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Comparative antimicrobial activity of non-fermented and fermented extracts of *Artemisia vulgaris* L. and *Cannabis sativa* L. on pathogenic organisms associated with cancer and diabetic wounds

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ABSTRACT

Wound infections in cancer and diabetic patients are a significant public health concern due to their complex etiology, delayed healing, and association with multidrug-resistant pathogens. Cancer-related wounds often result from surgical procedures, radiation, or tumor growth, while diabetic wounds are linked to neuropathy and vascular complications. The microbial diversity in these wounds is shaped by the host environment, often dominated by bacterial pathogens and opportunistic fungi. The increasing threat of antimicrobial resistance highlights the need for alternative therapies, such as plant-derived antimicrobials. *Artemisia vulgaris* and *Cannabis sativa* are rich in bioactive compounds with antimicrobial properties, but the impact of fermentation on their efficacy is underexplored. This study analyzed microbial profiles in wound infections from 30 cancer and diabetic patients and evaluated the antimicrobial efficacy of non-fermented and fermented extracts of *A. vulgaris* and *C. sativa*. Swab specimens were cultured to identify bacteria and fungi using standard microbiological methods. Extracts prepared by cold maceration were tested using disc diffusion techniques, Minimum Inhibitory Concentration (MIC), and Minimum Lethal Concentration (MLC). Zones of inhibition were measured, and statistical analysis determined significant differences ($P \leq 0.05$). Diabetic wounds showed a higher microbial burden, with *Staphylococcus aureus* being the most common isolate (40%), especially in diabetic wounds (46.67%). *Pseudomonas aeruginosa* was evenly distributed (20%), while *Enterococcus* species and *Candida albicans* were exclusive to diabetic wounds. Negative cultures (26.67%) were observed only in cancer wounds. Non-fermented extracts of *A. vulgaris* and *C. sativa* showed broader antimicrobial activity, with inhibition zones of 31.83 ± 0.44 mm and 29.20 ± 0.36 mm against *S. aureus* and *Enterococcus*, respectively. However, fermentation enhanced efficacy against specific pathogens like *E. coli* and *Candida albicans*. These findings highlight the complexity of wound microbiota and the potential of plant extracts, with fermentation selectively enhancing activity against certain pathogens.

Keywords: Antimicrobial activity, fermentation, multidrug-resistant, plant-derived antimicrobials, wound infections.

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INTRODUCTION

The emergence of drug-resistant pathogens has driven the search for alternative therapies, particularly those derived from natural products (Abreu et al., 2017). Medicinal plants such as *Artemisia vulgaris* L. and *Cannabis sativa* L. have been extensively studied for their pharmacological properties, including antimicrobial

and wound-healing effects. *A. vulgaris* is rich in flavonoids, terpenoids, and phenolic acids, which exhibit broad-spectrum antimicrobial activity (Singh et al., 2023). Similarly, *C. sativa* contains cannabinoids, terpenes, and other secondary metabolites with reported antibacterial, antifungal, and antiviral properties (Ribeiro et al., 2024). These bioactive compounds make both plants promising candidates for managing infections, particularly those associated with cancer and diabetic wounds, where microbial resistance poses significant clinical challenges (Aladejana et al., 2024).

Fermentation has been shown to enhance the bioactivity of plant extracts by breaking down complex molecules into more bioavailable forms, thereby increasing their therapeutic potential. Fermented extracts often exhibit higher antimicrobial activity due to the production of bioactive peptides, organic acids, and secondary metabolites during fermentation (Hussain et al., 2016). Studies have demonstrated that fermentation can significantly improve the antimicrobial and antioxidant activities of plant extracts, making it a viable strategy for optimizing their efficacy (Gupta and Abu-Ghannam, 2012; Leonard et al., 2021; Sindhu et al., 2016). A comparative analysis of non-fermented and fermented extracts of *A. vulgaris* and *C. sativa* in antimicrobial applications is essential for identifying more effective therapeutic interventions.

