

RESEARCH TITLE

Antibacterial activity of Hydro-ethanol extracts of *Acacia nilotica* fruit and *Punica granatum* peel against *E. coli* and *Staphylococcus aureus* in Gezira State, Sudan**Mona W. Abdalla^[1]; Yasir Mohammed Abdelrahim^[2]; Abdalla, A. I.^[3] and Mutaman A. A. Kehail^[4]**^[1] Department of Food Eng. and Technology, Faculty of Eng. and Tech., University of Gezira, Sudan^[2] Department of Molecular Biology, Faculty of Science, University of Gezira, Sudan^[3] Department of Envir. Health, Faculty of Environ. Health Science, University of Gezira, Sudan^[4] Department of Zoology, Faculty of Science, University of Gezira, SudanCorresponding author: Mutaman A. A. Kehail, k.mutaman@yahoo.comHNSJ, 2026, 7(10); <https://doi.org/10.53796/hnsj710/10>**Received at 05/09/2026****Accepted at 15/09/2026****Published at 01/10/2026****Abstract**

Plants as a source of medicinal compound have continued to play a vital role in the maintenance of human health since ancient times. The present study aimed to determine the antibacterial activities of *Acacia nilotica* fruit and *Punica granatum* peel against the local strains of *E. coli* and *Staphylococcus aureus*. The plant materials and the target microorganisms were brought from within Wad Medani City, Gezira State, Sudan. The standard methods of preparation of hydro-ethanol extracts, purifying and culturing microorganisms, and bioassay antibacterial activity tests were carefully followed. The results of this study revealed that, the lower concentration (1.14 mg/ml) of the hydro-ethanol extract of *A. nilotica* fruit produced a mean of 5.3±0.3 mm inhibition zone on *E. coli*, and 6.67±0.3 mm on *S. aureus*, whereas, the higher concentration (92.0 mg/ml) show inhibition zones of 12.67±0.67 mm on *E. coli* strain and 13.3±0.3 mm on *S. aureus* strain. The lower concentration (0.72 mg/ml) of the hydro-ethanol extract of pomegranate peel produced a mean of 6.7±0.67 mm inhibition zone on *E. coli*, and 6.7±0.67 mm on *S. aureus*, whereas, the higher concentration (89.32 mg/ml) show inhibition zones of 11.3±0.3 mm on *E. coli* strain and 12.0±0.0 mm on *S. aureus* strain. It was noticed that, *S. aureus* was relatively similar in its susceptible to *E. coli* strain against both plant extracts, but not against penicillin antibiotic. The study recommends running further studies that focuses on other local natural products within the study area, also on other pathogens.

Key Words: Antibacterial, *A. nilotica*, *P. granatum*, *E. coli*, *S. aureus*, Gezira, Sudan.

النشاط المضاد للبكتيريا للمستخلصات المائية الإيثانولية من ثمار السنط النيلي وقشور الرمان ضد الإشريكية القولونية والمكورات العنقودية الذهبية في ولاية الجزيرة، السودان

المستخلص

ما زالت النباتات مصدراً للمركبات الطبية وتؤدي دوراً مهماً في الحفاظ على صحة الإنسان منذ القدم. هدفت هذه الدراسة إلى تحديد النشاط المضاد للبكتيريا للمستخلصات المائية الإيثانولية من ثمار السنط النيلي (*Acacia nilotica*) وقشور الرمان (*Punica granatum*) ضد سلالات محلية من الإشريكية القولونية (*E. coli*) والمكورات العنقودية الذهبية (*Staphylococcus aureus*). جُمعت المواد النباتية والكائنات الدقيقة المستهدفة من مدينة ود مدني ولاية الجزيرة، السودان. وأُتبعَت الطرق القياسية في تحضير المستخلصات وتنقية الكائنات الدقيقة وزراعتها واختبار النشاط المضاد للبكتيريا. أظهرت النتائج أن التركيز الأدنى (1.14 ملغم/مل) من مستخلص ثمار السنط النيلي أحدث متوسط منطقة تثبيط قوه 5.3+0.3 ملغم ضد الإشريكية القولونية و6.67+0.3 ملغم ضد المكورات العنقودية الذهبية؛ بينما أحدث التركيز الأعلى (92.0 ملغم/مل) منطقتي تثبيط قوهما 12.67+0.67 ملغم و13.3+0.3 ملغم، على التوالي. وأحدث التركيز الأدنى (0.72 ملغم/مل) من مستخلص قشور الرمان متوسط منطقة تثبيط قوه 6.7+0.67 ملغم ضد كلتا السلالتين؛ بينما أحدث التركيز الأعلى (89.32 ملغم/مل) منطقتي تثبيط قوهما 11.3+0.3 ملغم ضد الإشريكية القولونية و12.0+0.0 ملغم ضد المكورات العنقودية الذهبية. ولوحظ تقارب قابلية السلالتين للتأثر بكل المستخلصين النباتيين، بخلاف استجابتهما للمضاد الحيوي البنسلين. وتوصي الدراسة بإجراء دراسات إضافية على منتجات طبيعية محلية أخرى ومسببات مرضية أخرى في منطقة الدراسة.

