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Investigation of Mineral Composition, Proximate Constituents, Anti-Nutritional Factor, Antimicrobial Activities and Phytochemical Screening of Aqueous Extract of *Tamarindus indica* Seed

Osigbemhe Izuagbe Gilbert, Kingsley Omoniyi Adebayo and Peace Amedu
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Abstract: *This study investigated the nutritional composition, mineral content, phytochemical constituents, anti-nutritional factors, and antimicrobial activities of aqueous extracts of Tamarindus indica seed. Proximate analysis revealed high levels of crude protein (30.75 ± 3.11%), crude fat (29.23 ± 0.12%), and carbohydrate (25.21 ± 0.02%), indicating the seed's potential as a nutrient-dense food component. The moisture content was 13.00 ± 2.01%, the ash content was 5.07 ± 2.03%, and the crude fiber was 9.73 ± 1.05%. Mineral analysis showed potassium as the most abundant mineral (60.13 mg/kg), followed by sodium (26.00 mg/kg), magnesium (4.22 mg/kg), calcium (3.60 mg/kg), and iron (3.10 mg/kg), with trace levels of manganese and zinc. Qualitative phytochemical screening confirmed the presence of flavonoids, alkaloids, terpenoids, tannins, glycosides, and saponins. Quantitative analysis indicated high levels of flavonoids (173.53 mg/kg), phenolics (95.88 mg/kg), saponins (22.88 mg/kg), and alkaloids (12.02 mg/kg). Anti-nutritional assessment showed tannins (170.00 ± 4.23 mg/100 g), oxalates (85.86 ± 6.32 mg/100 g), phytates (37.40 ± 2.32 mg/100 g), and saponins (30.23 ± 3.20 mg/100 g), all within acceptable safety margins. Antimicrobial activity was evaluated using the agar well diffusion method, with the highest inhibition observed against E. coli (6 mm at 100 mg/mL). Minimum Inhibitory Concentration (MIC) tests confirmed inhibition of E. coli and Staphylococcus at concentrations down to 50 mg/mL, and Salmonella at 75 mg/mL.These findings validate the nutritional and ethnomedicinal potential of Tamarindus indica seeds and support their use as a functional food ingredient and antimicrobial agent. Further studies are recommended to assess the bioavailability and in vivo efficacy of these bioactive compounds.*

Keywords: *Tamarindus indica, proximate composition, phytochemicals, anti-nutritional factors, antimicrobial activity.*

Osigbemhe Izuagbe Gilbert
Department of Chemistry, University of Benin, Benin City, Edo State, Nigeria,
Email: gilbert.osigbemhe@uniben.edu
Orcid id: https://orcid.org/0009-0004-5479-2442

Kingsley Omoniyi Adebayo
Department of Chemistry, Federal University Lokoja, Kogi State, Nigeria
Email: ***koadebayo@ksukabba.edu.ng***
Orcid id: https://orcid.org/0009-0004-5479-2442

Peace Amedu
Department of Chemistry, Federal University Lokoja, Kogi State, Nigeria
Email:

1.0 Introduction

Tamarind (Tamarindus indica), a part of the Fabaceae family and Caesalpinioideae subfamily (Stege et al., 2011), is widely recognized by different names across various cultures. In English, it is simply called tamarind, while it is referred to as "Tsamiya" in Hausa, "Awin" in Yoruba, "Chaleku" or "icheku" in Igbo, and "Ugbe" in Edo. It belongs to the third-largest family of flowering plants, the Leguminosae, which has 727 genera and about 19,327 species. Mahran et al. (1998)

showed that the fruit pulp had significant volatile components, such as carboxylic acids (38.2%) and furan derivatives (44.4%). Gums, pectin, sugars, tannins, alkaloids, flavonoids, sesquiterpenes, glycosides, tartaric acid, acetic acid, and succinic acid are all abundant in the plant's aerial portions. The seeds and pericarp also contain phenolic antioxidants.

In addition to its taste, tamarind is valued for its many health benefits and useful ingredients. Along with trace elements like sodium, zinc, and iron, the pulp and seeds are also good sources of minerals, including potassium, calcium, and phosphorus. Reduced sugars, tartaric acid, pectin, tannins, cellulose, and dietary fibre are all highly concentrated in the fruit, whether it is ripe or dried.

Tamarind seeds are an important source of proteins, lipids, carbs, and sugars. A study by Bhattacharyaa et al. (1997) investigated the tamarind's water absorption capacity, nitrogen solubility index, foaming ability, foam stability, and emulsifying capacity, among other functional properties. The tannin content in tamarind seeds, which is about 23%, makes them useful in leather processing, producing tough, deeply colored leather ideal for items such as soles and luggage. Additionally, the seed husk has shown potential as a natural fish poison. Tannins obtained from tamarind bark are traditionally used for setting dyes and preparing ink.

Tamarind has long been utilized in traditional treatment methods due to its nutritional and therapeutic benefits. The World Health Organization (WHO) states that tamarind includes all important amino acids except tryptophan. Various organic acids, including malic, succinic, and tartaric acids, are also present in tamarind (Ferrara, 2019). In traditional healing, different sections of the tamarind tree are employed to treat a wide range of conditions, from internal ailments to external wounds (Bhadoriya et al., 2011). These bioactive substances help explain why it is useful in controlling and preventing a wide range of medical conditions. Because tamarind contains tartaric and malic acids, its fruit pulp has a laxative effect. It aids in the alleviation of diarrhoea and stomach pain. It prevents liver damage and acts on bile secretion (Rodriguez-Amado et al., 2016).

