

Extraction, Characterization, and Biological Activities of Phytochemicals from Terminalia Arjuna (Arjuna) Plant

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Abstract

This study focuses on the comprehensive characterization and biological activities of phytochemicals from the bark of Terminalia Arjuna (Arjuna). The bark, known for its medicinal properties, was extracted using methanol and analyzed for its phytochemical profile. The study employed various spectroscopic methods including Fourier transform infrared (FTIR), ¹H NMR and ¹³C NMR spectroscopy. The antioxidant and anti-inflammatory activities were also evaluated while antimicrobial activity was assessed against both Gram-positive and Gram-negative bacteria. Additionally, the study included characterization by nuclear magnetic resonance (NMR) spectroscopy (¹H and ¹³C) and infrared (IR) spectroscopy, as well as the determination of acid value, peroxide value, and the presence of aflatoxins. The results provide valuable insights into the potential therapeutic applications of Terminalia arjuna and its phytochemical constituents.

Keywords: Arjuna, Phytochemicals, Medicinal properties, Characterization, Applications

1.Introduction

Medicinal plants [1] have been a cornerstone of traditional medicine for centuries, offering a rich source of bioactive compounds with diverse therapeutic properties. These plants have been extensively used in various forms to treat a wide range of ailments, from common infections to complex diseases. The importance of medicinal plants lies in their ability to provide natural, cost-effective, and often safer alternatives to synthetic drugs. One such plant, Terminalia Arjuna (Arjuna), has been revered in traditional medicine [2] for its numerous health benefits, particularly in the realm of cardiovascular health.

Terminalia Arjuna is a deciduous tree native to the Indian subcontinent, known for its rich bark, which has been used for centuries to treat various cardiovascular conditions, including anginal pain, hypertension, and congestive heart failure. The bark of the Arjuna tree is a treasure trove of phytochemicals, including flavonoids, phenolic compounds, tannins, and glycosides, which are known to exhibit a wide range of biological activities. These compounds have been shown to possess antioxidant, anti-inflammatory, and antimicrobial properties, making them valuable in the prevention and treatment of various diseases.



The bark of Terminalia Arjuna has been extensively studied for its medicinal properties, and its extracts have been found to possess inotropic, anti-ischemic, antioxidant, blood pressure lowering, antiplatelet, hypolipidemic, antiatherogenic, and antihypertrophic activities [3-5]. These properties make it an attractive candidate for the development of novel therapeutic agents for cardiovascular diseases.

This present study aims to extract, characterize, and evaluate the biological activities of phytochemicals from the bark of Terminalia Arjuna. The study employs various analytical techniques to identify and quantify the phytochemical compounds present in the bark extracts. The antimicrobial and antioxidant activities of these extracts are evaluated against human pathogenic microorganisms. The results of this study provide valuable insights into the potential therapeutic applications of Terminalia Arjuna and its phytochemical constituents.

2. Experimental

2.1. Chemicals, used

All chemicals used for the experimental were of AR grade. And, all the solutions were made in demineralized water.

2.2. Instruments used

FTIR study was carried out by using Perkin Elmer Spectrum instrument whereas ¹H NMR and ¹³C NMR have been conducted by NMR JEOL Delta Software instrument.

2.3. Preparation of T. Arjuna extract

The bark of Terminalia Arjuna was collected, washed, air-dried, finely ground into powder, and stored in airtight containers. The extraction involved cleaning, drying (shade-drying to prevent degradation), and grinding to increase surface area. The powder was mixed with distilled water (1:10 ratio) and heated at 60°C for 24 hours. After cooling, the extract was filtered through muslin cloth and Whatman filter paper, then stored at 4°C. This extract, used for biological assays, was prepared under strict quality control to ensure purity and reliability, and tested for heavy metals, acid value, and peroxide value.

