

Phytochemical, FTIR analysis, antimicrobial and antioxidant activity of Terminalia chebula Retz dried Fruits

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ABSTRACT

Conventional and contemporary health care, dietary supplements, nutritional supplements, and cultural cures all contain novel pharmaceuticals made from medicinal plants. One of the most significant herbs that is regularly used in the ancient system of medicine is Terminalia chebula Retz. Phytochemical analysis revealed that Terminalia chebula Retz contained most of the important phyto-constituents, such as alkaloid substances, protein chains, saponins, tannins, flavonoids, phenols, terpenoids, carbohydrates, triterpenoids, thiols, steroids, and glycosides. The dried fruits of Terminalia chebula Retz have an effective aqueous extract that scavenges free radicals in a dose-dependent way, as demonstrated by the DPPH scavenging movement lowering power tests, nitric oxide scavenging exertion, and ABTS radical-scavenging ability measured in vitro antioxidant activity. For the purpose of characterizing and identifying the chemicals or functional groups found in plants, FTIR is a useful instrument. There is renewed interest in using infrared spectra to identify biomolecules due to the high signal-to-noise ratio and excellent precision of Fourier transform infrared spectrometers in absorbance and wave number measurements. The results of this investigation carry the conventional application of Terminalia chebula Retz in medicinal products and demonstrate that the dried fruits of the plant contain bioactive chemicals that may be helpful in the manufacture of various therapeutic medicines.

Keywords: Terminalia chebula Retz, Phytochemical, Antioxidant, FTIR.

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1. Introduction

The resultant planet is rich with medicinal plants that are used as a storehouse of healing in almost every culture. Ever before the dawn of time, humans have been associated with plants and have employed them for a multitude of tasks. Primitive humans started to differentiate between plants with precise pharmacological action and those that were appropriate for medical purposes as a means for procuring food and effectively managing human misery. The human-plant communication has expanded, and numerous plants are now utilized as medications (Shakya 2016; Fan et al., 2006).

Since they are known for a variety of biological activities, from antibiotics to antitumors, the search for plants with beneficial properties is still ongoing. One of the most significant areas of conventional medicine worldwide is use of healing vegetation. According to previous reports (Aftab 2017; Nalini 2017; Piana 2013) plants are a particularly ancient source of pharmacologically active compounds. They have been an essential source of medicinally significant substances for humans for generations, and they serve as the "backbone" of conventional medicine (Husain A et al, 2022).

Phytochemicals are naturally occurring, physiologically active substances that thrive in plants and offer advantages to human health in the form of nutrients and therapeutic components (Cock 2015; Sultan 2023). They protect plants from disease and harm while enhancing their color, scent, and fragrance. In general, phytonutrients are the plant molecules that protect plants against environmental threats such pathogenic attack, UV exposure, dryness, pollution, and anxiety (Mathai K 2000). It has been demonstrated unequivocally that, when the foods they eat is substantial, they also play functions in protecting human health. Approximately 350 of the 4,500 phytochemicals that have thus far been identified are categorized based on their physical and chemical characteristics as well as their protective roles (Vemuri et al, 2019; Paul et al., 2003). A wide variety of dietary phytochemicals can be found in whole grains, nuts, legumes, fruits, and vegetables (Sharma DK et al, 2013).

Antioxidants are compounds that, when present in small amounts, stop or slow down the oxidation of biomolecules that are easily oxidized, like proteins, DNA, and lipids. They shorten oxidative damage by scavenging free radicals (Becker et al., 2004). Antioxidants can be divided into two main categories: synthetic and natural. Because epidemiological learn shown the frequent ingestion of natural antioxidants is

associated to a lower danger of tumor and cardiovascular sickness, consumers and scientific investigators are becoming more interested in natural antioxidants, particularly those found in fruits and vegetables (Bhatt et al., 2017). Three main categories of natural antioxidants discovered in fruits and vegetables are linked to their defensive properties: carotenoids, phenolics, and vitamins. Carotenoids are classified as lipophilic antioxidants, whereas ascorbic acid and phenolics are classified as hydrophilic antioxidants (Akhtar et al., 2019) (Gupta A, et al, 2011).

