Clinical Phytoscience

ORIGINAL CONTRIBUTION Open Access

Antimicrobial efficacy of chitosan-*Tridax procumbens* extract composite on selected wound surface microorganisms

I. F Ossamulu[1], I. O Usman[1], O. D Solomon[1] and T. Y Gara[1*]

Abstract

Background Antimicrobial resistance is a serious health problem that can be associated with wound surface microorganisms and delayed healing in wounds exposed to such microbes. This has led to the exploration of natural compounds with antimicrobial properties to alleviate these difficult situations. This study evaluated the antimicrobial effects of ethanol extract of *Tridax procumbens*, chitosan, and their composite on some wound surface-associated microorganisms; *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *A. niger*, *M. canis*, and *C. acremonium*.

Methods The phytochemical and antioxidant screening were conducted using standard methods. The sensitivity and susceptibility tests were determined using the agar well diffusion and double serial dilution methods respectively.

Result The quantitative phytochemical constituents of the extract include phenols, flavonoids, saponins, alkaloids, and tannins. However, the concentration of phenols was significantly ($p < 0.05$) higher than other phytoconstituents. The ethanol extract of *Tridax procumbens*-chitosan composite showed a significantly ($p < 0.05$) higher zone of inhibition against all the tested microorganisms (bacteria and fungi). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the composite that limited the growth and completely killed the selected bacteria were observed at 12.5 mg/ml and 25 mg/ml concentrations respectively. while the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of the composite for *M. canis* and *C. acremonium* were 12.5 mg/ml and 50 mg/ml whereas, that of *A. niger* were 6.25 mg/ml and 12 mg/ml respectively.

Conclusion The study suggests that ethanol extract of *T. tridax procumbens*-chitosan composite is more effective as an antimicrobial agent in combating wound surface-associated microbial organisms than the plant extract and chitosan being a single agent.

Keywords *Tridax procumbens*, Chitosan, Antioxidants, *A. niger*, *C. acremonium*

*Correspondence:
T. Y Gara
t.gara@futminna.edu.ng
[1]Department of Biochemistry, School of Life Science, Federal University of Technology, P.M.B 65, Minna, Niger State, Nigeria

Introduction

The invasion of an injury or the soft tissues around it by pathogenic organisms is known as wound microbial infection, and it is linked to a higher rate of morbidity and mortality [1]. One of the most frequent issues following an injury is infection. Due to exposure to the protective skin barrier, patients with wound infections from burns or diabetic foot ulcers are more susceptible to microbial infection [2]. The most prevalent gram-positive and gram-negative bacteria linked to wound infections are *Klebsiella pneumoniae*, P*seudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* [3]. Employing environmental spore pollution, fungi can potentially cause deep tissue damage [4].

The application of combination formulations in combating microorganisms on wound surfaces has been a subject of prior research. De S Araújo [5] and Alavi [6] explore the potential use of polysaccharide-based systems and micro- and nanoformulations in managing infections and promoting healing in chronic wounds. Additionally, probiotics are known for partially inhibiting antimicrobial activity and biofilm formation, offering a possible treatment option for infected chronic wounds [7]. Andersson [8], highlighted an ex-vivo wound infection model that effectively assesses the efficacy of topical formulations and provided a reliable approach for evaluating the effectiveness of combination therapies. Antibiotics have a major effect on lowering the issues brought on by microbial infections. Due to genetic alterations in the organisms that cause some germs to be susceptible to the harmful effects of antimicrobials, the rise in microbial resistance to a range of antimicrobial agents over time is a serious health problem [9]. To lessen the threat to public health, this issue must be addressed very soon since it has consequences for global health. Due to their production, strong and advantageous effects on the body, and lower toxicity when used to treat illnesses than manufactured medications, medicinal plants are used by almost 85% of the world's population today [10, 11]. According to the World Health Organization (WHO), several plants are utilized worldwide to treat various ailments. This is because bioactive substances found in plants, such as tannins, alkaloids, phenols, and flavonoids, have antibacterial, antioxidant, and anti-inflammatory qualities that can help fight bacterial and fungal infections [12]. It is essential to identify novel antimicrobial chemicals in these plants as potential therapeutic options due to the growing problem of antibiotic resistance in bacteria. *Tridax procumbens*, a kind of flowering plant in the Asteraceae family, is sometimes referred to as coat buttons or tridax daisy. It is typically thought of as a weed that invades different crops. On the other hand, several therapeutic benefits are recognized for it, such as antiviral, antioxidant, antibiotic efficacies, wound healing activity, insecticidal, and anti-inflammatory characteristics [10]. The second most prevalent polysaccharide that is separated from chitin, chitosan, is generally found in the hard-external skeleton of insects, mollusks, and shellfish [13]. It has been demonstrated to have strong antibacterial qualities and to work in concert with other antimicrobials to boost their antimicrobial potency [14]. This research focuses on combining *T. procumbens* ethanol leaf extract and chitosan to target clinically relevant wound microorganisms and investigate the synergistic properties of this composite. These innovative components could significantly contribute to developing new, potent, and natural antimicrobial therapies for wound infections, addressing a critical area of medical necessity.

Materials and methods

Sample collection and preparation of plant extract

The chitosan was obtained from Wisapple Biotech. Co., Ltd, Beijing. The plants *(Tridax procumbens*) were collected from the Oziokutu community, Kogi State, Nigeria, and identified at the herbarium, Department of Plant Biology, Federal University of Technology Minna, Nigeria. The voucher number is FUT/PLB/AST/001. The leaf and stem were separated, washed thoroughly with tap water, and then air dried under shade for 10 days. The dried plant was ground into powdered form using a mixer grinder and used for the extraction of phytochemicals.