According to several studies (Ray and Majumdar, 1976; Anon, 2008), tamarind fruits exhibit antifungal and antibacterial qualities. It has a strong fungicidal effect on Aspergillus niger and Candida albicans cultures. Tamarind has antibacterial qualities against Escherichia coli, Klebsiella pneumoniae, antiviral, anthelminthic (gets rid of worms), antiseptic, sunblock, and astringent, according to research by Daniyan and Muhammad (2008). Traditionally, tamarind has been used to treat a variety of illnesses, both simple and complicated. Boils, bacterial skin infections, and respiratory ailments like pneumonia, colds, asthma, and chest pain are all thought to be well-treated by it. It has also been used to treat gastrointestinal issues such as colic, indigestion, diarrhoea, dysentery, gallbladder or liver abnormalities, and constipation (both acute and chronic). Tamarind has also been used to treat conditions relating to the eyes, such as keratitis, irritation, dry eyes, and conjunctivitis. Gingivitis, sore throat, mouth sores, haemorrhoids, joint swelling, sprains, urinary stones, jaundice, pregnancy nausea and vomiting, paratyphoid fever, and wounds are among the other ailments they address. It is also recognized for its ability to lower fever and control the metabolism of cholesterol. Tamarind fruit pulp extracts have been reported to exhibit molluscicidal properties against aquatic snails such as *Bulinus truncatus* (Kloos et al., 2001). In countries like Vietnam and Burkina Faso, tamarind-based formulations have traditionally been utilized for purifying drinking water (Yapo et al., 2008). Additionally, tamarind extracts have been explored for their potential in managing

agricultural pests and microbial infections in crops (Siddhuraju & Becker, 2003). The bioactive compound lupeol, present in young tamarind leaves, has shown inhibitory effects against a range of phytopathogenic fungi (Patel et al., 2012; Sharma & Roy, 2015) and nematodes such as *Meloidogyne incognita* (Khan et al., 2007).Extracts from tamarind pulp that were tested for antibacterial activity showed less activity against A. niger and more activity against S. typhimurium and S. aureus (Jadhav et al., 2010).

The phytochemicals found in tamarind seeds include phenolic antioxidants, including epicatechin, 2-hydroxy-3′,4′-, methyl 3, 4-dihydroxybenzoate, and 3,4-dihydroxyphenyl acetate (Tsuda et al., 1994; Sudjaroenet al., 2005; El-Siddig et al., 2006, Andabatiet al., 2014). *Tamarindus indica* fruit has long been used as a laxative because of its high potassium acid, tartaric, and malic acid content. In vitro, tamarind seed extracts reduce lipid peroxidation, demonstrating their antioxidant properties (Tsuda et al., 1994). In West Africa, Fulani people consume tamarind-soaked fruits to relieve constipation. An effective way to treat wounds from Guinea worm infections is to make a decoction of *Tamarindus indica* leaves. The literature regularly mentions *Tamarindus indica* as a remedy for cuts, wounds, and abscesses. The venom's phospholipase A, protease, hyaluronidase, l-amino acid oxidase, and 5′-nucleotidase enzyme activity were all suppressed in a dose-dependent manner by tamarind seed extract. Tamarind extract prevented the indirect hemolysis brought on by the venom and the breakdown of the human fibrinogen's β-chain. *Tamarindus indica* extract is a viable substitute for serum therapy because it moderately extended the clotting time and significantly counteracted myotoxic effects, including edema and bleeding caused by the venom at varying doses. An isolated polysaccharide from Tamarindus indica has immunomodulatory properties, including leukocyte migration inhibition, cell proliferation inhibition, and phagocytic enhancement (Sreelekha et al., 1993). *Tamarindus indica* methanolic extracts demonstrated exceptional cytotoxic activity against the human amniotic epithelial cell line FL-cells (Al-Fatemiet al., 2007).

Therefore, this study aims to investigate the proximate composition, mineral content, anti-nutritional factors, phytochemical constituents, and antimicrobial activities of aqueous extracts of *Tamarindus indica* seed. Despite the broad pharmacological interest in Tamarindus indica, most research has focused on the fruit pulp and leaves, with limited studies exploring the seed, particularly using aqueous extraction. Additionally, comprehensive studies integrating proximate composition, mineral analysis, anti-nutritional factors, phytochemical screening, and antimicrobial activity remain scarce. This study, therefore, aims to evaluate the nutritional, anti-nutritional, and antimicrobial properties of aqueous extracts of Tamarindus indica seeds. The findings will help validate the traditional uses of the seed and support its application in food and pharmaceutical industries.

Understanding the nutritional and pharmacological profile of Tamarindus indica seed will contribute to its valorization as a functional food component and provide scientific validation for its traditional use in disease management.

2.0 Materials and Methods

The materials used in this study included seeds of *Tamarindus indica*, which served as the plant material for all analyses. The reagents and chemicals employed were of analytical grade and comprised distilled water, ethanol, hexane, methanol, nitric acid (HNO_3), hydrochloric acid (HCl), sodium hydroxide (NaOH), vanillin, sulfuric acid (H_2SO_4), Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3),

aluminum chloride ($AlCl_3$), sodium acetate, tannic acid, ammonium hydroxide, and quercetin standard.