In addition to phytochemical studies, the quest for sources of natural antioxidants is becoming increasingly important. Numerous phytochemicals with antioxidant capabilities have been discovered in plants, according to scientific publications and experimental research. Plant phenolic antioxidants that are most frequently detected include flavonoids, polyfunctional organic acids, coumarins, derivatives of cinnamic acid, and tocopherols. Numerous studies on free radicals have shown that plants high in antioxidants are crucial for preventing diseases linked to free radicals (Gupta 2012). The Perkin Elmer Spectrophotometer equipment was utilized to perform FTIR analysis and identify the characteristic peaks and their functional categories. FT-IR, is possibly one of a predominant accurate method for discover of many types of chemical bonds, or functional groups, that are existed in compounds. The annotated spectrum shows the wavelength of light received that distinguishes the chemical bond. Chemical bonding inside a molecule can be exploited to resolve the spectrum's infrared absorption (Shukla S, et al, 2016).

The functional group was examined using a Fourier Transfer Infra-Red Spectrophotometer, which provides information about the organic dye molecules' color absorption characteristics. Data about the aromatic, achromatic, and functional groups of the ring chains suggested that the dye molecule contained structural groups (Paul et al., 2003).

FT-IR is still regarded as a novel technique in the discipline of pharmacognosy for characterizing and distinguishing commercial components from adulterants. The methodology has been applied in a series of ways, comprising the monitoring of biotechnological processes, the classification, differentiation, and discrimination of similar microbial strains, plant species, and other creatures (Grube et al 2008). It is a main popular method for detecting the chemical components and clarifying the structure of compounds, and many nations' pharmacopoeia require the use of this technique in order to identify medications (Liu et al., 2006).

Since prehistoric times, humans have typically

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included therapeutic substances in their diets. Studies and information on the antioxidant and phytochemical features of many therapeutic plants are still scarce. In order to evaluate the therapeutic potentials associated with the herbal properties of *Terminalia chebula* Retz, this investigation focus on the phytochemical test, antioxidant activity, and FTIR examination of its dried fruits.

2. MATERIALS AND APPROACH

2.1. ACQUISITION OF THE SAMPLE:

Terminalia chebula Retz (Fig. 1) dried fruits were gathered in Tamil Nadu's Tiruchirappalli area. The raisins have been crushed over a smooth powder and accumulate in a dry environment and prepared for next stage.



Fig. 1. *Terminalia chebula* Retz

2.2. FABRICATION OF EXTRACTS:

2.2.1. AQUEOUS EXTRACTS:

200 g fruit-based fine particle and 1200 ml of H₂O were combined, and blend was constantly agitated still it diminished to a third and then filtered. Dryness was reached by evaporating the filtrate. Studies were conducted using the extracted paste form in vitro.

2.2.2. ETHANOL EXTRACTS:

For this investigation, dried fruit powder from *Terminalia chebula* Retz was utilized. For 12 hours, 20 g granules were steeped in ethanol. Afterwards, the extract was run via Whatmann filter paper No. 41 to get rid of any residual sediments and water traces in the filtrate. The filter paper was moistened with ethanol prior to filtration. Then the filtrate was intense and employed for additional research.

2.3. QUALITATIVE ANALYSIS OF PHYTOCHEMICALS:

The bioactive chemical elements of medicinal herbs beneath investigation were screened and

identified by chemical assays in extracts by established protocols since outlined by previous analysis (Brindha et al., 1982; Gunasekar et al., 2019).

