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Acacia nilotica: A Natural Antimicrobial Agent Compared to Standard Antibiotics

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Abstract

This study evaluated the antibacterial efficacy of *Acacia nilotica* extracts against six bacterial strains: *P. mirabilis*, *S. aureus* ATCC 6538, *E. faecium* ATCC 14936, *E. faecalis* ATCC 29212, *E. coli* ATCC 8739, and *Serratia* spp. The extracts were tested in varying concentrations (1/10, 1/100, and 1/1000) and compared to standard antibiotics, including Amoxicillin, Cefotaxime, Amikacin, and Ciprofloxacin.

The results revealed that alcohol-based extracts exhibited superior antibacterial activity compared to aqueous extracts, with the hot alcohol extract at a (1/10) concentration showing the most significant effects. *P. mirabilis* and *S. aureus* were the most susceptible strains, demonstrating inhibition zones up to 30 mm with alcohol extracts. In contrast, *Serratia* spp. and *E. coli* were largely resistant across all extract types and concentrations. *E. faecium* and *E. faecalis* showed moderate sensitivity, particularly to hot alcohol extract. High-concentration extracts (1/10) consistently produced measurable inhibition zones, while diluted extracts (1/100 and 1/1000) showed reduced or no activity, highlighting the importance of extract concentration for antimicrobial efficacy. Cold alcohol extract showed the highest activity against *S. aureus*, while hot alcohol extract was most effective against *P. mirabilis*. When compared with antibiotics, Amoxicillin and Ciprofloxacin demonstrated the highest antibacterial activity, with inhibition zones reaching up to 48 mm against *E. faecium*.

A. nilotica extracts showed significant antibacterial activity, even against resistant strains, though they were less potent. The results emphasize *A. nilotica's* promise as a natural antibacterial, with its effectiveness influenced by the extraction method and concentration.

Keywords: *Acacia nilotica*, Medicinal plants, Antibacterial efficacy, Natural antimicrobial agent

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Introduction:

For hundreds of years, between 75 and 80 percent of the world's population has relied on herbal medicines derived from medicinal plants, minerals, and organic matter for health care that is being promoted and growing in developed and developing nations (1). Plants have therapeutic qualities as a result of the presence of several active compounds such as volatile essential oils, alkaloids, tannins, glycosides, oleoresins, resins, steroids, phenols, and terpenes (2).

Bacterial infection is another source of problems for humankind. Though conventional medicines have effective antibiotic therapy for bacterial infection, they do have drug resistance problems. Hence, many known plants are used globally for the treatment of

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bacterial infections. Thus, healthcare practitioners must be aware of herbal antibiotics (3).

Acacia nilotica is a member of the *Fabaceae* family. It is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India (4,5). *Acacia nilotica* has different English names, like gum Arabic, Tomentose Babool, Black piquant, Black babul, Egyptian thorn, Prickly Acacia, Nile acacia, Scented thorn, and Scented-pod acacia; and different Arabic names: as Ummughilan, Usarequrz, and kaarad (6). *Acacia nilotica* is widely used. Almost all their parts are used in medication, including root, bark, leaves, flower, gum, and pods (7). The plant has anti-microbial, antiplasmodial, anticancer, antimutagenic, and antioxidant activity and is used for the treatment of cold, cough, sore throat, diarrhea, dysentery, tuberculosis, piles, hepatitis C virus, burns, and scalds (8).

The plant`s Seeds contain a high percentage of phenolic constituents consisting of m-digallic acid, gallic acid, protocatechuic and ellagic acids, tetrahydroxy flavan-3-ol, oligomer 3,4,7-trihydroxy flavan 3,4-diol, and 3,4,5,7-tetrahydroxy flavan-3-ol and epicatechol.

The mature seed also contains crude protein, crude fibre, crude fat, carbohydrates, potassium, phosphorus, and magnesium, iron, and manganese occurred in high concentrations, and it is a richer source of cystine, methionine, threonine, lysine, and tryptophan (9, 10).

A. nilotica showed antibacterial activity against most of the gram-positive and gram-negative bacteria, thereby suggesting a broad-spectrum antibacterial property. Its efficacy has been shown in the treatment of gonorrhoea, leucorrhoea, diarrhea, dysentery, and wounds (11).