2.4. Phytochemical analysis

Phytochemical analysis is vital in medicinal plant research for identifying and quantifying bioactive compounds. Techniques like IR spectroscopy, and NMR spectroscopy have been used to analyze these compounds, each offering unique insights: IR identifies functional groups, and NMR reveals molecular structures. Mass spectrometry provides molecular weight and fragmentation patterns, while elemental analysis determines elemental composition. Bioassays evaluate biological activities such as antioxidant, antimicrobial, and anti-inflammatory properties. In this study, FTIR, ¹H-NMR, and ¹³C-NMR spectroscopy were employed to characterize the molecular structure and composition of the plant extract.

A number of reaction tests such as, Salkowski reaction test for phytosterols, Liebermann-Burchard's test for triterpenoids, Foam test for saponins, Dragendroff's test for alkaloids, Lead



acetate test for flavonoids, Legal's test for lactones, Keller-Killiani test for glycosides, Ninhydrin test for proteins have been performed and the results are depicted in Table 1.

Table 1: Results of various reaction tests on *T. Arjuna* bark extract

S.N.	Name of Test	Results
1	Salkowski reaction test for phytosterols	Present
2	Liebermann-Burchard's test for triterpenoids	Present
3	Foam test for saponins	Present
4	Dragendroff's test for alkaloids	Present
5	Lead acetate test for flavonoids	Present
6	Legal's test for lactones	Present
7	Keller-Killiani test for glycosides	Present
8	Ninhydrin test for proteins	Present

2.5. Test for heavy metals

The test for heavy metals is a crucial aspect of quality control in the analysis of medicinal plants. Heavy metals, such as lead, cadmium, mercury, and arsenic, can have toxic effects on human health, and their presence in medicinal plants can pose serious risks to consumers. The test for heavy metals involves various techniques and methods, each with its own set of principles and applications. In the present study, we analyzed the levels of lead, cadmium, mercury, and arsenic, known as the most toxic metals, with the assistance of an external agency, reported in Table- 2.

Table 2: Results on tested heavy metals in *T. Arjuna* bark extract

S.N.	Name of Heavy Metal	Results	Limit
1	Lead	Less than 10 ppm	NMT 10 ppm
2	Cadmium	Less than 0.3 ppm	NMT 0.3 ppm
3	Mercury	Less than 1 ppm	NMT 1 ppm
4	Arsenic	Less than 3 ppm	NMT 3 ppm

2.6. Acid value

The acid value is a measurement of the number of free fatty acids in a sample, usually shown, in milligrams of potassium hydroxide required to neutralize the free acids in 1 g of sample. It is commonly used to determine the quality and freshness of fats and oils, as greater acid values indicate a higher level of free fatty acids, which lead to rancidity and off-flavors in the product.

2.7. Peroxide value

The peroxide value (PV) is measurement of the number of peroxides in given sample, usually depicted in milliequivalents of active oxygen per kg of sample. Peroxides are formed when fats and oils are exposed to oxygen, and their presence indicates the degree of oxidative rancidity in the sample. High peroxide values indicate a higher level of oxidative rancidity and can lead to off-flavors and a decrease in the nutritional value of the product.

2.8. Antimicrobial activity

Microbiological parameters [6-7] refer to various characteristics and attributes related to microorganisms present in a sample. The Total Viable Count (TVC) is a microbiological parameter that quantifies the total number of viable microorganisms present in each sample. These microorganisms include bacteria, yeast, and mold. The TVC is determined by culturing the sample on appropriate agar media under conditions that support the growth of microorganisms. After incubation, the colonies formed are counted, and the results are expressed as colony-forming units (CFUs) per unit volume or weight of the sample. TVC assessment provides an overall indication of the microbial load and hygiene status of the sample. Results are depicted in Table -3.

