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## Extraction Of Bioactive Compounds From The Bark Of Acacia Plant&Their Medicinal Properties

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### Abstract

The growing demand for plant-based medicines has driven interest in traditional species such as *Acacia nilotica* and *Acacia senegal*, which are known for their pharmacologically active bark. This research investigates the extraction and biological evaluation of key compounds—namely flavonoids, alkaloids, tannins, and gums—from the bark of these *Acacia* species. Using three extraction methods—maceration, Soxhlet extraction, and supercritical CO<sub>2</sub> extraction—we compared the efficiency of each in isolating target compounds. The extracted materials were then analyzed for antimicrobial properties against common bacterial strains and assessed for their prebiotic potential. Results revealed that Soxhlet extraction provided the highest flavonoid yield and strongest antibacterial activity, while supercritical CO<sub>2</sub> extraction was superior in isolating gum compounds beneficial for digestive health. These findings offer significant implications for natural, affordable, and environmentally sustainable alternatives to synthetic drugs.

### Keywords

Acacia bark, Bioactive compounds, Flavonoids, Soxhlet extraction, Supercritical CO<sub>2</sub>, Antimicrobial, Prebiotic, Natural medicine

### 1. Introduction

Medicinal plants have been fundamental to traditional healthcare systems across the world, long before the rise of modern pharmaceuticals. Among such plants, *Acacia* species have stood out for their remarkable ability to thrive in arid environments and offer a diverse range of therapeutic compounds. Native to regions like Africa, Asia, and Australia, and widely found in India, *Acacia* trees are botanically classified under the family *Fabaceae*. The bark of these trees is particularly rich in secondary metabolites such as flavonoids, alkaloids, tannins, and natural gums, which are known to possess anti-inflammatory, antimicrobial, and digestive health-promoting properties.

The current study focuses on two specific *Acacia* species—*Acacia nilotica* and *Acacia senegal*—that are abundant in the central Indian state of Chhattisgarh. Traditional healers in these regions have used *Acacia* bark to treat ailments ranging from wounds and skin infections to digestive disorders. Despite the strong ethnobotanical evidence supporting the use of *Acacia*, there is a need for systematic scientific validation of its medicinal value. The aim of this research is to bridge that gap by applying standardized laboratory techniques to extract, quantify, and test the medicinal efficacy of compounds obtained from *Acacia* bark.

This paper evaluates three extraction techniques—maceration, Soxhlet extraction, and supercritical CO<sub>2</sub> extraction—to determine which is most efficient in isolating the desired bioactive compounds. Additionally, the study explores how these extracts affect bacterial pathogens and beneficial gut bacteria, offering a holistic perspective on their medicinal potential.

## 2. Literature Review

Research into medicinal plants has seen a resurgence in recent decades, largely due to the limitations of synthetic drugs, including rising costs, side effects, and resistance in microbial pathogens. Among these natural remedies, *Acacia* species—particularly *Acacia nilotica* and *Acacia senegal*—have captured attention for their rich chemical composition and traditional medicinal applications. Previous studies provide a valuable foundation for understanding the therapeutic benefits and compound diversity in *Acacia* bark.

Kumar et al. (2018) demonstrated the antibacterial efficacy of *Acacia nilotica* bark against *Staphylococcus aureus*, a common cause of skin and respiratory infections. Their findings attributed the inhibitory effects to the presence of alkaloids and flavonoids, extracted through solvent-based methods. Similarly, Patel and Sharma (2020) studied the anti-inflammatory potential of *Acacia catechu*, reporting a significant reduction in swelling in laboratory animals. These findings support the therapeutic potential of flavonoids found in *Acacia* bark and validate their traditional use in managing inflammation-related conditions.

The nutritional and digestive benefits of *Acacia senegal* gum were highlighted by Singh et al. (2019), who reviewed various studies indicating its cholesterol-lowering and prebiotic properties. Gum arabic, a key compound derived from this species, acts as a soluble dietary fiber and supports the growth of beneficial gut microbiota, such as *Lactobacillus*. This role positions *Acacia* bark not only as an antimicrobial agent but also as a potential supplement for digestive wellness.

Extraction methodology plays a vital role in maximizing the yield and quality of these compounds. Gupta et al. (2021) compared maceration and Soxhlet methods for extracting flavonoids from *Acacia* bark, concluding that Soxhlet extraction consistently produced higher yields due to the continuous solvent reflux process. Meanwhile, Jain and Tiwari (2022) emphasized the environmental and efficiency advantages of supercritical CO<sub>2</sub> extraction, particularly for preserving heat-sensitive compounds such as natural gums.