الكلمات المفتاحية: النشاط المضاد للبكتيريا، السنط النيلي، الرمان، الإشريكية القولونية، المكورات العنقودية الذهبية، الجزيرة، السودان.

INTRODUCTION

Escherichia coli (abbreviated as *E. coli*), is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (Tenaillon *et al.*, 2010). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for food contamination incidents that prompt product recalls (CDC, 2012). Multidrug resistance in *E. coli* has become a worrying issue in human and in veterinary medicine worldwide. The most problematic mechanisms in *E. coli* correspond to the acquisition of genes coding for extended-spectrum β -lactamases, carbapenemases, 16S rRNA methylases, plasmid-mediated quinolone resistance genes, and *mcr* genes. *E. coli* show resistances to tetracyclines, phenicols, sulfonamides, trimethoprim, and Fosfomycin, because of the massive use of antimicrobial agents in medicine (Poirel *et al.*, 2017).

Staphylococcus aureus is a gram-positive spherically shaped bacterium and facultative anaerobic, nonmotile and does not form spores. When viewed with microscope, they formed large round golden-yellow colonies on blood agar plate. An estimated 21% to 30% of the human population are long-term carriers of *S. aureus* (Tong *et al.*, 2015), which can be found as part of the normal skin microbiota, in the nostrils, and as a normal inhabitant of the lower reproductive tract of females (Hoffman, 2012). *S. aureus* has developed an adaptive mechanism to tolerate hypothiocyanous acid (HOSCN), a potent oxidant produced by the human immune system (Loi *et al.*, 2023).

Antibiotic resistance in *S. aureus* was uncommon when penicillin was first introduced in 1943. Hospital *S. aureus* isolates were penicillin-resistant. Minor skin infections can be treated with triple antibiotic ointment (Bonomo *et al.*, 2007). One topical agent that is prescribed is mupirocin, a protein synthesis inhibitor that is produced naturally by *Pseudomonas fluorescens* and has seen success for treatment of *S. aureus* (Archer *et al.*, 2011).

Vachellia nilotica known as *Acacia nilotica*, is a flowering tree in the family Fabaceae. It is native to Africa, the Middle East and the Indian subcontinent (Kyalangalilwa *et al.*, 2013). In the present commercial market, gum Arabic is defined as the dried exudate from the trunks and branches of *Senegalia (Acacia) senegal* or *Vachellia (Acacia) seyal* in the family Leguminosae (Fabaceae). The gum of *A. nilotica* is also referred to in India as *Amaravati gum* (Rojas-Sandoval, 2023).

The pomegranate (*Punica granatum*) is a fruit-bearing, deciduous shrub in the family Lythraceae, it is thought to have originated from Iran also considered to be native to Indian Subcontinent and Afghanistan (Holland *et al.*, 2009). The edible portion of raw pomegranate is 78% water, 19% carbohydrates, 2% protein, and 1% fat. A 100 g, and provides 11% of the Daily Value for vitamin C, 14% for vitamin K, and 10% for folate, while the seeds are a rich source of dietary fiber (Stallings *et al.*, 2019).

The antibacterial activities of some of the local natural products against the local drug resistance strains of *E. coli* and *S. aureus* bacteria are not well updated in Gezira State, Sudan. The current study tried to present a scientific effort to understand to how extend the local materials can be used to solve the problem caused by the drug-resisted bacteria.

MATERIALS AND METHODS

Study materials

The *A. nilotica* fruits, and pomegranate (*P. granatum* L.) peel, were brought from the local market of Wad Medani City, Gezira State, Sudan.

The identified strains of *E. coli* (O25:H4) and *S. aureus* (SO-1977) were brought from the Microbiology Department, Central Medial Laboratory, University of Gezira.

Preparation of ethanol extracts

This part was run in the Food Analysis Lab., Fac. Of Engineering and Technology, University of Gezira. Three g of each plant dried powder were dissolved separately in 20 ml ethanol in a 50-ml conical flask for 24 hours, and then filtered. The ethanol extract was poured in a clean Petri-dish, and let to evaporate under room temperature.

The weight of the dried extract of each product were used to quantify the concentration of the stock extracted materials (in gm/ml), and hence, the dilutions prepared from these extracts.