Fermentation significantly enhances the bioactive compound profile of plant-based extracts, improving their nutritional and functional properties. Lactic acid bacteria (LAB), particularly *Lactobacillus plantarum*, play a pivotal role in this transformation. Studies have demonstrated that fermentation with *L. plantarum* increases the concentration of phenolic compounds, flavonoids, and antioxidants in various plant substrates. For instance, fermentation of myrtle berries with *L. plantarum* resulted in a fivefold increase in total phenol concentration and a tenfold increase in anthocyanins (Curiel et al., 2015). Similarly, fermentation of kiwifruit pulp with LAB, including *L. plantarum*, led to an increase in phenolic compounds, enhancing the fruit's antioxidant properties (Chen et al., 2022). Additionally, *L. plantarum* fermentation has been shown to improve the nutrient content and organic acid production in soy flour, enhancing its rheological and physicochemical properties (Teleky et al., 2020). These modifications not only boost the health benefits of fermented plant foods but also improve sensory attributes and extend shelf life. The metabolic activities of *L. plantarum* during fermentation are crucial for these enhancements, as they biotransform plant constituents into more bioavailable and bioactive forms. Consequently, utilizing *L. plantarum* in the fermentation of plant-based foods presents a promising strategy to naturally enrich bioactive compounds, thereby augmenting their functional and health-promoting qualities.

Cancer and diabetic wounds represent complex clinical conditions that are often complicated by infections with multidrug-resistant pathogens (Matta-Gutierrez et al., 2021). These wounds harbor a diverse microbial population, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, which delay healing and increase the risk of systemic infections (Rahim et al., 2017). The high prevalence of biofilm-forming pathogens in these wounds further exacerbates the challenge, underscoring the need for novel antimicrobial agents (Kadam et al., 2019). Natural plant extracts, especially those with enhanced activity through fermentation, offer a sustainable and potentially more effective alternative to conventional antimicrobial treatments for managing such infections (Gyawali and Ibrahim, 2014).

This study aimed to compare the antimicrobial activity of non-fermented and fermented extracts of *A. vulgaris* and *C. sativa* against pathogenic organisms commonly associated with cancer and diabetic wounds. The findings will contribute to understanding the role of fermentation in enhancing the therapeutic potential of medicinal plants and provide insights into alternative strategies for managing infections in these clinically challenging conditions.

MATERIALS AND METHODS

Sample collection

In this experiment, a total of 30 swab specimens were aseptically collected from human subjects with the assistance of a physician. The specimens comprised 15 swabs from diabetic wounds and 15 swabs from cancer wounds. All subjects were either attending the Diabetic Clinic or the Cancer Clinic at Jos University Teaching Hospital, Jos, Nigeria.

The wound area was first disinfected with 70% isopropyl alcohol and allowed to dry completely. A sterile swab stick was then used to gently wipe the wound surface in a circular motion, covering a significant area of the wound. The swab stick was returned to its sterile packaging and securely sealed. The collected specimens were transported to the laboratory for analysis within 30 minutes (Cheesbrough, 2006).

Isolation and identification of microorganisms

In the laboratory, the samples were inoculated onto appropriate culture media. For bacterial isolation, the samples were inoculated onto nutrient agar (NA) for general bacterial growth, MacConkey agar (MAC) for Gram-negative bacteria, and blood agar (BA) to observe hemolytic activity. Plates were incubated aerobically at 37°C for 24–48 hours.

For fungal isolation, samples were inoculated onto Sabouraud Dextrose Agar (SDA) supplemented with 1 mL of gentamicin to inhibit bacterial growth. The SDA plates were incubated at 25°C for 5–7 days. Colonies

with distinct morphological features were sub-cultured on NA for bacteria and SDA for fungi to obtain pure isolates.

Microscopic examination was performed using a compound microscope with oil immersion. Gram staining was conducted for bacterial identification, and lactophenol cotton blue staining was used for fungal characterization. Biochemical tests for bacterial identification included catalase, coagulase, oxidase, and IMViC (Indole, Methyl Red, Voges-Proskauer, and Citrate Utilization) tests. Additionally, sugar fermentation tests were performed to evaluate the carbohydrate utilization patterns of the bacterial isolates (Forbes et al., 2016).

Collection and processing of plant materials

The plants used in this study included *Artemisia vulgaris* L. and *Cannabis sativa* L. *A. vulgaris* was harvested from the A-Z Biotechnology Limited Medicinal Plants Plantation in Rayfield, Jos, Plateau State, Nigeria. The plant was authenticated by the Herbarium Unit of the Department of Plant Science, University of Jos, with Voucher Number: JUHN21000361. *C. sativa* leaves were obtained from the National Drug Law Enforcement Agency Command Headquarters, Jos, Nigeria.