Extraction process

Plant materials were extracted using a cold maceration process with 95% ethanol. 250 ml of 95% ethanol was poured into a 1000 ml beaker containing 100 g of the plant extract for 72 h. For a complete and efficient extraction, the solution was allowed to stand for three days. After that, it was filtered through a muslin cloth and Whatmann filter paper No 1. The filtrate was evaporated to dryness in a water bath at 45 °C, and stored in air tight containers till further use.

Quantitative determination of the phytochemicals

The ethanol extract of *Tridax procumbens* was investigated quantitatively for the presence of flavonoids, phenols, alkaloids, saponins, and tannins using the spectrophotometric method [15–17].

In vitro antioxidant assays

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging assay as described by Zahra et al., [18]. Briefly, different concentrations of extracts and ascorbic acid (50, 100, 250, and 500 µg/ml) were prepared from stock solutions (1000 µg/ml), prepared by weighing and dissolving 0.01 g of the

extracts and ascorbic acid, respectively in 10 ml of methanol. Thereafter, 2 ml of 0.004% DPPH in methanol added to 1 ml of various concentrations of plant extracts and ascorbic acid, respectively. The reaction mixtures were incubated at 25 °C for 30 min. The absorbance of each test mixture was read against blank at 517 nm using double beam Shimadzu UV-1800 series spectrophotometer. The experiment was performed in triplicates. The percentage antioxidant activity was calculated using the formula below:

$$\%\text{Inhibition} = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \times 100$$

Ferric reducing antioxidant power (FRAP) assay

Estimation of antioxidant activity of the plant extracts via ferric reducing antioxidant power assay was conducted according to the method of Zahra et al., [18]. Stock solutions of plant extracts and ascorbic acid (1000 µg/ml) were prepared, from which different concentrations of 50,100, 250, and 500 µg/ml were prepared. In this assay, 1 ml of each plant extracts and ascorbic acid concentration was mixed with 1 ml of 0.2 M sodium phosphate buffer and 1 mL of 1% potassium hexacyano ferrate (III). The reaction mixtures were incubated at 50 °C for 20 min. Thereafter, 1 ml of 10% TCA was added. The reaction mixtures were then centrifuged at for 10 min at room temperature. Then 1 ml of each supernatant obtained was mixed with 1 ml of distilled water and then 0.2 ml of 0.1% ferric chloride was added. The blank was prepared in the same extracts as samples except that the extracts were replaced by distilled water. The absorbance of the test mixtures was read at 700 nm. The percentage antioxidant activity was calculated using the formula below:

$$\%\text{activity} = \frac{\text{Asample} - \text{Ablank}}{\text{Asample}} \times 100$$

Antimicrobial assay

Preparation of sample concentration

Plant extract (0.2, 0.4, 0.6, 0.8, and 1) g were weighed into five different containers labeled as A, B, C, and D, and 5 ml of DMSO was added to each of the containers. The resulting concentration was (0.04, 0.08, 0.12, 0.16, and 0.2) g/ml. Similar concentrations were also prepared for the chitosan sample and the chitosan-plant extract composite in a ratio of 1:1.

Preparation of nutrient broth for bacteria

0.2 ml of the test organism (*S. aureus*, *E. coli*, *K. pneumoniae* and *P. aeroginosa)* cultured overnight was transferred into 20 ml of sterile nutrient broth and the culture was incubated at 37 °C for 3 h to standardize the culture to 106 CFU/ml McFarland. A loopful of the standardized inoculum was used for the antibacterial assay [19].

Preparation of potato dextrose broth for fungi

0.2 ml of the test organism (*A. niger*, *M. canis*, and *C acremonium)* cultured overnight was transferred into 20 ml of sterile potato dextrose broth and the culture was incubated at 37 °C for 3 h to standardize the culture to 106 CFU/ml McFarland. A loopful of the standardized inoculum was used for the antibacterial assay [19].

Preparation of media

Nutrient agar (NA) (20 g) of standard measurement was weighed and poured into a sterile conical flask and 1 L of distilled water was added to the conical flask (28 g/1000 ml) also for potato dextrose agar, 24 g of potato dextrose agar powder was dissolved in 1 L of distilled water mixed and dissolved completely. The media was autoclaved for 15 min at 121 °C. After the autoclaving, the media was removed and cooled to 37 °C before pouring into a sterile petri dish. These procedures were carried out according to the method described in [20].

Sensitivity tests of ethanol extract of T. procumbens, chitosan and their composite on bacteria

The sensitivity tests (zones of inhibition) were carried out using the "Agar well diffusion method" as reported by Maqbool et al. [21]. For ethanol extract of *T. procumbens*, about 25 ml of nutrient agar (NA) was poured into 4 Petri dishes labeled as *S. aureus*, *E. coli*, *Klebsiella*, and *Pseudomonas*. The plates were allowed to stand until the NA became firmly gel. Each of the respective bacteria was then inoculated into their respective plates and a sterile stainless-steel cork borer of 1 mm diameter was used to make 5 wells (A, B, C, D, and E) on each of the Petri dishes. 0.2 ml of ethanol extract of *T. procumbens* in the container labeled A (0.04 g/ml), B (0.08 g/ml), C (0.12 g/ml), D (0.16 g/ml) and E (0.2 g/ml) were dispensed into the wells A, B, C, D, and E respectively. The precaution was taken to avoid spillage of the extract on the surface of the media. The inoculated plates were left for 1 h to allow the extract to diffuse into the agar. The culture plates were then incubated at 37 °C for 24 h. The diameter of the zone of inhibition around the bacteria was measured and recorded after incubation. The same procedure was followed for the sensitivity test of chitosan and their composite. Each of the analyses was done in triplicate.