The present study is undertaken to evaluate the antimicrobial potential of ethanolic and water extracts of *Acacia nilotica* seeds

Material and Method

Plant Preparation

Acacia nilotica seeds have been purchased from a Herbalist in Sebha city. The plant was in the form of dry seeds. The plant was identified by the Department of Pharmacognosy (Faculty of Pharmacy, University of Benghazi). The plant was cleaned and washed with distilled water, then dried on a flat plate for 72 hours, and then crushed in a special electrical mill in the laboratory until we obtained coarse powder.

Preparation of plant extracts

The dried and grinded plant material of *Acacia nilotica* (40 g) was hot extracted by Soxhlet apparatus using Ethanol (M) as solvent. The resulting solution was filtered and concentrated under reduced pressure (Rotatory evaporator). The residues were weighed and calculated as % of the yield.

The dried powdered plant (40 g) was cold extracted by maceration with ethanol for 24 hrs. with frequent shaking. The extract was filtered and evaporated under reduced pressure using a rotary evaporator. The obtained residue was dissolved in its solvent and kept in a tightly closed container for further analysis.

The water extract of the plant was obtained by a decoction of (40gm) of the dried grinded plant with water for about 30 min. The extract was decanted and concentrated under vacuum pressure.

$$\textbf{Extractive Value} = \frac{Weight of extrac (g)}{\text{Weight of Sample (g)}} \times 100$$

$$= \frac{0.48g}{40g} \times 100 = 1.2\%$$

Preparation of Inoculum

The antimicrobial properties of plant extracts were tested against Gram-positive bacteria and Gram-negative bacteria (*Enterococcus faecium ATCC 14936, Proteus mirabilis, Staphylococcus aureus ATCC 6538, Enterococcus faecalis ATCC 29212, Serratia Spp, E. coli ATCC 8739).* All the strains of bacteria were provided by the medical microbiology laboratory of the Department of Laboratory Medicine in the Faculty of Public Health, Benghazi University.

The Gram-positive and Gram-negative bacteria were pre-cultured in Mueller-Hinton agar overnight at 37°C. Afterward, each strain was adjusted at a concentration of 10^8 cells/ml using a 0.5 McFarland standard (12).

Different concentrations of the *Acacia nilotica* extract (water extract, cold ethanol extract, and hot ethanol extract) were prepared by serial dilution. (0.1, 0.100, and 0.1000) in sterile test tubes.

The method involved the use of sterile filter paper discs impregnated with *Acacia nilotica* extracts, prepared for each type of solvent extraction and concentration. These discs were aseptically placed on Mueller-Hinton agar plates previously inoculated with bacterial suspensions using sterile swabs. The inoculated plates were then incubated at 37°C for 18–24 hours. Following incubation, the antibacterial activity was assessed by measuring the diameter (in millimetres) of the zone of inhibition formed around each disc.

Results

In this study, Figure 1 shows the effects of *Acacia nilotica* extracts (in cold water, cold alcohol, and hot alcohol) on various bacterial strains, measured by the inhibition zone in millimeters (mm).

In General Effectiveness: The antibacterial activity varies across bacterial strains and types of *Acacia nilotica* extract. Cold alcohol extract (C. alcohol) and hot alcohol extract (H. alcohol) generally show stronger antibacterial effects compared to water extract (Cold water extract).

The Comparison of Extracts showed that *E. faecium ATCC 14936* the inhibition zones are relatively similar across all three extracts, with H. alcohol slightly outperforming the others. *P. mirabilis* and *S. aureus ATCC 6538* show the highest inhibition zones (25 mm), particularly with cold alcohol and H. alcohol, indicating significant sensitivity to these extracts.

E. faecalis ATCC 29212 demonstrates a lower inhibition zone (12-18 mm), suggesting reduced susceptibility, particularly to water extract. No inhibition is observed for *Serratia spp.*, indicating resistance to *Acacia nilotica* extracts. For *E. coli ATCC 8739*, H. alcohol performs slightly better, followed by cold alcohol and cold water.

Strain susceptibility showed that *P. mirabilis* and *S. aureus* are the most susceptible strains. *Serratia spp.* appears completely resistant, as shown by the absence of an inhibition zone. The results suggest that the method of extraction influences the antibacterial efficacy of *Acacia nilotica*. Alcohol-based extracts, especially hot alcohol, tend to be more effective than water-based extracts. Variations in inhibition zones highlight the importance of strain-specific testing when considering natural extracts for antibacterial use.

Figure 2 represents the effect of cold-water extract of *Acacia nilotica* at different concentrations (Cold Water extract 1/10, Cold Water extract/100, and Cold Water extract/1000) on various bacterial strains, measured by the inhibition zone in millimeters (mm).