2.4. IN VITRO ANTIOXIDANT ASSAY

The DPPH (Gyamfi et al., 2002) eliminating potential assay, (Singhal et al., 2011) nitric oxide metabolism and ABTS⁺ radical scavenging property (Stratil et al., 2006) were employed to examine the antioxidant action of *Terminalia chebula* Retz.

2.5. FTIR Spectroscopic Analysis

The Fourier transform infrared spectrophotometer (FTIR) is a particularly frequently employed practical instrument to detect the numerous kinds of chemical bonds (functional groups) that exist in compounds. Dried powders with many solvent-extracted varieties of each plant item were used for FTIR analysis. Ten milligrams of the dried extract powder were encapsulated in one hundred milligrams of KBr pellet to create the translucent sample disc. An FTIR Spectroscope (Shimadzu, IR Affinity1, Japan) with a magnification of 4 cm⁻¹ and a scan range of 400 to 4000 cm⁻¹ was used to examine a powdered sample from each plant specimen (Nithyadevi et al., 2015).

3. Results and discussion

Table 1. Phytochemical analysis of *Terminalia chebula* Retz dried fruits

S.NO	PHYTOCHEMICALS	ETHANOLIC EXTRACT	AQUEOUS EXTRACT
1	Flavonoids	+	+
2	Phenol	+	+
3	Tannins	-	+
4	Saponins	+	+
5	Coumarins	+	+
6	Quinone	+	+
7	Alkaloids	-	-
8	Glycosides	+	+
9	Sugar	-	+
10	Volatile Oils	+	+

Figure 1. Phytochemical screening of aqueous extract of *Terminalia chebula* Retz dried fruits



Figure 2. Phytochemical screening of ethanol extract of *Terminalia chebula* Retz dried fruits

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S.NO	CONCENTRATION (µg/ml)	% OF SCAVENGING ACTIVITY	
		AQUEOUS EXTRACT	ASCORBIC ACID (STANDARD)
1	50	16.05 ± 0.02	11.07 ± 0.01
2	100	24.06 ± 0.03	20.04 ± 0.01
3	150	32.08 ± 0.01	31.06 ± 0.01
4	200	44.06 ± 0.01	38.05 ± 0.04
5	250	56.08 ± 0.01	53.04 ± 0.04

Table 2: DPPH scavenging action of *Terminalia chebula* Retz dried fruits



Evaluation was carried out in triplicates. Values are expressed as mean ± SD

S.NO	CONCENTRATION (µg/ml)	% OF INHIBITION	
		AQUEOUS EXTRACT	ASCORBIC ACID (STANDARD)
1	50	15.02 ± 0.01	13.06 ± 0.01
2	100	21.05 ± 0.03	18.05 ± 0.03
3	150	34.07 ± 0.02	29.06 ± 0.02
4	200	52.06 ± 0.04	49.03 ± 0.02
5	250	63.06 ± 0.03	58.04 ± 0.04

Table 3. ABTS⁺ Radical scavenging action of *Terminalia chebula* Retz dried fruits

Evaluation was executed in triplicates. Values are expressed as mean ± SD

S.NO	CONCENTRATION (µg/ml)	% OF SCAVENGING ACTIVITY	
		AQUEOUS EXTRACT	ASCORBIC ACID (STANDARD)
1	50	10.06 ± 0.03	8.04 ± 0.02
2	100	17.05 ± 0.04	15.05 ± 0.02
3	150	28.04 ± 0.04	23.07 ± 0.02
4	200	40.02 ± 0.01	33.02 ± 0.01
5	250	52.07 ± 0.02	45.07 ± 0.01

Table 4. Nitric oxide scavenging action of *Terminalia chebula* Retz dried fruits

Examination was carried out in triplicates. Values are expressed as mean ± SD

S.NO	CONCENTRATION (µg/ml)	FLOWER	ASCORBIC ACID (STANDARD)
		AQUEOUS EXTRACT % OF INHIBITION	
1	50	20.05 ± 0.01	15.04 ± 0.03
2	100	31.04 ± 0.02	26.07 ± 0.02
3	150	39.03 ± 0.02	36.06 ± 0.03
4	200	56.03 ± 0.03	50.04 ± 0.01
5	250	71.05 ± 0.02	63.06 ± 0.03