Sensitivity tests of ethanol extract of T. procumbens, Chitosan and their composite on fungi

The sensitivity tests (zones of inhibition) for fungi were carried out using the "Agar well diffusion method" as described by Maqbool et al. [21]. For *T. procumbens* extract, about 25 ml of potato dextrose agar (PDA) was

poured into 3 Petri dishes labeled *A. niger*, *M. canis*, and *C. acremonium*. The plates were allowed to stand until the PDA became firmly gel. Each of the respective fungi was then inoculated into their respective plates and a sterile stainless-steel cork borer of 1 mm diameter was used to make 5 wells (A, B, C, D, and E) on each of the Petri dishes. 0.2 ml *T. procumbens* extract in the container labeled A (0.04 g/ml), B (0.08 g/ml), C (0.12 g/ml), D (0.16 g/ml), and E (0.2 g/ml) were dispensed into the wells A, B, C, D, and E respectively. The precaution was taken to avoid spillage of the extract on the surface of the media. The inoculated plates were left for 1 h to allow the extract to diffuse into the agar. The culture plates were then incubated at room temperature for 48 h. The diameter of the Zone of inhibition around the walls was measured and recorded after incubation. The same procedure was followed for the sensitivity test of chitosan and the *T. procumbens* extract and chitosan composite. Each of the analyses was done in triplicate.

Determination of minimum inhibitory concentration (MIC) of the composite for both bacteria and fungi

The MIC of the composite against the test bacteria was determined using the broth dilution method as described by [22]. 2 ml of fresh nutrient broth was prepared and dispensed into five test tubes. A stock solution (100 mg/ml) of the chitosan-extract composite (1:1) was prepared and 2 ml of the solution was double-fold serial diluted across the test tubes to obtain the following concentration of (50, 25, 12.5, 6.25, and 3.125) mg/ml. These concentrations were prepared for each of the test bacterium (*E. coli*, *S. aureus*, *P. aeruginosa* and *K. pneuminiae*). A loop full of individual bacteria was collected and inoculated into their respective prepared concentrations. The tubes were then incubated in a culture box at a 37 °C temperature for 24 h. After incubation, the tube with the least concentration of the extract and no detectable growth of the microorganism was recorded as the MIC. The same procedure was followed to determine the MIC of the fungi, but potato dextrose broth was used instead of nutrient broth, and the incubation process took place at room temperature.

Table 1 Quantitative phytochemical composition of ethanol extract of *T. procumbens*

Phytochemical constituents	Concentrations (mg/100 g)
Phenols	439.96 ± 0.68
Flavonoids	202.30 ± 0.36
Tannins	9.36 ± 0.35
Saponins	127.66 ± 0.60
Alkaloids	21.65 ± 0.39

Values are mean ± standard error of the mean (SEM) of the triplicate experiment

Determination of minimum bactericidal/fungicidal concentration (MBC/MFC) of the composite

The MBC/MFC of the composite against the test organisms was determined using the method described by Nguyen et al.,[23]. Samples that did not show any visible growth after the incubation period from the tubes used for MIC, were streaked out on NA (for bacteria) and PDA (for fungi). The sample plates were then incubated for 24 h (bacteria) and 48 h (fungi) to determine the minimum concentration of the composite that would kill the organisms. The lowest concentration of the composite that kills 99% of the bacteria and fungi was regarded as the minimum bactericidal/fungicidal concentration.

Statistical analysis

The data were entered into an excel spreadsheet for statistical analysis using Statistical Package for Social Science (SPSS) version 22. Descriptive statistics, one-way ANOVA, was utilized for statistical analysis. Descriptive statistics were employed for the calculation of the group mean of inhibition zone diameter as mean ± SEM. The one-way ANOVA was performed to determine the significant difference among group means. Statistically significant differences were declared at a *p-value* of less than 0.05.

Result and discussion

Quantitative phytochemical constituents

The quantitative phytochemical constituents of the ethanol extract of *T. procumbens* are shown in Table 1. Phenols (439.96 ± 0.68) mg/100 g were significantly ($p < 0.05$) higher in concentration when compared to the other phytoconstituents. However, this is followed by flavonoids (202.30 ± 0.36) mg/100 g, and then saponins (127.66 ± 0.60) mg/100 g. Alkaloids (21.65 ± 0.39) mg/100 g and tannins (19.36 ± 0.35) mg/100 g were the least observed phytoconstituent. The medicinal importance of *Tridax procumbent* is attributed to the presence of various bioactive constituents in the plant such as phenols, flavonoids, saponins, alkaloids, and tannins which are known to play significant roles in enhancing the physiological survival of the plants and also fight against microbial infections in humans [24]. These phytochemicals found in the ethanol extract of *T. procumbens* agree with the findings of Nakum et al. [25], who evaluated the in-vitro antioxidant activities and phytochemical analysis in different solvents extracted from *T. procumbens*. The significantly high phenols concentration in T. procumbens is in agreement with the study of Akinola et al. [26] who evaluated some phenolic compounds in *T. procumbens* by chromatographic and spectrophotometric methods. However, Nakum et al. [25], reported saponins as the major phytoconstituents in *Tridax procumbens*. This observed variation in the phytochemical concentration of

T. procumbens may be influenced by the developmental stages of the plant species, method of analysis, and/or method of extraction [27]. Phytochemicals can degrade over time and this can be a result of factors such as temperature, light and exposure to oxygen thereby reducing their efficacy. Proper storage conditions can be helpful in preserving the stability of the bioactive compounds.

