

Antioxidant and antimicrobial activities of diverse parts of *Acacia nilotica* plant extracted by different solvents

Ghadir A. El-Chaghaby ^{1*}, S. Rashad ¹, H.G.M. El-Sayed ¹ and A.A.A. Abdel-Wareth ²

¹ Regional Center for Food and Feed (RCFF), Agricultural Research Center, 12619 Giza, Egypt

² Department of Animal and Poultry Production, Faculty of Agriculture, South Valley University, 83523 Qena, Egypt

Abstract

The present study investigates the effect of three different extraction solvents on the antioxidant and antibacterial activities of *Acacia nilotica* fruits, bark, and leaves. The solvents used included ethanol, acetic acid, and distilled water. The extraction was done by ultrasonication for 2 hours at 25°C. The total antioxidant capacity was determined by the colorimetric “phosphomolibdenum method”. The total phenolic content of plant parts used in the present investigation was measured using the Folin–Ciocalteu assay and the total flavonoids were determined by aluminum chloride assay. The antibacterial activities of plant extracts against *Bacillus subtilis*, *Escherichia Coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were determined by the disc diffusion method. The data revealed that for all plant parts the highest values of total antioxidant capacity, total phenolic compounds, total flavonoids, and potent inhibitory effects against the tested bacteria were achieved using ethanol as extraction solvent followed by acetic acid, whereas, the lowest values were recorded for water extracts. The results also revealed that *Acacia* bark ethanolic extract recorded significantly higher total antioxidant capacity (14293 mgAAE/100g), total phenolics (14053.98 mgGAE/100g) and total flavonoids (218.33 mgQE/Kg) as compared to all other extracts. All ethanolic extracts exerted high inhibition percentages ranging from 46-71% for fruits, 42-62% for bark, and 50-62% for leaves. The study also revealed that the bacterial inhibition of the extracts is positively correlated with their phenolic contents. Based on the results obtained during the present work, *Acacia* extract could be further investigated as a natural animal feed or food additive alternative to synthetic ones.

Keywords: *Acacia* extracts; bioactivity; Acetic acid; Ethanol; Water.

1. Introduction

Plants offer a wide variety of opportunities for several applications owing to their natural bioactive constituents such as vitamins, phenolic compounds, flavonoids and many other plant-derived compounds (El-Chaghaby *et al.*, 2019). Significant physiological activity and commercial worth are possessed by these bioactive components found in plants. Therefore,

it is crucial to extract these bioactive components from plants, especially for sectors like food processing, animal production, pharmaceutical engineering, and bioengineering (Shen *et al.*, 2023).

Synthetic antioxidants have been used extensively as food additives to prevent or postpone food oxidation and to extend food shelf life. Nowadays, the scientific community is becoming more interested in recovering safer antioxidants from natural sources as alternatives to chemical ones that are potentially harmful.


*Corresponding author: Ghadir A. El-Chaghaby

Email: ghadiraly@yahoo.com

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These naturally occurring antioxidants include phenolic chemicals that come from different kinds of plants (Kaderides *et al.*, 2021). Animal scientists are facing challenges as a result of consumers' increasing preference for safe animal products, which has sparked interest in the use of natural feed additives. Keeping in mind the advantages of phenolics, they can be a useful natural feed supplement for animals. Additionally, agricultural byproducts are a great source of antioxidant and phenolic chemicals that may be included in feed (Mahfuz, Shang, and Piao, 2021). Thus, researchers are increasingly interested in the extraction of bioactive compounds from different types of plant biomass in their efforts to find natural alternatives to chemical compounds, especially those used in food or feed. The extraction of bioactive compounds from plants is dependent on several parameters including the solvent type, extraction method, extraction time and others. Ultrasonic assisted extraction has been reported to be one of the most effective extraction methods (Rashad *et al.*, 2023).