Table 3: Results of *T. Arjuna* bark extract

S.N.	Test Parameters	Testing Results	Observations
1	Total Bacterial Count	150 cfu/g	-
2	Total Fungal Count	65 cfu/g	-
3	<i>E. coli</i>	Absent	Positive control growth observed
4	<i>Salmonella</i>	Absent	Positive control growth observed
5	<i>Shigella</i>	Absent	Positive control growth observed
6	<i>Pseudomonas aeruginosa</i>	Absent	Positive control growth observed
7	Total Yeast and molds count	6.5 cfu/g	Positive control growth observed
8	Total Aerobic Microbial Count	15 cfu/g	Positive control growth observed
9	Test for Aflatoxin	Negative	-
10	Antioxidant activity	Positive	-
11	Anti-inflammatory activity	Positive	-

2.9. Anti-oxidant and anti-inflammatory activity

Antioxidant activity is vital for defending cells against oxidative stress, which occurs when free radicals like superoxide anion and hydroxyl radicals cause damage to lipids, proteins, and DNA, leading to chronic diseases such as cancer and cardiovascular disorders. Antioxidants, both enzymatic (superoxide dismutase, catalase) and non-enzymatic (vitamins C and E), neutralize these free radicals by donating electrons or hydrogen atoms, preventing cellular damage. This protective function underscores their importance in maintaining cellular health and highlights their significance in research areas like nutrition, medicine, and pharmacology. Anti-inflammatory activity involves compounds that reduce inflammation by inhibiting pro-inflammatory molecules like cytokines and enzymes such as cyclooxygenase. Chronic inflammation contributes to diseases like arthritis and cancer. Anti-inflammatory agents include steroidal drugs (corticosteroids) that inhibit transcription factors and non-steroidal drugs (NSAIDs) like ibuprofen that inhibit COX enzymes. Natural compounds, such as polyphenols, omega-3 fatty acids, curcumin, and resveratrol, also exhibit anti-inflammatory properties by modulating signaling pathways and reducing oxidative stress. Recent research focuses on the anti-inflammatory effects of these natural compounds found in fruits, vegetables, herbs, and spices.

Results for both above activities for Arjuna are depicted in Table-3.

2.10. Test for Aflatoxins

To test for aflatoxins, samples were collected and prepared according to standard protocols. The analysis was conducted using high-performance liquid chromatography (HPLC) with fluorescence detection. Samples were first extracted using an appropriate solvent, followed by purification through solid-phase extraction (SPE) columns. The purified extracts were then subjected to HPLC analysis, where aflatoxins were separated and quantified based on their retention times and fluorescence characteristics compared to known standards. Quality control measures, including the use of spiked samples and blanks, were implemented to ensure accuracy and precision of the results.

3. Results and Discussion

The IR spectrum [8-9] of arjuna bark extract (Figure-1) shows peaks at 3371.05 cm⁻¹ (O-H stretching in alcohols/phenols or N-H stretching in amines), 2925.94 cm⁻¹ and 2855.42 cm⁻¹ (C-H stretching in alkanes), 1621.58 cm⁻¹ (C=C stretching in alkenes or aromatic compounds, or amide C=O stretching), 1521.14 cm⁻¹ (N-H bending or aromatic C=C stretching), 1446.85 cm⁻¹ (C-H bending in methylene groups), 1383.66 cm⁻¹ (C-H bending in methyl groups), 1287.28 cm⁻¹, 1238.05 cm⁻¹, and 1202.09 cm⁻¹ (C-O stretching in alcohols/ethers or C-N stretching in amines), 1151.90 cm⁻¹ (C-O-C stretching in esters/ethers), 1077.84 cm⁻¹ and 1027.82 cm⁻¹ (C-O or C-N stretching), 929.12 cm⁻¹ and 873.37 cm⁻¹ (C-H bending in aromatics), and 755.19 cm⁻¹ and 711.95 cm⁻¹ (C-H out-of-plane bending in aromatic rings), 578.25 cm⁻¹ and 529.26 cm⁻¹ (bending or skeletal vibrations), indicating the presence of

hydroxyl, methylene, methyl, aromatic, amide, amine, ether, and ester groups, reflecting the complex composition of biochemical substances in the extract.