Table 5. Reducing power scavenging activity of *Terminalia chebula* Retz dried fruits

Evaluation was executed in triplicates. Values are expressed as mean ± SD

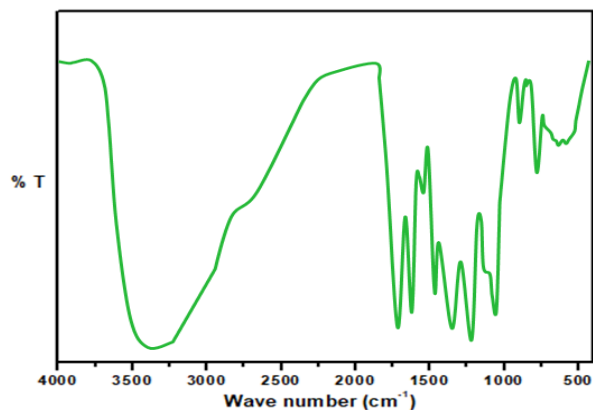


Fig 3: FTIR Spectrum of *Terminalia chebula* Retz dried fruits

Table 6: FTIR Peak Values of *Terminalia chebula* Retz dried fruits

S.No	Peak frequency cm ⁻¹	Bond	Functional group
1	3367.61	O-H stretch	Alcohol/phenol
2	3331.57	O-H Stretch	Alcohol/phenol
3	3286.31	O-H Stretch	Alcohol/phenol
4	1711.35	C=O Stretch	Ketone
5	1615.98	NH ₂ scissoring (1° amines)	Amines
6	1536.35	N-H (2°-amide)band	Nitriles
7	1449.11	α-CH ₂ bending	Aldehyde & ketone
8	1347.83	O-H bending	Alcohol & phenol
9	1214.42	C-N	Amines
10	1035.41	C-N stretch	Amines
11	874.24	NH ₂ bond	Amines
12	836.15	=C-H & =CH	Alkanes
13	763.56	O-H bend	Alcohol & phenol
14	603.64	C-H deformation	Alkynes
15	552.78	-	Unknown

According to the initial phytochemical analyses, the powdered dried fruits of *Terminalia chebula* Retz contain the following compounds: alkaloids, flavonoids, tannins, protein, terpenoids, sterols, carbohydrates, and glycosides (Table 1). It is well known that flavonoids improve vascularity and prevent or delay the start of cell necrosis, which both minimize lipid peroxidation. Hence, is deliberation that any drug that put off lipid peroxidation would raise the longevity of collagen fibrils through boosting flow, strengthening collagen fibers, averting cell damage, and encouraging DNA synthesis. According to reports, flavonoids also have antibacterial and pesticidal properties. Additionally, terpenoids have been shown to support wound healing, which is thought to be the cause of wound contraction and a faster rate of epithelialization. Additionally, terpenoids may have anti-HIV and anti-platelet aggregation properties. The primary ingredient in many plant extracts, tannins

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function as a scavenger of free radicals. Because it is an acetylated mannose polymer, tannin is utilized in medicine. Treatment for ulcers and diabetes involves the use of cardiac glycosides (Karunyadevi et al 2009). The alkaloids found in these leaves include benzoquinazoline and aporphine, two families that are well-known for their diverse range of therapeutic applications.

Alkaloids and their derivatives have a significant biological role in the treatment of pain, spasticity, and bactericidal effects. Nonetheless, alkaloids are mostly found in huge quantities in plants and have a significant physiological impact on humans. The main categories of alkaloids are quinine, nicotine, ephedrine, morphine, and strychnine (Stary et al., 1998; Meher et al., 2018). Because saponins naturally repel microorganisms, they are a suitable option for treating infections caused by yeast and fungi. According to Santhi et al., 2011, these substances acted as usual antibiotics, assisting the body in fending off illness and microbial assault.