Acacia nilotica plant is one of the plants that are widely found in Egypt, it is a member of the Fabaceae family and has a variety of medicinal uses. The plant is rich in compounds with diverse bioactive properties. Approximately 1350 species make up the vast genus *Acacia*, which is grown in tropical and subtropical climates across Africa, Asia, Australia, and the Caribbean. *Acacia* is a member of the Fabaceae (Leguminosae) family. It is an extremely important plant for the production of gum, fuel wood, charcoal, and animal feed (Diab *et al.*, 2022; Khalaf *et al.*, 2023). *Acacia nilotica* is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa (Abdalla *et al.*, 2020; Ahmed *et al.*, 2020; Gaara *et al.*, 2020). Among its various biological activities, many bioactive secondary constituents such as gallic acid, isoquercetin, terpenes, phenolic glycosides, volatile essential oils, ascorbic acid, carotene, calcium, magnesium, and selenium

have been identified in the plant (Aremu *et al.*, 2020; Badry *et al.*, 2021).

Although several studies have reported the bioactivity of different *Acacia* parts, but still there is a lack of studies regarding the evaluation of solvent effect on the antioxidant and antimicrobial activities of different parts of *Acacia nilotica* plant. So, the present study focuses on the effect of different solvents on the antioxidant and antimicrobial activities of the different parts of *Acacia nilotica*.

2. Materials and Methods

2.1. Samples collection

The *Acacia nilotica* tree leaves, fruits, and barks were freshly collected from the field located in South Valley University Plant Farm, Qena, Egypt in March 2022 (Photo, 1 a, b and c). Manually selected random samples were weighed right away, and they were then sun-dried. Before being used, dried materials were crushed into particles smaller than 1 mm and kept at room temperature in sealed plastic bags.

2.2. *Acacia nilotica* extracts preparation

Various solvents were used to prepare plant extracts of *A. nilotica* as follows: Ethanol, Acetic acid, and aqueous extract. *A. nilotica* fruits, bark and leaves extracts were obtained by weighing 5 grams of plant powder into 250 ml flask containing 100 ml of either ethanol (96%), Acetic acid (70%), or distilled water. The solvents used were chosen based on preliminary trials done in our laboratory. The extraction was done by ultrasonication for 2 hours at 25°C using an ultrasonic water bath. After extraction the samples were filtered and the solvent was evaporated using a rotary evaporator at 40°C (El-Chaghaby *et al.*, 2019).

2.3. Determination of the total antioxidant capacity of *Acacia* extracts

The total antioxidant capacity of the extracts was determined by the phosphomolybdenum method (Prieto *et al.*, 1999). The results were calculated from a standard curve using ascorbic acid as a

reference antioxidant material and results were expressed as mg ascorbic acid equivalent/100g of extract (mg AAE/100g).

2.4. Determination of the total phenolic content of Acacia extracts

The total phenols content of the extracts was determined using the Folin-Ciocalcau method (Turkmen *et al.*, 2006). A calibration curve of gallic acid was prepared and the results were determined from regression equation of the calibration curve and expressed as mg gallic acid equivalents per 100g of extract (mgGAE/100g).

2.5. Determination of the total flavonoids of Acacia extracts

The total flavonoids content of the extracts was determined by the aluminium chloride test (Mohdaly *et al.*, 2010) using quercetin as standard and the results were calculated as mg quercetin equivalent/Kg of extract (mgQE/Kg).

2.6. Antibacterial activity of Acacia extracts

The antibacterial activity of Acacia was done using the disc diffusion method following the procedure described by (Malabadi *et al.*, 2012). The studied bacteria stains were *Bacillus subtilis*,

Escherichia Coli, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Every extract was soaked onto paper discs. 10^8 cfu/ml of pure bacterial cultures were swabbed onto Muller-Hinton agar plates. Plates with culture and media were split into four equal sections, and discs that had already been made were positioned on each section. For twenty-four hours, the plates were incubated at 37°C. Then, the inhibition zone diameters against each kind of test microorganism were measured. The antibacterial tests were carried out at the Microbiology unit in the Microanalytical center, Cairo University.