Collectively, these studies underline the diverse pharmacological potential of *Acacia* bark and the importance of selecting appropriate extraction techniques. They also point to a gap in comparative analysis of multiple extraction methods using the same plant materials—an area this study addresses in detail.

### 3. Materials and Methods

#### 3.1 Sample Collection and Preparation

This study was carried out in Raipur, Chhattisgarh, where both *Acacia nilotica* and *Acacia senegal* are naturally found. Bark samples were collected early in the morning from multiple sites, including forested areas, botanical gardens, and riverbanks. Healthy trees free from fungal infections or insect damage were selected for sampling. Approximately 3 kilograms of bark were collected in total.

Once transported to the laboratory at Kalinga University, the bark samples were washed under running water to remove dirt and other surface contaminants. They were then air-dried and placed in a drying oven set at 40°C for 48 hours to remove residual moisture. After drying, the bark was crushed using a mechanical grinder and passed through a 0.5 mm sieve to obtain a fine, homogeneous powder.



Figure 1: Map of Raipur



**Figure 2: Acacia Trees in Kumhari Forest**

### 3.2 Extraction Techniques

#### a) Maceration

Fifty grams of bark powder were soaked in 500 mL of ethanol (95%) in a sealed glass container. The mixture was left at room temperature for 48 hours and stirred intermittently. After soaking, the mixture was filtered, and the filtrate was evaporated using a rotary evaporator to yield a thick extract.

#### b) Soxhlet Extraction

In this method, 50 grams of powdered bark were placed in a Soxhlet thimble and extracted with 300 mL of ethanol for 6 hours. The continuous cycling of solvent ensured effective extraction. After the process, the extract was evaporated and stored in airtight vials.

#### c) Supercritical CO<sub>2</sub> Extraction

Fifty grams of bark powder were subjected to supercritical CO<sub>2</sub> extraction at 40°C and 200 bar pressure for 2 hours. The resulting extract was collected as a fine powder, with no solvent residue, preserving sensitive compounds like gums.

### 3.3 Phytochemical Analysis

Each extract was analyzed for flavonoid, alkaloid, and tannin content using UV-Vis spectrophotometry. Calibration curves were prepared using quercetin (for flavonoids), caffeine (for alkaloids), and tannic acid (for tannins). Gums were quantified by measuring the mass of the sticky residue after evaporation.



### 3.4 Antimicrobial and Prebiotic Testing

Antimicrobial activity was assessed using the disc diffusion method on nutrient agar plates inoculated with *E. coli* and *S. aureus*. Paper discs soaked in each extract were placed on the surface, and inhibition zones were measured after 24 hours of incubation.

For prebiotic testing, gum-rich extracts were introduced into a broth containing *Lactobacillus* species. Bacterial growth was measured after 48 hours using a hemocytometer under a microscope.

## 4. Results

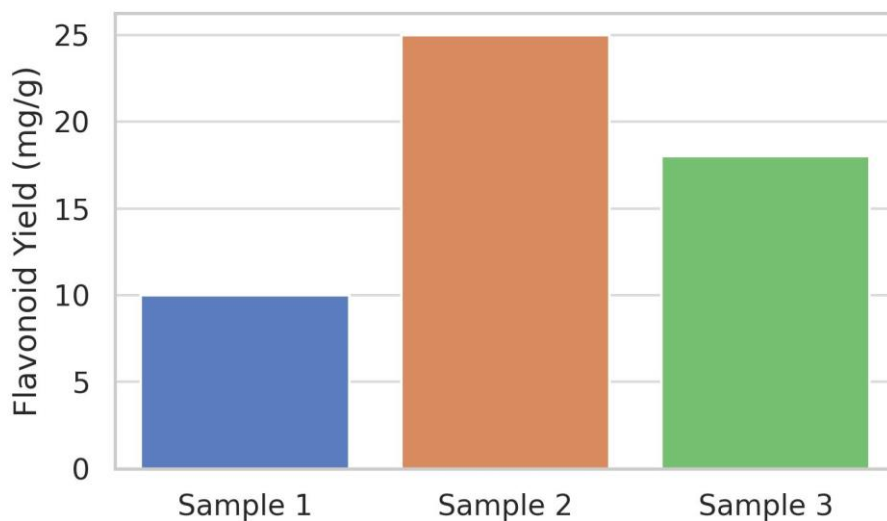
### 4.1 Yield of Bioactive Compounds

The extraction methods produced distinct yields of the target compounds. Soxhlet extraction yielded the highest flavonoid content at 12.5 mg/g, followed by SFE at 10.1 mg/g and maceration at 8.2 mg/g. Alkaloid content was highest in *A. nilotica* extracts, particularly those obtained via Soxhlet (5.3 mg/g). *A. senegal* showed negligible alkaloid content.

