Phytochemical Analysis and Investigation of the Antimicrobial and Cytotoxic Potential of Andrographis Paniculata Against WHO Classified Oral Pathogens

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**Abstract:** Andrographis paniculata, the "King of Bitters," is rich in andrographolide and flavonoids, known for antimicrobial and anticancer properties. This study evaluates its phytochemical composition, antimicrobial, and cytotoxic potentials. Ethanolic, ethyl acetate, and aqueous extracts were analyzed for antioxidant activity, phenolic content, and flavonoids. FTIR confirmed polyphenols and flavonoids. The ethanolic extract showed strong antibacterial effects, particularly against *S. aureus* and *S. mutans*, with low MIC values. It also exhibited dose-dependent cytotoxicity against KB cells (IC50: 38.4 µg/mL). These findings highlight A. paniculata's potential for developing antimicrobial and anticancer agents.

**Keywords:** fourier transform infrared spectroscopy (FTIR), cytotoxicity, antimicrobial, phytochemical analysis, andrographis paniculata

# Introduction

Andrographis paniculata, commonly known as the "king of bitters," is a widely used medicinal herb in traditional Asian practices[(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/vUTkDU/r3dY+u2ng+QdRe). It is an annual herb that is extremely bitter in taste and has been extensively used in Ayurvedic, Siddha, and traditional medicine systems in India[(Ganapathy et al. 2021)](https://paperpile.com/c/vUTkDU/lXoVN). Approximately 26 distinct polyherbal formulations containing this plant are documented in Ayurveda as widely used remedies for the treatment of various disorders [(Kumar et al., 2004)](https://paperpile.com/c/vUTkDU/9YRn). In recent years, the leaves and stems of the plant have been used extensively for extraction of active phytochemicals, including diterpenes, flavonoids and stigmasterols [(Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/vUTkDU/SUUd3+HsdvX);[(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/vUTkDU/UQsJ+ZXym). The most important bioactive compounds of A. paniculata are andrographolide (AG), 14-Deoxy-11,12-didehydroandrographolide (DDAG), and neoandrographolide (NAG) [(Valdiani et al., 2022)](https://paperpile.com/c/vUTkDU/S0fe). A. Paniculata contains therapeutically active secondary metabolites that include lactones, diterpenes, flavonoids, quinic acid, xanthones, noriridoids, and other compounds [(Naomi et al., 2022)](https://paperpile.com/c/vUTkDU/4m9V). These active molecules have been reported to exhibit various activities such as antibacterial, anti- biofilm, antiviral, antiparasitic, cytotoxic, anti-inflammatory, anti- fungal and many more to report [(Naomi et al., 2022; Valdiani et al., 2022)](https://paperpile.com/c/vUTkDU/S0fe+4m9V). Andrographolide can inhibit the virulence factors of invasive microbes and regulate the host immune system. Controlled clinical trials have demonstrated that A. paniculata is a safe and effective treatment for acute respiratory tract infections, such as the common cold and sinusitis and scientifically proven oxidative therapies[(Marya et al., 2022)](https://paperpile.com/c/vUTkDU/4ysZx) [(Jain & Verma, 2022; Marya et al., 2022)](https://paperpile.com/c/vUTkDU/4ysZx+u8FgU) [(Wadhwani et al., 2022)](https://paperpile.com/c/vUTkDU/Qb528). Therefore, A. paniculata, particularly andrographolide (a major terpenoid), holds great potential as a candidate for antimicrobial drug development.

The plant has been reported to have excellent antioxidant potential. In recent surveys, methanolic extracts of the plant has demonstrated anti-oxidant and anti- inflammatory activities [(Adel et al., 2023)](https://paperpile.com/c/vUTkDU/uKNRm)[(Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/vUTkDU/8FSb)[(Solanki et al., 2023)](https://paperpile.com/c/vUTkDU/uxANT). Andrographolide, the diterpenoid, shares these properties with other diterpenoids such as neoandrographolide (NEO), 14- deoxyandrographolide (14DAP), and 14-deoxy-11,12-didehydroandrographolide (14DAP11-12), which also exhibit significant antioxidant and anti-inflammatory activities [(Mussard et al., 2020)](https://paperpile.com/c/vUTkDU/Q0pg).