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Because of their wide range of biological functions, steroids have garnered a lot of study. It works by preventing the absorption of LDL and total cholesterol from the small intestine, which lowers the levels of these two types of cholesterol in plasma. As a result, they provide defense against cardiovascular disease and reduce the risk of atherosclerosis. Proteins and carbohydrates were essential components of the food supplement for herbivores and plant growth. It uses a

variety of defense mechanisms, such as the synthesis of antimicrobial proteins, to protect itself against microbial infections (Walter 2012).

Many secondary metabolites, including alkaloids, flavonoids, glycosides, phenols, saponins, steroids, and others, may be liable for the medicinal qualities of medicinal plants (Arora 2013). Thus, the antimicrobial action originates in the current learn can partially clarified by the existence of these phytochemicals (alkaloids, anthraquinones, cardiac glycosides, proteins, tannins, terpenoids and sugars).

The DPPH method, which was unquestionably created almost 50 years ago (Blois 1958) is frequently used to evaluate a compound's ability to function as a source of hydrogen or free radical scavenger in addition to its antioxidant capacity. The IC₅₀ value, which is used to understand the results of the DPPH procedure, is the concentration of substrates that causes a 50% decrease in DPPH activity. The ability of dried fruit extracts species *Terminalia chebula* Retz to scavenge DPPH radicals was shown in Table 2.

Terminalia chebula Retz dried fruit aqueous extracts demonstrated superior 1,1-diphenyl 1-2-picrylhydrazyl (DPPH) free radical scavenging capabilities. A maximal scavenging activity of 250 µg/ml was found to be ineffective. The least quantity of activity was observed at a concentration of 50 µg/ml. The greater percentage of phenolic antioxidants in the extract of water may be the reason for its greater radical scavenging action, as phenolic compounds are hydrogen-donating antioxidants. The IC₅₀ value was found to be 192 µg/ml.

Hydrogen-donating antioxidants reduce the number of stable free radical DPPH. The results of the scavenging experiment indicated that the leaf extract was quite active. The extracts' ability to scavenge DPPH radicals was marginally greater than that of ascorbic acid. This implies that the isolates have capacity to supply protons to free radicals and could potentially manipulate as main antioxidants since their antioxidant effect on DPPH is believed to be due to their ability to donate hydrogen to free radicals (Krishna Kumar et al., 2012).

By calculating the quantity that blocks absorbance at 734 nm, which indicates the decrease of the radical cation, one can evaluate the decolorization of ABTS•⁺. The ABTS•⁺ was produced by letting the chromophore undergo the reaction (Wolfenden et al., 1982). A few chemical components found in the leaf extracts of *Terminalia chebula* Retz's dried fruits may inhibit potassium persulfate's activity, which would lower the production of ABTS•⁺. The ABTS•⁺ test is a great way to measure the antioxidant activity of antioxidants that

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break chains and donate hydrogen. Phenolic compounds found in medicinal plants are crucial for ABTS⁺ scavenging (Pietta et al 1998).

The ABTS⁺ scavenging assay, which requires a fast reaction time and employs a specific absorbance (734 nm) at a wavelength beyond the visible area, is an index that shows the antioxidant ability of the test substances (Kumaravel et al., 2013). It was demonstrated that *Terminalia chebula* Retz's dried fruits had an efficient aqueous extract capable of scavenging radicals; additionally, the increase in the radical scavenging capacity was concentration-dependent. At 250 µg/ml, the extract exhibited 52.07% inhibition, whereas regular ascorbic acid demonstrated 45.07 percent inhibition. The pulp extract's IC₅₀ was 240 µg/ml, while standard ascorbic acid's was 277 µg/ml. This indicates that the aqueous pulp extract has an important effect ability to scavenge the ABTS⁺ radical. Here, the extract's phenolic components were directly responsible for its capacity to scavenge free radicals.