The equipment used for various analyses included a Soxhlet extractor, a moisture analyzer, a UV-Visible spectrophotometer (Jenway 6100, Dunmow, Essex, UK), an atomic absorption spectrophotometer (AVANTA GBC, version 2.02), a laboratory oven, an autoclave, an incubator, and standard laboratory glassware.

2.2 Collection and Identification of Plant Material

Fresh seeds of *Tamarindus indica* were collected on May 23, 2024, from the International Market in Lokoja, Kogi State, Nigeria. The plant was identified and authenticated by a botanist at the Department of Botany, University of Benin, Edo State, Nigeria. A voucher specimen was deposited in the university's herbarium with the identification number: Herbarium No. 0273.

2.3 Preparation of Plant Sample

The collected seeds were washed thoroughly to remove any debris and then air-dried in shade at room temperature to prevent photodegradation of active compounds. The dried seeds were ground into fine powder using an electric blender and stored in airtight zip-lock bags until further analysis.

2.4 Extraction of Sample

For aqueous extraction, 10.00 g of the powdered seed material was macerated in 100 mL of distilled water. The mixture was left to stand at room temperature for 72 hours with intermittent shaking. After extraction, the mixture was filtered through Whatman No. 1 filter paper, and the filtrate was stored in airtight containers at 4°C for subsequent analyses.

2.5 Proximate Analysis

The proximate composition of the seed powder—including moisture content, crude protein, crude fiber, crude fat, ash content, and carbohydrate—was determined using standard methods described by the Association of Official Analytical Chemists (AOAC, 2019). All analyses were performed in triplicate, and the mean values were reported.

2.6 Quantitative Phytochemical Analysis

2.6.1 Total Phenolic Content

The total phenolic content was determined using the Folin–Ciocalteu method as described by Singleton and Rossi (1965), with slight modifications. A 1.0 mL aliquot of the extract (250 µg/mL) was mixed with 1.0 mL Folin–Ciocalteu reagent and allowed to react for 5 minutes. Then, 15.0 mL of 20% Na_2CO_3 was added. The mixture was incubated for 2 hours at room temperature, and absorbance was measured at 760 nm. Results were expressed as tannic acid equivalents (TAE).

2.6.2 Alkaloid Content

Alkaloid content was determined using Harborne's method (1973). A total of 5.0 g of the sample was extracted with 100 mL of 20% acetic acid in ethanol for 2 hours. The mixture was filtered and concentrated to one-fourth of its volume. Concentrated ammonium hydroxide was added dropwise to precipitate the alkaloids. The precipitate was washed with 1% NH_4OH, dried, and weighed. Alkaloid content (%) was calculated using the following equation,

$$Alkaloid\ (\%) = \frac{Weight\ of\ residue}{Weight\ of\ the\ sample} \times \frac{100}{1} \quad (1)$$

2.6.3 Flavonoid Content

Flavonoid content was determined following Ilahy et al. (2011). A 30 µL aliquot of methanolic extract was mixed with 90 µL methanol, 6 µL of 10% $AlCl_3$, 6 µL of 1 M sodium acetate, and additional methanol to make 170 µL. The mixture was incubated for 30 minutes at room temperature. Absorbance was measured at 415 nm, and results were expressed as quercetin equivalents (QE) per gram of sample.

2.6.4 Saponin Content

Saponin was quantified using a vanillin–sulfuric acid colorimetric method. A 50 µL aliquot of the extract was mixed with 250 µL vanillin reagent and 2.5 mL of 72% H_2SO_4. The mixture was incubated at 60°C for 10 minutes, then cooled in ice water. Absorbance was recorded at 570 nm. Saponin concentration was calculated using a standard curve and expressed in parts per million (ppm).

2.6.5 Tannin Content

Tannin content was determined spectrophotometrically using the Folin–Denis method. A 0.2 mL sample was extracted with 20 mL of 50% methanol, heated at 80°C for 1 hour, and filtered. To 1 mL of filtrate, 2.5 mL Folin–Denis reagent, 10 mL of 17% Na_2CO_3, and distilled water were added to make 50 mL. Absorbance was measured at 760 nm. Tannin content was expressed as TAE.

2.7 Mineral Content Analysis

Mineral elements—including magnesium, calcium, potassium, sodium, iron, copper, and manganese—were analyzed using atomic absorption spectrophotometry (AAS) following standard AOAC (2006) procedures. Samples were digested with a mixture of nitric acid and perchloric acid before analysis.

2.8 Anti-Nutritional Factor Analysis

Anti-nutritional factors, including phytate, oxalate, tannin, and saponin, were analyzed using standard methods described by Khan et al. (2023) and Sewell et al. (2016). Both qualitative and quantitative evaluations were carried out. Results were expressed in mg/100 g of dry weight.

2.9 Antimicrobial Screening

2.9.1 Test Microorganisms

The antimicrobial activity was tested against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*. Pure cultures of these organisms were obtained from a microbiology laboratory and maintained on nutrient agar slants.

2.9.2 Culture Media

The following media were used: Mueller–Hinton agar (MHA), Mueller–Hinton broth (MHB), nutrient agar (NA), and potato dextrose agar (PDA). All media were prepared according to the manufacturer's instructions and sterilized at 121°C for 15 minutes.

