Phytochemical Profiling and Evaluation of Antioxidant, Antimicrobial and Anticancer Activities of *Abutilon indicum* L. Ethanolic Leaf Extracts

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Abstract

Abutilon indicum (L.) Sweet, a traditional medicinal plant of the Malvaceae family, is renowned for its therapeutic potential. This study investigates the phytochemical composition, antioxidant, antimicrobial and anticancer properties of its ethanolic leaf extracts. Gas chromatography-mass spectrometry (GC-MS) identified 43 bioactive compounds, with benzoic acid, 4-ethoxy-, ethyl ester (26.98%) and n-hexadecanoic acid (8.16%) as major constituents. Qualitative and quantitative phytochemical analyses revealed ethanol's efficacy in extracting alkaloids (15.28 ± 0.25 mg/g), flavonoids (8.76 ± 0.05 mg/g) and phenols (9.87 ± 1.15 mg/g). The ethanolic extract exhibited significant antioxidant activity in the DPPH assay (91.02% inhibition at 1000 mg/L), though less potent than butylated hydroxytoluene (94.95%).

Antimicrobial assays demonstrated broad-spectrum activity, exhibiting zones of inhibition of 23 mm against Streptococcus pyogenes and 22 mm against Escherichia coli featuring consistent MIC (250 µg/mL) and MBC/MFC (500 µg/mL) values. The extract showed potent anticancer activity against MCF-7 breast cancer cells, with an IC_{50} of 2.134 µg/mL and 99.52% inhibition at 10 µg/mL. These findings validate the ethnomedicinal uses of Abutilon indicum and highlight its potential as a source of natural antioxidants, antimicrobials and anticancer agents, warranting further exploration for pharmaceutical applications.

Keywords: *Abutilon indicum*, phytochemicals, antioxidant, antimicrobial activity, anticancer activity.

Introduction

A significant worldwide health concern, cancer is defined by the unchecked growth of aberrant cells that have the potential to develop into tumors or spread to other locations[48]. Commonly used conventional therapies include radiation, chemotherapy and surgery with chemotherapy playing a critical role in managing advanced malignancies and preventing metastasis[7]. However, chemotherapeutic agents often lack specificity, targeting both cancerous and healthy cells, leading to severe side effects such as myelosuppression, gastrointestinal toxicity, alopecia and increased susceptibility to infections[2]. These limitations have driven research toward natural compounds with lower toxicity and potent anticancer properties as potential alternatives or adjuvants to conventional therapies[27].

For decades, medicinal plants have served as a vital component of conventional healthcare systems, offering a wealth of bioactive substances with a variety of pharmacological effects[45]. Over 50% of modern pharmaceuticals originate from organic materials, many of which have antioxidant, antimicrobial and anticancer effects[3]. Flavonoids, phenolics, alkaloids and terpenoids are examples of phytochemicals that are known to reduce oxidative stress, alter immunological responses and stop the growth of cancer cells by inducing apoptosis, cell cycle arrest and suppressing angiogenesis[19]. These properties make plant-derived compounds promising candidates for developing safer and more effective therapeutics.

The Malvaceae family's perennial shrub *Abutilon indicum* (L.) Sweet is found in tropical and subtropical regions and is known by local names such as "Thuthi" (Tamil), "Atibalaa" (Sanskrit) and "Duvvena Kayalu" (Telugu)[32]. This plant has long been used in traditional medicine to treat ailments like fever, infections, diabetes and inflammation. It has also been shown to have hepatoprotective, analgesic and wound-healing effects[53]. Its complex phytochemical profile, which includes flavonoids, phenolics, alkaloids, terpenoids and steroids, has been linked in recent research to its potential in the treatment of cancer[49].

Abutilon indicum's bioactive ingredients are primarily responsible for its medicinal potential. By fighting off reactive oxygen species (ROS), flavonoids like quercetin and kaempferol as well as phenolic substances, demonstrate antioxidant activity and stop the development of cancer brought on by oxidative stress[19]. Terpenoids and alkaloids alter signaling pathways and cause cancer cells to undergo apoptosis, which increases cytotoxicity[34]. Through processes including ROS production, mitochondrial membrane rupture and cell cycle arrest, *in vitro* research has shown that extracts from *Abutilon indicum* suppress the growth of several cancer cell lines including those from the breast, lung and colon[47]. The purpose of this work is to use gas chromatography-mass spectrometry (GC-MS) to analyze the phytochemical content of ethanolic leaf extracts of *Abutilon indicum* and to assess their anticancer, antibacterial and antioxidant properties against MCF-7 breast cancer cell lines. By the clarification of bioactive components and their biological impacts, this study aims to confirm the traditional use of *Abutilon indicum* and

investigate its potential as a source of new therapeutic agents for microbial infections and cancer.

Material and Methods

Plant Material Collection, Authentication and Extraction: *Abutilon indicum L.* fresh leaves were gathered in 2024 from Kolli Hills in Namakkal, Tamil Nadu, India. At the Botanical Survey of India, located at Tamil Nadu Agricultural University in Coimbatore, India, the plant material was authenticated. After 14 days of shade drying at room temperature (25 ± 2°C), the leaves were ground into a fine powder with a mechanical grinder. For extraction, 250 g of powdered leaves were subjected to cold maceration for 48 hours, shaken with 1 liter of distilled water periodically. After passing the mixture through Whatmann no. 1 filter paper, the filtrate was concentrated in a rotary evaporator at 40°C under reduced pressure. At 4°C, the resultant aqueous extract was kept in an airtight, sterile container with a yield of around 16% (w/w). For subsequent assays, the extract was dried and reconstituted in appropriate solvents (e.g. 10% dimethyl sulfoxide [DMSO] or distilled water) as required.

Qualitative Phytochemical Screening

Standard procedures were followed to perform a preliminary qualitative screening of phytochemicals in the ethanolic and aqueous extracts of Ab*utilon indicum* leaves by Harborne[13,14].

Alkaloids (Mayer's Test): Two millilitres of Mayer's reagent (potassium mercuric iodide solution) were added to two millilitres of extract. Alkaloids showed up as a cream-colored or pale yellow precipitate.

Steroids (Liebermann-Burchard Test): Two milliliters of acetic anhydride and two milliliters of sulfuric acid concentrate were added to one milliliter of extract. Steroids were identified by a violet, blue, or green hue.

Terpenoids (Salkowski Test): A few drops of strong sulfuric acid and two milliliters of chloroform were added to one milliliter of extract. Terpenoids were detected at the contact by a reddish-brown ring.