The plant materials were washed with distilled water to remove debris, air-dried at room temperature (25-30°C) for two weeks, and pulverized into a fine powder using a Vitamix A3500 electric blender. The powdered materials were stored in airtight 120-J series containers.

Extraction of plant materials

Non-Fermented Extract Preparation

To prepare the non-fermented extract, 50 g of powdered plant material was macerated in 500 mL of distilled water (1:10 w/v) for 72 hours with occasional shaking. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 60°C. The concentrated extract was stored at 4°C for future analysis (Harborne, 1998).

Fermented Extract Preparation

The fermentation protocol was adapted from a previously described method (Martins et al., 2013) with modifications. Fifty grams of powdered plant material was mixed with 500 mL of distilled water and inoculated with a 10% (w/v) starter culture of *Lactobacillus plantarum*. The mixture was incubated under anaerobic conditions at 37°C for seven days in a fermentation flask. The pH of the medium was monitored daily to ensure microbial activity.

After fermentation, the mixture was filtered, and the filtrate was concentrated using a rotary evaporator at 60°C. The concentrated extract was stored at 4°C for subsequent use.

Preparation and reconstitution of plant extracts

The plant extracts (*A. vulgaris* and *C. sativa*) were reconstituted by dissolving them in 10% dimethyl sulfoxide (DMSO) using a modified method (Abiy and Berhe, 2016). Specifically, 0.8 g of each extract was dissolved in 4 mL of distilled water to prepare a stock solution of 200 mg/mL. Double-fold serial dilutions were then performed to prepare concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.13 mg/mL, and 1.57 mg/mL. The reconstituted extracts were stored at 2–8°C until further use.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of isolates from diabetic and cancer wounds, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus spp.*, *Escherichia coli*, and *Candida albicans*, was determined using the disc diffusion method (Gadisa and Tadesse, 2021).

Inoculums were prepared by emulsifying 4–5 colonies of the isolates in sterile nutrient broth and adjusting the turbidity to a 0.5 McFarland standard. Mueller-Hinton agar plates were inoculated by spreading the bacterial suspension evenly with a sterile cotton swab.

Sterile filter paper discs (6 mm diameter) were impregnated with 10–20 µL of plant extract solutions at different concentrations and air-dried to remove excess solvent. The discs were aseptically placed on the agar surface, ensuring proper spacing. Positive controls included standard antibiotics (chloramphenicol) or antifungal agents (fluconazole), while the solvent alone served as a negative control. Plates were incubated at 37°C for 24 hours.

After incubation, the diameters of inhibition zones were measured in millimeters. Experiments were performed in triplicates to ensure consistency, and results were compared to control values to assess the extracts' efficacy.

Determination of Minimum Inhibitory Concentrations (MIC)

The MIC of the plant extracts was determined using the tube dilution method (Dudley et al., 2022). A 100 µL inoculum adjusted to the McFarland standard was added to test tubes containing 9 mL of pre-sterilized nutrient broth and 1 mL of plant extract at varying concentrations. Tubes were incubated overnight, and turbidity changes were visually assessed using a spectrophotometer. The

MIC was defined as the lowest concentration of the extract that prevented visible growth.

Determination of Minimum Lethal Concentrations (MLC)

The MLC was determined by inoculating a loopful of the mixture from MIC tubes with no turbidity onto solid media. Plates were incubated at 37°C for 24 hours, and the MLC was defined as the lowest concentration showing no visible colonies.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism software (version 8.0). One-way analysis of variance (ANOVA) was used to compare significant differences between samples, with values expressed as means ± standard deviations. All determinations were conducted in triplicate, and confidence limits were set at 95% ($P \leq 0.05$).

RESULTS

Microbial species associated with cancer and diabetic wounds

The distribution of microbial isolates from wound sites of cancer and diabetic patients, as shown in Table 1, revealed notable differences in microbial prevalence between the two groups. Negative cultures were observed exclusively in cancer wounds (26.67%) and were completely absent in diabetic wounds, suggesting a higher microbial burden in the latter. Among the bacterial isolates, *S. aureus* was the most frequently identified pathogen, accounting for 40.00% of the total isolates, with a higher prevalence in diabetic wounds (46.67%) compared to cancer wounds (33.33%).

