *C. albicans*. Biochemical Engineering Journal 175: 108139. https://doi.org/10.1016/j.bej.2021.108139

Madikizela B, Aderogba MA, Finnie JF, Van SJ (2014). Isolation and characterization of antimicrobial compounds from *Terminalia phanerophlebia* Engl. & Diels leaf extracts. Journal of Ethnopharmacology 156: 228-234. https://doi.org/10.1016/j.jep.2014.09.003

Mechqoq H, Hourfane S, Yaagoubi MEl, Hamdaoui AEl, Msanda F et al. (2022). Phytochemical screening, and in vitro evaluation of the antioxidant and dermocosmetic activities of four Moroccan plants: *Halimium antiatlanticum*, *Adenocarpus artemisiifolius*, *Pistacia lentiscus* and *Leonotis nepetifolia*. Cosmetics 9 (5): 94. https://doi.org/10.3390/cosmetics9050094

Mohammadi F, Montazer M, Mianehro A, Eslahi N, Mahmoudi RM (2023). Preparation of antibacterial cellulose fabric via copper (II) oxide and corn silk (*Stigma maydis*) nanocomposite. Starch 75 (1-2): 2200156. https://doi.org/10.1002/star.202200156

Morshed S, Islam SM (2015). Antimicrobial activity and phytochemical properties of corn (*Zea mays* L.) silk. SKUAST Journal of Research 17 (1): 8-14.

Mota KSDL, Pereira FDO, De Oliveira WA, Lima IO, Lima EDO (2012). Antifungal activity of *Thymus vulgaris* L. essential oil and its constituent phytochemicals against *Rhizopus oryzae*: interaction with ergosterol. Molecules 17 (12): 14418-14433. https://doi.org/10.3390/molecules171214418

Najmi A, Javed SA, Al Bratty M, Alhazmi HA (2022). Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. Molecules 27 (2): 349. https://doi.org/10.3390/molecules27020349

Nawaz H, Aslam M, Muntaha ST (2019). Effect of solvent polarity and extraction method on phytochemical composition and antioxidant potential of corn silk. Free Radicals and Antioxidants 9 (1): 5-11. https://doi.org/10.5530/fra.2019.1.2

Nessa F, Ismail Z, Mohamed N (2012). Antimicrobial activities of extracts and flavonoid glycosides of corn silk (*Zea mays* L). International Journal of Biotechnology for Wellness Industries 1 (2): 115.

Poloni NM, Carvalho G, Nunes CVS, Francis DA, Nunes MJL et al. (2021). Widespread distribution of resistance to triazole fungicides in Brazilian populations of the wheat blast pathogen. Plant Pathology 70 (2): 436-448. https://doi.org/10.1111/ppa.13288

Rivero-Pérez MD, Muñiz P, González-Sanjosé ML (2007). Antioxidant profile of red wines evaluated by total antioxidant capacity, scavenger activity, and biomarkers of oxidative stress methodologies. Journal of Agricultural and Food Chemistry 55 (14): 5476-5483. https://doi.org/10.1021/jf070306q

Rodrigues BB, Kakde UB (2019). Post harvest fungi associated with *Solanum lycopersicum* (tomato) fruits collected from different markets of Mumbai. Online International Interdisciplinary Research Journal 9 (1): 52-60.

Rozlianah FS, Sariah M (2010). Characterization of Malaysian isolates of *Fusarium* from tomato and pathogenicity testing. Research Journal of Microbiology 5 (9): 926-932. https://doi.org/10.3923/jm.2006.266.272

Saad HA, Shukr SM, Ali EH (2021). Study of the effect of alcoholic extracts of maidenhair fern and corn silk in breaking up kidney stones out of living body. Research Journal of Pharmacy and Technology 14 (10): 5489-5497. https://doi.org/10.52711/0974-360X.2021.00958

Sepehri G, Derakhshanfar A, Zadeh FY (2011). Protective effects of corn silk extract administration on gentamicin-induced nephrotoxicity in rat. Comparative Clinical Pathology 20: 89-94. https://doi.org/10.1007/s00580-009-0943-3

Singh J, Inbaraj BS, Kaur S, Rasane P, Nanda V (2022a). Phytochemical analysis and characterization of corn silk (*Zea mays*, G5417). Agronomy 12 (4): 777. https://doi.org/10.3390/agronomy12040777

Singh J, Rasane P, Nanda V, Kaur S (2022b). Bioactive compounds of corn silk and their role in management of glycaemic response. Journal of Food Science and Technology 60 (6): 1695-1710. https://doi.org/10.1007/s13197-022-05442-z

Tamokou JDD, Kuiate, JR, Tene M, Nwemeguela TJK, Tane P (2011). The antimicrobial activities of extract and compounds isolated from *Brillantaisia lamium*. Iranian Journal of Medical Sciences 36 (1): 24-31.