The antibacterial activity of the cold-water extract was observed to diminish with decreasing concentration, indicating a dose-dependent effect. Notably, only certain bacterial strains exhibited zones of inhibition, even at higher extract concentrations, suggesting selective antibacterial activity. *Proteus mirabilis* demonstrated the highest susceptibility, with a zone of inhibition measuring 22 mm. This was followed by *Enterococcus faecium* ATCC 14936 (20 mm) and *Staphylococcus aureus* ATCC 6538 (18 mm), both of which showed considerable sensitivity to the extract. In contrast, *Enterococcus faecalis* ATCC 29212, *Serratia* spp., and *Escherichia coli* ATCC 8739 exhibited no inhibition zones, indicating resistance to the cold-water extract.

At a 1:100 dilution of the cold-water extract, only *Proteus mirabilis* exhibited a measurable zone of inhibition (14 mm), which was reduced compared to the zone observed at the undiluted 0.1 concentration, indicating a clear concentration-dependent antibacterial effect. At a further diluted concentration (1:1000), no antibacterial activity was observed against any of the tested bacterial strains, suggesting that the extract loses its efficacy at very low concentrations. Among all isolates, *P. mirabilis* consistently demonstrated the highest susceptibility across all tested concentrations. In contrast, *Enterococcus faecium* and *Staphylococcus aureus* exhibited susceptibility only at the highest concentration (0.1), while *Enterococcus faecalis*, *Serratia* spp., and *Escherichia coli* remained resistant at all concentrations tested. These findings indicate that the antibacterial activity of the *Acacia nilotica* cold-water extract is strongly concentration-dependent and that certain bacterial species may possess intrinsic resistance to this extract.

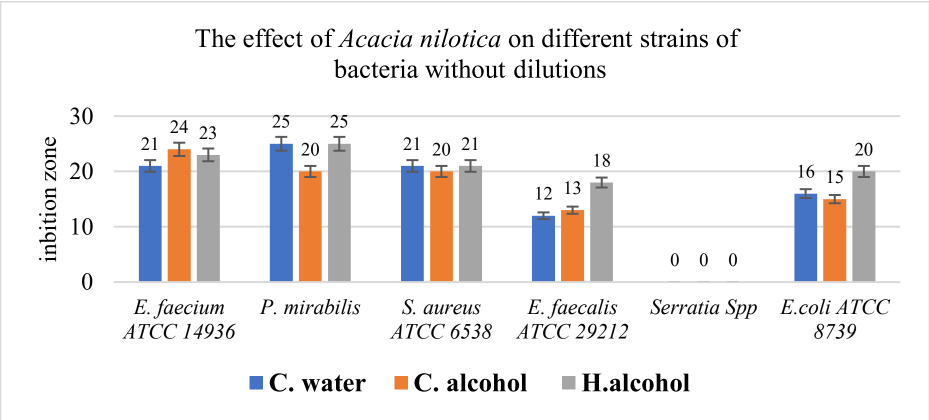

Figure (1). The effect of *Acacia nilotica* on different strains of bacteria without dilutions

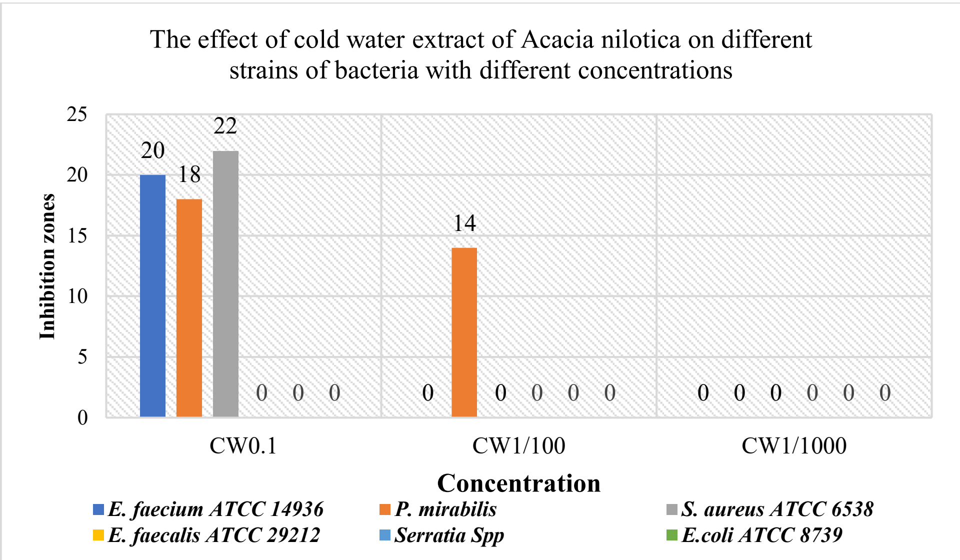

Figure (2). The effect of the cold-water extract of *Acacia nilotica* on different concentrations