P. aeruginosa exhibited an equal distribution between the two groups (20.00% each), underscoring its role as a common wound pathogen irrespective of the underlying condition. Enterococcus species were isolated solely from diabetic wounds (20.00%), potentially reflecting differences in host susceptibility or wound environments. Similarly, *E. coli* was identified in both groups but was slightly more prevalent in cancer wounds (20.00%) than in diabetic wounds (13.33%). Additionally, *C. albicans* was found exclusively in diabetic wounds (6.67%), suggesting a predisposition to fungal infections in this group.

Antimicrobial activities of the plant extracts

Table 2 presents the antimicrobial susceptibility of isolates treated with various concentrations of non-fermented and fermented *A. vulgaris* extracts against different bacterial and fungal strains. The non-fermented extracts demonstrated greater antimicrobial efficacy, as indicated by larger zones of inhibition across all tested concentrations. For example, at the highest concentration of 200 mg/mL, the non-fermented extract showed inhibition zones of 31.83±0.44 mm against *S. aureus* and 29.20±0.36 mm against Enterococcus species. In comparison, the fermented extract exhibited slightly lower inhibition zones of 28.13±0.41 mm and 32.67±0.88 mm, respectively. This trend was consistent across other bacterial strains and *C. albicans*.

Table 1. Frequency of isolation of bacteria and fungi obtained from wound sites of cancer and diabetic patients.

	Number of isolates (%)		
	Cancer wounds	Diabetic wounds	Total
Negative culture	4 (26.67)	0 (0.00)	4 (13.33)
Staphylococcus aureus	5 (33.33)	7 (46.67)	12 (40.00)
Pseudomonas aeruginosa	3 (20.00)	3 (20.00)	6 (20.00)
Enterococcus species	0 (0.00)	2 (20.00)	2 (6.66)
Escherichia coli	3 (20.00)	2 (13.33)	5 (16.67)
Candida albicans	0 (0.00)	1 (6.67)	1 (3.33)

The inhibitory effect of both extracts diminished with decreasing concentrations, indicating a dose-dependent activity. Similarly, Table 3 outlines the antimicrobial susceptibility of isolates treated with different concentrations of non-fermented and fermented *C. sativa* extracts. The non-fermented extract exhibited notable antimicrobial activity across all tested concentrations, with the largest inhibition zones observed at 200 mg/mL against Enterococcus species (19.33±0.67 mm) and *S. aureus* (15.73±0.29 mm). This highlights the broad-spectrum antimicrobial activity of the non-fermented *C. sativa* extract, particularly against Gram-positive bacteria like *S. aureus*.

In contrast, the fermented *C. sativa* extract showed

Table 2. Antimicrobial susceptibility of isolates to *A. vulgaris* extracts.