2.7. Statistical analysis

The data were expressed as means \pm standard error (SE) and analyzed using SAS software 2005. One-way analysis of variance (ANOVA) and Turkey's multiple comparisons were carried out to test any significant differences between the means. Differences between the means at the 5% confidence level were considered significant. Correlations between variables were computed using the regression model in SPSS 11.0 for windows.



Photo 1. The *Acacia nilotica* tree leaves, flowers, and fruits growing in Qena, Egypt (Photo, 1 a, b and c).

3. Results and discussion

3.1. Total antioxidant capacity, total phenols and total flavonoids of *Accacia* extracts

The effect of different solvents on the antioxidant capacity, total phenols and total flavonoids of *Acacia* fruits, bark and leaves are given in tables (1), (2) and (3), respectively. The data revealed that for all plant parts the highest values of TAA, TP and TF were achieved using ethanol as extraction solvent followed by acetic acid. On the other hand, the water extracts showed the lowest values for total antioxidant capacity, total phenols and total flavonoids. Phenols and flavonoids represent classes of secondary metabolites possessing strong antioxidant activity. Previous studies have proven that different solvent extracts of *A. nilotica* exhibit different antioxidant capacities with polar solvent extracts being the most efficient (Yadav *et al.*, 2018).

According to Diem *et al.* (2013), the use of water as solvent results in a decrease of the total antioxidant capacity and total phenolic contents. Solvent plays a crucial role in extraction process by weakening of the solute-matrix interactions as well as better swelling of the plant matrix that results from the adsorption of solvent molecules on the hydroxyl and carboxyl groups of cellulose fibers (Zuorro *et al.*, 2019). Because of polyphenols are linked to the cell-wall matrix their water solubility may be reduced. Thus, several authors displayed those alcohols are suitable solvents for the extraction of phenolic and flavonoids compounds (Lefahal *et al.*, 2018). Also, the superiority of ethanol and acetic acid over water in extracting phenolic and flavonoids compounds is due to the solubility of these compounds in solvents less polar than water (Windson *et al.*, 2014).

Table 1. Antioxidant capacity, total phenols and total flavonoids activity of *Acacia* fruit extracts

Properties	Ethanol extract	Acetic acid extract	Aqueous extract
<i>Total Antioxidant Capacity (mgAAE/100g)</i>	9650.75 ^a ± 100.00	9321.75 ^b ± 60.80	3107.25 ^c ± 28.57
<i>Total phenols (mgGAE/100g)</i>	14127.47 ^a ± 64.22	13712.47 ^b ± 56.35	4570.82 ^c ± 47.72
<i>Total Flavonoids (mgQE/Kg)</i>	507.50 ^a ± 2350.54	178.50 ^b ± 9.27	80.30 ^c ± 5.15

Table 2. Antioxidant capacity, total phenols and total flavonoids activity of *Acacia* bark extracts

Properties	Ethanol extract	Acetic acid extract	Aqueous extract
<i>Total Antioxidant Capacity (mgAAE/100g)</i>	14293.00 ^a ± 100.16	13844.00 ^b ± 73.11	4465.81 ^c ± 33.73
<i>Total phenols (mgGAE/100g)</i>	14053.98 ^a ± 95.27	13644.98 ^b ± 75.11	4705.17 ^c ± 42.73
<i>Total Flavonoids (mgQE/Kg)</i>	218.33 ^a ± 16.92	133.33 ^b ± 5.38	53.33 ^c ± 3.39

Table 3. Antioxidant capacity, total phenols and total flavonoids activity of *Acacia* leaves extracts

Properties	Ethanol extract	Acetic acid extract	Aqueous extract
<i>Total Antioxidant Capacity (mgAAE/100g)</i>	13211.25 ^b ± 55.71	12882.25 ^b ± 66.77	3755.76 ^a ± 52.90
<i>Total phenols (mgGAE/100g)</i>	15376.10 ^b ± 8085.90	14847.10 ^b ± 26.34	3535.02 ^a ± 25.66
<i>Total Flavonoids (mgQE/Kg)</i>	513.66 ^a ± 6.91	480.77 ^b ± 5.38	81.48 ^c ± 5.75

Values are expressed as mean ± standard deviation (n = 3)

These results are important in practical as according to Manuelian *et al.*, (2021) dietary supplementation with plant extracts into animals and poultry diets is very advantageous for animal production yields as well as for the quality of the resulting food products, including changes in

fatty acid content and oxidative state that typically improve associated features such as flavor and color. Plant extracts effects vary but are frequently similar to those of the synthetic antioxidant VitE, indicating that they might be

used as a partial alternative for this vitamin in the diet.