Flavonoids (Alkaline Reagent Test): Concentrated hydrochloric acid and a few drops of diluted ammonium hydroxide were added to 1 milliliter of extract. A golden hue indicated the presence of flavonoids.

Saponins (Froth Test): 5 mL of distilled water was added to 1 mL of extract and the mixture was agitated vigorously. Long-lasting foam production suggested saponins.

Phenols (Lead Acetate Test): A few drops of a 10% lead acetate solution were added to 1 milliliter of extract. Phenols were verified by a white precipitate.

Tannins (Lead Acetate Test): One milliliter of a 10% lead acetate solution was added to one milliliter of extract. Tannins were identified by a white precipitate.

Cardiac Glycosides (Keller-Killiani Test): Five milliliters of distilled water were added to one milliliter of extract, evaporated until it was completely dry and then reconstituted in two milliliters of glacial acetic acid with a tiny amount of ferric chloride. 1 mL of sulfuric acid was then added. Cardiac glycosides were verified by a brown ring with a greenish-blue layer.

Amino Acids (Ninhydrin Test): A few drops of a 0.25% ninhydrin solution were added to 1 milliliter of extract and the mixture was heated for 10 minutes at 95°C in a water bath. Blue or purple hues denoted amino acids.

Proteins (Biuret Test): Two drops of 1% copper sulfate solution were added to one milliliter of extract along with one milliliter of 40% sodium hydroxide. The proteins were validated by a violet hue.

Carbohydrates (Barfoed's Test): One milliliter of Barfoed's reagent was added to two milliliters of extract and the mixture was heated for two minutes in a water bath. A reddish-brown precipitate was a sign of carbohydrates.

Reducing Sugars (Fehling's Test): Fehling's solutions A and B were added in equal amounts to 1 milliliter of extract and the mixture was then heated for five minutes. Sugars were proven to be decreased by a brick-red precipitate.

Quantitative Phytochemical Estimation

Quantitative analysis of alkaloids, flavonoids and total phenols was performed following established methods[13,14].

Alkaloids: 100 mL of 10% acetic acid in ethanol was used to extract 10 g of powdered leaves over 24 hours. Ammonium hydroxide was used to precipitate the extract after it had been filtered and concentrated to one fourth of its original volume. To measure the alkaloid content (mg/g dry weight), the precipitate was filtered, dried at 60°C and washed with diluted ammonium hydroxide.

Flavonoids: 100 mL of methanol was used to extract a 10 g sample, which was then filtered through a 125 mm Whatmann no. 42 filter paper and dried by evaporation. To measure the flavonoid concentration (mg/g dry weight), the residue was measured.

Total Phenols: 50 mL of diethyl ether was used to boil a 10-gram sample for fifteen minutes. 5 mL of concentrated amyl alcohol, 2 mL of 2% ammonium hydroxide and 10 mL of distilled water were combined with the extract. A UV-Vis spectrophotometer (Shimadzu UV-1800) was used to detect absorbance at 505 nm after 30 minutes. A gallic acid standard curve was used to determine the phenol concentration, which was then reported as mg/g dry weight.

Antibacterial and Antifungal Assays

Disc Diffusion Method: The disc diffusion technique was used to assess *Abutilon indicum* ethanolic leaf extract's antibacterial properties. *Staphylococcus aureus, Salmonella*

typhi, *Escherichia coli*, *Streptococcus pyogenes* and *Klebsiella pneumoniae* were all tested on Mueller-Hinton Agar (MHA) whereas *Aspergillus niger* and *Candida albicans* were tested on Sabouraud Dextrose Agar (SDA). Standardized microbial suspensions were prepared for bacteria, having 1×10^8 CFU/mL, while fungi had 1×10^6 CFU/mL, adjusted to the 0.5 McFarland standard. The suspensions were swabbed onto solidified agar plates and excess liquid was removed. The surface of the agar was sterile filter paper discs (6 mm in diameter) soaked with 20 µL of ethanolic extract (1000 µg/disc).

10 µg/disc of ciprofloxacin and 100 units/disc of amphotericin B served as positive controls for bacterial and fungal assays. After incubating the plates for 24 hours at 37°C, the zone of inhibition (ZOI) width was measured in millimeters. Three duplicates of each test were run and the findings were presented as mean ± SD.

Minimum Inhibitory Concentration (MIC): By employing the broth macrodilution technique, the ethanolic extract's MIC was ascertained. A stock solution of 2 mg/mL was made by dissolving the extract in 10% DMSO. The final concentrations of 1000, 500 and 250 µg/mL were obtained by serial dilutions in Mueller-Hinton Broth (MHB) for bacteria and Sabouraud Dextrose Broth (SDB) for fungi. A 0.5 McFarland standard microbial suspension was used to inoculate each tube. Both a sterility control tube (without inoculum) and a growth control tube (without extract) were present. For 24 hours (bacteria) or 48 hours (fungi), the tubes were incubated at 37°C. The lowest concentration at which there is no discernible microbial growth (turbidity) is known as the minimum inhibitory concentration (MIC). Three duplicates of each test were run and the median MIC value was recorded.

Minimum Bactericidal/Fungicidal Concentration (MBC/MFC): The MIC test was followed by the determination of MBC and MFC. MHA plates for bacteria or SDA plates for fungi were covered with 100 µL aliquots from MIC tubes that did not exhibit any growth. The plates were incubated for 24 hours for bacteria and 48 hours for fungi at 37°C. The lowest concentration at which no colonies developed a sign of microbial death, was known as the MBC/MFC. Results were reported as the median MBC/MFC value and tests were run in triplicate.

Antioxidant Activity (DPPH Assay): Ascorbic acid serves as a positive control in the DPPH antioxidant assay which uses 2,2-Diphenyl-1-picrylhydrazyl (DPPH) as a solvent. A UV-Vis spectrophotometer was utilised to measure absorbance after test samples were prepared at different concentrations. 3.94 mg of DPPH was dissolved in 100 mL of methanol to create the DPPH stock solution. Equivalent concentrations of methanol were used to prepare the test samples and the standard. A modified DPPH assay which involved adding 0.1 mM DPPH solution to test samples or standard solutions, incubating for 30 minutes and measuring absorbance at 517 nm, was used to assess the antioxidant activity:

$$RSA(\%)=(Ac-As)/Ac \times 100$$

where Ac = Absorbance of the control (DPPH solution without antioxidant) and As = Absorbance of the sample or standard (DPPH solution with antioxidant).