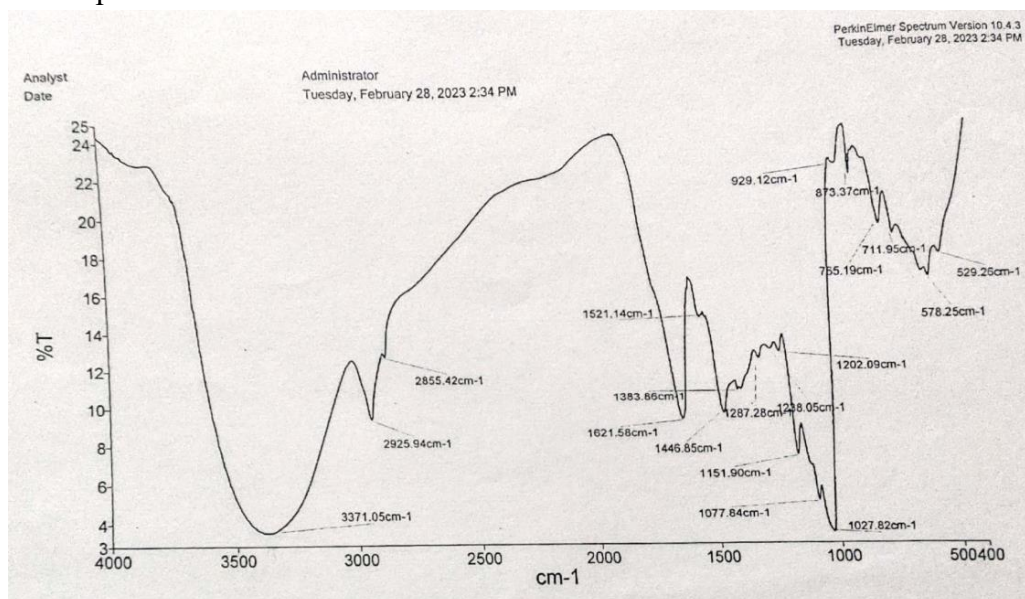


Figure 1: FTIR spectra of the extract

The ¹H NMR spectrum (Figure-2) of the Arjuna bark extract, which features chemical shifts from 5.291 to 3.309 ppm, reveals a rich and complex chemical composition primarily indicative of glycosides, phenolic compounds, and possibly other oxygenated aliphatic structures. The peak at 5.291 ppm is characteristic of an anomeric proton in glycosides, a common structural feature in plant-derived compounds where the sugar unit is bonded to another molecule through a glycosidic bond. This assignment is supported by the chemical shift typical of protons attached to a carbon that is bonded to an oxygen atom in a cyclic structure. The peaks at 4.717 and 4.656 ppm [10] suggest the presence of secondary alcohols or methane protons adjacent to electronegative atoms, likely part of sugar units. These shifts are consistent with the environments of protons in glycosides where oxygen or nitrogen atoms are nearby, causing downfield shifts. Such peaks further corroborate the presence of glycosidic linkages and possibly sugar alcohols, common in plant extracts.