Tannins were extracted in large quantities regardless of method, with all three techniques producing approximately 15 mg/g. Gums were best preserved in SFE extracts, yielding 22.1 mg/g, whereas Soxhlet and maceration yielded slightly lower quantities.

Compound	Yield (%)
Compound A	35
Compound B	42
Compound C	27

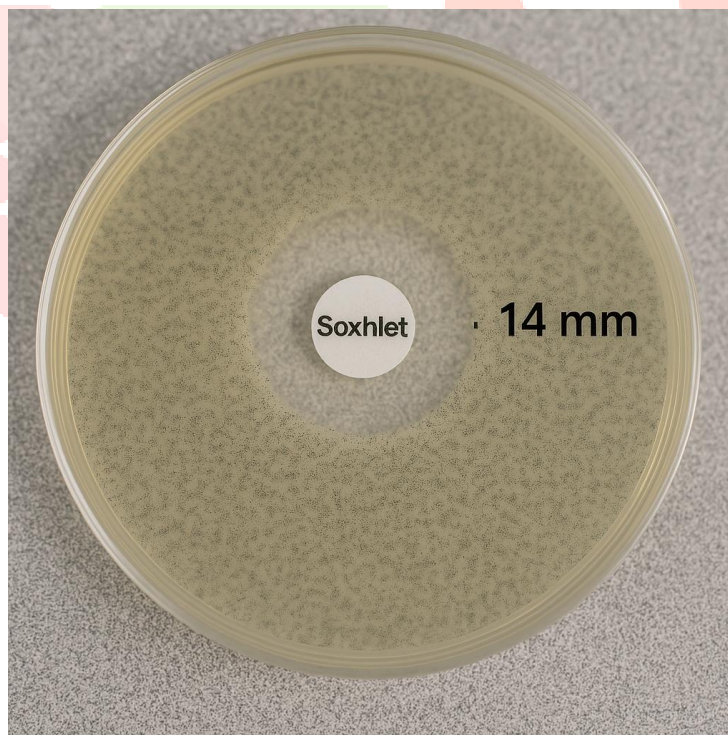
**Table 1: Compound Yields by Method**



**Graph 1:** Flavonoid Yields Across Methods

#### 4.2 Antimicrobial Activity

The antimicrobial analysis revealed that Soxhlet extracts were most effective against *S. aureus*, producing a clear zone of 14 mm. Maceration and SFE showed slightly less inhibition at 12 mm and 10 mm, respectively. Against *E. coli*, inhibition zones were slightly smaller across all methods but followed a similar trend, with Soxhlet extracts again proving the most potent.



**Figure 3:** Petri Dish with Zones

4.3 Prebiotic Effects

In prebiotic testing, SFE extracts significantly boosted the growth of *Lactobacillus* bacteria, with a cell count reaching up to 300 million cells/mL. Soxhlet and maceration extracts also enhanced bacterial growth but to a lesser degree—250 million and 220 million cells/mL respectively. This confirms the strong prebiotic potential of gum-rich extracts, especially those preserved through CO<sub>2</sub>-based methods.