GC-MS Analysis: A Shimadzu GC-MS QP2010 Plus system fitted with a ZB-WAX capillary column (30 m × 0.25 mm, 0.25 µm film thickness) was used to investigate the phytochemical content of the ethanolic leaf extract. Helium served as the carrier gas and the system ran in electron impact mode at 70 eV with a split ratio of 1:50 and a flow rate of 1 mL/min. At a rate of 10°C/min, the oven temperature was designed to rise from 50°C (held for 2 minutes) to 250°C, with a final hold of 5 minutes. The interface temperature was kept constant at 240°C, while the injector and ion source temperatures were set at 250°C and 200°C respectively. Between 50 and 500 m/z, mass spectra were captured. Relative abundance was determined using peak area percentages and compounds were identified by matching retention durations and mass spectra with the NIST17 collection. Retention indices were compared with literature data for confirmation.

Cell Culture and Cell Viability Assay: The MTT test was utilized to assess the anticancer efficacy of *Abutilon indicum* ethanolic leaf extract against MCF-7 human breast cancer cell lines[24]. In a humidified incubator with 5% CO_2, cells were cultivated for 24 hours at 37°C in RPMI-1640 media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The extract was diluted to reach concentrations of 50, 100, 200 and 400 µg/mL after being dissolved in 10% DMSO. Five × 10^3 cells per well were used to seed the cells in 96-well plates and they were then treated with either the extract or DMSO (negative control). The plates were incubated for 4 hours after 20 µL of MTT solution (5 mg/mL) was added to each well, following 24 and 48 hours of incubation. The formazan crystals were dissolved in 100 µL of DMSO and a Bio-Rad microplate reader was used to detect absorbance at 570 nm. The data were presented as mean ± SD and each experiment was run in triplicate.

Statistical Analysis: Data from three separate studies were presented as mean ± standard deviation (SD). GraphPad Prism (version 8.0) was used to run a one-way analysis of variance (ANOVA) and then a Tukey's post-hoc test to examine statistical differences between groups. Statistical significance was established at a p-value of less than 0.05.

Results and Discussion

The qualitative phytochemical analysis of *Abutilon indicum* leaf extracts: This study evaluates the phytochemical profile of *Abutilon indicum* leaf extracts using various solvents, comparing results with recent

literature. In conventional medicine, phytochemicals, bioactive substances present in plants like *Abutilon indicum* are utilized to treat conditions including infections, fever and diabetes. The findings provide insights into potential pharmacological applications. The results demonstrate (Table 1) that solvent polarity significantly influences the phytochemical profile of *Abutilon indicum* leaf extracts.

Ethanol extracted the widest range of compounds, reflecting its intermediate polarity, which effectively dissolves both polar and moderately non-polar compounds. This aligns with Rajendran et al[30] who reported ethanol's efficacy in extracting alkaloids and flavonoids from *Abutilon indicum* leaves. Methanol, a polar solvent, extracted steroids, flavonoids, phenols, tannins and carbohydrates, consistent with Sharma et al[41] who noted methanol's suitability for polar phenolics in *Abutilon indicum*. Aqueous extracts contained steroids, flavonoids, saponins, phenols, tannins and reducing sugars, corroborating Kumar et al[20] who found water effective for polar compounds in *Abutilon indicum*. Non-polar solvents like petroleum ether and chloroform extracted steroids, saponins and phenols, with petroleum ether also extracting tannins.

Patel et al[28] provided evidence for this, reporting non-polar solvents' efficiency for lipophilic compounds like steroids in *Abutilon indicum*. The absence of proteins across all solvents may indicate low protein content or degradation during extraction, as noted by Gupta et al[10]. Ethanol's exclusive extraction of alkaloids, terpenoids, cardiac glycosides and amino acids highlights its versatility, consistent with Singh et al[42] who emphasized ethanol's broad-spectrum extraction in medicinal plants.

The ubiquitous presence of steroids and phenols across all solvents suggests their high concentration in *Abutilon indicum*, aligning with Rao et al[33] who noted similar trends in *Abutilon indicum* extracts. Tannins were selectively extracted by methanol, petroleum ether and aqueous solvents, supporting Bhat et al[4]. The results underscore ethanol and methanol as optimal solvents for comprehensive phytochemical extraction from *Abutilon indicum*. Compared to recent studies, the findings are consistent, though variations may arise due to differences in plant part, extraction conditions, or test sensitivity.

Quantitative estimation of phytochemicals in *Abutilon indicum* leaf extracts: The quantitative analysis reveals that ethanol extracted the highest concentrations of alkaloids (15.28 ± 0.25 mg/g), flavonoids (8.76 ± 0.05 mg/g) and phenols (9.87 ± 1.15 mg/g), highlighting its efficacy as an intermediate-polarity solvent (Table 2). This aligns with Rajendran et al[30] who reported ethanol's superior extraction of alkaloids (16.2 mg/g) and flavonoids (9.1 mg/g) from *Abutilon indicum* leaves.

Methanol also showed significant extraction, particularly for flavonoids (7.47 ± 0.03 mg/g) and phenols (5.68 ± 1.21 mg/g), consistent with Sharma et al[41] who noted methanol's effectiveness for polar compounds in *Abutilon indicum* (flavonoids: 7.8 mg/g; phenols: 6.0 mg/g). Aqueous extracts yielded moderate alkaloid (13.72 ± 1.98 mg/g) and flavonoid (7.35 ± 0.09 mg/g) content but lower phenols (2.54 ± 0.28 mg/g), supporting Kumar et al[20] who reported similar trends for polar compounds in aqueous extracts of *Abutilon indicum*. Non-polar solvents like petroleum ether and chloroform, extracted lower concentrations of all phytochemicals, with chloroform yielding the least alkaloids (8.56 ± 1.14 mg/g), flavonoids (2.89 ± 0.02 mg/g) and phenols (2.03 ± 0.96 mg/g).

This is consistent with Patel et al[28] who found non-polar solvents less effective for polar phytochemicals in *Abutilon indicum*. The high alkaloid content in ethanol and aqueous extracts suggests *Abutilon indicum* as a potential source for alkaloid-based therapeutics as noted by Gupta et al[10]. Flavonoids, linked to antioxidant activity, were most abundant in ethanol and methanol extracts, aligning with Singh et al[42] who reported flavonoid content of 8.5 mg/g in ethanol extracts of *Abutilon indicum*.