2, 2-Diphenyl-2-Picryl hydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP) of ethanolic extract of *T. procumbens*

Figure 1 shows the scavenging efficacy of ethanolic extract of *T. procumbens* on 2, 2-Diphenyl-2-Picryl Hydrazyl (DPPH) Radicals. The percentage of inhibition of ascorbic acid and ethanol extract of *T. procumbens* were dose-dependent where the scavenging activities increased as their concentration increased. The ethanol extract of *T. procumbens* also demonstrated visible antioxidant effects in reducing Fe_3^+ to Fe_2^+ as shown in Fig. 2. However, its activity on ferric ions was found to be concentration dependent thus, increasing as its concentration increased. The DPPH and ferric-reducing power of the extract showed a dose-dependent scavenging activity. This observed scavenging activity of ethanol extract of *Tridax procumbens* could be explained by the presence of different phytochemicals found in the plant as they are known to be effective against free radical by chelating trace elements involved in free-radicals production, suppress the formation of reactive oxygen species and provide oxidative stress tolerance in plants [28]. Earlier researchers reported the existence of a direct relationship between phytochemicals and antioxidant activities [10, 25, 29]. Phenolics and flavonoids have been observed to possess the ability to restrict free radical formation and hold the capacity to donate hydrogen atoms. The bioactive compounds present in the extract are in varying concentrations which can influence their antioxidant properties [27]. Thus, the high concentration of phenols and flavonoids in the ethanol extract of *Tridax procumbens* may have possibly worked synergistically to elicit a stronger antioxidant activity.

Zone of Inhibition of ethanol extract of *T. procumbens*, chitosan and their composite on the selected bacteria

The zone of inhibition demonstrated by ethanol extract of *T. procumbens*, chitosan, and their composite against the four selected bacteria are shown in Table 2. The selected strains of bacteria were sensitive to ethanol extract of *T. procumbens* with *K. pneumonia* having the highest zone of inhibition (29.00 ± 0.50) mm. The chitosan demonstrated a visible zone of inhibition on *E. coli*, *S. aureus*, and *K. pneumoniae* but no zone of inhibition was observed on *P. aureginosa* (0.00 ± 0.00) mm. The composite demonstrated a significant ($p < 0.05$) higher

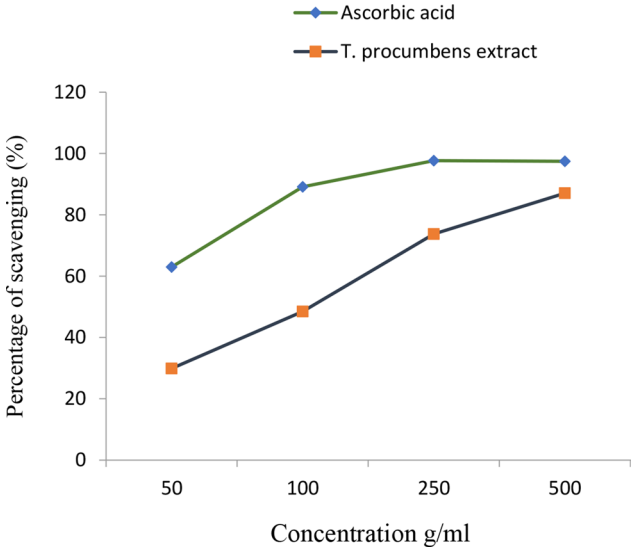

Fig. 1 DPPH radical scavenging activity of ethanol extract *T. procumbens*

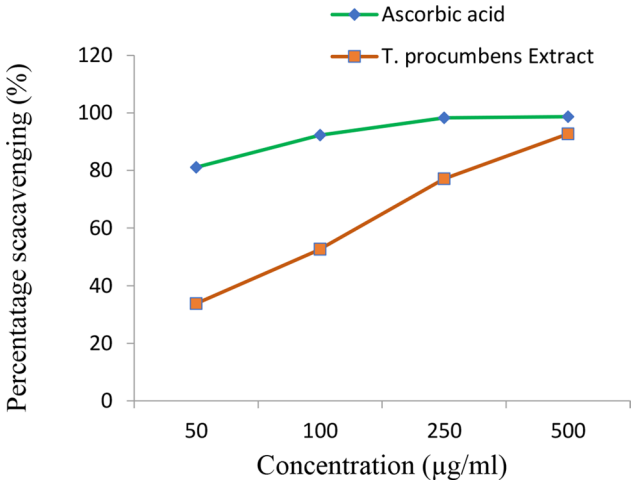

Fig. 2 Ferric reducing antioxidant power of ethanol extract of *T. procumbens*

Table 2 Zone of Inhibition of ethanol extract of *T. procumbens*, chitosan, and their composite on the selected bacteria