The cytotoxic activity of the plant for preventing cancer cell proliferation is contributed by the presence of α-alkylidene γ-butyrolactone, the D12 (13), double bond, C-14 hydroxyl, and the D8 (17), double bond in andrographolide [(Naomi et al., 2022)](https://paperpile.com/c/vUTkDU/4m9V). The plant has successfully contributed in anti- cancer mechanisms such as cell cycle and apoptosis, regulation of cytochrome P400 and P450 and cytokine inhibition [(Malik et al., 2021; Suriyo et al., 2014)](https://paperpile.com/c/vUTkDU/Tjn9+a3q0).

This study critically evaluated the antimicrobial therapeutic potential of ethanolic extract of A. paniculata in inhibiting oral microbes as well as cytotoxic potential against KERATIN- forming tumor cell line HeLa (KB) cells. The evaluation of secondary metabolites through FTIR to identify pure compounds with antimicrobial properties has added significant value to this study. Despite the promise of A. paniculata as a source of antimicrobial agents and safe treatment for many infectious diseases, further empirical research is necessary.

# Materials and Methods

## Collection of plant materials, processing and extraction

Fresh leaves of A. paniculata were collected from forest regions in Palayamkottai, Tamil Nadu, India. The samples underwent initial sieving and cleaning to remove dirt and extraneous plant materials. Botanical authentication was performed by experts at St. Xavier's College, Palayamkottai, Tirunelveli, Tamil Nadu (India). The leaves were then subjected to thorough washing, wiping, air drying, and fine grinding with the help of electric blender. The dry weight of the leaves was measured both before and after washing and complete drying. The resulting powdered samples were sieved to ensure uniformity and prevent lump formation.

The extraction was performed using a Soxhlet apparatus. Approximately 100 g of dried and powdered plant material was combined with 1 liter of various solvents, including ethanol, ethyl acetate, and water, each at a concentration of 0.1 g/mL. The mixture was prepared at room temperature with constant shaking in a 500 ml conical flask for 24 h. The solution was filtered and solvent was evaporated using rotary evaporator (R-200; BUCHI, Flawil, Switzerland) coupled with a Buchi Vac V-500 pump. The extracts were resuspended in respective solvents and stored at 4ºC [(Alara et al., 2019; Stamm et al., 1976)](https://paperpile.com/c/vUTkDU/BX7K+mX7s).

## Phytochemical studies

## Determination of Antioxidant Activity Using the 2,2-Diphenyl-1- picrylhydrazyl (DPPH) Radical Scavenging Method

Radical scavenging action of ethanolic extracts of A. paniculata against the stable 1, 1-diphenyl-2- picrylhydrazyl (DPPH) radical was measured spectrophotometrically at 517 nm [5,10]. DPPH solution was prepared by adding DPPH (12.5 mg) in 50 mL of methanol. 1.5 mL sample,(0.1 mg/mL, prepared in 50 mM phosphate buffer, pH 7.5) was allowed to react with 1.5 mL of methanolic solution of DPPH for 30 min in darkness at room temperature. Decrease in absorbance were determined at 517 nm. Ascorbic acid was used as a standard. The reaction was performed in triplicate. Scavenging activity was the calculated by following formula; where Q represents the DPPH scavenging activity.

Q(%)= [Absorbance of control - Absorbance of sample/ Absorbance of control] X 100

## Determination of Antioxidant Activity Using the ABTS Free Radical Scavenging Method

Stock solutions of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) at 7.4 mM and potassium persulfate at 2.46 mM were prepared according to the protocols of Barapatre et al. [(Barapatre et al., 2015)](https://paperpile.com/c/vUTkDU/NJzS). To make the working solution, equal quantities of the two stock solutions were mixed and left to rest for 12 hours at room temperature in the dark. Subsequently, the mixture was diluted by combining 1 mL of the ABTS solution with 60 mL of methanol. Fresh ABTS solutions were prepared for each assay. For the assay, 150 μL of 0.1 mg/ml extract was reacted with 2850 μL of the ABTS solution for 2 hours in the dark. Absorbance was then measured at 734 nm using a spectrophotometer. The total antioxidant activity (TAA) was calculated and expressed as ascorbic acid equivalents (mg AAE/g extract) in reference to a standard curve.