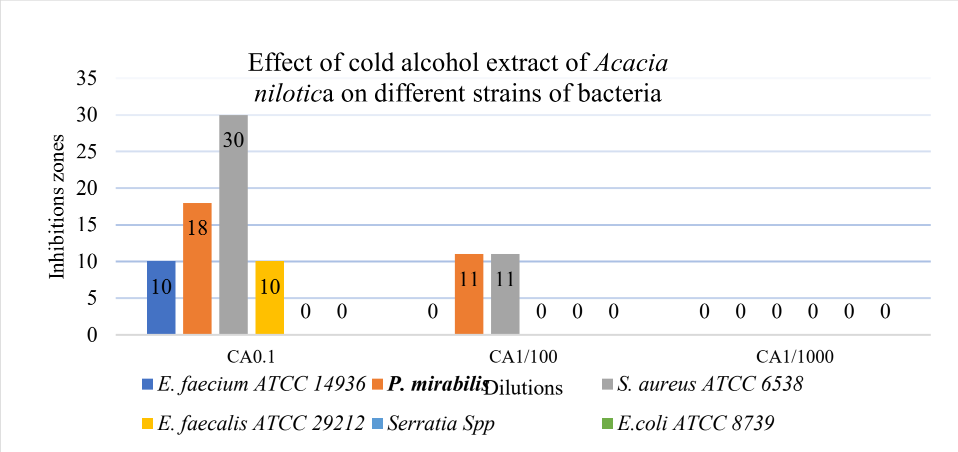

Figure (3). Effect of cold alcohol extract of *Acacia nilotica* on different strains of bacteria

Figure 3 depicts the effect of cold alcohol extract (CA) of *Acacia nilotica* at different concentrations (CA0.1, CA1/100, and CA1/1000) on various bacterial strains

The highest concentration (CA0.1) shows significant activity against some bacterial strains, while the effect diminishes or disappears entirely at lower concentrations.

S. aureus ATCC 6538 shows the largest inhibition zone (30 mm), indicating it is highly susceptible to the cold alcohol extract*. mirabilis* and *E. faecalis ATCC 29212* exhibit moderate inhibition zones (18 mm and 10 mm, respectively). *E. faecium ATCC 14936*, *Serratia spp.*, and *E. coli ATCC 8739* show no inhibition, suggesting resistance to the extract even at the highest concentration.

At CA1/100, the inhibition zones for *P. mirabilis* and *S. aureus* are reduced to 11 mm each, showing diminished activity compared to CA 0.1. At CA1/1000, no inhibition zones are observed for any strain, indicating a complete loss of antibacterial activity at this concentration.

S. aureus ATCC 6538 is the most susceptible strain, followed by *P. mirabilis* and *E. faecalis*.

E. faecium, *Serratia spp.*, and *E. coli* show resistance across all tested concentrations. Similar patterns of reduced activity with dilution are observed. The extract's efficacy is highly dependent on both concentration and bacterial strain, emphasizing the need for precise dosing in potential applications.

Figure 4 shows the effect of the hot alcohol extract (HA) of *Acacia nilotica* on the inhibition of different bacterial strains, tested at three different dilutions: HA0.1, HA1/100, and HA1/1000. The bacterial strains analyzed are represented by different bars in the chart, and the inhibition is measured in terms of the zone of inhibition (in millimeters).

The effectiveness at HA0.1: shows the highest inhibitory effect at this concentration. *Proteus mirabilis* (orange bar) exhibits the largest zone of inhibition (~30 mm), indicating strong susceptibility. *E. faecalis ATCC 29212* (gray bar) also shows significant inhibition (~27 mm).

Other bacteria, such as *E. faecium* (blue bar), *Serratia spp.* (yellow bar), and *S. aureus* (light gray bar) display varying levels of inhibition (~19-15 mm). *E. coli* (green bar) shows the least inhibition (~10 mm). The antibacterial activity decreases at this dilution. The extract is moderately effective against *P. mirabilis* (~12 mm) and *E. faecalis ATCC 29212* (~18 mm).