Reactive peroxynitrite (ONOO⁻), which is created when NO and O₂ combine, increases toxicity and damage and can cause significant harmful interactions with biomolecules. In our investigation, we found that the water-soluble leaf extract of the dried berries of *Terminalia chebula* Retz exhibited a significant nitric oxide radical scavenging action (Table 4). The proven significance of NO as a mediator in the genesis of inflammation may support the use of *Terminalia chebula* Retz dried fruits for the therapy of inflammation-related diseases (Lee et al; 2007; Mandeville et al 2018). A quantity-related increase in NO scavenging ability was demonstrated by the aqueous pulp extract, with the highest effectiveness observed at a dosage of 250 µg/ml, where 56.08 percent scavenging was observed. Nitric oxide (NO) is a potent pleiotropic mediator that plays a role in several physiological functions. Peroxynitrous acid (ONOOH), a severe oxidant and possibly deadly chemical that nitrates aromatic molecules like tyrosine, is created when it reacts with the superoxide anion. The nitric oxide radical generated by sodium nitroprusside is measured using the Griess reduction method. Sodium nitroprusside naturally generates nitric oxide at physiological pH, which combines with leaf extract to prevent the formation of anions (Sangameswaran et al., 2009).

In the reduction of power test, the extracts' antioxidants helped reduce Fe³⁺ to Fe²⁺ by one electron. Even the ascorbic acid reference value was surpassed by the extract, which showed the highest reducing capacity. Reducing power action is commonly used to evaluate a natural antioxidant's capacity to supply electrons

(Dorman et al., 2003). Several investigations have shown a clear correlation between the antioxidant qualities and reducing capacity of certain plant extracts (Yildirim et al., 2000). The decreasing energy utilization of the plant's aqueous extracts of leaves increased steadily from 50 µg to 250 µg of extract. Compared to the standard ascorbic acid (63.06 at 250 µg/ml), pulp extract showed higher absorbance (71.05 at 250 µg/ml). Subsequent investigation showed that the reducing power activity of the dried fruit extract was substantially greater than that of ascorbic acid (Table 5).

Following analysis of the FTIR spectrum, the results of the FTIR spectrum pattern were shown in (Fig. 3 & Table 6). According to the FT-IR investigations of *Terminalia chebula* Retz, the following functional group infra-red spectrum shows the frequency regions 3367.61 & 3331.57 & 3286.31 cm⁻¹, which reflect the O-H stretching and suggest the existence of alcohols/phenol. The signal located at 1711.35 cm⁻¹ suggested the presence of ketone's C=O stretching. The signal at 1615.98 cm⁻¹ indicated the existence of amines with NH₂ stretching. The signal at 1536.35 cm⁻¹ revealed the presence of primary nitriles with N-H bending.

The signal at 1517.60 cm⁻¹ suggested the presence of nitrogenous substances with N-O stretching. The presence of ketone and aldehyde is indicated by the α-CH₂ bending in the periodicity range 1449.11 cm⁻¹ peak. When it comes to O-H stretching, the peak at 1347.83 cm⁻¹ indicates the presence of phenol and alcohol. The peak at 1214.42 & 1035.41 cm⁻¹, which represents the C-N stretch, confirms the presence of amines. The signal at 874.24 cm⁻¹ indicates the presence of amines and the NH₂ bond. The signal at 836.15 cm⁻¹ indicated the presence of alkanes with =C-H & =CH stretching. The existence of alkynes with C-H dislocation is indicated by the signal recorded at 603.64 cm⁻¹.