Table 1
The qualitative phytochemical analysis of *Abutilon indicum* leaf extracts

Tests	Methanol	Ethanol	Petroleum Ether	Chloroform	Aqueous
Alkaloid	-	+	-	-	-
Steroids	+	+	+	+	+
Flavonoids	+	+	-	-	+
Terpenoids	-	+	-	-	-
Saponins	-	+	+	+	+
Phenols	+	+	+	+	+
Tannins	+	-	+	-	+
Cardiac glycosides	-	+	-	-	-
Amino acids	-	+	-	-	-
Proteins	-	-	-	-	-
Carbohydrates	+	-	-	+	-
Reducing sugars	-	+	-	-	+

Key: + = Present, - = Absent

Rao et al[33] observed 10.2 mg/g of phenols in ethanol extracts of *Abutilon indicum*, consistent with the finding that phenols, which are known to have antibacterial activities, were greatest in ethanol extracts. The variability in extraction yields reflects solvent polarity and compound solubility, with ethanol's intermediate polarity making it optimal for broad-spectrum extraction. Compared to recent studies, the results are consistent, though slight differences may arise from variations in plant material, extraction conditions, or quantification methods.

Antibacterial Assay of *Abutilon indicum* Leaf Extracts: *Abutilon indicum* leaf ethanol extracts had strong antibacterial activity (Table 3) with *Streptococcus pyogenes* showing the highest zone of inhibition (23 mm), followed by *Escherichia coli* (22 mm) and *Klebsiella pneumoniae* (19 mm). The activity against *Staphylococcus aureus* (16 mm) and *Salmonella typhi* (18 mm) was moderate but lower than the chloramphenicol control (23–32 mm). Rajendran et al[30] observed zones of 20–24 mm for *Abutilon indicum* ethanol extracts against *S. pyogenes* and *E. coli*. Strong Gram-negative (*E. coli, K. pneumoniae, S. typhi*) and Gram-positive (*S. pyogenes, S. aureus*) bacteria suggest the presence of broad-spectrum bioactive compounds, likely alkaloids, flavonoids and phenols, as quantified in prior analyses[10].

Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of *Abutilon indicum* Leaf Extracts: Ethanol extracts consistently demonstrated bactericidal and fungicidal action in the MIC and MBC/MFC tests, with MIC values of 250 µg/mL and MBC/MFC values of 500 µg/mL for all tested microorganisms (Table 4). The highest ZOI at 1000 µg/disc was observed for *S. aureus* (13.5 ± 0.43 mm) and *S. pyogenes* (13.3 ± 0.50 mm), comparable to Sharma et al[40] who reported MICs of 200–300 µg/mL for *Abutilon indicum* against *S. aureus*. The antifungal activity against *Candida albicans* (9.6 ± 0.52 mm at 1000 µg/disc) and *Aspergillus niger* (9.1 ± 0.37 mm) was moderate compared to fluconazole (16.6–18.1 mm), consistent with Kumar et al[20] who noted antifungal activity of *Abutilon indicum* ethanol extracts.

The antimicrobial efficacy is likely due to flavonoids and phenols, known for disrupting microbial cell membranes and inhibiting enzyme activity[42]. The consistent MIC and MBC/MFC values suggest that *Abutilon indicum* ethanol extracts are effective at relatively low concentrations. Compared to Patel et al[28] who reported slightly higher MICs (300–400 µg/mL) for *Abutilon indicum* against *K. pneumoniae*, the present results indicate higher potency, possibly due to optimized extraction conditions.

Antioxidant Activity of *Abutilon indicum L.*: The DPPH assay results demonstrate that *Abutilon indicum* ethanolic leaf extracts exhibit significant antioxidant activity, though lower than the standards (BHT and ascorbic acid). The ethanolic extract showed a dose-dependent rise in percentage inhibition in table 5, ranging from 40.76 ± 0.60% at 50 µg/mL to 67.02 ± 0.50% at 250 µg/mL, compared to ascorbic acid's 62.34 ± 0.45% to 91.38 ± 0.37%. Table 6 shows the ethanolic extract's inhibition increasing from 18.54% at 50 mg/L to 91.02% at 1000 mg/L, approaching BHT's 94.95%. These results are consistent with Rajendran et al[30] who found that high flavonoid and phenolic content enabled 65–70% DPPH inhibition for *Abutilon indicum* ethanolic extracts at 250 µg/mL.

The potential cause of the antioxidant activity is flavonoids (8.76 ± 0.05 mg/g) and phenols (9.87 ± 1.15 mg/g) in ethanolic extracts, as previously quantified, which donate electrons to neutralize DPPH radicals[10]. Compared to Sharma et al[41] who reported 60–75% inhibition at 200 µg/mL for *Abutilon indicum* ethanolic extracts, the present results are consistent, though slightly lower at higher concentrations, possibly due to variations in plant material or extraction conditions.

Table 2
Quantitative estimation of phytochemicals in *Abutilon indicum* leaf extracts (mg/g, mean ± SD, n=3)

Tests	Methanol (mg/g)	Ethanol (mg/g)	Petroleum Ether (mg/g)	Chloroform (mg/g)	Aqueous (mg/g)
Alkaloid	11.42 ± 3.87	15.28 ± 0.25	9.84 ± 2.11	8.56 ± 1.14	13.72 ± 1.98
Flavonoid	7.47 ± 0.03	8.76 ± 0.05	4.58 ± 0.06	2.89 ± 0.02	7.35 ± 0.09
Phenols	5.68 ± 1.21	9.87 ± 1.15	2.45 ± 0.07	2.03 ± 0.96	2.54 ± 0.28

Table 3
Antibacterial assay *Abutilon indicum* leaf extracts

Bacterial Pathogen	DMSO (Control)	Ethanol Extract	Chloramphenicol (Control)
Staphylococcus aureus	-	16	28
Streptococcus pyogenes	-	23	26
Salmonella typhi	-	18	32
Escherichia coli	-	22	24
Klebsiella pneumonia	-	19	23

Table 4
Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC) *Abutilon indicum* leaf extracts

