SHORT COMMUNICATION

# Profiling the Bioactive Richness (Flavonoids, Phenolic Acids and Vitamins) of *Acacia* Leaves

Sagi Raju[1], Devanaboyina Nagalakshmi[2], Nagireddy Nalini Kumari[2], Boini Sravanthi[2]*, Kolli Vijay[3]

## Abstract

Phytochemicals derived from plants have gained considerable attention for their health-promoting properties, including antioxidant, anti-inflammatory, and disease-preventing capabilities. This study focuses on the phytochemical profiling of Acacia leaf extracts, highlighting their potential as nutraceutical and animal health supplements. Using LC-MS/MS, we identified and quantified key flavonoids, phenolic acids, and vitamins present in Acacia leaves. Significant levels of bioactive flavonoids such as quercetin, rutin, hesperetin, naringenin, and luteolin were detected, with quercetin-3-rutinoside (rutin) showing the highest concentration. Phenolic profiling revealed abundant levels of gallic acid, p-coumaric acid, and dihydroxybenzoic acid, compounds known for their antioxidant and antimicrobial properties. In addition to phytochemicals, Acacia leaves were found to be rich in essential vitamins, particularly water-soluble B-complex vitamins (thiamine, niacin, pantothenic acid) and fat-soluble vitamins E and K1. These findings demonstrate the multifunctional value of Acacia leaf extracts in promoting health and productivity in both humans and animals. The results support further exploration of Acacia as a sustainable source of natural antioxidants and micronutrients for functional food, pharmaceutical, and animal feed applications.

**Key words:** Acacia leaf extract, Flavonoids, LC-MS/MS profiling, Phenolic acids, Phytochemicals.

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## Introduction

Phytochemicals, derived from plants through metabolic processes, offer a wealth of health benefits and are increasingly recognized for their potential in preventing chronic diseases and safeguarding cellular health against oxidative damage **(**Martel *et al.,* 2019). These compounds, including polyphenols, flavonoids, and carotenoids, found abundantly in various plant sources, play a significant role in human nutrition, offering protection against a wide array of diseases ranging from cancer to diabetes (Lin *et al.,* 2016). Moreover, their inclusion in diets is associated with improved health outcomes, making them invaluable components of a balanced nutrition plan. Flavonoids, found in a range of natural sources including fruits, vegetables, tea, and wine, are valued for their health benefits. Efforts to isolate these compounds are underway due to their antioxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties. They are now integral components in nutraceutical, pharmaceutical, medicinal and cosmetic applications for their ability to modulate key cellular enzyme function (Diwan and Panche, 2016). Phenolic compounds, found widely in plants and plant-based foods and beverages, are secondary metabolites with diverse structures and functions. They can be classified into water-soluble compounds like phenolic acids, flavonoids, and quinones, and water-insoluble compounds such as condensed tannins and lignins. These compounds exhibit a wide variety of structures, ranging from simple molecules like vanillin and gallic acid to more complex polyphenols like stilbenes and flavonoids. Phenolic compounds are recognized as one of the most important and abundant groups of compounds in the plant kingdom (Sarkar *et al.,* 2022).

Certain plant leaves, such as acacia present promising opportunities in animal feeding and health promotion. Rich in essential nutrients and bioactive compounds, these leaves serve as valuable dietary supplements for livestock, contributing to growth, digestion, and overall well-being. Acacia leaves, rich in protein, fibre, vitamins and minerals, are widely used in livestock nutrition (Maji and Modak, 2021). Incorporating acacia leaves into animal diets requires careful consideration of species variation and processing

[1]Department of Animal Nutrition, College of Veterinary Science, Warangal-506005, PVNRTGVU, Telangana, India

[2]Department of Animal Nutrition, College of Veterinary Science, Rajendranagar, Hyderabad-500030,PVNRTGVU, Telangana, India

[3]Department of Livestock Production and Management, College of Veterinary Science, Warangal-506005, PVNRTGVU, Telangana, India

**Corresponding Author:** Dr. Boini Sravanthi, M.V.Sc. Scholar, Department of Animal Nutrition, College of Veterinary Science, Rajendranagar, Hyderabad-500030, PVNRTGVU, Telangana, India. e-mail: boinisravanthi1998@gmail.com

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methods to optimize nutritional benefits while avoiding adverse effects. The potential of *Acacia nilotica* leaf meal as an alternative feed for ruminants is showcasing its high nutritional value (Raju *et al.,* 2025). Consulting with experts in animal nutrition can provide valuable insights into the safe and effective utilization of these plant resources in promoting animal health and productivity. Overall, the objectives aimed to connect the medicinal properties of *Acacia nilotica* plant through phytochemical and vitamin extraction, which has rich bioactive compounds offering potential solutions for improving human health, agricultural productivity and animal welfare.