2.9.3 Agar Well Diffusion Assay

The agar well diffusion method was used to assess antimicrobial activity. Standardized bacterial inocula were swabbed onto MHA plates. Wells (6 mm in diameter) were bored into the agar and filled with 0.2 mL of extract concentrations (12.5, 25, 50, and 100 mg/mL). Plates were allowed to stand for 1 hour to allow diffusion and then incubated at 37°C for 24 hours. Zones of inhibition were measured in millimeters.

2.9.4 Minimum Inhibitory Concentration (MIC)

MIC was determined by the tube dilution method using MHB. Serial dilutions of the extract were made in test tubes. Each tube was inoculated with a standardized suspension of the test organism and incubated at 37°C for 24 hours. The MIC was recorded as the lowest concentration that showed no visible growth.

2.9.5 Minimum Bactericidal Concentration (MBC)

After the MIC test, aliquots from tubes with no visible growth were subcultured onto nutrient agar plates. Plates were incubated at 37°C for 24 hours. MBC was defined as the lowest concentration of extract that prevented visible growth on the agar plate.

3.0 Results and Discussion

Table 1 presents the proximate composition of *Tamarindus indica* seed, offering a comprehensive overview of its nutritional profile. The analysis includes moisture content,

ash content, crude fiber, crude protein, crude fat, and carbohydrate content, each expressed as a percentage of the seed's dry weight.

The moisture content of 13.00 ± 2.01% indicates that the seed has moderate water content, which is favourable for storage stability as lower moisture levels generally reduce the risk of microbial spoilage and prolong shelf life. The ash content, measured at 5.07 ± 2.03%, reflects the total mineral matter in the seed, suggesting a good presence of inorganic nutrients that contribute to dietary mineral intake. The seed contains 9.73 ± 1.05% crude fiber, which supports gastrointestinal health by enhancing bowel movement and preventing constipation. Dietary fibre also plays a role in lowering cholesterol levels and regulating blood sugar, contributing to the seed's potential as a functional food.

Table 1: Proximate Composition of *Tamarindus indica* Seed

Sample	% Moisture	% Ash	% Crude Fiber	% Crude Protein	% Crude Fat	% Carbohydrate
T. indica	13.00 ± 2.01	5.07 ± 2.03	9.73 ± 1.05	30.75 ± 3.11	29.23 ± 0.12	25.21 ± 0.02

A notably high crude protein content of 30.75 ± 3.11% underscores the seed's value as a plant-based protein source. This makes it particularly useful in regions where protein deficiency is common and where plant-derived proteins are an important dietary component. Proteins also play vital roles in tissue repair, enzymatic activities, and immune functions.

The crude fat content is also relatively high, recorded at 29.23 ± 0.12%, suggesting the seed may be a valuable source of energy and essential fatty acids. Fats contribute to cellular structure, hormone production, and the absorption of fat-soluble vitamins.

Lastly, the carbohydrate content of 25.21 ± 0.02% indicates that the seed contributes a significant amount of energy to the diet, complementing the fat and protein content. Carbohydrates are essential for energy metabolism and cellular function.

Altogether, the proximate analysis of *Tamarindus indica* seed reveals a nutrient-rich composition characterized by high protein and fat contents, along with appreciable levels of fiber and carbohydrates. These properties highlight its potential utility in addressing nutritional deficiencies, especially as an affordable plant-based protein and energy source. The findings support its use in traditional diets and open up possibilities for its incorporation into functional food products and dietary supplements.

Table 2 presents the mineral composition of *Tamarindus indica* seed, analyzed in three replicates and expressed in milligrams per kilogram (mg/kg). This data reveals the presence of essential macro- and micro-elements that contribute to the nutritional and physiological functions of the seed when consumed as part of the human diet.

Table 2: Mineral Composition of Tamarind Seed (mg/kg)

S/N	Parameter	Rep 1	Rep 2	Rep 3
1	Calcium	3.410	3.360	3.600
2	Potassium	58.340	60.130	58.720
3	Iron	3.100	2.530	2.360
4	Zinc	0.400	0.350	0.310
5	Magnesium	3.718	4.224	3.145
6	Sodium	23.490	26.000	24.129
7	Manganese	0.740	0.560	0.631

Among the minerals quantified, potassium was present in the highest concentration, ranging between 58.340 and 60.130 mg/kg across the three replicates. Potassium is a vital electrolyte that plays a central role in fluid balance, nerve transmission, and muscle contraction. The high potassium content complements the nutritional profile observed in Table 1, where the seed was found to be rich in protein and fat, further confirming the potential of the seed as a functional food ingredient.

Sodium, another important electrolyte, showed substantial levels ranging from 23.490 to 26.000 mg/kg. The presence of both potassium and sodium in moderate to high quantities supports ionic regulation, cardiovascular health, and maintenance of osmotic balance in the human body. Notably, the sodium-to-potassium ratio is favourable, which is beneficial for managing hypertension and reducing the risk of heart disease.

Magnesium was recorded between 3.145 and 4.224 mg/kg, indicating the seed's contribution to enzymatic functions, energy metabolism, and muscle function. Similarly, calcium concentrations ranged from 3.360 to 3.600 mg/kg, providing a moderate source of this mineral crucial for bone formation, blood clotting, and neuromuscular signaling.

The presence of iron (2.360–3.100 mg/kg) signifies the seed's role in hemoglobin synthesis and oxygen transport. While the iron levels are modest, they are still meaningful, particularly in diets where iron-rich animal products are limited. Zinc (0.310–0.400 mg/kg), though present in lower amounts, is important for immune function, DNA synthesis, and cellular repair. Manganese, detected in trace amounts between 0.560 and 0.740 mg/kg, also supports enzymatic activity and contributes to bone development and antioxidant defense mechanisms.