Antibacterial activity tests

It was run in the Microbiology Lab., Fac. Of Engineering and Technology, University of Gezira, using Disc Diffusion method. Five diluted concentrations (w/v; gm/ml) of each product were prepared from the stock concentration, in addition to penicillin (produced by Cityphar Pharmaceutical Industry, Sudan, production date: June 2021) antibiotics at 200 (mg/ml) as +ve control and distilled water (0.0 concentration) as -ve control. The Lab. was first sterilized completely by 70% ethanol and UV-light.

Isolation of pure culture

A nichrome inoculating loop was used to inoculate the infective bacteria on suitable media. The inoculum was spread into four quadrants of the nutrient agar plate with the help of a nichrome inoculation loop. The loop was sterilized between each successive quadrant streak. The purpose of this technique was to dilute the inoculum sufficiently on the surface of agar medium so that well-defined colonies of bacteria can be obtained. The isolated colonies were sub-cultured individually to obtain pure culture isolates. The pure colonies thus obtained and can be picked up for confirmatory biochemical tests (if necessary).

Preparation of standard inoculum

Pure colonies were picked up with the help of a straight wire and emulsified in a test tube containing saline, and the turbidity was matched with 0.5% McFarland standard containing 1×10^7 CFU/ml approximately.

Media preparation

For S. aureus strain:

Weight of 28 g of nutrient agar powder (0.5% of peptone, 0.3% yeast extract, 1.5% agar, and 0.5% NaCl) was suspended in 1.0 L distilled water. This mixture was heated, stirred to fully dissolve all components, and autoclaved at 121 °C for 15 minutes. Then allowed to cool but not solidify. When the mixture cooled to 45-50 °C, 5% (v/v) sterile defibrinated blood (that has been warmed to room temperature) was added and mixed well. Air bubbles were avoided and pH was adjusted to 7.2 – 7.6. The whole mixture was then dispensed into sterile plates before solidified (Turista and Puspitasari, 2019).

For E. coli strain:

Ten g tryptone, 5 g yeast extract, 5 g NaCl, and 15 g agar were added and the volume was adjusted to 1000 ml with distilled water. The mixture was autoclaved in conical flask at 121°C for 30 minutes, let to cool to about 60°C and then poured into sterile plastic petri dishes. The plates were allowed to cool and solidify at room temperature and pH of 7.0 (Son and Taylor, 2021).

Each media was then poured in sterile Petri dishes (size of 100 mm × 15 mm), left to solidify and incubated at 37°C under aerobic conditions. Each bacteria strain was cultured in

its proper media before the antibacterial agents were added, in Biosafety Level 2 (BSL-2) cabinet, provided with fan, ultra violet lamp and flame. The prepared plates were stored at 4°C before used.

Bioassay

Using sterile cotton wood swab, *S. aureus* and *E. coli* strains were streaked at the surface of the prepared media (on the Petri-dishes) inside the level-two safety cabinet. After 10 minutes, the excessive solution whenever noticed was aspirated completely out of each Petri-dish. Small disk papers (5 mm diameter) each of which soaked for 5 minutes in only one concentration of the prepared extracts were placed onto the media seeded with the tested bacterial strain, in addition to the negative and the positive control. These procedures were performed under strict aseptic condition. Three replicates of each concentration were done. The Petri-dishes were incubated at 37°C for 48 h in an incubator. The formation of *S. aureus* and *E. coli* colonies at the negative control Petri-dishes considered the sign to calculate the inhibition zones formed in the rest of the Petri-dishes. The distance (in mm) from the center of the disc paper to the nearest colony was taken as an inhibition zone for each concentration tested. The diameter (mm) of each produced inhibition zone was measured by transparent ruler.

Statistical analysis

The antimicrobial activity was expressed as inhibition zone (in mm) and the Least Significant Difference (LSD) was used to evaluate the obtained results.

RESULTS

A. nilotica fruit

The inhibition zones exerted by the hydro-ethanol extract of *A. nilotica* fruit were recorded in Table (1). The lower concentration (1.14 mg/ml) produced a mean of 5.3 ± 0.3 mm inhibition zone on *E. coli*, and 6.67 ± 0.3 mm on *S. aureus*, whereas, the higher concentration (92.0 mg/ml) show inhibition zones of 12.67 ± 0.67 mm on *E. coli* strain and 13.3 ± 0.3 mm on *S. aureus* strain. The positive control produced a mean of 15.3 ± 0.3 mm inhibition zone on *E. coli* strain and 13.3 ± 0.3 mm on *S. aureus* strain.

It was noticed that, *S. aureus* was relatively similar in its susceptible to *E. coli* strain against *A. nilotica* fruit extract, but not against penicillin antibiotic.