## Materials and Methods

### Profiling of Phenolic Acids and Flavonoids by LCMS

The individual phenolic compounds and flavonoids in Acacia leaf extracts was analysed by Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS) with 80% methanol as described by Weidner *et al.* (2000) and Chen *et al. (*2001).

### Profiling of Vitamins by LC-MS/MS

Water-soluble and fat-soluble vitamins extraction procedure from Acacia leaf extracts was followed as described by Santos *et al*. (2012).

## Results And Discussion

Phytochemical screening of the extracts identified the presence of various flavonoids and phenolic acids in acacia leaf extracts.Flavonoids and phenolic compounds, found in various plant parts, act as natural antioxidants. They are known for their ability to scavenge free radicals and exhibit antioxidant properties, attributed to their reducing and chelating abilities (Joseph *et al.,* 2016). Table 1 summarizes the phenolic and flavonoid content in acacia leaf extract. Flavonoids found in a variety of natural sources, which are prized for their health benefits. Their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties, along with their ability to modulate cellular enzymes, have led to their inclusion in numerous nutraceutical, pharmaceutical, medicinal, and cosmetic products. Flavonoids play a vital role in disease defence (Rajanandh and Kavitha, 2010) with a reflecting its high-quality flavonoid content. Environmental and genetic factors, as well as seasonal variations, influence flavonoid concentrations (Kumar and Roy, 2018). Plant foods rich in flavonoids, like flavanols, play a crucial role in protecting against conditions like coronary heart disease and dementia (Fang *et al.,* 2013). Quercectin and Rutin showed highest concentration in acacia leaves. Rutin, a potent plant-based antioxidant known as quercetin-3-rutinoside, offers various health benefits, including managing diabetes, oxidative stress, microbial infections, cancer, and cardiovascular issues (Rauf *et al.,* 2017). In Acacia leaf extraction, another major compound was Hesperetin. It is a cholesterol lowering flavanoid found in a number of citrus juices. It appears to reduce cholesteryl ester mass and inhibit apo B secretion up to 80% (Mulvihill *et al.,* 2016). Acacia leaves contained significant proportions of Naringenin, Luteolin, Quercetin, and Hesperetin.

Phenolic compounds, widespread in plants and derived foods, vary from water-soluble phenolic acids and flavonoids to water-insoluble tannins and lignins. They range from simple molecules like vanillin to complex polyphenols, playing a vital role in plant biology (Nollet and Gutierrez-Uribe, 2018). p-Coumaric acid, a hydroxy derivative of cinnamic acid, is a prevalent isomer in nature, particularly found in the cell walls of gramineous plants. Similar results were found in a study conducted by Sharma *et al.* (2019) which concludes that p-Coumaroyl trifolin A, a hydroxycinnamate that was high in Acacia leaf-meal harvested in the hot-dry-season is effective in absorbing ultraviolet-B radiation than flavonoids that have a greater ability to absorb visible light and UV-A wavelengths. Classified as both a phytochemical and nutraceutical, it exhibits antioxidant and antimicrobial properties, contributing to human health by reducing low-density lipoprotein peroxidation. Coumaric acid was found to be abundant in acacia leaf extracts of acacia (Jiang *et al.,* 2020). Gallic acid's antioxidant and anti-inflammatory properties are attributed to its involvement in various signaling pathways, including those regulating inflammation, apoptosis, and NF-κB signalling (Bai *et al.,* 2021). Its derivatives show promise in preventing and managing disorders, making them potential dietary supplements due to their safety and stability. Gallic acid is the second major compound found in leaf extracts of Acacia (Mohamed and Hafez, 2023). Acacia leaves predominantly featured Dihydroxybenzoic acid, Gentisic acid, and Gallic acid. The higher phenolic compounds reported for Acacia might be attributed to its defence mechanism that probably code for more phenolic biosynthesis stress resistance genes ( Ul Haq *et al.,* 2019).