In comparison with the proximate analysis in Table 1, the mineral content of *Tamarindus indica* seed aligns with its classification as a nutrient-dense plant product. The seed's richness in proteins and fats (30.75% and 29.23%, respectively), combined with its moderate mineral content, suggests that it can serve both macronutrient and micronutrient roles in food formulations. The combined presence of magnesium, iron, and zinc further enhances its nutritional significance, particularly in populations susceptible to mineral deficiencies. Overall, the mineral analysis underscores the seed's nutritional and health-promoting potential, making it suitable for dietary diversification and functional food development. The consistent presence of essential elements across all replicates also demonstrates the reliability and uniformity of the seed's mineral profile.

Table 3 presents the qualitative phytochemical composition of *Tamarindus indica* seed, revealing the presence and relative abundance of key bioactive compounds using standard phytochemical screening procedures. Each compound is marked by its relative intensity: strong presence (+++), moderate presence (++), low presence (+), or absence (–).

Table 3: Qualitative Phytochemical Composition of Tamarind Seed

Phytochemicals	Presence
Flavonoids	+++
Terpenoids	+++
Tannins	++
Steroids	++
Saponins	+
Phlobatannins	+
Alkaloids	+++
Carbohydrates	++
Glycosides	++
Volatile oil	++
Coumarin glycosides	+
Cardiac glycosides	+++
Anthraquinone	+

The phytochemical profile of *Tamarindus indica* seed indicates a rich abundance of secondary metabolites, particularly flavonoids, terpenoids, alkaloids, and cardiac glycosides, all marked with a strong presence (+++). These compounds are well known for their potent antioxidant, anti-inflammatory, antimicrobial, and cardioprotective properties, underscoring the pharmacological significance of the seed.

The presence of tannins, steroids, volatile oils, carbohydrates, and glycosides at moderate levels (++) further enhances the medicinal and nutritional potential of the seed. Tannins, for instance, while traditionally classified as anti-nutrients, possess notable antimicrobial and free-radical scavenging abilities. Steroids may contribute to hormonal balance and immune function, while glycosides and volatile oils are known to exhibit a wide range of bioactivities, including antimicrobial, expectorant, and anti-hypertensive effects.

Compounds such as saponins, phlobatannins, coumarin glycosides, and anthraquinone were detected in low quantities (+). Saponins have been documented to exhibit cholesterol-lowering, immune-stimulating, and anticancer effects. However, as also indicated in Table 1 and Table 5, their anti-nutritional effects—such as protein precipitation and gastrointestinal irritation—require processing techniques (e.g., heating, fermentation) to minimize adverse impacts. The detection of anthraquinones suggests mild laxative potential and supports traditional use of tamarind in treating digestive ailments.

When compared with the proximate composition in Table 1, the phytochemical richness adds to the already substantial nutritional value of *T. indica* seed, which was shown to have high levels of crude protein (30.75%) and fat (29.23%). This reinforces the argument that the seed provides both macronutritional and medicinal benefits. The phytochemicals identified could also play a role in preserving the seed during storage by acting as natural antimicrobials and antioxidants. Also, correlating the findings in Table 2, the presence of mineral elements such as iron, zinc, calcium, and magnesium complements the phytochemicals with enzymatic cofactors necessary for bioactivity and metabolic reactions. For example, flavonoids and alkaloids often require trace elements like zinc and magnesium for full expression of their antioxidant potential.

In summary, the data in Table 3 highlight the multifunctional nature of *Tamarindus indica* seed, combining nutritive compounds with a dense array of phytochemicals. This makes the seed a candidate for development into functional foods, dietary supplements, or phytopharmaceutical formulations, especially in regions where malnutrition and microbial infections are prevalent. The results support its traditional medicinal applications and justify further pharmacological studies and product development initiatives.

Table 4 presents the quantitative phytochemical screening of *Tamarindus indica* seed, offering detailed concentration values (in mg/kg) for five major classes of bioactive compounds. These include alkaloids, tannins, total phenolics, saponins, and flavonoids, assessed across three replicates to ensure consistency and reproducibility of results.

Table 4: Quantitative Phytochemical Screening of Tamarind Seed (mg/kg)

S/N	Phytochemical	Rep 1	Rep 2	Rep 3
1	Alkaloid	8.468	12.024	10.541
2	Tannins	1.499	1.193	2.735
3	Phenolics	94.350	90.172	95.880
4	Saponins	22.880	22.660	22.630
5	Flavonoids	162.553	173.525	157.746

The most abundant phytochemicals quantified in the *Tamarindus indica* seed extract were flavonoids, ranging from 157.75 to 173.53 mg/kg. These compounds are well-documented for their antioxidant, anti-inflammatory, and antimicrobial properties, suggesting a strong potential for disease prevention and therapeutic use. The high flavonoid content aligns well with the qualitative data presented in Table 3, where flavonoids were recorded as strongly present (+++). This rich flavonoid profile supports the use of *T. indica* seed in traditional medicine for treating infections, inflammation, and metabolic disorders.

Phenolic compounds, which include flavonoids as a subgroup, also appeared in significant concentrations (90.17–95.88 mg/kg). Phenolics are recognized for their free radical scavenging activity, and their presence in high amounts reinforces the antioxidant capability of the seed. Together, the high levels of flavonoids and phenolics highlight the seed's potential role as a functional food ingredient or natural preservative in food and pharmaceutical applications.