According to the findings of the FT-IR analysis of the research spectrum, the crude powder of *Terminalia chebula* Retz contained functional elements such alcohols/phenols, alkanes, alkynes, amines, ketones, aldehyde, and nitriles. Fourier Transform Infrared, or FT-IR, is the recommended method for infrared spectroscopy. Infrared spectroscopy involves the passage of infrared energy through a material. A portion of the infrared light is absorbed by the sample, while the remainder is transmitted. The resulting spectrum, which displays the molecule absorption and transmission, creates a molecular profile of the substance. Like fingerprints, no two different molecular combinations have the same infrared spectrum. As a result, infrared spectroscopy can be used for a variety of analysis types.

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It can identify materials that are unknown. It can determine the quality or homogeneity of a sample. It can determine the number of elements in a mixture (Sharma D, et al, 2011).

Fingerprinting (marker ingredient analysis) employing chemical and verified spectroscopic and chromatographic methods has grown increasingly significant for standardization in herbal medicine formulations. It is possible to do a metabolomic fingerprinting assessment of a herbal product using appropriate FT-IR techniques (Giri et al 2010). These methods also provide useful information about the chemometric patterns identified of herbal remedies and their quantitative and qualitative composition (Arora 2013).

4. Antimicrobial activity

The antimicrobial activity of pure *Terminalia chebula* Retz was evaluated towards Gram (+ve), and Gram (-ve) bacteria and fungus such as *candida albicans* and *aspergillus flavus*. Fig. 4.1 and Table 4.2 present the results. The results showed that the antibacterial effect against bacteria and fungi was superior. When juxtaposed to the negative control, Figure 4.1 shows the potential for *Terminalia chebula* Retz to demonstrate antimicrobial activity as well as the cell damage brought on by bacteria in the present nanoparticles.

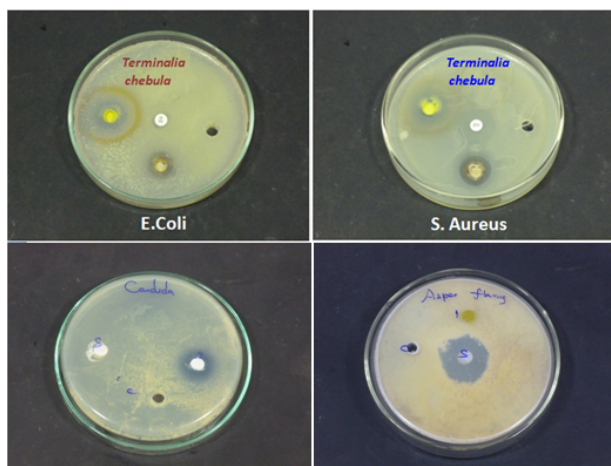


Fig. 4.1. Antimicrobial activity of *Terminalia chebula* against Gram (+ve), and Gram (-ve) bacteria and fungus such as *candida albicans* and *aspergillus flavus*.

Table. 4.2 Antimicrobial action of *Terminalia chebula*

Microbes	Zone of inhibition (mm)		
	Control (DMSO) (C)	Standard (ketoconazole) (S)	<i>Terminalia chebula</i>
Gram +	5	10	17
Gram -	7	12	14
<i>Candida albicans</i>	6	8	11
<i>Aspergillus flavus</i>	Resistant	11	Resistant

5. Conclusion

Terminalia chebula Retz is successfully extracted. The findings of this investigation validate the presence of bioactive substances that enhance human health in the extract of dried fruits from *Terminalia chebula* Retz. Therefore, the dried fruits of *Terminalia chebula* Retz contain significant bioactive components that are commonly employed to enhance human health. With the use of improved screening techniques, research on the creation and characterisation of novel natural medications must proceed. The antimicrobial activity of *Terminalia chebula* Retz was investigate towards Gram (-ve), Gram (+ve), and Gram (-ve) bacteria and fungus. The antimicrobial studies display the excellent activities against the microbes.

Conflicts of interest statement

All authors declare that there is no conflict of interest.

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Informed consent

Not applicable.

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