Table 1. Inhibition zone (mm) of *E. coli* and *S. aureus* strains caused by *A. nilotica* fruit hydro-ethanol extract

Conc (mg/ml)	<i>E. coli</i>	<i>S. aureus</i>
	Mean $\pm$ SE	Mean $\pm$ SD
0.0	0 $\pm$ 0 ^g	0 $\pm$ 0 ^f
1.14	5.3 $\pm$ 0.3 ^f	6.67 $\pm$ 0.3 ^e
4.40	6.82 $\pm$ 0.17 ^e	7.7 $\pm$ 0.67 ^d
10.22	9.16 $\pm$ 0.17 ^d	10.3 $\pm$ 0.3 ^c
30.66	10.67 $\pm$ 0.67 ^c	11.7 $\pm$ 0.67 ^b
92.0	12.67 $\pm$ 0.67 ^b	13.3 $\pm$ 0.3 ^a
200 (Penicillin)	15.3 $\pm$ 0.3 ^a	13.3 $\pm$ 0.3 ^a

- Different letters reflected different significant levels

Pomegranate peel

The inhibition zones exerted by the hydro-ethanol extract of pomegranate peel were represented in Table (2). The lower concentration (0.72 mg/ml) produced a mean of 6.7 ± 0.67 mm inhibition zone on *E. coli*, and 6.7 ± 0.67 mm on *S. aureus*, whereas, the higher concentration (89.32 mg/ml) show inhibition zones of 11.3 ± 0.3 mm on *E. coli* strain and 12.0 ± 0.0 mm on *S. aureus* strain.

It was noticed that, *S. aureus* was relatively similar in its susceptible to *E. coli* strain toward pomegranate peel extract, but not against penicillin antibiotic.

Table 2. Inhibition zone (mm) of *E. coli* and *S. aureus* strains caused by pomegranate peel hydro-ethanol extract

Conc (mg/ml)	<i>E. coli</i>	<i>S. aureus</i>
	Mean+SE	Mean+SD
0.0	0+0 ^e	0+0 ^e
0.72	6.7+0.67 ^d	6.7+0.67 ^d
2.16	7.5+0.5 ^d	8.0+0.67 ^d
6.48	9.3+0.3 ^c	9.7+0.67 ^c
19.44	10.7+0.67 ^b	10.7+0.67 ^c
58.32	11.3+0.3 ^b	12.0+0.0 ^b
200 (penicillin)	15.3+0.3 ^a	13.3+0.3 ^a

- Different letters reflected different significant levels

Discussion

To combat the threat of antimicrobial resistance, it is important to discover innovative and effective alternative antibacterial agents. The present work evaluated the antibacterial activity of the crude extracts of five natural products, assuming that, the local strains and the local product are not well studied. May. And as suggested by Singh and Prakash (2012), these products can be easily incorporated in diet without producing any side effect in contrast to the antibiotics.

Many natural products extract that contain flavonoids and terpenoids were found to be active on various *E. coli* virulence traits. Some of the effects were strikingly specific whereas some were due to non-specific mechanisms (Dubreui, 2020).

All the tested extracts of the local plant materials showed comparable potent antimicrobial activities against the local strains of *E. coli* and *S. aureus* bacteria, and agreed to greet extend to the local, regional and international reports.

Sidiq *et al.*, 2017 stated that, *A. nilotica* can be a potential source of new antimicrobials, effective against antibiotic-resistant strains of *E. coli* and *Salmonella*.

In India, *Acacia nilotica* extracts exhibited antimicrobial activities with zones of inhibition ranging from 6 to 22 mm and exhibited appreciable activity against all the clinically important bacterial (*S. aureus*, *Bacillus subtilis*, and *E. coli*) and fungal species (*Aspergillus niger*, *Fusarium oxysporium* and *Dreschlera avenacea*). Overall maximum Inhibition zone (22 mm) was observed in extract of methanol and chloroform (75:25) against *Bacillus subtilis* and minimum (4 mm) in extract of methanol and chloroform (50:50) against *Dreschlera avenacea* (Kaur *et al.*, 2016). In Sudan, the plant shows promising potential as an alternative antibacterial agent for resistant bacterial strains (Abdelgader *et al.*, 2024).

The water-methanol Pomegranate peel extract demonstrated a remarkable inhibition zone against common food-borne pathogens: *Listeria monocytogenes*, *S. aureus*, *E. coli* and *Yersinia enterocolitica* (Singh *et al.*, 2019).

CONCLUSION

All the tested extracts of the local plant materials showed comparable potent antimicrobial activities against the local strains of *E. coli* and *S. aureus* bacteria. Further studies should focus on the other local natural products and other pathogens within the study area.

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Conflicts of Interest

The authors declare no conflicts of interest.

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