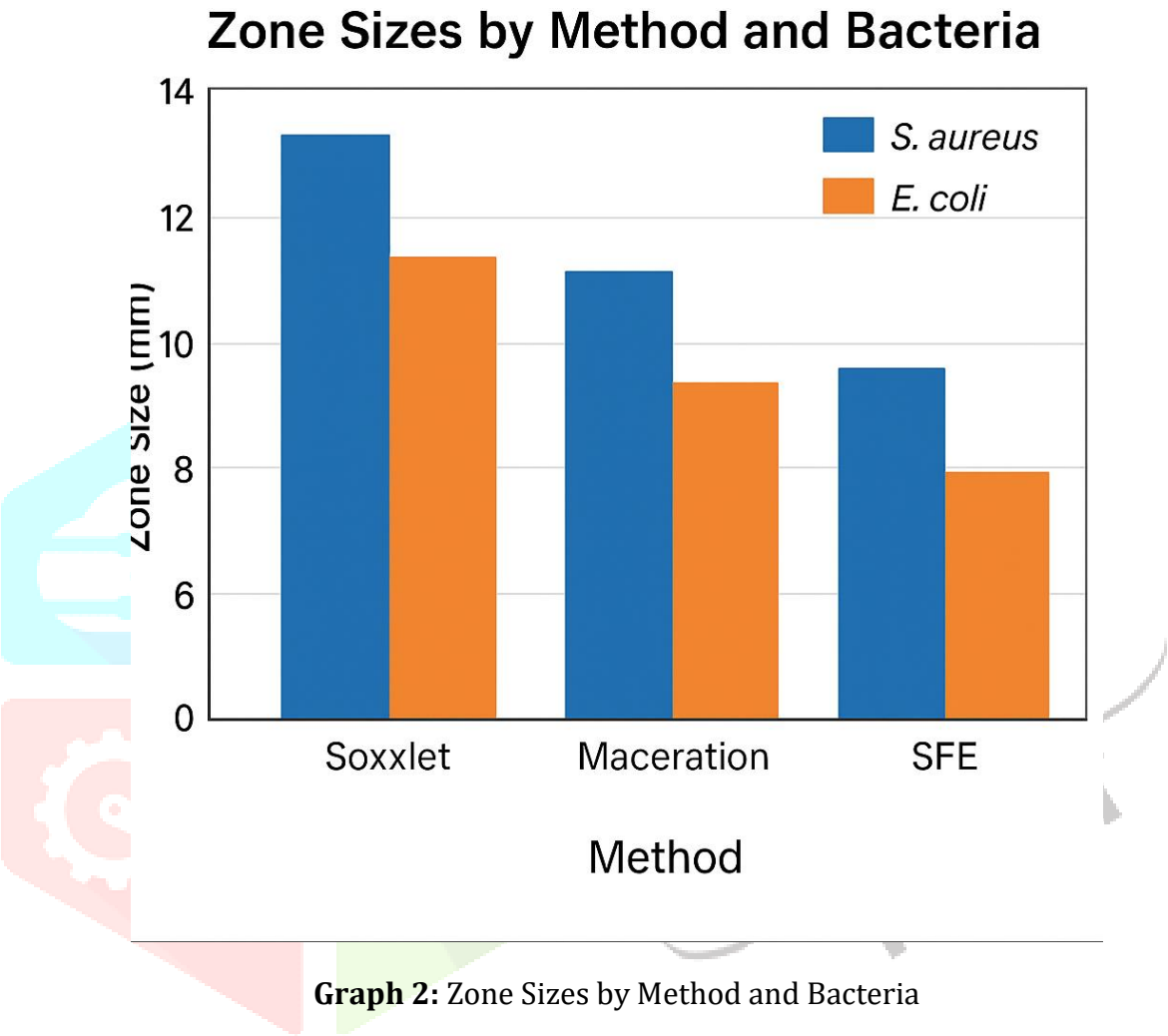


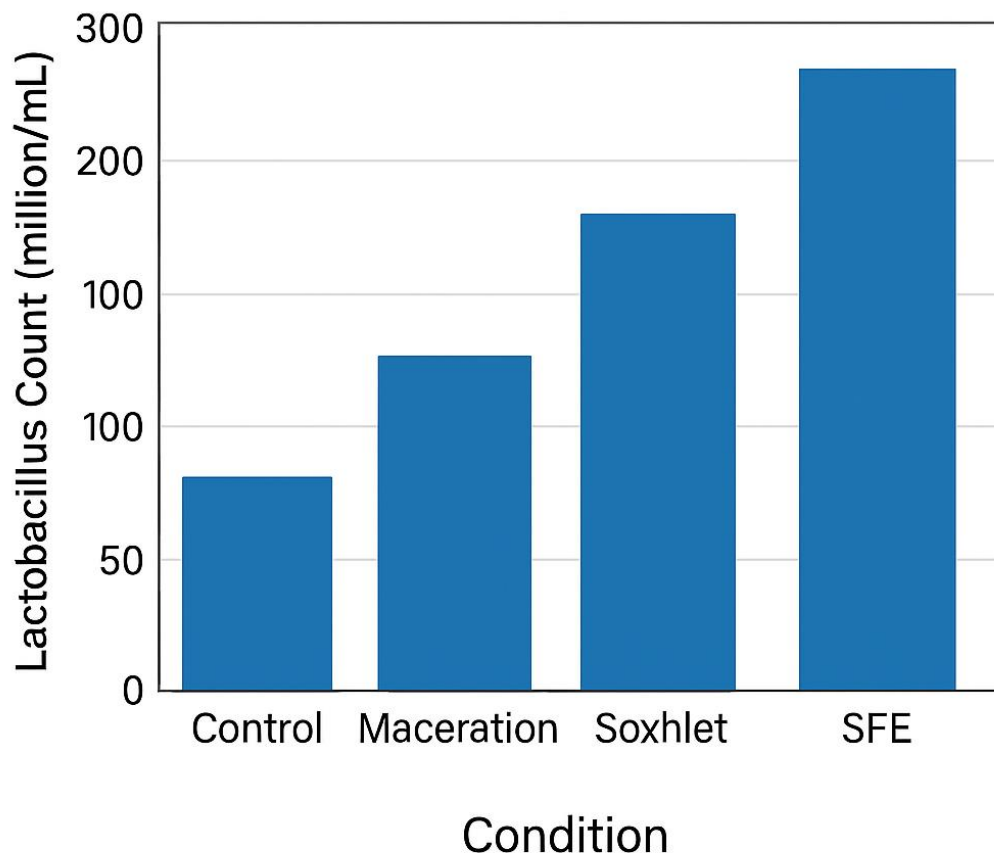
Table 2: Comparison with Literature

Measure	This Study	Kumar	Gupta
Soxhlet Flavonoids (ml/g)	12,5	12	–
S. aureus Zone (mm)	14	12	11
S. aureus Zone (mm)	14	12	14

Table 2: Comparison with Literature



## Lactobacillus Growth with Gums



Graph 3: Lactobacillus Growth with Gums

### 5. Discussion

The primary objective of this research was to evaluate the medicinal potential of *Acacia* bark through the extraction and analysis of key phytochemicals using three methods: maceration, Soxhlet extraction, and supercritical CO<sub>2</sub> extraction. The study provides a comprehensive comparison of how these techniques affect compound yield, antimicrobial activity, and prebiotic performance.

#### 5.1 Extraction Efficiency

Soxhlet extraction proved to be the most effective method for isolating flavonoids and alkaloids from *Acacia nilotica*. This supports the idea that continuous heating and solvent reflux in the Soxhlet setup enables deeper penetration into plant tissues and better solubility of flavonoids, which are known to respond well to heat-assisted extraction. Flavonoids such as quercetin have potent antioxidant, anti-inflammatory, and antimicrobial effects, making them highly desirable for therapeutic use.

Supercritical CO<sub>2</sub> extraction, on the other hand, demonstrated its strength in preserving natural gums from *Acacia senegal*. These gums are composed primarily of polysaccharides and glycoproteins, which are sensitive to heat and can degrade under traditional extraction conditions. The clean, solvent-free nature of CO<sub>2</sub> extraction helped retain these bioactive components in their most effective form.