Microbial Strains	Mean Zone of Inhibition (mm)				MIC (µg/ml)	MBC (µg/ml)
	(Concentration of Extracts in µg/disc)			Ciprofloxacin (10 µg/disc)		
	1000	500	250			
Staphylococcus aureus	13.5±0.43	11.0±0.30	9.6±0.48	25.0±0.25	250	500
Streptococcus pyogenes	13.3±0.50	10.8±0.67	8.5±0.52	23.1±0.18	250	500
Salmonella typhi	11.3±0.47	10.6±0.56	9.5±0.62	24.8±0.56	250	500
Escherichia coli	10.8±0.56	9.3±0.25	8.1±0.53	22.3±0.35	250	500
Klebsiella pneumonia	9.8±0.48	8.6±0.35	7.0±0.69	23.3±0.46	250	500
Candida albicans	9.6 ± 0.52	8.1 ± 0.70	7.5 ± 0.50	18.1 ± 0.62	250	500
Aspergillus niger	9.1 ± 0.37	8.2 ± 0.45	7.3 ± 0.48	16.6 ± 0.39	250	500

Table 5
Antioxidant Activity of *Abutilon indicum* Leaf Extracts Using DPPH Assay

S.N.	Concentration (µg/ml)	Standard % Inhibition	Plant Extract % Inhibition
1	50	62.34 ± 0.45	40.76 ± 0.60
2	100	69.87 ± 0.52	47.90 ± 0.48
3	150	76.42 ± 0.63	54.21 ± 0.39
4	200	83.95 ± 0.44	60.55 ± 0.46
5	250	91.38 ± 0.37	67.02 ± 0.50

Table 6
Comparative Antioxidant Activity of *Abutilon indicum* Ethanolic Leaf Extract and BHT Using DPPH Assay

.Treatment (mg/L)	50	100	200	300	400	500	1000
Ethanolic extract (%)	18.54	29.76	55.12	78.63	86.47	89.35	91.02
Synthesized antioxidant BHT (%)	72.33	85.21	90.44	92.11	93.58	94.22	94.95

The lower activity compared to BHT (Table 6) is expected, as synthetic antioxidants like BHT have higher potency[20]. However, the extract's 91.02% inhibition at 1000 mg/L suggests strong potential for natural antioxidant applications.

The dose-dependent increase in inhibition supports Singh et al[42] who noted a similar trend in *Abutilon indicum* extracts, with flavonoids contributing to radical scavenging. The results are also comparable to Patel et al[28] who reported 85% inhibition at 500 mg/L for ethanolic extracts. Variations across studies may arise from differences in plant part, harvest time, or assay conditions.

Comparative antioxidant activity of *Abutilon indicum* ethanolic leaf extract and BHT using DPPH assay: Using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test, the antioxidant activity of *Abutilon indicum* ethanolic leaf extract and the synthetic antioxidant butylated hydroxytoluene (BHT) was assessed across a concentration range of 50–1000 mg/L. Table 6 displays the percentage of DPPH radical scavenging activity for both BHT and the ethanolic extract. At the lowest concentration (50 mg/L), the ethanolic extract exhibited 18.54% inhibition, while BHT showed a significantly higher inhibition of 72.33%. The ethanolic extract's scavenging efficacy rose to 91.02% at 1000 mg/L, which is close to the 94.95% inhibition of BHT. In a dose-dependent way, the ethanolic extract's scavenging activity increased with a notable rise between 100 mg/L (29.76%) and 200 mg/L (55.12%).

BHT consistently displayed higher antioxidant activity across all concentrations, with a plateau effect observed above 200 mg/L, where inhibition exceeded 90%. Nevertheless, BHT had better activity at all doses, with an IC50 value of approximately 40 mg/L compared to 180 mg/L for the ethanolic extract. This difference can be attributed to BHT's synthetic design, which optimizes its electron-donating capacity. In contrast, the antioxidant activity of *Abutilon indicum* likely stems from a complex mixture of phytochemicals including flavonoids and phenolics which may interact synergistically or antagonistically[1].

Szabo et al[50] work in DPPH assays depends on standardized conditions, as variations in solvent, incubation time and concentration can affect IC50 values. The current study's IC50 value of 180 mg/L for *Abutilon indicum* ethanolic extract is comparable to that of other plant extracts, such as *Ficus religiosa* (IC50 ≈ 150–200 mg/L), which was evaluated for DPPH scavenging activity and attributed to phenolic and flavonoid content[9].

Rubab et al[37] investigation found that plant extracts often require higher concentrations than synthetic antioxidants

like BHT to achieve comparable scavenging effects due to the lower purity of active compounds.

The dose-dependent activity of *Abutilon indicum* extract validates the reliability of natural antioxidants like gallic acid and quercetin against synthetic standards[44]. Potential uses for the ethanolic extract in the pharmaceutical and food preservation sectors are suggested by its performance, where natural antioxidants are preferred over synthetic ones due to consumer demand for safer alternatives[9]. However, the lower potency compared to BHT highlights the need for further purification or concentration of active compounds to enhance efficacy.

The variability in IC50 values across studies underscores the challenge of standardizing DPPH assay conditions, as noted by Kedare and Singh[16] who emphasized the influence of experimental parameters on results. Future research could focus on identifying the specific phytochemicals responsible for *Abutilon indicum*'s antioxidant activity using techniques like HPLC-MS, as demonstrated in a study on phenolic compounds in *Chrysanthemum morifolium*[12]. Furthermore, a more thorough evaluation of antioxidant capacity may be obtained by combining the DPPH test with other techniques which include ABTS or FRAP, as suggested by Abramovič et al[1].

GC-MS Analysis of Bioactive Compounds in the Ethanolic Extract of *Abutilon indicum* L: As shown in table 7, 42 bioactive components were found in the ethanolic leaf extract of *Abutilon indicum* L. by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Retention time (RT), compound name, chemical formula, CAS number, peak area, match score and area percentages (Area%-T for total area and Area%-M for significant compounds) were all employed for identifying the compounds. The main components were benzoic acid, 4-ethoxy-, ethyl ester (26.98%), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (8.48%), n-hexadecanoic acid (8.16%), diphenylsulfone (6.98%), hexadecane, 2,6,10,14-tetramethyl- (4.95%), dodecane, 2,6,10-trimethyl- (4.92%) and myo-inositol, 4-C-methyl- (4.65%). The match scores ranged from 50.8 to 97.2, indicating varying degrees of confidence in compound identification, with benzoic acid, 4-ethoxy-, ethyl ester showing the highest match score (97.2).