Organisms	T. procumbens (mm)	Chitosan (mm)	T. procumbens-chitosan (mm)
E. coli	18.00 ± 0.30^b	15.00 ± 0.30^a	21.50 ± 0.10^c
S. aureus	18.00 ± 0.60^a	18.00 ± 0.20^a	22.00 ± 0.20^b
P. aureginosa	17.50 ± 0.30^b	0.00 ± 0.00^a	24.00 ± 0.50^c
K. pneumoniae	29.00 ± 0.50^b	16.00 ± 0.40^a	30.00 ± 1.00^b

Values are mean ± standard error of the mean (SEM) of a triplicate experiment. Values across the row with different letters as superscripts are considered significant at $p < 0.05$

zone of inhibition against all the selected strains of bacteria compared to *T. procumbens* extract and chitosan sample. The highest (30.00 ± 1.00) mm zone of inhibition demonstrated by the composite was on *K. pneumonia*. There was no significant ($p < 0.05$) difference in the zone of inhibition observed between the composite and ethanol extract of *T. procumbens.* The efficiency of the antimicrobial agents used depended on their components and the nature of the surface organism selected. In this study, the ethanol extract of *Tridax procumbens*, chitosan, and *Tridax procumbens*-chitosan composite exhibited antibacterial activity against both the gram-negative and gram-positive bacterial strains, with the *Tridax procumbens*-chitosan composite having the most distinctive activity against *E. coli*, *S. aureus*, and *P. aureginosa* bacterial strains. *K. Pneumoniae* strain was found to be the most sensitive gram-negative bacteria to ethanol extract of *Tridax procumbens* and *Tridax procumbens*-chitosan composite.

The incubation conditions for the growth of bacteria could have influenced the observed antibacterial activity since, the experiments were conducted at 37 °C [30], which is suitable for the growth of many bacteria and these conditions might fully replicate the environmental conditions of a wound site in the human body, where temperatures are generally closer to 37 °C. The susceptibility of all the bacteria microorganisms to the ethanol extract of *Tridax procumbens* may be a result of the presence of the bioactive constituents of the plant. David et al. [31], reported that phytochemicals repressed microbial growth through inhibition of bacteria colonization, lowering the surface tension of extracellular medium, lysing bacterial membrane, or inactivating essential enzymes such as DNAse and RNAse. Chitosan showed zones of inhibition on *E. coli*, *S. aureus*, and *K. pneumoniae* organisms as similarly reported by Ferreira et al. [32]. The effects of chitosan on *E. coli* and *K. Pneumoniae* (gram-negative bacteria) have been described to be associated with the electrostatic interaction between the membrane lipopolysaccharide and the positive charge (NH_3^+) from chitosan resulting in disruption of their outer membrane. This effect may also lead to the leakage of cytosolic contents and subsequent death of the microbial cells [33].

Table 3 Zone of Inhibition of ethanol extract of *T. procumbens*, chitosan, and their composite on the selected fungi

Organisms	T. procumbens (mm)	Chitosan (mm)	T. procumbens-chitosan (mm)
A. niger	15.00 ± 0.50^{b}	12.00 ± 0.50^{a}	19.50 ± 0.70^{c}
C. acremonium	21.00 ± 0.20^{a}	17.50 ± 0.40^{a}	27.50 ± 1.30^{b}
M. canis	14.00 ± 0.30^{a}	13.00 ± 0.20^{a}	18.00 ± 0.20^{b}

Values are mean ± standard error of the mean (SEM) of a triplicate experiment. Values down the column with different letters as superscripts are considered significant at $p < 0.05$

The effect of chitosan on *S. aureus* (gram-positive bacteria) may involve interaction with membrane teichoic acid and peptidoglycan, leading to the destruction of the cell membrane and failure of the membrane-bound enzymatic activities which can affect protein synthesis [34]. The effect of chitosan could also involve the formation of barriers on the microbial cell wall inhibiting the penetration of nutrients into the cells and subsequent death [35]. Chitosan revealed no activity against the *P. aeruginosa* strain. This gram-negative bacterium has been reported to exhibit multidrug resistance toward several antimicrobial agents [36]. The resistance of *P. aeruginosa* towards chitosan may be linked to its reported lower membrane permeability to a fold of about 100 compared to *E. coli*, possession of an efflux pump system on their membrane that may have possibly expelled out chitosan [37, 38]. The significantly higher vulnerability of the selected microorganisms to the composite may have resulted from the joint action of both the plant bioactive constituents and chitosan. However, the method of delivery may affect the synergistic effect of the composite observed. Saponins possess detergent-like properties and might increase the permeability of bacterial cell membranes [39]. Similarly, Aboody et al. [40] reported the ability of flavonoids to disrupt microbial efflux pumps thereby increasing their susceptibility to antimicrobial agents. This mutual action of saponins and flavonoids may have enabled chitosan molecules in the composite to efficiently reach their target site in *P. aureginosa* compared to the use of chitosan as a single antimicrobial agent.