Other strains show no measurable inhibition, indicating resistance or negligible effectiveness. Effectiveness at HA1/1000: No significant inhibition is observed for any bacterial strain at this high dilution, indicating the loss of antibacterial activity due to reduced concentration of active compounds. The hot alcohol extract of *Acacia nilotica* demonstrates concentration-dependent antibacterial activity. *P. mirabilis* and *E. faecalis ATCC 29212* are the most susceptible strains, particularly at higher concentrations (HA0.1).

Figure 5 shows the effect of four different antibiotics—Amoxicillin (AMX), Cefotaxime (CXL), Amikacin (AK), and Ciprofloxacin (CIP)—on the inhibition of various bacterial strains. Amoxicillin (AMX): Displays the highest inhibition zones among the tested antibiotics.

E. faecium ATCC 14936 and *P. mirabilis* are highly susceptible with zones of ~48 mm and 44 mm, respectively. *S. aureus* ATCC 6538 and *E. faecalis* ATCC 29212 show moderate inhibition (~40 mm and ~36 mm, respectively). *E. coli* ATCC 8739 and Serratia spp. exhibit the least susceptibility (~20 mm and 26 mm, respectively). Cefotaxime (CXL) shows relatively lower antibacterial activity compared to AMX.*P. mirabilis* shows moderate susceptibility (~26 mm).

Other bacterial strains exhibit inhibition zones in the range of ~14-20 mm, with Serratia spp. (green bar) being least susceptible (~14 mm). *E. faecium* ATCC 14936 (blue bar) shows the highest susceptibility (~36 mm).

Figure 6 compares the effects of different treatments (natural extracts and control antibiotics) on the inhibition of various bacterial strains, measured as inhibition zones in millimeters. Each bacterial strain is represented by color-coded bars, including cold water extract (CW), cold alcohol extract (CA), hot alcohol extract (HA), and different antibiotics.

In Figure 6, natural Extracts showed that cold and hot alcohol extracts exhibit moderate antibacterial activity, particularly at higher concentrations (0.1). Dilutions (1/100 and 1/1000) lose efficacy, suggesting concentration-dependent effects. Among extracts, cold alcohol (CA0.1) and hot alcohol (HA0.1) are the most effective, especially against *Enterococcus faecium* and *Staphylococcus aureus*.

Antibiotics: Amoxicillin and Ciprofloxacin demonstrate the strongest antibacterial activity, with consistent inhibition across all strains. Cefotaxime and Amikacin show moderate effectiveness, with lower inhibition zones compared to Amoxicillin and Ciprofloxacin.

Bacterial Susceptibility:

Enterococcus faecium and *Proteus mirabilis* are the most susceptible to both natural extracts and antibiotics. *Serratia spp.* and *E. coli* consistently show lower inhibition zones, indicating possible resistance or lower susceptibility.

This figure demonstrates the effectiveness of natural extracts and antibiotics against various bacterial strains. While natural extracts, particularly cold and hot alcohol extracts, show potential antibacterial activity, antibiotics like Amoxicillin and Ciprofloxacin are more potent and broad-spectrum. These findings support further exploration of *Acacia nilotica* extracts as complementary antimicrobial agents, especially against antibiotic-resistant strains.

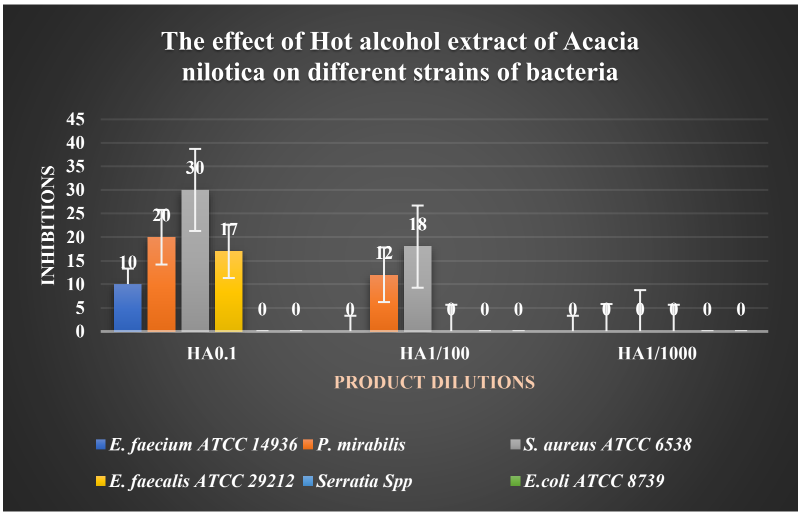

Figure 4. The effect of the hot alcohol extract of *Acacia nilotica* on different strains of bacteria