3.2. Antibacterial activity of *Accacia* extracts

The unwanted effects of synthetic antibacterial agents, such as the presence of antibiotic residues and toxic metabolites in meat and byproducts that contribute to the problem of antibiotic resistance in humans, are a cause for worry in the long-term use of synthetic goods. Such difficulties have highlighted the usage of plant extracts, which are

thought to be reasonably safe and economical. Thus, plant extracts are utilized as feed supplements for animals to increase livestock output by boosting digestibility, nutrient absorption, and pathogen destruction in the animal intestine. Phytochemicals, also known as phytochemicals, are commonly employed in traditional and alternative animal antibiotics (Kuralkar and Kuralkar, 2021).

Table 4. Antimicrobial activity of *Acacia* fruit extracts

Bacteria strain	Standard antibacterial Ampicillin	Ethanol extract	Acetic acid extract	Aqueous extract
<i>Bacillus subtilis</i>	26	12 (46) ⁺	10 (38) ⁺	7 (27) ⁺
<i>Staphylococcus aureus</i>	21	15 (71) ⁺⁺⁺	12 (57) ⁺⁺	9 (42) ⁺
<i>Escherichia Coli</i>	25	13 (52) ⁺⁺	11 (44) ⁺	6 (24) ⁺
<i>Pseudomonas aeruginosa</i>	26	17 (65) ⁺⁺	14 (53) ⁺⁺	9 (35) ⁺

Values in parentheses are the inhibition percentages compared to standard antibiotic + Weak inhibition, ++Moderate inhibition, +++ Strong inhibition

Table 5. Antimicrobial activity of *Acacia* bark extracts

Bacteria strain	Standard antibacterial Ampicillin	Ethanol extract	Acetic acid extract	Aqueous extract
<i>Bacillus subtilis</i>	26	11(42) ⁺	10 (38) ⁺	6 (23) ⁺
<i>Staphylococcus aureus</i>	21	13 (62) ⁺⁺	11 (52) ⁺⁺	7 (33) ⁺
<i>Escherichia Coli</i>	25	12 (48) ⁺	10 (40) ⁺	7 (28) ⁺
<i>Pseudomonas aeruginosa</i>	26	13 (50) ⁺⁺	12 (46) ⁺	8 (31) ⁺

Values in parentheses are the inhibition percentages compared to standard antibiotic + Weak inhibition, ++Moderate inhibition

Table 6. Antimicrobial activity of *Acacia* leaves extracts

Bacteria strain	Standard antibacterial Ampicillin	Ethanol extract	Acetic acid extract	Aqueous extract
<i>Bacillus subtilis</i>	26	13 (50) ⁺	11 (42) ⁺	7 (27) ⁺
<i>Staphylococcus aureus</i>	21	13 (62) ⁺⁺	10 (48) ⁺	8 (38) ⁺
<i>Escherichia Coli</i>	25	13 (52) ⁺⁺	11 (44) ⁺	6 (24) ⁺
<i>Pseudomonas aeruginosa</i>	26	14 (54) ⁺⁺	12 (46) ⁺	9 (35) ⁺⁺

Values in parentheses are the inhibition percentages compared to standard antibiotic+ Weak inhibition, ++Moderate inhibition

In the present work, the antibacterial activity of different *Accacia* extracts was also investigated. The results of the antibacterial activity of different solvent extracts for *Acacia* fruits, bark and leaves are given in tables 4, 5 and 6, respectively. The different extracts showed selective inhibitory activities against the tested bacteria stains. Tested strains included two Gram negative bacteria (*Escherichia coli* and