Zone of Inhibition of ethanol extract of *T. procumbens*, chitosan and their composite on the selected fungi

Table 3 showed the zones of inhibition of ethanol extract of *T. procumbens*, chitosan, and their composite on the selected fungi microorganisms. All three samples demonstrated a significant zone of inhibition on the selected fungi. However, higher zones were observed on *C. acremonium* for the entire three samples. Although, there is a significant ($p < 0.05$) difference in the zone of inhibition (27.50 ± 1.30) mm demonstrated by the composite on *C. acremonium* when compared to that of chitosan (17.50 ± 0.40) mm and ethanol extract of *T. procumbens* (21.50 ± 0.20) mm respectively. Based on the result obtained, *T. procumbens*-Chitosan composite showed a higher zone of inhibition against the selected fungi. This, therefore, suggests that the composite was a more effective antifungal agent compared to chitosan and the ethanol extract of *T. procumbens*. While the results indicate that the extract composite is effective under laboratory conditions, the efficacy in a real-world setting may vary. The higher temperatures and different environmental conditions of a human wound could influence both microbial resistance and the activity of

the antimicrobial agents. This synergistic activity may be associated with hydrophobic or hydrogen interaction between the membrane proteins in the fungi and the composite leading to structural changes in the membrane and impeding the growth of the fungi [34, 41]. This observation is similar to studies by Al-mamum et al. [42], who reported a positive synergistic action of *T. procumbens* on bone morphogenetic protein (BMP)–2.

Minimum inhibitory and bactericidal concentration of the *T. procumbens*-chitosan composite on the selected bacteria

The minimum inhibitory and bactericidal concentrations (MIC) of ethanol extract of *T. procumbens*-chitosan composite on the selected bacteria are shown in Table 4. The MIC of ethanol extract of *T. procumbens*-chitosan composite that limited the growth of the microorganisms was observed at 12.5 mg/ml concentration across all the tested bacteria. However, the minimum bactericidal concentration (MBC) that completely kills the selected microorganisms was observed at 25 mg/ml of the composite across all the tested bacteria. In the MIC and MBC study, all the bacteria strains had the same lowest amount of *T. procumbens*- chitosan composite that greatly inhibited their growth and subsequently resulted in microbial death. The synergistic effect of the various components of the composite may have effectively interfered with the synthesis of the bacteria cell surface, stimulated oxidative stress, and thus, inhibited the growth of the bacteria [43, 44]. When the metabolic pathway in a living organism is repressed or the course of replication and transcription are subdued, the population of bacterial infection is drastically reduced [45, 46].

Minimum inhibitory and fungicidal concentration of ethanol extract *T. procumbens*-chitosan composite on the selected fungi

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the composite against *A. niger*, *C. acremonium*, and *M. canis* fungi further confirm the activity of the composite on the fungi isolates (Table 5). The MIC values observed were between (6.25–12.5) mg/ml. while the MFC was between (12.5–50) mg/ml. *A. niger* had the most effective MIC and MFC at 6.25 mg/ml and 12 mg/ml respectively. The MIC and MFC of the composite on *C. acremonium* and *M. canis* are significantly higher than that observed for *A. niger*. The effect of the composite may be associated with the ability of the composite to penetrate the thick cell wall exhibited by fungi and bind to mRNA in the nuclei of the microorganism to inhibit its function [47, 48]. Chitosan is also reported to possess positive charges at several sites that can interact with the negative charges on the membrane lipids leading to permeability changes, loss of cellular content, and subsequent cell death [33].

Table 4 The minimum inhibitory and bactericidal concentration of the ethanol extract *T. procumbens*-chitosan composite on the selected bacteria

Bacteria	MIC (mg/ml)	MBC (mg/ml)
E. coli	12.5	25
S. aureus	12.5	25
P. aureginosa	12.5	25
K. pneumoniae	12.5	25

MIC = Minimum Inhibitory Concentrations, MBC: Minimum Bactericidal Concentrations

Table 5 The minimum inhibitory and fungicidal concentration of the *T. procumbens*-chitosan composite on the selected fungi

Fungi	MIC (mg/ml)	MFC (mg/ml)
A. niger	6.25	12
C. acremonium	12.5	50
M. canis	12.5	50

MIC = Minimum Inhibitory Concentrations, MFC: Minimum Fungicidal Concentrations

Conclusion

This study provided evidence to show that ethanol extract of *T. procumbens*, chitosan, and *T. procumbens*- chitosan composite exhibited varying antimicrobial activities against the selected bacterial and fungi pathogens; *E. coli*, *S. aureus*, *P. aureginosa*, *K. pneumoniae* bacterial pathogens and *A. niger*, *C. acremonium*, *M. canis* in wound infection. Chitosan was not as effective as the ethanol extract of *T. procumbens.* However, the antimicrobial activity of the *T. procumbens*-chitosan composite defined by its higher zone of inhibition and lower MIC, MBC, and MFC demonstrated remarkably more effective activities in suppressing the growth of all the selected bacteria and fungi strains as an of the interaction between the two anti-microbial agents. It has also shown that the bioactive compounds contained in the extract possess potential medicinal properties that work together with chitosan to produce a synergistic treatment effect on wound infections. Conducting in vivo studies or utilizing models that mimic human wound conditions will offer a more comprehensive understanding of the extract composite's effectiveness in real-world situations.

Acknowledgements
Not applicable.

Author contributions
All the authors contributed. They read and approved the final manuscript.

Funding
None.

Data availability
Not applicable.