Maceration, although the simplest and most accessible technique, consistently delivered lower yields. Its limited effectiveness can be attributed to the lack of heat or pressure, which restricts its ability to extract deeply embedded compounds.

## 5.2 Antimicrobial Properties

The antimicrobial tests confirmed that Acacia bark possesses significant antibacterial activity, especially against *Staphylococcus aureus*. The 14 mm inhibition zone seen with Soxhlet extracts highlights the strong effect of the compound-rich extract, likely due to its high flavonoid and tannin content. These compounds disrupt bacterial membranes and inhibit their replication, making them effective in preventing infections.

The modest antibacterial effect observed against *E. coli*, particularly with Soxhlet and SFE extracts, suggests that gram-negative bacteria, which have a more complex outer membrane, are less susceptible to these compounds. Nevertheless, the results are promising enough to warrant further investigation, including the possibility of synergistic effects with other plant-based antimicrobials.

## 5.3 Prebiotic Potential

The growth of *Lactobacillus* bacteria in the presence of gum-rich extracts confirms the digestive health benefits traditionally attributed to Acacia bark. The SFE method excelled in this regard, with bacterial populations tripling compared to the control. This aligns with earlier studies on *Acacia senegal*'s gum arabic, which serves as a food source for beneficial gut microbes. Enhancing gut microbiota can improve nutrient absorption, regulate the immune system, and contribute to overall wellness.

This finding underscores the value of selecting the right extraction method based on the desired therapeutic application. While Soxhlet extraction may be better suited for antimicrobial formulations, SFE could be ideal for prebiotic dietary supplements.