Leaf-derived vitamins act as natural boosters, safeguarding animal health while lifting growth, fertility, and overall productivity. Table 2 summarizes the water soluble and fat soluble vitamins content in acacia leaf extract. Acacia leaf extract rich in water soluble vitamins such as thiamine, niacin, pantothenic acid and some other water soluble vitamins. Higher concentration of vitamin E (Bindawa *et al.,* 2022) followed by vitamin K1 was noticed among fat soluble vitamins. Vitamin E is a group of eight antioxidant lipophilic molecules, four of which are tocopherols and four of which are tocotrienols. It is mostly found in green vegetables, grains, nuts and various vegetable oils, as well as in eggs and milk. Although it is commonly known today for its antioxidant properties, the first biological role attributed to vitamin E was its necessity for fetal survival. Today vitamin E is known to possess many biological properties, including antioxidant activity and the ability to modulate protein function and gene expression (Bindawa *et al.,* 2022).

**Table 1:** LC-MS profile of flavonoids and phenolic compoundsidentified from methanol extract of acacia leaves

| Acacia extract | Compounds | Conc (µg/g of acacia) |
|---|---|---|
| Flavonoids | Umbelliferone | 1.661 |
| | Apigenin | 4.427 |
| | Galangin | 0.006 |
| | Naringenin | 171.673 |
| | Kaemperol | 0.071 |
| | Luteolin | 240.318 |
| | Fisetin | 0.020 |
| | Eriodictyol | 0.003 |
| | Catechin | 676.696 |
| | Epicatechin | 128.692 |
| | Hesperetin | 1134.245 |
| | Quercetin | 7248.917 |
| | Epigallocatechin | 4.683 |
| | Myricetin | 49.981 |
| | Rutin | 3.628 |
| Phenolic acids | Benzoic acid | 0.174 |
| | p-hydroxy benzoic acid | 18.910 |
| | Salicylic acid | 4.667 |
| | 3-Hydroxy benzoic acid | 4.693 |
| | t-Cinnamic acid | 16.009 |
| | 2,4-dihydroxybenzoic acid | 10.386 |
| | Gentisic acid | 16.303 |
| | Protocatechuic acid | 4.041 |
| | p-Coumaric acid | 680.493 |
| | o-Coumaric acid | 220.212 |
| | Vanillic acid | 7.343 |
| | Gallic acid | 5485.043 |
| | Caffeic acid | 35.096 |
| | Ferulic acid | 272.826 |
| | Syringic acid | 1.307 |
| | Sinapic acid | 1.849 |
| | Ellagic acid | 4.371 |
| | Chlorogenic acid | 0.214 |

*Each value is average of duplicate analysis

**Table 2:** LC-MS profile of water soluble and fat solublevitamins identified from acacia leaves

| Type | Name | Amount (ng/g) |
|---|---|---|
| Water Soluble Vitamins | Thiamine | 136.165 |
| | Riboflavin | 0.111 |
| | Niacin | 21.727 |
| | Pantothenicacid | 29.036 |
| | Pyridoxin | 0.745 |
| | Biotin | 0.183 |
| | Folic acid | 0.121 |
| | Cyanocobalamine | 0.372 |
| Fat Soluble Vitamins | Vitamin D1 | 0.147 |
| | Vitamin D2 | 1.698 |
| | Tocopherol | 89348.834 |
| | Vitamin K1 | 1055.799 |
| | Vitamin K2 | 2.544 |

In general, the comprehensive phytochemical and vitamin profiling of *Acacia* leaf extracts confirms their rich composition of bioactive compounds, including flavonoids, phenolic acids, and essential vitamins. Key flavonoids contribute significantly to the antioxidant and therapeutic potential of *Acacia* leaves. Additionally, the presence of water-soluble and fat-soluble vitamins underscores the nutritional value of these leaves. With further research and appropriate processing, *Acacia* leaf extracts can be effectively utilized in the development of natural health products, contributing to sustainable agriculture, improved animal welfare, and preventive healthcare solutions.

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