Declarations

Ethical approval/consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 22 August 2023 / Accepted: 24 July 2025
Published online: 01 August 2025

References

1. Omole A, Stephen E. Antibiogram Profile of Bacteria Isolated from Wound Infection of Patients in Three Hospitals in Anyigba, Kogi Sate, Nigeria. FUTA J Res Sci. 2014;2:258–266.
2. Dryden MS. Complicated Skin and Soft Tissue Infection. Journal of Antimicrobial Chemotherapy. 2010;65:35–44. https://doi.org/10.1093/jac/dkq302
3. Pallavali RR, Degati VL, Lomada D, Reddy MC, Durbaka VRP. Isolation and in vitro evaluation of bacteriophages against MDR bacterial isolates from septic wound infections. PLoS ONE 2017;12(7):179–245
4. Kalan L, Grice EA, Fungi in the Wound Microbiome. Advances in wound care. 2018;7(7):247–255. https://doi.org/10.1089/wound.2017.0756
5. De S Araújo, G. R., Viana, N. B., Gómez, F., Pontes, B., & Frases, S. (2019). The mechanical properties of microbial surfaces and biofilms. Cell Surf. 2019;5:100028.
6. Alavi, M., Karimi, N., & Salimikia, I. (2019). Phytosynthesis of zinc oxide nanoparticles and its antibacterial, antiquorum sensing, antimotility, and antioxidant capacities against multidrug resistant bacteria. J Ind Eng Chem. 2019;72:457–473. https://doi.org/10.1016/j.jiec.2019.01.002
7. Brognara, L., Salmaso, L., Mazzotti, A., Di Martino, A., Faldini, C., & Cauli, O. (2020). Effects of Probiotics in the Management of Infected Chronic Wounds: From Cell Culture to Human Studies. Curr Clin Pharmacol. 2020;15(3):193–206.
8. Andersson, M., Madsen, L. B., Schmidtchen, A., & Puthia, M. (2021). Development of an Experimental ex vivo wound model to evaluate antimicrobial efficacy of topical formulations. Int J Mol Sci. 2021;22(9):5045.
9. Chandra H, Bishnoi P, Yadav A, Patni B, Mishra AP, Nautiyal AR. Antimicrobial Resistance and the Alternative Resources with Special Emphasis on Plant-Based Antimicrobials-A Review. Plants (Basel, Switzerland). 2017;6(2):16
10. Andriana Y, Xuan TD, Quy TN, Minh TN, Van TM, Viet TD. Antihyperuricemia, antioxidant, and antibacterial activities of Tridax procumbens. Foods. 2019;8(1):21.
11. Atef NM, Shanab SM, Negm SI, Abbas YA. Evaluation of antimicrobial activity of some plant extracts against antibiotic susceptible and resistant bacterial strains causing wound infection. Bull Natl Res Cent. 2019;43(1):1–11. https://doi.org/10.1186/s42269-019-0184-9
12. De Silva GO, Abeysundara AT, Aponso MMW. "Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants," Am J Essent Oil. 2017;5(2):29–32.
13. Tsurkan MV, Voronkina A, Khrunyk Y, Wysokowski M, Petrenko I, Ehrlich H. Progress in chitin analytics. Carbohydr Polym. 2020;252:117204.
14. Hemmingsen LM, Skalko-Basnet N, Joraholmen MW. The expanded role of chitosan in localized antimicrobial therapy. JMar Drugs. 2021;19(12):697.
15. Chlopicka J, Pasko P, Gorinstein S, Jedryas A, Zagrodzki P. Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. LWT Food Sci Technol. 2012;46(1):548 – 55. 37.
16. Stanković MS. Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. L. extracts. Kragujevac J Sci. 2011;33:63–72.
17. Tease GE and Evans WC. Textbook of pharmacognosy. 12th ed. London: Tindall and Co; 1983. p. 343 – 83.
18. Zahra N, Saeed M, Shahzad K, Firdous S, Ahmad I, Ashraf M, Abidi SH, Syed Q. DPPH Assay and Reducing Power Activity of Water Extract of (*Mentha longifolia*) Mint. LGU J Life Sci. 2022;6(01):38–47. https://doi.org/10.54692/lgujls.2022.0601198
19. Babayi HI, Kolo JI, Ijah UJJ. The antibacterial activities of methanolic extracts of Eucalyptus camaldulensis and *Terminalia catapa* against some pathogenic microorganisms. Biokemistri. 2004; 16:106–111.
20. Aujla IS, Paulitz TC. An Improved Method for Establishing Accurate Water Potential Levels at Different Temperatures in Growth Media. Front Microbiol. 2017;8:1497. https://doi.org/10.3389/fmicb.2017.01497
21. Maqbool HS, Visnuvinayagam AA, Zynudheen MP, Safeena K, Sathish Kumar. Antibacterial activity of beetroot peel and whole radish extract by modified well diffusion assay. Int J Curr Microbiol Appl Sci. 2020;9(1): 1222–1231.
22. Schumacher A, Vranken T, Malhotra A, Arts JJC, Habibovic P. In vitro antimicrobial susceptibility testing methods: agar dilution to 3D tissue-engineered models. Eur J Clin Microbiol Infect Dis. 2018;37(2):187–208.
23. Nguyen NT, Grelling N, Wetteland CL, Del Rosario RM, Liu H. Antimicrobial Activities and Mechanisms of Magnesium Oxide Nanoparticles (nMgO) against Pathogenic Bacteria, Yeasts, and Biofilms. Sci Rep. 2018;8(1). https://doi.org/10.1038/s41598-018-34567-5
24. Hayat M, Abbas M, Munir F, Hayat MQ, Keyani R, Amir R. Potential of plant flavonoids in pharmaceutics and nutraceutics. J Biomol Biochem. 2017;1(1): 11–16.
25. Nakum S, Umed B. Comparative study on in-vitro antioxidant activities by ABTS, DPPH, FRAP assessment methods and phytochemical analysis in different solvents extracted from *Tridax procumbens*. World Sci News. 2022;170:16–31.
26. Akinola AO, Adelowo FE. Chromatographic and spectrophotometric determination of some phenolic compounds in Tridax procumbens Stem. Chemistry Africa, 4, 103–113.
27. Adegbaju, O. D., Otunola, G. A., & Afolayan, A. J. (2020). Effects of growth stage and seasons on the phytochemical content and antioxidant activities of crude extracts of *Celosia argentea* L. Heliyon. 2021;6(6). https://doi.org/10.1016/j.heliyon.2020.e04086
28. Carbonell-Capella JM, Barba FJ, Esteve MJ, Frígola A. Quality parameters, bioactive compounds and their correlation with antioxidant capacity of commercial fruit-based baby foods. Food Sci Technol Int. 2014;20(7):479–487.
29. Iswarya Velu AR, Gopalakrishnan D, Manivannan B, Sathiavelu M, Arunachalam S. Comparison of antioxidant activity and total phenolic content of Amaranthus tristis and Celosia argentea var spicata. Asian Pac J Trop Biomed. 2012;1–4
30. Son, M. S., & Taylor, R. K. (2020). Growth and Maintenance of *Escherichia coli* Laboratory Strains. Curr Protoc. 2020;1(1):e20. https://doi.org/10.1002/cpz1.20
31. Musyimi DM, Neema P. Phytochemicals and antibacterial activities of leaf extract of *Tridax procumbens* on *Staphylococcus aureus* and *Escherichia coli*. East Afr Sch J Biotechnol Genet. 2019;1(6):2663–2686.
32. Ferreira RL, Da Silva BCM, Rezende GS, Nakamura-Silva R, Pitondo-Silva A, Campanini EB, Brito MCA, Da Silva EML, De Melo Freire CC, Da Cunha AF, Da Silva Pranchevicius MC. High Prevalence of Multidrug-Resistant *Klebsiella pneumoniae* Harboring Several Virulence and β-Lactamase Encoding Genes in a Brazilian Intensive Care Unit. FFront Microbiol. 2019;9. https://doi.org/10.3389/fmicb.2018.03198
33. Yan D, Li Y, Liu Y, Li N, Zhang X, Yan C. Antimicrobial Properties of Chitosan and Chitosan Derivatives in the Treatment of Enteric Infections. Molecules. 2021;26(23):7136. https://doi.org/10.3390/molecules26237136
34. Kravanja G, Primožič M, Knez Ž, Leitgeb M. Chitosan-Based (Nano)Materials for novel biomedical applications. Molecules. 2019;24(10):1960. https://doi.org/10.3390/molecules24101960
35. Devlieghere F, Vermeulen A, Debevere J. Chitosan: Antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. Food Microbiol. 2004;21:703–714.
36. Bălăşoiu M, Bălăşoiu AT, Mănescu R, Avramescu C, Ionete O. Pseudomonas aeruginosa resistance phenotypes and phenotypic highlighting methods. Curr Health Sci J. 2014;40(2):85–92.
37. Langendonk RF, Neill DR, Fothergill JL. The Building Blocks of Antimicrobial Resistance in Pseudomonas aeruginosa: Implications for current resistance-breaking therapies. Front Cell Infect Microbiol. 2021;11: https://doi.org/10.3389/fcimb.2021.665759
38. Hassan MA, Omer AM, Abbas E, Baset WMA, Tamer TM. Preparation, physicochemical characterization and antimicrobial activities of novel two phenolic chitosan schiff base derivatives. Sci Rep. 2018;8(1):11416.
39. Arabski M, Węgierek-Ciuk A, Czerwonka G, Lankoff A, Kaca W. Effects of saponins against clinical E. coli strains and eukaryotic cell line. Journal of Biomedicine & Biotechnology. 2021;2(8):6216–6240.
40. Aboody MSA, Mickymaray S. Anti-fungal efficacy and mechanisms of flavonoids. Antibiotics. 2020;9(2):45.

41. Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, Hatab SR. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Frontier Microbiology. 2018;9:1639. https://doi.org/10.3389/fmicb.2018.01639
42. Al-Mamun A. *Tridax procumbens* flavonoids stimulated synergistic effects on bmp-2-induced bone regeneration in critical-sized calvarial defect. Journal of Dental Oral Health. 2019;6:1–8.
43. Ali HI, HA, Salih HA, Al-jezani. Preservative study of the AgNPs effect on the materials and embryonic development in albino rats. Iraqi Journal of Agricultural Sciences. 2019;50(6):1605–1612
44. Patrick GL. An Introduction to Medicinal Chemistry; Oxford University Press: New York, NY, USA, 2013.
45. Bermingham A, Derrick JP. The folic acid biosynthesis pathway in bacteria: Evaluation of potential for antibacterial drug discovery. Bioessays. 2002;24:637–648.
46. Hong W, Zeng J, Xie J. Antibiotic drugs targeting bacterial RNAs. Acta Pharmaceutica Sinica B, 2014;4(4):258–265. https://doi.org/10.1016/j.apsb.2014.06.012
47. Madigan MT, Martinko J.M. 2006. Brock Biology of Microorganisms. Pearson-Prentice Hall, Upper Saddle River, New Jersey, USA.
48. Sudarshan N, Hoover DG, Knorr, D. Antibacterial action of chitosan. Food Biotechnology. 1992;6(3):257–272. https://doi.org/10.1080/08905439209549838

Publisher's note