Saponins were also present in moderate amounts (22.63–22.88 mg/kg), consistent with their moderate detection in Table 3 (+). These compounds are known for their hypocholesterolemic, antimicrobial, and immunomodulatory effects. While saponins can exhibit anti-nutritional characteristics at very high levels, the concentration observed here falls within the beneficial range (10–50 mg/kg), as supported by previous nutritional safety studies. This suggests a dual functionality—therapeutic benefit alongside manageable dietary impact. Alkaloids ranged from 8.47 to 12.02 mg/kg, showing appreciable levels that correspond to their strong presence (+++) in Table 3. Alkaloids have well-documented antibacterial, antimalarial, and analgesic activities, and their presence supports the antimicrobial results observed in Tables 6 and 7, where *Tamarindus indica* seed extract showed inhibitory effects against *E. coli*, *Staphylococcus*, and *Salmonella*.

Tannins were present in the lowest quantities (1.19–2.74 mg/kg), consistent with their moderate qualitative presence in Table 3 (++). Despite being considered anti-nutritional due to their protein-binding ability, tannins also contribute antioxidant, anti-inflammatory, and astringent properties. The moderate levels reported here, especially when interpreted alongside the proximate data in Table 1, suggest a safe and potentially beneficial concentration. The protein content (30.75%) and fiber content (9.73%) may interact with tannins, but at this level, any negative effect on protein digestibility is likely minimal.

When compared with Table 1, the phytochemical concentrations reinforce the nutritional and therapeutic potential of the seed. The presence of high protein and fat contents aligns well with the significant bioactive compound levels, making the seed not only a nutrient-dense but also a phytochemically active component. Likewise, the results complement the mineral composition in Table 2, where elements like iron, calcium, and zinc support the metabolic roles of flavonoids and phenolics in cellular repair and immune response.

In summary, the quantitative data from Table 4 confirm that *Tamarindus indica* seed is rich in bioactive compounds, particularly flavonoids and phenolics, which confer significant antioxidant, antimicrobial, and functional health benefits. These findings justify its continued exploration in both nutraceutical development and functional food formulations, while also validating its traditional medicinal use.

Table 5 presents the quantified concentrations of four major anti-nutritional factors—phytate, oxalate, tannin, and saponin—in the aqueous extract of *Tamarindus indica* seed. These compounds are naturally occurring in many plant-based foods and are known to influence

the bioavailability of nutrients and overall digestibility of food products. While they are often associated with negative nutritional implications, several of these compounds also possess valuable pharmacological and health-promoting effects, depending on their concentration and dietary context.

Table 5: Anti-Nutritional Factors in Aqueous Extract of *Tamarindus indica* Seed

Component	Value (mean ± SD) (mg/100g)
Phytate	37.40 ± 2.32
Oxalate	85.86 ± 6.32
Tannin	170.00 ± 4.23
Saponin	30.23 ± 3.20

Among the anti-nutritional factors quantified, tannins recorded the highest concentration (170.00 ± 4.23 mg/100 g), followed by oxalates (85.86 ± 6.32 mg/100g), phytates (37.40 ± 2.32 mg/100g), and sa ponins (30.23 ± 3.20 mg/100g). The high tannin content indicates a significant presence of polyphenolic compounds that can bind to proteins and minerals, reducing their digestibility and bioavailability. However, this concentration is consistent with the findings of Tables 3 and 4, where tannins were detected moderately (++ in Table 3) and quantified between 1.19 to 2.74 mg/kg in Table 4. The discrepancy in units suggests that the values in Table 5 represent the total tannin content in the raw extract, encompassing both free and bound forms. Despite their anti-nutritional reputation, tannins have demonstrated antioxidant, antimicrobial, and anti-inflammatory properties, indicating a dual nutritional and pharmacological role.

Oxalates were present at 85.86 ± 6.32 mg/100g, a concentration that, while moderately high, remains below the toxicity threshold typically cited in literature (200 mg/100g) for plant-based foods. Oxalates can bind calcium to form insoluble calcium oxalate, potentially affecting calcium absorption and contributing to kidney stone formation in susceptible individuals. However, when evaluated alongside the calcium content in Table 2, which ranged between 3.36 and 3.60 mg/kg, the oxalate-to-calcium ratio suggests that while some binding may occur, the overall dietary impact may be minimized through food processing or combining the seed with calcium-rich foods.

Phytate content was 37.40 ± 2.32 mg/100g, which falls within the lower to moderate range typically reported for legumes and oilseeds (30–90 mg/100g). Phytates can reduce the bioavailability of minerals such as iron, zinc, and calcium by chelating them in the gastrointestinal tract. This finding correlates with the moderate levels of iron (2.36–3.10 mg/kg), zinc (0.31–0.40 mg/kg), and magnesium (3.15–4.22 mg/kg) recorded in Table 2, suggesting some inhibition of absorption may occur. Nonetheless, phytates also exhibit anticancer, antioxidant, and cholesterol-lowering properties, supporting their inclusion in functional food applications when levels are managed.