Extract	Concentration (mg/ml)	Zones of inhibition (mm)				
		S. aureus	***P. aeruginosa***	***Enterococcus Sp***	***E. coli***	***C. albicans***
Non-fermented	200	31.83±0.44^{a}	20.23±0.19^{d}	29.20±0.36^{b}	22.00±0.10^{c}	14.17±0.33^{e}
	100	27.07±0.18^{a}	17.13±0.41^{c}	27.40±0.23^{a}	19.10±0.38^{b}	11.10±0.21^{d}
	50	22.00±0.58^{b}	12.20±0.15^{d}	24.07±0.34^{a}	15.37±0.23^{c}	6.17±0.17^{e}
	25	11.20±0.47^{b}	8.17±0.38^{c}	19.33±0.24^{a}	7.40±0.27^{c}	4.17±0.27^{d}
	12.5	7.67±0.33^{b}	5.00±0.00^{c}	11.33±.0.20^{a}	4.10±0.21^{c}	0.00±0.00^{d}
	6.25	3.00±0.00^{b}	5.00±0.00^{a}	5.20±.25^{a}	3.00±0.00^{b}	0.00±0.00^{c}
	3.13	0.00±0.00^{b}	0.00±0.00^{b}	3.00±0.00^{a}	0.00±0.00^{b}	0.00±0.00^{b}
	1.57	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	L.S.D	0.66				
Fermented	200	28.13±0.41^{b}	23.40±0.56^{c}	32.67±0.88^{a}	28.27±0.88^{b}	20.50±0.45^{d}
	100	22.13±0.69^{c}	19.17±0.09^{d}	29.00±1.16^{a}	26.90±0.10^{b}	17.43±0.26^{e}
	50	18.63±0.35^{b}	16.63±0.15^{c}	26.23±0.39^{a}	17.33±0.24^{bc}	11.37±.023^{d}
	25	10.33±0.44^{bc}	10.20±0.25^{bc}	21.17±0.22^{a}	12.47±0.26^{b}	9.07±0.35^{c}
	12.5	8.00±0.00^{c}	7.60±0.35^{c}	15.10±0.32^{a}	11.07±0.23^{b}	5.00±0.00^{d}
	6.25	4.00±0.00^{c}	6.10±0.10^{b}	8.37±.027^{a}	6.43±0.23^{b}	2.00±0.00^{d}
	3.13	4.00±0.00^{a}	0.00±0.00^{c}	4.40±0.21^{a}	3.00±0.00^{b}	0.00±0.00^{c}
	1.57	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	L.S.D	1.06				

At P≤0.05 there was a significant difference in antimicrobial activity of *A. vulgaris* non-fermented and fermented extracts on isolated organisms. Values are presented as mean ± standard error of means. Ranking was done across the organisms at different concentrations and values with the same super script are not significant. L.S.D = least significant difference.

Table 3. Antimicrobial susceptibility of isolates to *C. sativa* extracts.

Nature of extract	Concentration (mg/ml)	Zones of inhibition (mm)				
		S. aureus	***P. aeruginosa***	***Enterococcus Sp***	***E. coli***	***C.albicans***
Non -fermented	200	15.73±0.29^{b}	11.33±0.55^{d}	19.33±0.67^{a}	13.47±0.26^{c}	9.57±0.33^{e}
	100	13.07±0.07^{b}	9.47±0.26^{c}	15.57±0.29^{a}	12.53±0.27^{b}	6.00±0.00^{d}
	50	8.57±0.29^{b}	8.23±0.23^{b}	11.13±0.49^{a}	9.20±0.36^{a}	6.00±0.00^{c}
	25	7.97±0.26^{b}	3.00±0.00^{e}	9.27±0.27^{a}	5.13±0.13^{c}	4.00±0.00^{d}
	12.5	3.00±0.00^{c}	3.00±0.00^{c}	5.00±0.00^{a}	4.00±0.00^{b}	0.00±0.00^{d}
	6.25	0.00±0.00^{c}	0.00±0.00^{c}	3.00±0.00^{b}	4.00±0.00^{a}	0.00±0.00^{c}
	3.13	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	1.57	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	L.S.D	0.60				
Fermented	200	12.13±0.49^{b}	11.37±0.27^{b}	20.38±1.01^{a}	19.00±1.16^{a}	11.20±0.25^{b}
	100	12.33±0.33^{c}	8.00±0.00^{d}	16.37±0.27^{a}	16.06±0.67^{b}	8.00±0.00^{d}
	50	7.00±0.00^{c}	8.00±0.00^{b}	13.09±0.49^{a}	13.37±0.37^{a}	5.00±0.00^{d}
	25	5.17±0.16^{b}	5.23±0.23^{b}	12.10±0.39^{a}	11.00±0.58^{a}	3.00±0.00^{c}
	12.5	0.00±0.00^{d}	3.00±0.00^{c}	7.37±0.59^{a}	6.00±0.00^{b}	3.00±0.00^{d}
	6.25	0.00±0.00^{c}	0.00±0.00^{c}	3.00±0.00^{b}	5.00±0.00^{a}	0.00±0.00^{c}
	3.13	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	1.57	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	L.S.D	0.98				

At P≤0.05 there was a significant difference in antimicrobial activity of *C. sativa* non-fermented and fermented extracts on isolated organisms. Values are presented as mean ± standard error of means. Ranking was done across the organisms at different concentrations and values with the same super script are not significant. L.S.D = least significant difference.

reduced antimicrobial activity, particularly at higher concentrations, potentially due to fermentation altering its bioactive compounds. Despite this reduction, the fermented extract retained some efficacy, inhibiting Enterococcus species (20.38±1.01 mm) and *E. coli* (19.00±1.16 mm) at 200 mg/mL. These findings underscore the potential of *C. sativa* extracts as antimicrobial agents, with non-fermented extracts demonstrating greater potency and emphasizing the importance of processing methods in enhancing

therapeutic properties.

Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) of test extracts

The MIC and MLC data for the test extracts against bacterial and fungal isolates from wound sites of cancer and diabetic patients revealed differences in antimicrobial efficacy based on the extract type and fermentation status (Table 4).

Fermented extracts of *A. vulgaris* (F. *A. vulgaris*) demonstrated superior antimicrobial activity, as reflected in their lower MIC and MLC values compared to non-fermented extracts (NF *A. vulgaris*). For example, the MIC and MLC of F *A. vulgaris* against *S. aureus* were 3.13 mg/mL and 12.5 mg/mL, respectively, whereas those for NF *A. vulgaris* were 6.25 mg/mL and 50.0 mg/mL.

Similarly, fermented *C. sativa* (F *C. sativa*) showed enhanced activity against *E. coli* and *C. albicans*, with MIC values of 6.25 mg/mL and 12.5 mg/mL, respectively, compared to higher MICs for the non-fermented extract. In general, non-fermented extracts required higher concentrations to inhibit and kill microbial isolates, highlighting the potential enhancement of bioactivity through fermentation.

The data emphasize the promising antimicrobial potential of fermented extracts, particularly against Enterococcus species and *E. coli*, for which the MIC and MLC values were notably lower.

Table 4. Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) of test extracts on bacteria and fungi obtained from wound sites of cancer and diabetic patients.

Isolate	NF ***A. vulgaris***		F ***A. vulgaris***		NF ***C. sativa***		F ***C. sativa***	
	MIC Mg/ml	MLC Mg/ml	MIC Mg/ml	MLC Mg/ml	MIC Mg/ml	MLC Mg/ml	MIC Mg/ml	MLC Mg/ml
S. aureus	6.25	50.0	3.13	12.5	12.5	50.0	25.0	50.0
P. aeruginosa	6.25	50.0	6.25	25.0	12.5	50.0	12.5	50.0
***Enterococcus* Spp**	3.13	25.0	3.13	12.5	6.25	50.0	6.25	25.0
E. coli	6.25	12.5	3.13	6.25	6.25	25.0	6.25	12.5
C. albicans	25.0	100.0	6.25	50.0	25.0	100.0	12.5	100.0

NF = Non-fermented, F = Fermented.

DISCUSSION

The observed differences in microbial prevalence between wound sites of cancer and diabetic patients align with and expand upon findings from other studies. The higher microbial burden in diabetic wounds, indicated by the absence of negative cultures, is consistent with research by Kalan et al. (2019), which attributes this to impaired immunity and prolonged hyperglycemia in diabetic patients, fostering microbial colonization. The predominance of *Staphylococcus aureus* as the most frequently isolated pathogen, particularly in diabetic wounds, corroborates findings by Dunyach-Remy et al. (2016), who identified *S. aureus* as a leading cause of diabetic foot infections due to its adaptability and ability to form biofilms.

The equal distribution of *Pseudomonas aeruginosa* across both groups underscores its role as a common opportunistic pathogen, echoing the findings of Botelho et al. (2019), who emphasized its ubiquity in chronic wound infections regardless of underlying health conditions. The exclusive isolation of *Enterococcus* spp. in diabetic wounds aligns with the findings of Konar and Das (2013), who suggested that *Enterococcus* thrives in disrupted microbiota environments, such as those in diabetic wounds. Similarly, the presence of *Escherichia coli* in both groups, with a slight preference for cancer wounds, corresponds with studies by Abd-El-Raouf et al. (2020), which reported *E. coli* as a less common but significant pathogen in cancer-associated wounds. The exclusive isolation of *Candida albicans* from diabetic wounds supports the work of Chellan et al. (2010), which highlighted the increased susceptibility of diabetic patients to fungal infections due to hyperglycemia and immune dysfunction.