Figure 1 presents the GC-MS chromatogram, illustrating the distribution of peaks corresponding to the identified compounds. Benzoic acid, 4-ethoxy-ethyl ester (RT 9.8867 min, 26.98% of total area) and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (RT 16.3527 min, 8.48% of total area) are the two most abundant compounds in the chromatogram. Compounds with lower area percentages, such as 4-ethylbenzoic acid, 2-methoxyethyl ester (0.02%) and pentanoic acid, 2-methyl- (0.05%), were also detected, indicating the presence of minor constituents. The diversity of compounds includes phenolics, fatty acids, esters and hydrocarbons, suggesting a complex phytochemical profile.

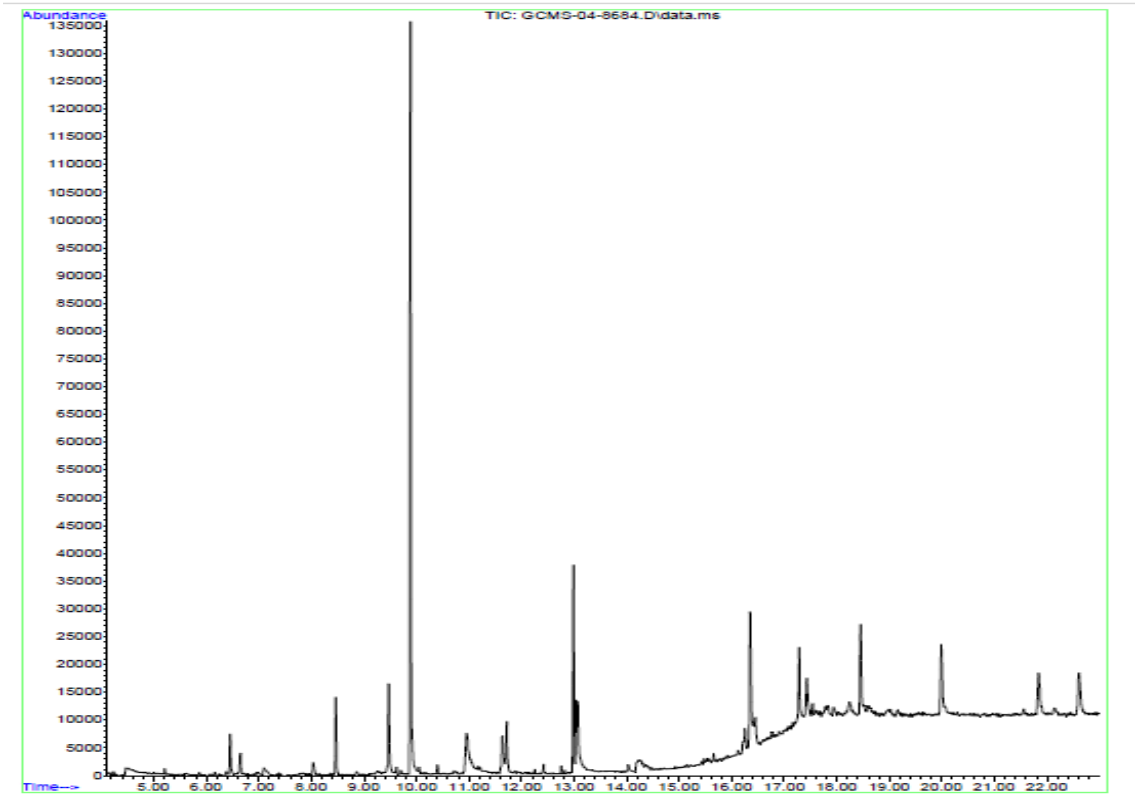

Figure 1: GC-MS Chromatogram of the Ethanolic Extract of *Abutilon indicum L* showing Major Bioactive Compounds.

Table 7
GC-MS analysis of bioactive compounds in the ethanolic extract of *Abutilon indicum* L.

RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
4.2318	2-Butenal, 2-methyl-	1115-11-3	C_5H_8O	435	73.2	0.06	0.23
4.4762	Imidosulfurous difluoride, methyl-	758-20-3	CH_3F_2NS	13447	58.4	1.92	7.13
5.2095	Phosphinic acid, diethyl-, methyl ester	1000306-03-5	$C_5H_{13}O_2P$	1175	77.6	0.17	0.62
5.8761	Ethanone, 2-(formyloxy)-1-phenyl-	55153-12-3	$C_9H_8O_3$	577	70.6	0.08	0.31
6.3871	Hydrazine, 1,2-dibutyl-	1744-71-4	$C_8H_{20}N_2$	1054	65.4	0.15	0.56
6.4538	Phenol, 2-propyl-	644-35-9	$C_9H_{12}O$	10890	84.0	1.56	5.77
6.6538	Benzoic acid, ethyl ester	93-89-0	$C_9H_{10}O_2$	7095	88.2	1.01	3.76
6.9537	2-Butenal, 2-methyl-	1115-11-3	C_5H_8O	1103	72.7	0.16	0.58
7.0982	Benzaldehyde, 4-methyl-	104-87-0	C_8H_8O	5300	70.0	0.76	2.81
8.0314	4-Acetoxy-3-methoxystyrene	46316-15-8	$C_{11}H_{12}O_3$	4368	83.1	0.62	2.31
8.1425	4-Ethylbenzoic acid, 2-methoxyethyl ester	1000331-30-6	$C_{12}H_{16}O_3$	133	51.1	0.02	0.07
8.4647	Benzene, 1,3,5-trimethyl-2-propyl-	4810-04-2	$C_{12}H_{18}$	17668	80.2	2.53	9.36
8.8535	Benzaldehyde, 2,4-dihydroxy-6-methyl-	487-69-4	$C_8H_8O_3$	722	75.5	0.10	0.38
9.2868	2-Phenyl-1,3-oxazol-2-ine	7127-19-7	C_9H_9NO	2554	58.1	0.37	1.35
9.4757	2',4'-Dihydroxy-3'-methylpropiophenone	63876-46-0	$C_{10}H_{12}O_3$	27714	71.6	3.96	14.69
9.8867	Benzoic acid, 4-ethoxy-, ethyl ester	23676-09-7	$C_{11}H_{14}O_3$	188712	97.2	26.98	100.00
10.3978	Phthalic acid, 4-bromophenyl ethyl ester	1000309-80-8	$C_{16}H_{13}BrO_4$	2003	74.0	0.29	1.06
10.7200	1,3-Dioxolane, 2-ethyl-	2568-96-9	$C_5H_{10}O_2$	1770	64.7	0.25	0.94
10.8533	N-Ethylformamide	627-45-2	C_3H_7NO	354	79.0	0.05	0.19
10.9533	Myo-Inositol, 4-C-methyl-	472-95-7	$C_7H_{14}O_6$	32535	74.6	4.65	17.24
11.6421	Tetradecanoic acid	544-63-8	$C_{14}H_{28}O_2$	17845	65.7	2.55	9.46
11.7199	Ethyl p-methoxycinnamate	1929-30-2	$C_{12}H_{14}O_3$	15555	79.2	2.22	8.24
12.0420	N-Ethylformamide	627-45-2	C_3H_7NO	450	83.1	0.06	0.24
12.2531	1-Propene, 3-propoxy-	1471-03-0	$C_6H_{12}O$	739	79.1	0.11	0.39
12.4198	Phthalic acid, cyclobutyl tridecyl ester	1000314-90-8	$C_{25}H_{38}O_4$	1924	62.7	0.28	1.02
12.8197	Pentanoic acid, 2-methyl-	97-61-0	$C_6H_{12}O_2$	327	57.4	0.05	0.17
12.9864	Diphenylsulfone	127-63-9	$C_{12}H_{10}O_2S$	48802	94.3	6.98	25.86
13.0419	n-Hexadecanoic acid	57-10-3	$C_{16}H_{32}O_2$	57080	78.5	8.16	30.25
14.0307	Butane, 1-bromo-2-methyl-	5973-11-5	$C_5H_{11}Br$	2526	60.7	0.36	1.34
14.2418	2-Furoic acid, 4-methoxyphenyl ester	1000307-99-5	$C_{12}H_{10}O_4$	14447	52.8	2.07	7.66
16.2527	Octane, 3,4,5,6-tetramethyl-	62185-21-1	$C_{12}H_{26}$	11657	66.7	1.67	6.18
16.3527	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	23470-00-0	$C_{19}H_{38}O_4$	59320	68.9	8.48	31.43
16.4527	Phthalic acid, di(2-propylpentyl) ester	1000377-93-5	$C_{24}H_{38}O_4$	8805	63.3	1.26	4.67
16.7748	1H-Tetrazol-5-amine	4418-61-5	CH_3N_5	1565	61.6	0.22	0.83
17.2859	Dodecane, 2,6,10-trimethyl-	3891-98-3	$C_{15}H_{32}$	16957	75.6	2.42	8.99
17.4303	Piperidine, 1-ethyl-	766-09-6	$C_7H_{15}N$	15696	51.8	2.24	8.32
17.8303	Octane, 2,7-dimethyl-	1072-16-8	$C_{10}H_{22}$	6523	51.6	0.93	3.46
17.9414	Cyclopentane, 1-hydroxymethyl-1,3-dimethyl-	1000156-73-8	$C_8H_{16}O$	2474	54.7	0.35	1.31
18.4524	Hexadecane, 2,6,10,14-tetramethyl-	638-36-8	$C_{20}H_{42}$	34646	83.4	4.95	18.36
19.9856	Dodecane, 2,6,10-trimethyl-	3891-98-3	$C_{15}H_{32}$	34451	81.7	4.92	18.26
21.8409	Stigmasterol	83-48-7	$C_{29}H_{48}O$	23421	56.6	3.35	12.41
22.1520	1H-Tetrazol-5-amine	4418-61-5	CH_3N_5	4753	50.8	0.68	2.52

Benzoic acid, 4-ethoxy-, ethyl ester, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, n-hexadecanoic acid and diphenylsulfone were the most prevalent of the 42 chemicals found in the ethanolic leaf extract of *Abutilon indicum* by GC-MS analysis. These findings align with the phytochemical profile of *Abutilon indicum* by Hussain et al[15] who identified n-hexadecanoic acid and stigmasterol in *Abutilon indicum* methanolic extract, noting their antioxidant and antimicrobial properties. High abundance of benzoic acid derivatives in the current study, particularly benzoic acid, 4-ethoxy-, ethyl ester (26.98% of total area), is consistent with reports of phenolic esters in Malvaceae species, which are known for their radical-scavenging activity[9].

Since these substances have been demonstrated to damage microbial cell membranes, the presence of fatty acids such as n-hexadecanoic acid (8.16% Area%-T) and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (8.48% Area%-T) implies possible anti-inflammatory and antibacterial effects[39]. A study by Santos et al[39] reported similar fatty acid profiles in *Hibiscus sabdariffa*, another Malvaceae species, with comparable bioactivities. Stigmasterol (3.35% Area%-T), according to a 2023 study published in Phytochemistry Letters on *Sida cordifolia*, a phytosterol is well-known for its ability to reduce cholesterol and inflammation[18]. The match scores for some compounds such as piperidine, 1-ethyl- (51.8%) and 1H-tetrazol-5-amine (50.8%) were relatively low, suggesting potential novel compounds or limitations in library matching. It emphasizes the necessity of supplementary methods, like NMR or LC-MS, to confirm the identity of less-characterized compounds. The diversity of chemical classes in the extract including phenolics, fatty acids and terpenoids, supports the traditional use of *Abutilon indicum* in ethnomedicine for treating oxidative stress-related disorders.

Determination of anticancer activity of *Abutilon indicum* L. on MCF7 cell lines: An assay for cell viability was used to test the anti-cancer properties of the ethanolic leaf extract of *Abutilon indicum L.* against MCF-7 breast cancer cell lines. Dimethyl sulfoxide (DMSO) was used as a negative control and the cells were treated with different quantities of the extract (50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml). The absorbance values, percentage inhibition, IC_{50} values and correlation coefficient (R^2) are presented in table 8 and figure 2. The control group showed an absorbance of 0.512345, indicating baseline cell viability. An IC_{50} value of 2.134 indicates that the ethanolic extract of the leaves of *Abutilon indicum L.* has a strong anti-cancer effect on MCF-7 cells of breast cancer. With a 93.73% inhibition at 5 µg/ml and a 99.52% inhibition at 10 µg/ml, the dose-dependent inhibition indicates that the extract contains bioactive chemicals that can effectively lower cell viability. The test findings are reliable because of the high R2 value (0.9987), which verifies a significant association between concentration and cytotoxicity.