Saponins were recorded at 30.23 ± 3.20 mg/100 g, a concentration that aligns closely with the values found in Table 4 (22.63–22.88 mg/kg) and their weak qualitative presence in Table 3 (+). Although saponins may reduce protein digestibility and cause gastrointestinal irritation at high doses, they are also recognized for their hypocholesterolemic, antimicrobial, anti-inflammatory, and immunomodulatory effects. The value observed here falls within the tolerable and potentially beneficial range (10–50 mg/100g), supporting the idea that *T. indica* seeds may serve not only as a nutritional supplement but also as a source of bioactive compounds for pharmaceutical or nutraceutical use.

When compared to Table 1, which showed high levels of crude protein (30.75%), fat (29.23%), and carbohydrate (25.21%), the anti-nutritional factors in Table 5 may have some modulating

effect on nutrient utilization, particularly for protein and mineral absorption. However, none of the values recorded exceed known safety thresholds, and traditional processing methods such as boiling, roasting, fermentation, or soaking could substantially reduce these compounds, enhancing the seed's nutritional quality. Finally, Table 5 confirms that *Tamarindus indica* seed contains appreciable levels of anti-nutritional compounds, particularly tannins and oxalates. However, these compounds also possess pharmacological properties that may enhance the functional and therapeutic value of the seed extract. The results underscore the importance of balancing nutritional benefits with processing methods to reduce anti-nutrient levels, thereby maximizing the health-promoting potential of the seed as a food or nutraceutical ingredient.

Table 6 presents the minimum zone of inhibition (MZI) values in millimeters, used to assess the antimicrobial activity of *Tamarindus indica* seed aqueous extract against selected pathogenic bacteria—*Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*. The agar well diffusion method was used to measure bacterial growth inhibition at varying extract concentrations (100, 75, 50, and 25 mg/mL), alongside a positive control (standard antibiotic) and a negative control (distilled water). This table helps to understand the concentration-dependent antibacterial potential of the plant extract and serves as a critical indicator of its therapeutic relevance.

Table 6: Minimum Zone of Inhibition (mm)

Isolate	100 mg/mL	75 mg/mL	50 mg/mL	25 mg/mL	Positive Control	Negative Control
E. coli	6	4	2	0	10	0
Staphylococcus	5	3	1	0	10	0
Salmonella	2	3	0	0	10	0

The data reveal a clear trend of dose-dependent inhibition, as the antimicrobial activity increases with concentration. At 100 mg/mL, the extract exhibited moderate zones of inhibition against *E. coli* (6 mm), *Staphylococcus aureus* (5 mm), and *Salmonella typhi* (2 mm). A notable reduction in inhibitory effect was observed at 75 mg/mL, with inhibition zones dropping to 4 mm, 3 mm, and 3 mm, respectively. At 50 mg/mL, only *E. coli* and *Staphylococcus* exhibited inhibition zones (2 mm and 1 mm, respectively), while *Salmonella* showed no inhibition. At 25 mg/mL, no bacterial inhibition was recorded across all test organisms. In contrast, the positive control (antibiotic) showed a consistent zone of 10 mm for all isolates, confirming the susceptibility of the bacteria and validating the efficacy of the assay. The negative control produced no zones of inhibition, confirming that distilled water alone had no antibacterial activity and reinforcing that the observed inhibition was due to the extract.

The comparative potency of the extract against *E. coli* suggests that Gram-negative bacteria may be more sensitive to the phytochemical components in *Tamarindus indica* seed extract at higher concentrations. *Staphylococcus aureus*, a Gram-positive bacterium, also showed measurable sensitivity, although to a slightly lesser degree. *Salmonella typhi* displayed the least sensitivity overall, with no inhibition at concentrations below 75 mg/mL. This resistance could be attributed to the structural features of its outer membrane or its natural resistance mechanisms.

Linking these results with the phytochemical data in Tables 3 and 4, the extract's observed antibacterial effects can be partially attributed

to the high levels of flavonoids (162.553–173.525 mg/kg), phenolics (90.172–95.880 mg/kg), and alkaloids (8.468–12.024 mg/kg), all of which are well-documented for their antimicrobial activity. Flavonoids and phenolics, for example, are known to disrupt microbial cell walls and interfere with nucleic acid synthesis, while alkaloids can intercalate into bacterial DNA or inhibit protein synthesis. Saponins, also present at moderate levels (22.63–22.88 mg/kg), may contribute by increasing membrane permeability in microbial cells. The qualitative phytochemical screening (Table 3) further supports these observations, indicating the presence of alkaloids, flavonoids, glycosides, and cardiac glycosides—all of which have been linked to antimicrobial efficacy in previous studies.

Comparing the results of Table 6 with the nutritional and anti-nutritional profiles in Tables 1 and 5, it becomes evident that the *Tamarindus indica* seed is not only rich in nutritional compounds such as protein (30.75%) and fat (29.23%), but also contains significant bioactive compounds capable of offering antimicrobial defence. Interestingly, some anti-nutritional factors like tannins (170.00 ± 4.23 mg/100g) and saponins (30.23 ± 3.20 mg/100g) also play roles in antimicrobial mechanisms by inhibiting microbial enzymes or forming complexes with cell wall proteins.

The implications of these findings suggest that aqueous extracts of *Tamarindus indica* seed possess modest but measurable antibacterial activity at higher concentrations, particularly against *E. coli* and *Staphylococcus aureus*. This supports its traditional use in managing infectious diseases, especially gastrointestinal and skin infections. However, the relatively weak inhibition zones compared to standard antibiotics indicate that while the extract may not serve as a standalone antimicrobial agent, it may enhance the efficacy of conventional treatments or be integrated into alternative therapies when properly standardized and possibly combined with other bioactive agents.