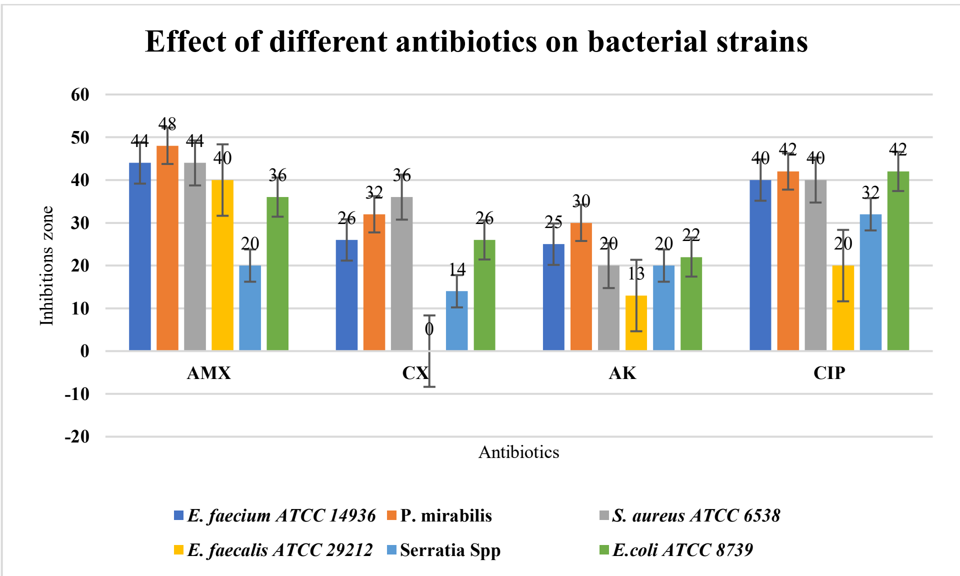

Figure (5). Effect of different antibiotics on bacterial strains

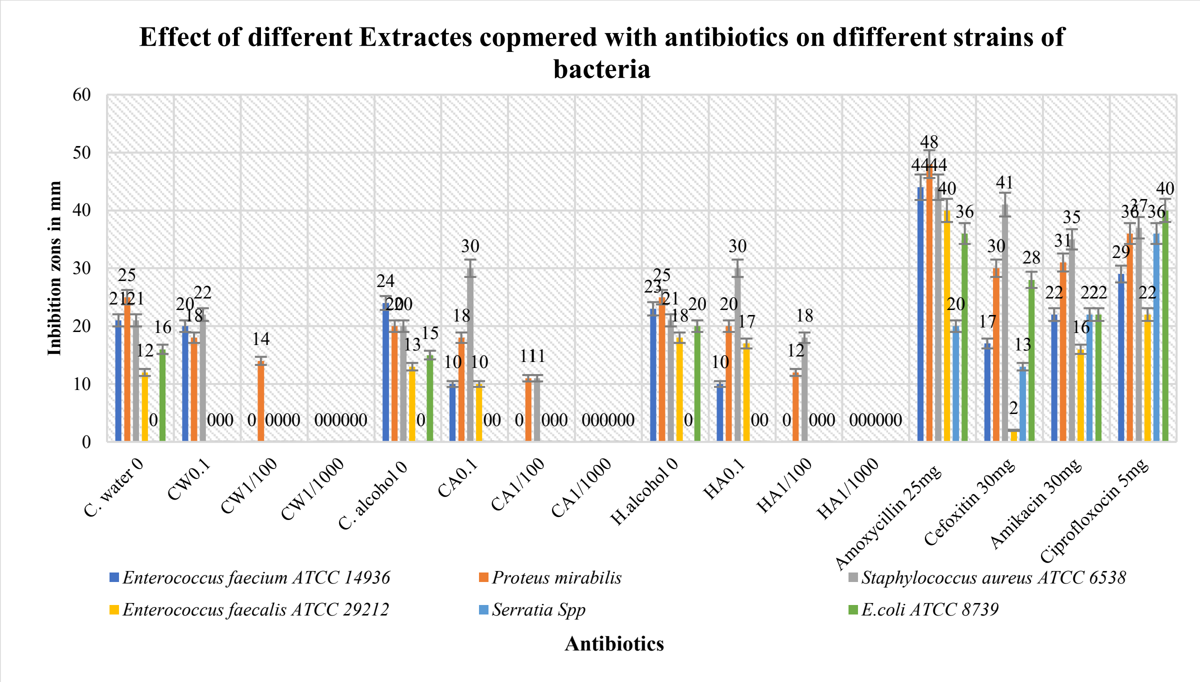

Figure (6). Effect of different Extracts compared with antibiotics on different strains of bacteria

Discussion

Vachellia nilotica, more commonly known as *Acacia nilotica*, and by the vernacular names of gum Arabic tree, babul, thorn mimosa, Egyptian acacia, or thorny acacia, is a flowering tree in the family Fabaceae. It is native to Africa, the Middle East, and the Indian subcontinent.