The findings on the antimicrobial susceptibility of *A. vulgaris* revealed higher zones of inhibition exhibited by non-fermented extracts against bacterial strains such as *Staphylococcus aureus* and *Enterococcus* spp., supporting earlier work by Hossain et al. (2017). Hossain attributed this effect to the degradation or transformation of phenolic compounds during fermentation, which often diminishes antimicrobial activity. This reduction is evident in the lower inhibition zones observed for fermented extracts in this study, particularly against *S. aureus* (28.13 ± 0.41 mm) compared to the non-fermented extract (31.83 ± 0.44 mm).

However, contrasting studies, such as those by Leonard et al. (2021), suggest that fermentation can enhance certain bioactive properties by releasing bound phenolics or generating new antimicrobial metabolites. For example, fermented *A. vulgaris* demonstrated higher inhibition against *Enterococcus* spp. (32.67 ± 0.88 mm). These differences may reflect variations in fermentation

conditions, microbial strains used, or the composition of the plant extract. The dose-dependent activity observed in this study aligns with findings by Aribi et al. (2015), who noted that higher concentrations of plant extracts yielded stronger inhibitory effects. Similarly, the antimicrobial efficacy of non-fermented and fermented *C. sativa* extracts aligns with existing research. Notable inhibitory effects of non-fermented extracts, particularly against *Enterococcus* spp. (19.33 ± 0.67 mm) and *S. aureus* (15.73 ± 0.29 mm) at 200 mg/mL, corroborate Ribeiro et al. (2024), who reported the broad-spectrum antimicrobial potential of raw *C. sativa* extracts due to intact phenolic and terpenoid compounds.

In contrast, the reduced efficacy of fermented extracts observed in this study is consistent with findings by Leonard et al. (2021), which attributed such reductions to the degradation or modification of active compounds during fermentation. However, the retained activity of fermented extracts, particularly against *Enterococcus* spp. (20.38 ± 1.01 mm) and *E. coli* (19.00 ± 1.16 mm), diverges from the broader notion of reduced efficacy post-fermentation and supports research by Limón et al. (2015), which suggested that fermentation under specific conditions could enhance antimicrobial activity by releasing new bioactive metabolites. The dose-dependent trends observed in both extract types are consistent with the findings of Li et al. (2017), who emphasized the importance of concentration in achieving effective microbial inhibition. The differential impacts on Gram-positive bacteria (*S. aureus*) versus Gram-negative bacteria (*E. coli*) further underscore the variable bioactivity of plant extracts and highlight the significance of processing methods.

The results of the Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) for fermented and non-fermented extracts both align with and diverge from previous studies on fermentation's impact on antimicrobial efficacy. The enhanced antimicrobial activity of fermented *A. vulgaris* and *C. sativa* supports findings by Limón et al. (2015), who reported that fermentation increases the bioavailability of antimicrobial compounds through enzymatic conversion of inactive precursors into active metabolites. The lower MIC (3.13 mg/mL) and MLC (12.5 mg/mL) values of fermented *A. vulgaris* against *S. aureus* compared to its non-fermented counterpart (MIC: 6.25 mg/mL, MLC: 50.0 mg/mL) highlight the increased potency achieved through fermentation. This trend is consistent across other isolates, such as *E. coli* and *C. albicans* for fermented *C. sativa*.

However, these findings contrast with studies by Sadh et al. (2018), which suggested that fermentation could degrade bioactive compounds, reducing their efficacy. The significant activity observed against *Enterococcus* spp. and *E. coli* in this study agrees with the research by Azi et al. (2021), which emphasized the broad-spectrum antimicrobial effects of biotransformed plant metabolites. The higher MIC and MLC values of non-fermented extracts reflect their comparatively limited antimicrobial potential.

CONCLUSION

This study explored the therapeutic potential of *A. vulgaris* and *C. sativa* extracts as antimicrobial agents, with non-fermented forms exhibiting broader activity. However, fermentation may enhance specificity against certain pathogens. These findings underscore the need for tailored approaches in utilizing plant extracts to manage wound infections in cancer and diabetic patients. Future research should investigate the effects of fermentation on bioactive compounds to optimize the antimicrobial properties of plant-based therapies.

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