Pseudomonas aeruginosa) and two Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). The recorded inhibition zone diameters for the different extracts indicated that water extracts were the least efficient against all studied Gram positive and Gram-negative bacteria. In contrary, ethanolic and acetic acid extracts exerted moderate inhibitory effect against all tested organisms. The antibacterial

activity of plant extracts is usually related to their content of phytochemical compounds such as phenols, flavonoids, alkaloids, tannins, glycosides, etc. (Debalke *et al.*, 2018). According to Olatunde *et al.* (2021) the antibacterial activity exerted by plant extracts might be attributed to polyphenolic chemicals, which have the capacity to interact with bacterial cell membranes via hydrophobic-hydrophobic interactions, hence increasing membrane leakage and destructing cell structure. Bacterial mortality is primarily caused by the excessive loss of essential cell ions and molecules as a result of cell rupture.

3.3. Correlation between the total phenolic content and antibacterial activity of *Accacia* extracts

It has been generally agreed that the antibacterial activity of plant extracts against a variety of microorganisms is usually attributed to their phytochemical constituents, especially phenolic compounds and flavonoids. In the present study, as shown in Figures (2, 3, 4) the results indicated

a positive correlation between the phenolic content of different *Accacia* parts' extracts and both Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) with high correlation coefficient values as presented in Table (6). According to Safari & Ahmady-Asbchin, (2019), it appears that plants produce phenolic compounds in response to microbial infection, therefore it makes sense that they have been discovered to be efficient antibacterial agents *in vitro* against a variety of pathogenic microbes. Phenolic compounds, can act as antimicrobial agents through a variety of mechanisms, including the inhibition of nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, attachment and biofilm formation, inhibition of the porin on the cell membrane and permeability changes, which might result in cell destruction, and pathogenicity attenuation.