Tang S, Bremner P, Kortenkamp A, Schlage C, Gray AI et al. (2003). Biflavonoids with cytotoxic and antibacterial activity from *Ochna macrocalyx*. Planta Medica 69 (3): 247-253. https://doi.org/10.1055/s-2003-38478

Tantratian S, Sae-ngow A, Pradistsuwan C, Prakitchaiwattana C, Pukahuta C (2019). Survival of *Kluyveromyces marxianus* with stigmasterol as subjected to freezing stress. Journal of Bioscience and Bioengineering 128 (1): 39-43. https://doi.org/10.1016/j.jbiosc.2018.12.003

Tian J, Chen H, Chen S, Xing L, Wang Y et al. (2013). Comparative studies on the constituents, antioxidant and anticancer activities of extracts from different varieties of corn silk. Food and Function 4 (10): 1526-1534. https://doi.org/10.1039/c3fo60171d

Van, S, Holl D (2017). Antimicrobial natural product research: a review from a South African perspective for the years 2009–2016. Journal of Ethnopharmacology 208: 236-252. https://doi.org/10.1016/j.jep.2017.07.011

Velazquez DVO, Xavier HS, Batista JEM, De Castro CC (2005). *Zea mays* L. extracts modify glomerular function and potassium urinary excretion in conscious rats. Phytomedicine 12 (5): 363-369. https://doi.org/10.1016/j.phymed.2003.12.010

Vida N, Svobodová H, Rárová L, Drašar P, Šaman D et al. (2012). Polyamine conjugates of stigmasterol. Steroids 77 (12): 1212-1218. https://doi.org/10.1016/j.steroids.2012.07.009

Wang C, Zhang S, Hou R, Zhao Z, Zheng Q et al. (2011). Functional analysis of the kinome of the wheat scab fungus *Fusarium graminearum*. PLoS Pathogens 7 (12): e1002460. https://doi.org/10.1371/journal.ppat.1002460

Wang KJ, Zhao JL (2019). Corn silk (*Zea mays* L.), a source of natural antioxidants with α-amylase, α-glucosidase, advanced glycation and diabetic nephropathy inhibitory activities. Biomedicine and Pharmacotherapy 110: 510-517. https://doi.org/10.1016/j.biopha.2018.11.126

Wang S, Sun L, Zhang W, Chi F, Hao X et al. (2020). *Bacillus velezensis* BM21, a potential and efficient biocontrol agent in control of corn stalk rot caused by *Fusarium graminearum*. Egyptian Journal of Biological Pest Control 30: 9. https://doi.org/10.1186/s41938-020-0209-6

Xu P, Wang X, Lin T, Shao Q, Peng J et al. (2022). A strategy for pinpointing natural bioactive components using two-dimensional bioassay profilings combined with comprehensive two-dimensional countercurrent chromatography × high-performance liquid chromatography. Analytical Chemistry 94 (37): 12715-12722. https://doi.org/10.1021/acs.analchem.2c02196

Yang T, Guang HJ, Yu Y, Li G, Guo X et al. (2019). Comparison of phenolics, flavonoids, and cellular antioxidant activities in ear sections of sweet corn (*Zea mays* L. *saccharata* Sturt). Journal of Food Processing Preservation 43 (1): e13855. https://doi.org/10.1111/jfpp.13855

Yinusa I, George NI, Shuaibu UOA, Ayo RG (2014). Bioactivity of stigmasterol isolated from the aerial part of *Spillanthes acmella* (Murr) on selected microorganism. International Journal of Current Microbiology and Applied Science 3 (2): 475-479.

York T, Van VSF, De WH (2012). An antimicrobial evaluation of plants used for the treatment of respiratory infections in rural Maputaland, KwaZulu-Natal, South Africa. Journal of Ethnopharmacology 144 (1): 118-127. https://doi.org/10.1016/j.jep.2012.08.038

Zhang B, Zhang Y, Li H, Deng Z, Tsao R (2020). A review on insoluble-bound phenolics in plant-based food matrix and their contribution to human health with future perspectives. Trends in Food Science and Technology 105: 347-362. https://doi.org/10.1016/j.tifs.2020.09.029