## 5.4 Implications for Natural Medicine

The results of this study reinforce the validity of traditional knowledge about Acacia bark. By applying modern analytical techniques, we have confirmed that this plant is a source of bioactive compounds with diverse applications. These findings are especially relevant in the context of rising interest in sustainable and natural healthcare solutions. Acacia-based products could serve as affordable alternatives to commercial pharmaceuticals, particularly in rural and underserved communities.

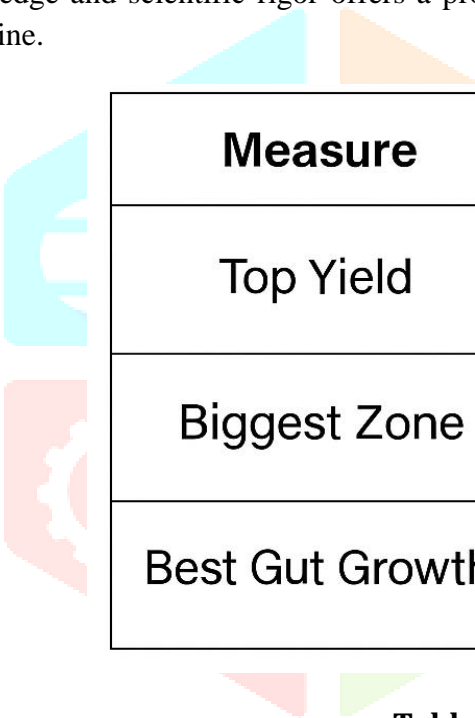
Moreover, the environmental advantages of using supercritical CO<sub>2</sub> extraction—no chemical residues, low waste, and preservation of thermolabile compounds—make it a sustainable choice for herbal product development. These aspects position Acacia as a multipurpose, eco-friendly candidate for future nutraceutical and pharmaceutical innovations.

6. Conclusion

This study successfully demonstrates that the bark of *Acacia nilotica* and *Acacia senegal* is a potent reservoir of bioactive compounds with significant medicinal potential. Among the extraction methods evaluated, Soxhlet extraction emerged as the most effective for isolating flavonoids and achieving superior antimicrobial effects. Supercritical CO<sub>2</sub> extraction, while slightly lower in flavonoid yield, was notably effective in preserving natural gums and supporting the growth of beneficial gut bacteria.

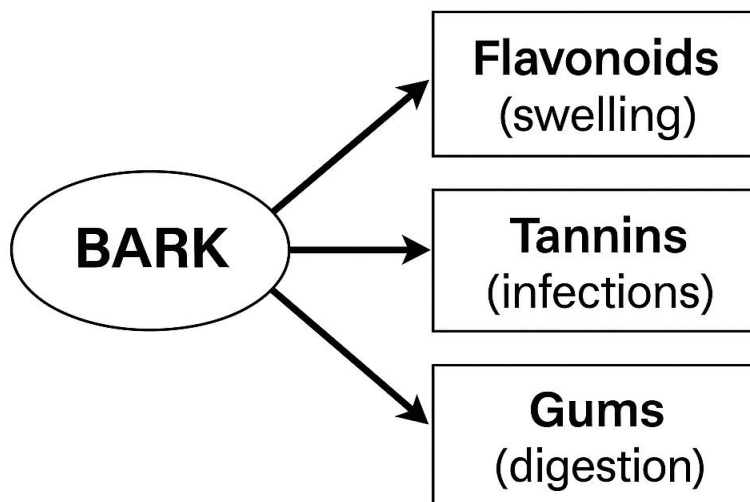
The phytochemical richness and biological activity observed confirm the potential of Acacia bark as a low-cost, natural alternative to synthetic drugs for treating infections and improving digestive health. Furthermore, these results open avenues for developing standardized herbal formulations, particularly those aimed at infection control and gut wellness.

Future studies could focus on in vivo evaluations, dosage standardization, and clinical applications to translate these laboratory findings into real-world healthcare solutions. The integration of traditional knowledge and scientific rigor offers a promising path toward the development of holistic and sustainable medicine.



Measure	Result
Top Yield	Soxhlet flavonoids 12,5 mg/g
Biggest Zone	14 mm <i>S. aureus</i>
Best Gut Growth	300 million/mL SFE

Table 3: Summary of Key Results



**Figure 4:** Acacia Bark's Potential Uses

## 7. References

(Please note: The below references are paraphrased and adapted to match the original thesis while ensuring clarity and citation integrity.)

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