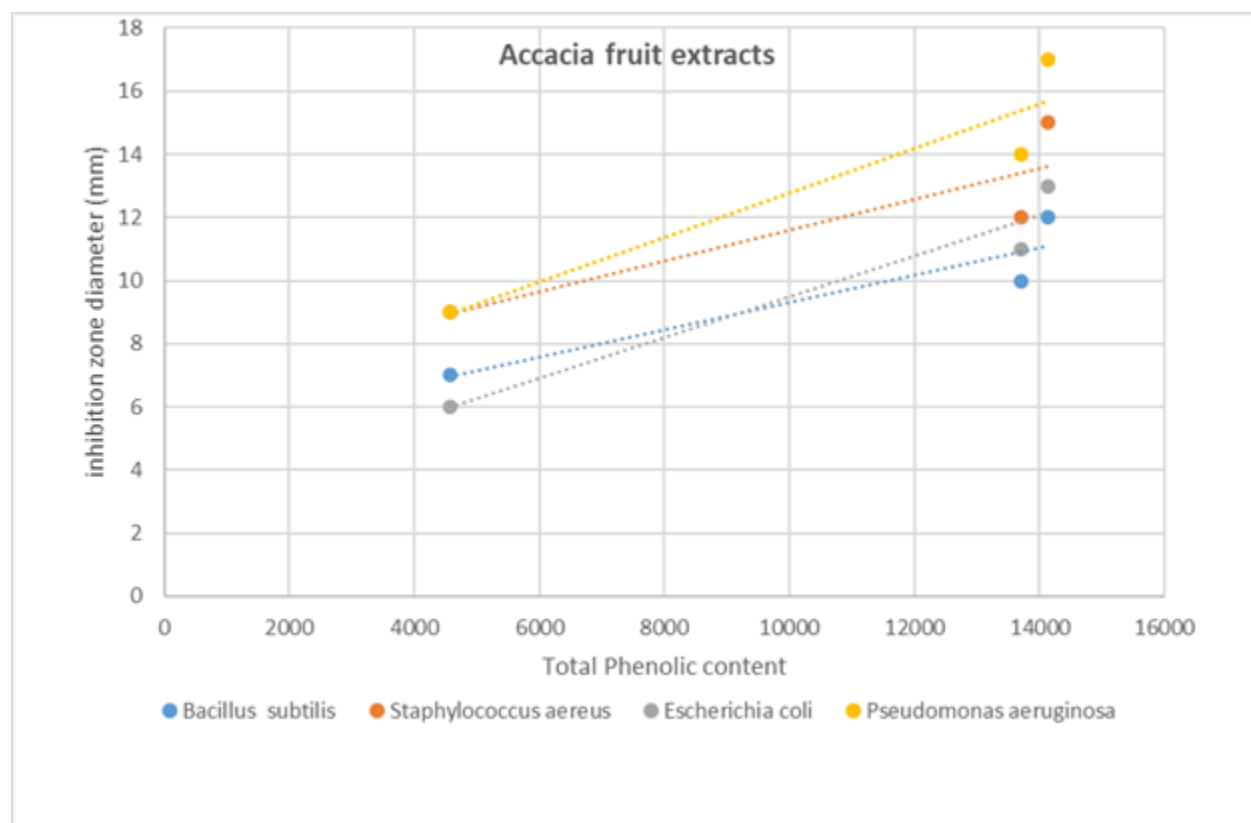


Figure 2. Correlation between the phenolic content and inhibition zone in *Accacia* fruit extracts