In Libya, it is mainly found in southern Libya (Fezzan region), particularly near Sabha, Murzuq, and Kufra. It prefers arid and semi-arid zones and often grows in wadis (dry riverbeds) and areas with seasonal water; it grows well in sandy to loamy soils. The traditional Medicine in Libya used the Bark and pods for treating diarrhea, cough, infections, and skin diseases, and gum exudate (gum arabic) was used as a demulcent and for wound healing.

The present study evaluated the antibacterial efficacy of different extracts of *Acacia nilotica*—namely cold water, cold alcohol, and hot alcohol extracts—against a range of Gram-positive and Gram-negative bacterial strains and compared their activity to standard antibiotics. The results revealed that the alcohol-based extracts, especially hot alcohol, demonstrated stronger antibacterial activity compared to the aqueous extract. This aligns with previous research indicating that alcohol serves as a more efficient solvent for extracting phenolic compounds, tannins, and flavonoids, which are known for their antimicrobial properties (13,14).

The pronounced activity of alcohol-based extracts aligns with recent studies. For instance, Yassin *et al.* (2023) demonstrated that ethanolic extracts of *Acacia. nilotica* pods possess significant antibacterial properties against *S. aureus*, *E. coli*, and *P. aeruginosa*, attributing this to the presence of bioactive compounds like tannins and flavonoids (15).

Among the bacterial strains tested, *P. mirabilis* and *S. aureus* ATCC 6538 were the most susceptible to the extracts, showing inhibition zones up to 30 mm in the case of *S. aureus* (cold alcohol extract) and 30 mm for *P. mirabilis* (hot alcohol extract at 0.1

concentration). These findings are consistent with prior studies where *Acacia nilotica* exhibited notable inhibition against *S. aureus* and Gram-negative organisms such as *P. aeruginosa* and *E. coli* (16,17). However, in this study, *E. coli* and *Serratia spp.* showed either low or no susceptibility to the extracts, indicating potential strain-specific resistance or insufficient extract concentration.

Interestingly, *E. faecium* and *E. faecalis* exhibited moderate susceptibility to alcohol extracts but remained less responsive to aqueous extracts. This result suggests that the antibacterial compounds in *Acacia nilotica* may be hydrophobic in nature, hence more efficiently extracted with ethanol. Earlier phytochemical analyses have shown that ethanol extracts of *Acacia nilotica* bark and pods contain tannins, saponins, and alkaloids that are potent antimicrobials (18).

The concentration-dependent response observed in this study is also significant. None of the extracts retained their antibacterial activity at the 1/1000 dilution, and only the highest concentrations (0.1) exhibited meaningful inhibition. This supports the dose-dependent nature of plant-based antimicrobials, as documented by Biswas *et al*. (2002), who highlighted the necessity of high bioactive compound concentrations for therapeutic efficacy (19).

In comparison to standard antibiotics, the natural extracts demonstrated moderate activity. Antibiotics such as Amoxicillin and Ciprofloxacin produced larger inhibition zones across most strains, notably up to 48 mm for *E. faecium* and 44 mm for *P. mirabilis*. This outcome was anticipated given the purified and potent nature of synthetic antibiotics. However, the ability of *Acacia. nilotica* extracts to inhibit pathogenic strains, particularly those with known antibiotic resistance, positions it as a candidate for combination therapy or as a natural alternative in antimicrobial development.

Conversely, *Serratia spp.* exhibited resistance across all extract types, suggesting possible intrinsic resistance mechanisms. This observation is supported by Alwash and Abedsalih (2023), who found that aqueous solutions of *A. nilotica* gum had limited efficacy against *Klebsiella pneumoniae*, indicating variability in susceptibility among Gram-negative bacteria (20).