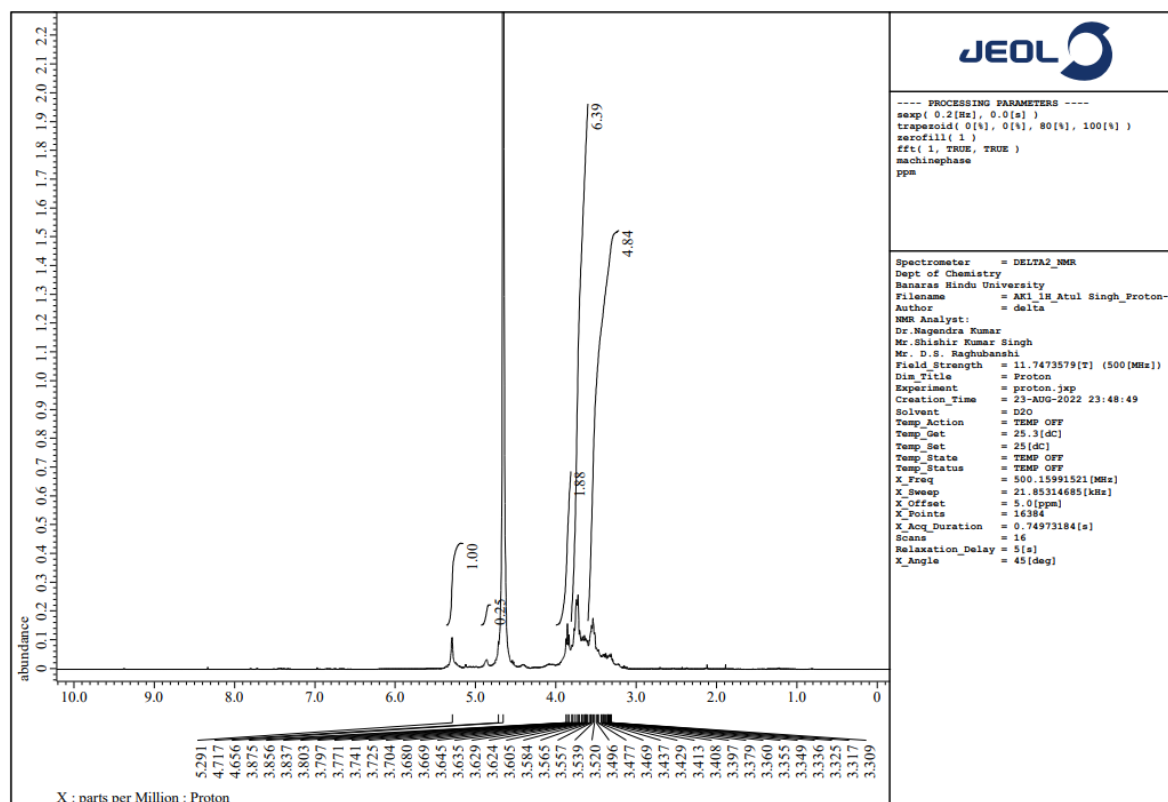


Figure 2: ^1H NMR spectra of *Terminalia arjuna* extract

The extensive series of peaks between 3.875 and 3.309 ppm indicates a large number of methylene and methane protons in various environments, typically attached to oxygen atoms, as seen in ethers, alcohols, and sugars. This range is particularly rich in protons from polyol structures, which include complex carbohydrates and glycosides. The multitude of closely spaced peaks reflects the complexity and diversity of the aliphatic proton environments, suggesting a mixture of different sugar units and other oxygenated compounds, which is common in the extracts of medicinal plants like Arjuna bark. Hence, the ^1H NMR spectrum suggests that the extract contains a significant amount of glycosides, supported by the presence of anomeric protons and numerous oxygenated aliphatic protons. The peaks within the aromatic region also suggest possible contributions from phenolic compounds or flavonoids, which are known for their broad biological activities. The presence of such a diverse array of compounds in the Arjuna bark extract aligns well with its traditional use in herbal medicine, providing a complex profile that could be responsible for its therapeutic properties.

The ^{13}C NMR spectrum of the Arjuna bark (Figure-3) extract reveals a diverse array of chemical shifts, indicating the presence of various organic compounds within the extract. The shifts spanning from 60 to 76 ppm likely correspond to carbon atoms in aliphatic chains, such as those found in fatty acids, contributing to the extract's lipid profile and potential cardioprotective effects. Additionally, shifts in the range of 71 to 73 ppm suggest the presence of aromatic or heterocyclic ring systems, characteristic of polyphenolic compounds like

flavonoids and tannins abundant in Arjuna bark. Notably, a shift around 99.694 ppm signifies a unique functional group or moiety within the extract, possibly indicative of specific bioactive compounds like esters or ketones. Overall, the spectrum reflects the complex phytochemical composition of Arjuna bark, underpinning its traditional medicinal uses, particularly in supporting cardiovascular health. Further analysis can elucidate the specific compounds responsible for its therapeutic effects. These studies revealed detailed information about the hydrogen and carbon atoms in the molecules, helping to map out the structure of complex organic compounds. The identification of compounds like gallic acid, a potent antioxidant, was significant, as it underscored the plant's potential in neutralizing free radicals and protecting against oxidative stress.