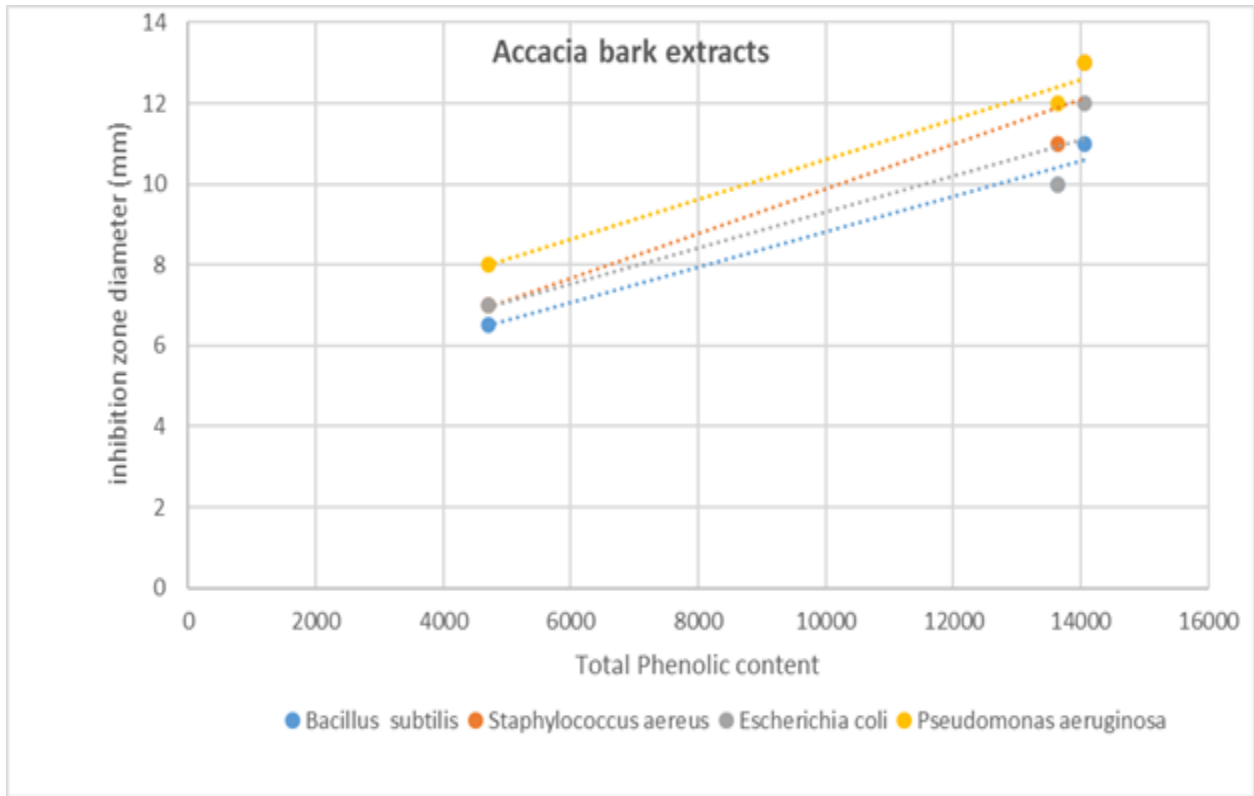


Figure 3. Correlation between the phenolic content and inhibition zone in *Accacia* bark extracts

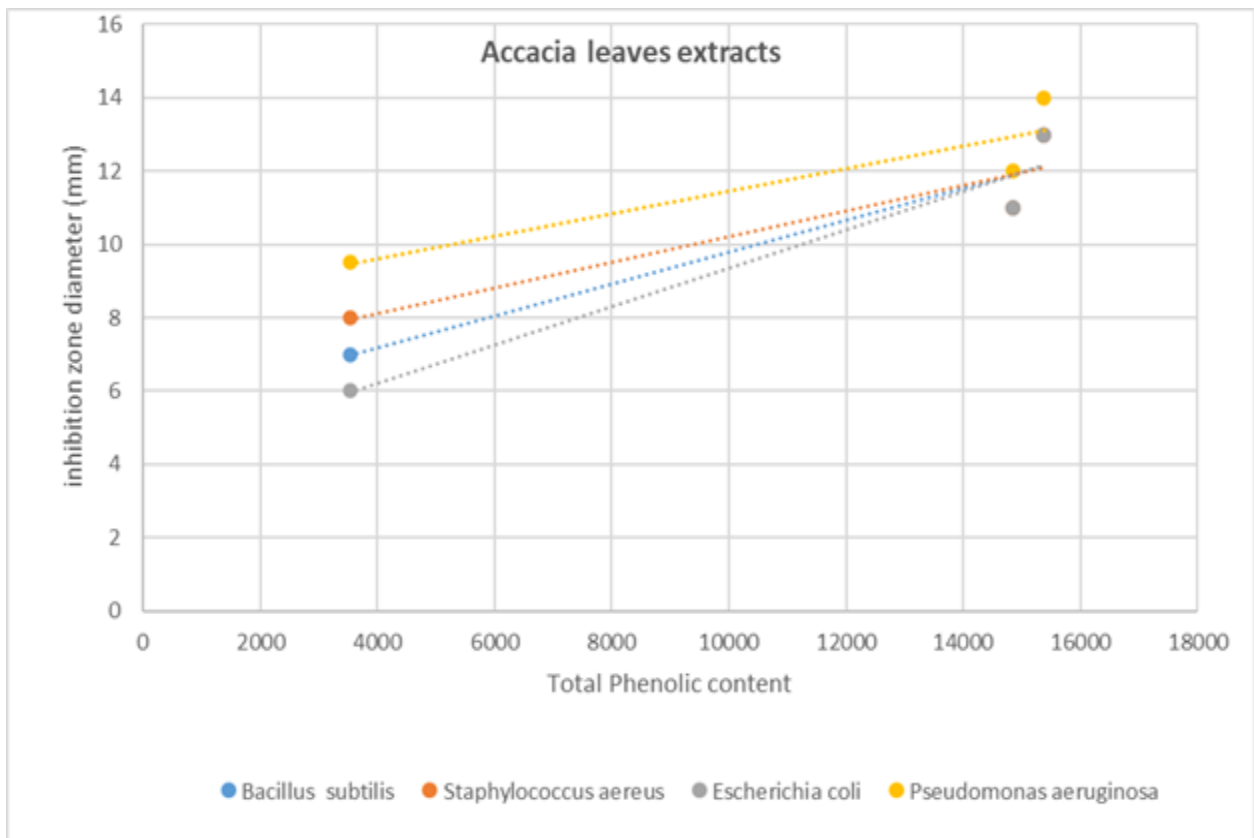


Figure 4. Correlation between the phenolic content and inhibition zone in *Accacia* leaves extracts