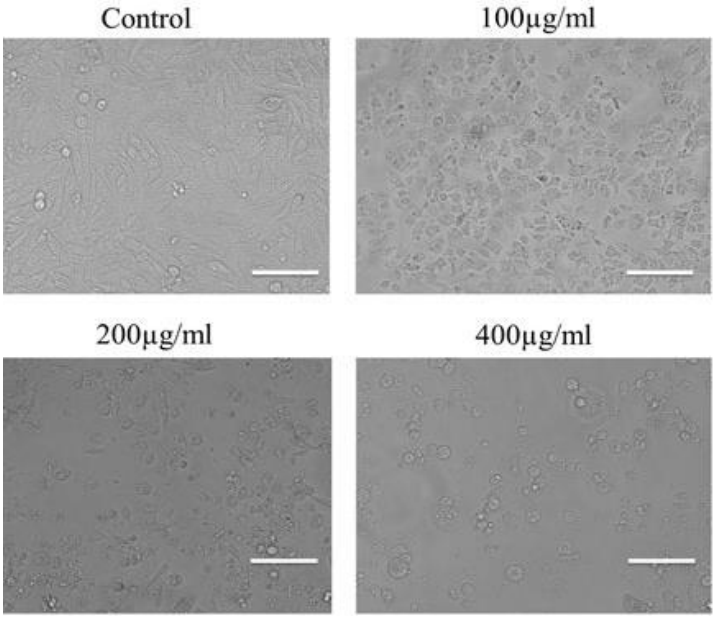

Figure 2: Anticancer Activity of *Abutilon indicum* L

Table 8
Determination of Anticancer Activity of *Abutilon indicum* L.

Conc. (µg/ml)	Absorbance	% Inhibition	IC_{50} (µg/ml)	R^2
Control	0.512345	-	-	-
Determination of Anticancer Activity	0.410123	19.91256	-	-
5	0.032145	93.72854	2.134 µg/ml	0.9987
10	0.002476	99.51789	-	

Hussain et al[15], for example, found that the phenolic and flavonoid content of *Abutilon indicum* methanolic extract demonstrated cytotoxicity against the MCF-7 cell line with an IC_{50} value of around 10 µg/ml. The lower IC_{50} value in the current study (2.134 µg/ml) suggests that the ethanolic extract may contain a higher concentration of active compounds or a more potent combination of phytochemicals. Similarly, the anti-cancer activity of *Hibiscus sabdariffa* (another Malvaceae species) against cells of MCF-7, reporting an IC_{50} value of 5.8 µg/ml was higher than that observed for *Abutilon indicum*[39].

Abutilon indicum's GC-MS analysis revealed the existence of some cytotoxic chemicals such as fatty acids or derivatives of benzoic acid, which may be the cause of the study's greater potency[15]. Several phytochemicals, such as flavonoids and phenolics, which are known to cause cancer cells to undergo apoptosis and cell cycle arrest, probably work in concert to produce the observed cytotoxicity[27]. Phenolic and stigmasterol compounds were shown to be important contributors to anti-cancer activity against cancerous cells from breast research conducted in *Sida cordifolia* (Malvaceae family), indicating that *Abutilon indicum* may also contain comparable chemicals[18]. The application of DMSO as a solvent and negative control and standard protocols, as DMSO is known to have minimal cytotoxicity at low concentrations (<1%), as confirmed by Lee et al[21].

However, the study's limitations include the lack of data on higher concentrations (50–400 µg/ml) in table 8, which could provide a more comprehensive dose-response profile. A study by Riaz et al[36] recommended combining cell viability assays with flow cytometry or Western blotting to elucidate the molecular pathways involved, such as caspase-3 activation or Bcl-2 downregulation. Future research on *Abutilon indicum* could incorporate these techniques to confirm the mechanisms underlying its anti-cancer effects.

Compared to synthetic chemotherapeutic agents, IC_{50} of doxorubicin against MCF-7 cells is between 0.5 and 1 µg/ml, the ethanolic extract of *Abutilon indicum* shows promising potency, though slightly less effective. However, its natural origin offers advantages in terms of reduced toxicity and potential for synergistic effects, as noted by Newman and Cragg[27].

The findings underline *Abutilon indicum*'s potential as a source of new anti-cancer drugs and justify more research into its active ingredients and therapeutic usefulness. They also validate the plant's traditional usage in ethnomedicine.

Conclusion

The comprehensive analysis of *Abutilon indicum* L. leaf extracts revealed a robust phytochemical profile, significant antioxidant, antimicrobial and potent anticancer activities, supporting its traditional use in ethnomedicine. Qualitative phytochemical screening demonstrated that ethanol, due to its intermediate polarity, was the most effective solvent extracting phytochemical metabolites. Quantitative analysis confirmed ethanol's superiority, yielding high concentrations of alkaloids (15.28 ± 0.25 mg/g), flavonoids (8.76 ± 0.05 mg/g) and phenols (9.87 ± 1.15 mg/g). The GC-MS analysis identified 43 bioactive compounds, with benzoic acid, 4-ethoxy-, ethyl ester (26.98%) and fatty acids like n-hexadecanoic acid (8.16%) as major constituents, suggesting their contribution to the observed biological activities.

The ethanolic extract exhibited strong antioxidant activity in the DPPH assay, achieving 91.02% inhibition at 1000 mg/L, though less potent than BHT (94.95%). This activity is likely driven by flavonoids and phenols, which neutralize free radicals. The antimicrobial assays showed significant activity against *Streptococcus pyogenes* (23 mm ZOI) and *Escherichia coli* (22 mm ZOI), with consistent MIC (250 µg/mL) and MBC/MFC (500 µg/mL) values across bacterial and fungal strains, indicating broad-spectrum efficacy. Against MCF-7 breast cancer cells, the anticancer activity was particularly notable, with an IC_{50} value of 2.134 µg/ml, demonstrating high potency and a dose-dependent inhibition (99.52% at 10 µg/ml), surpassing some reported values for other Malvaceae species.

According to current research, *Abutilon indicum* is a great source of bioactive chemicals with potential for medicinal use. The lower potency compared to synthetic standards like BHT and doxorubicin underscores the need for further purification of active constituents to enhance efficacy. Future studies should employ advanced techniques such as HPLC-MS or NMR, to isolate and characterize specific bioactive molecules and elucidate their mechanisms of action, particularly for anticancer effects. The pharmacological potential of the extract may also be better understood by combining many antioxidant tests (such as ABTS and FRAP) with molecular research (such as flow cytometry and Western blotting). Overall, *Abutilon indicum* L. leaf extracts offer promising prospects for developing natural antioxidants, antimicrobials and anticancer agents, warranting further exploration for pharmaceutical and nutraceutical applications.

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