Table 7 presents the minimum inhibitory concentration (MIC) results for the aqueous extract of *Tamarindus indica* seed against three pathogenic bacteria: *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*. The MIC represents the lowest concentration of the extract at which visible bacterial growth is completely inhibited, thereby reflecting the bacteriostatic potential of the plant extract.

Table 7: Minimum Inhibitory Concentration (MIC) Result

Bacteria	100 mg/mL	75 mg/mL	50 mg/mL	25 mg/mL
E. coli	+	+	+	–
Staphylococcus	+	+	+	–
Salmonella	+	+	–	–

In this table, the symbol "+" indicates visible growth (no inhibition), while "–" indicates no growth (complete inhibition). The MIC values demonstrate that *E. coli* and *Staphylococcus aureus* were inhibited at a concentration of 25 mg/mL, while *Salmonella typhi* required a minimum concentration of 50 mg/mL for inhibition. These results confirm that the antimicrobial activity of the aqueous extract is both organism-specific and dose-dependent.

When compared with the zone of inhibition values in Table 6, the MIC results validate the observed sensitivity patterns. In Table 6, both *E. coli* and *Staphylococcus* showed zones of inhibition at concentrations as low as 50 mg/mL, although reduced in diameter, with complete loss of inhibition at 25 mg/mL. This corresponds well with the MIC data, which shows that bacterial growth was only fully inhibited at or above 25 mg/mL. *Salmonella*,

which showed no inhibition at 50 mg/mL in Table 6, aligns with its MIC value of 50 mg/mL in Table 7, highlighting its relatively higher resistance to the extract.

The MIC results also correlate with the phytochemical composition reported in Tables 3 and 4. The strong presence of antimicrobial phytochemicals—particularly flavonoids (up to 173.525 mg/kg), phenolics (up to 95.880 mg/kg), and alkaloids (up to 12.024 mg/kg)—can be attributed to the observed inhibitory effects. These bioactive compounds are known to exert their antimicrobial properties through mechanisms such as cell wall disruption, protein denaturation, and inhibition of DNA replication in bacteria. Furthermore, the modest but significant levels of saponins (22.880–22.660 mg/kg) and tannins (1.193–2.735 mg/kg) also contribute to microbial inhibition by altering membrane permeability and interacting with microbial enzymes.

Linking these findings with Table 1, which shows the nutritional composition of the seed (notably high protein at 30.75% and fat at 29.23%), it is evident that *Tamarindus indica* seed offers both nutritional benefits and functional bioactivity. This duality enhances its potential as a functional food component or a nutraceutical agent. The results in Table 5, which document moderate levels of anti-nutritional factors such as tannins (170.00 ± 4.23 mg/100g), oxalates, and phytates, support the antimicrobial findings since these compounds—though considered anti-nutrients—also exhibit antimicrobial effects.

Taken together, the MIC data in Table 7 reinforce the view that *Tamarindus indica* seed extract possesses dose-dependent antimicrobial activity, particularly against *E. coli* and *Staphylococcus aureus*. However, compared to standard antibiotics (positive control in Table 6), the efficacy is lower, suggesting that while the extract holds promise for complementary antimicrobial use, it is not a direct substitute for conventional antibiotics. Nevertheless, the data affirm its traditional use for managing infections and support further exploration into its therapeutic potential, especially in developing plant-based antimicrobial formulations or preservatives for food and pharmaceutical applications.

4.0 Conclusion

The study investigated the proximate composition, mineral content, phytochemical constituents, anti-nutritional factors, and antimicrobial activity of aqueous extracts of *Tamarindus indica* seed. The findings revealed that the seed contains appreciable amounts of crude protein (30.75%), crude fat (29.23%), and carbohydrate (25.21%), indicating a rich nutritional profile. Mineral analysis showed high levels of potassium and sodium, with moderate concentrations of calcium, magnesium, iron, and trace elements such as zinc and manganese, all of which are essential for metabolic, cardiovascular, and immune functions. Qualitative and quantitative phytochemical screening confirmed the presence of important bioactive compounds including flavonoids, alkaloids, phenolics, tannins, and saponins. These compounds are associated with antioxidant, antimicrobial, and therapeutic properties.

The study also recorded moderate levels of anti-nutritional factors such as tannins, oxalates, phytates, and saponins. Although these compounds may interfere with nutrient absorption, their concentrations were within safe consumption limits and could be further reduced through conventional processing techniques. The antimicrobial assessment showed that the aqueous extract exhibited inhibitory activity against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*, with the highest sensitivity observed at 100 mg/mL concentration, suggesting potential for therapeutic or preservative applications.

In conclusion, the aqueous extract of *Tamarindus indica* seed possesses a rich nutritional profile and contains significant

phytochemicals and minerals that support both dietary and medicinal use. Despite the presence of anti-nutritional factors, their levels do not pose significant toxicity risks and may be mitigated through proper processing. The extract's antimicrobial activity further reinforces its ethnopharmacological relevance. It is recommended that future research focus on in vivo studies to validate the bioavailability and therapeutic efficacy of the seed components. Additionally, integrating appropriate processing methods will enhance its application as a functional food ingredient or in pharmaceutical formulations.

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Declaration

Consent for publication

Not applicable

Availability of data

Data shall be made available on demand.

Competing interests

The authors declared no conflict of interest

Ethical Consideration

Not applicable

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Authors' Contribution

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