Table 6. Correlation coefficient (R^2) for antioxidant and antibacterial activities of Accacia extracts

Bacterial strain	Accacia fruit extract	Accacia bark extract	Accacia leaves extract
<i>Bacillus subtilis</i>	0.8691	0.9699	0.9161
<i>Staphylococcus aureus</i>	0.7825	0.9156	0.8699
<i>Escherichia coli</i>	0.9423	0.8693	0.9428
<i>Pseudomonas aeruginosa</i>	0.8876	0.9772	0.8337

4. Conclusion

The results of the present investigation revealed that ethanolic extracts of different Acacia parts exhibited better antioxidant activities and higher phenolic and flavonoid content followed by the acetic acid extracts. The results highlighted that the plant's different parts can be used as a source of natural antioxidants and antibacterial compounds. Acacia plant contains many phytochemicals that are beneficial for general health. Future work could be addressed to investigate the isolation of bioactive compounds from Acacia to expand the plant extracts used in food and animal production.

Authors' Contributions

All authors are contributed in this research

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Institutional Review Board Statement

All Institutional Review Board Statements are confirmed and approved.

Data Availability Statement

Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate

Not applicable

Consent for Publication

Not applicable.

Conflicts of Interest

The authors disclosed no conflict of interest.

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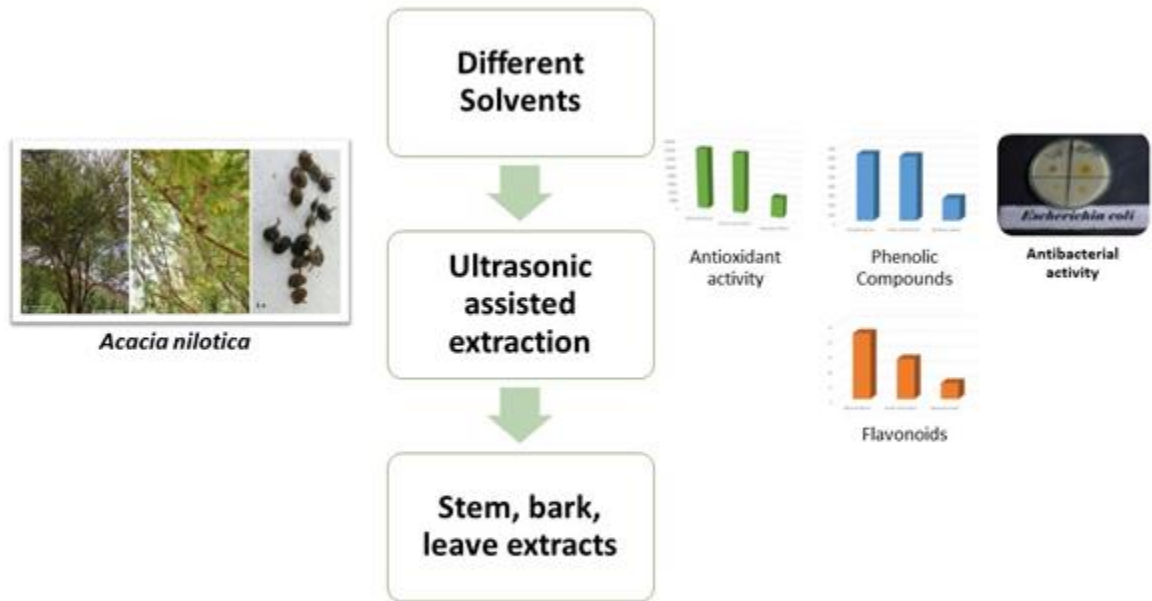
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The Graphical abstract