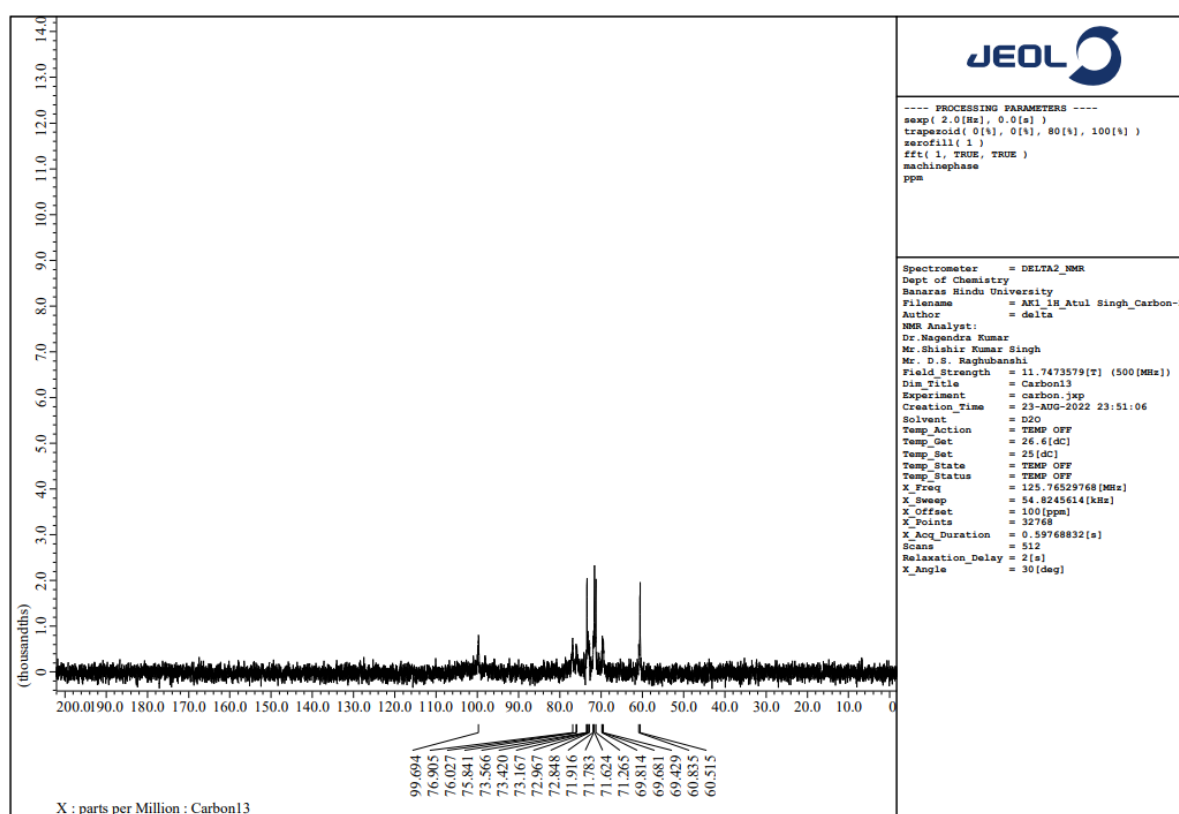


Figure 3: ^{13}C NMR spectra of *Terminalia arjuna* extract

Further reaction tests - Salkowski reaction test, Liebermann-Burchard's test, Foam test, Dragendroff test, Lead acetate test, Legal's test, Keller-Killiani test, Ninhydrin test confirm the presence of phytosterols, triterpenoids, saponins, alkaloids, flavonoids, lactone, glycosides, and proteins in the extract and the results are depicted in Table-1.