The concentration-dependent antibacterial activity observed, particularly the diminished efficacy at higher dilutions, underscores the importance of extract concentration in therapeutic applications. This phenomenon was also noted by Al-Rajhi *et al*. (2023), who reported that higher concentrations of *A. nilotica* flower extract exhibited significant anti-*Helicobacter pylori* activity (21).

Overall, the results confirm that *Acacia nilotica* possesses broad-spectrum but variable antibacterial properties, with alcohol-based extracts showing the most promise. The activity is clearly strain-dependent and affected by extract concentration. These findings support further pharmacological investigation and potential development of *Acacia nilotica* as a supplementary antimicrobial agent, particularly in regions where traditional medicine is integral to healthcare.

Conclusion:

The data highlights the potential use of *Acacia nilotica* extract as a natural antimicrobial agent, particularly against *P. mirabilis* and *E. faecalis*. However, its efficacy diminishes with dilution, indicating the need for sufficient concentrations to achieve therapeutic effects. Further studies are necessary to identify the active compounds and evaluate their clinical applicability.

While *Acacia nilotica* extracts are less potent than standard antibiotics, they demonstrate promising activity against specific strains (e.g., *Staphylococcus aureus*), positioning them as potential complementary agents or alternatives in antibiotic-resistant cases. Further studies are needed to optimize extraction methods, clarify mechanisms of action, and validate safety.

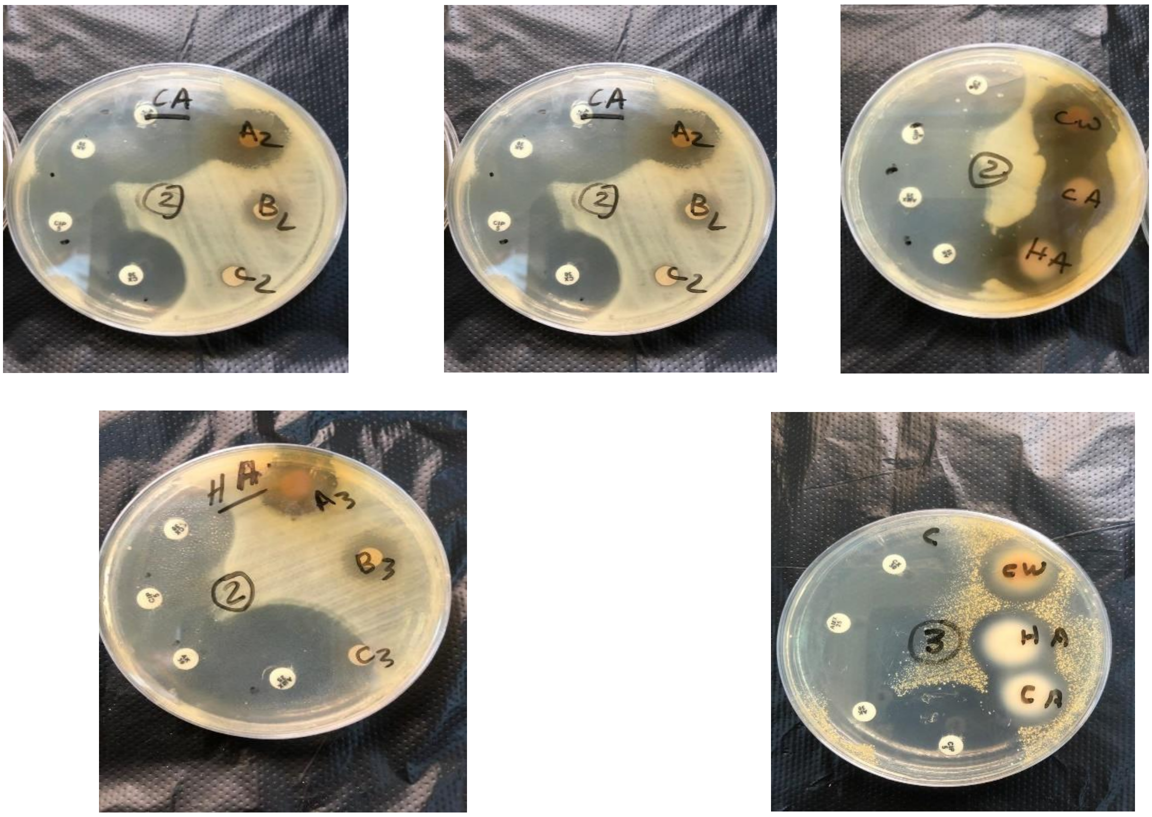

Figure (7). Effect of different Extracts compared with antibiotics on different strains of bacteria

Conflict of interest: NIL

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