As per the results, depicted in Table-2, lead, cadmium, mercury, and arsenic are found within acceptable limits, it indicates that the product meets safety standards and poses no immediate health risks to consumers. This is particularly important in foods, beverages, and medicinal products, where the presence of these metals above permissible levels can have serious health

implications. The acid value of above extract was found to be 0.067 which suggests that the sample contains a minimal amount of free fatty acids, which is indicative of its freshness and low degree of oxidation or hydrolysis. This is particularly desirable in fats and oils intended for consumption, as it ensures the product's sensory and nutritional qualities are maintained. The peroxide value of the extract (1.34 meq/Kg) is relatively low which suggests that the sample has undergone some oxidative rancidity. This can result in the development of off-flavors, odors, and a decrease in the nutritional quality of the product.

As per the studies carried out (depicted in Table-3), total viable counts including total bacterial and fungal count has been found within prescribed limits which indicates that the microbial load in the sample is within the specified range and does not pose a significant risk to human health or product quality. This result suggests that the sample meets microbiological safety standards and is suitable for consumption or further processing. Total viable counts within limits imply that the microbial population present in the sample is at an acceptable level, minimizing the likelihood of microbial spoilage or contamination. It indicates proper hygiene practices during production, handling, and storage, ensuring the microbiological quality of the product. *E. coli*, *Salmonella*, *Shigella*, and *Pseudomonas aeruginosa* were absent in the sample, and positive controls yielded expected results, indicating no contamination by these pathogenic bacteria. This absence ensures product safety and quality, particularly in food, pharmaceuticals, and cosmetics, and reflects effective production and hygiene practices. Meeting regulatory standards, the absence of these pathogens eliminates the risk of related illnesses, ensuring consumer health. Total yeast and mold counts were within acceptable limits, with positive control growth observed, confirming the sample meets microbiological safety standards and the testing method's reliability. Proper handling, processing, and storage are suggested by these counts, minimizing microbial growth risks and ensuring product quality and safety. The total aerobic microbial count was 15 colony-forming units (CFU), with positive control growth observed, indicating low microbial contamination. This low count suggests favorable handling and storage conditions. Positive control growth validates the testing method's accuracy. Despite the low contamination level, ongoing monitoring and proper hygiene practices are essential to maintain product safety and quality.

The sample exhibits both antioxidant and anti-inflammatory activities, indicating its potential health benefits. Antioxidants help neutralize oxidative damage caused by free radicals, reducing the risk of diseases like cancer, cardiovascular disorders, and neurodegenerative conditions. Anti-inflammatory properties suggest the sample can modulate the body's inflammatory response, potentially alleviating conditions like arthritis and cardiovascular diseases. These activities imply the presence of beneficial compounds such as vitamins, polyphenols, flavonoids, and carotenoids. The positive results indicate the sample's potential use as a functional ingredient in food, nutraceutical, or pharmaceutical products aimed at promoting health and combating inflammation-related disorders. In the present study, a test for aflatoxins is found negative, it indicates that the sample does not contain detectable levels of aflatoxins.



4. Conclusions

The heavy metal analysis, acid value, and peroxide value tests indicate that the sample meets regulatory standards, ensuring safety and quality. Heavy metals like lead, cadmium, mercury, and arsenic are within permissible limits, suggesting the sample is free from toxic contaminants. The low acid value (<1) and peroxide value (1.34 meq/kg) indicate minimal oxidation, suggesting the sample is fresh and has high nutritional value. Microbiological analysis confirmed the absence of harmful pathogens such as *E. coli*, *Salmonella*, *Shigella*, and *Pseudomonas aeruginosa*, with yeast and mold counts within acceptable limits, affirming good microbial quality. The sample tested negative for aflatoxins, ensuring safety from these potent toxins. Chemical tests revealed the presence of bioactive compounds, including proteins, amino acids, alkaloids, glycosides, flavonoids, lactones, and phytosterols, suggesting potential therapeutic applications. Positive antioxidant and anti-inflammatory activities indicate the sample's potential to protect against oxidative stress and alleviate inflammation, making it a promising ingredient for food, pharmaceuticals, and nutraceuticals. Spectroscopic analysis of *Terminalia Arjuna* bark extract confirmed a complex biochemical profile, including phenolic compounds, flavonoids, and gallic acid, contributing to its medicinal and therapeutic benefits.

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