## Determination of Antioxidant Activity Using the Reducing Power (RP) Method

To estimate the reducing power of the extract, a 1 mL of 0.1 mg/ml of each dissolved extract was combined with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of a 1% (w/v) potassium hexacyanoferrate solution. This mixture was incubated at 50°C for 30 minutes. Following incubation, 2.5 mL of 10% (w/v) aqueous trichloroacetic acid (TCA) was added, and the mixture was centrifuged for 10 minutes. A 2.5 mL aliquot of the supernatant was then mixed with 2.5 mL of water and 0.5 mL of a 0.1% FeCl3 solution. The absorbance of the final solution was measured at 700 nm. Results were expressed as ascorbic acid equivalent (AAE) values [mg AAE/g extract], with ascorbic acid serving as the positive control [(Mussard et al., 2020)](https://paperpile.com/c/vUTkDU/Q0pg).

## Total Phenolic Content

Total Phenolic Content (TPC) was analyzed by Folin-Ciocalteu assay. Gallic acid was used as reference. Standard gallic acid solution was prepared by dissolving 10 mg of gallic acid in 10 mL of methanol (1 mg/mL). Various concentrations of gallic acid solutions in methanol (25, 50, 75, and 100 μg/mL) were prepared from the standard solution. To each concentration, 5 mL of 10% Folin-Ciocalteu reagent and 4 mL of 7% Na2CO3 were added making a final volume of 10 mL. After incubating for 30 mins at 40ºC in a water bath, absorbance were measured at 765 nm and calibration curve was plotted [(Phuyal et al., 2020)](https://paperpile.com/c/vUTkDU/u5AI). For analysing the TPC of the extracts, About 0.25 mL of crude extract (0.1 mg/mL) and 1 mL of Folin Ciocalteu’s reagent were diluted with 10-fold water. After incubation of solution in the dark for 5 minutes, 2 mL of sodium carbonate (7.5% w/v) was mixed followed by optical density measurement at 765 nm. Findings were reported as mg gallic acid equivalents per gram of dry weight (mg GAE/g extract) with reference to standard curve.

## Total Flavonoid Content

The total flavonoid content of the leaves of A. paniculata was measured by mixing the 100 μL of leaf extract (0.1 mg/ml) and 100 μL of 2% Aluminium Chloride (AlCl3 ), incubating them for 10 mins, followed by measuring the absorbance at 420 nm. Quercetin from a concentration of 0.2- 1 mg/ml was used as a standard. Total flavonoid concentration was calculated as a percentage of total quercetin equivalents per gram of extract (mg QE/g) [(Barapatre et al., 2015)](https://paperpile.com/c/vUTkDU/NJzS).

## Characterization of the extracts using FTIR

Five to ten milligrams of the solvent dried extracts of A. paniculata analysed for FT- IR analysis. FT-IR experiments were performed using Bruker Alpha II (Bruker, USA) to identify the characteristic functional groups in the sample. The spectra was measured within a range of 4000-400 cm−1, with a resolution of 4 cm−1 [(Wongsa et al., 2022)](https://paperpile.com/c/vUTkDU/pKHw).

## Antimicrobial activity against oral pathogens

The antimicrobial properties of A. paniculata extracts were tested against Staphylococcus mutans (MTCC 890), Enterobacter faecalis (ATCC 29212), Lactobacillus acidophilus (Clinical), Staphylococcus aureus (Clinical), and Candida albicans (Clinical). E. faecalis and S. aureus were pre-cultured in Mueller Hinton broth (MHB) overnight in a rotary shaker at 37°C. Whereas, S. mutans in Mannitol Salt Agar (MSA) and L. acidophilus in ChromAgar MRSA Medium. Afterward, each strain was adjusted at a concentration of 10 8 cells/ml using 0.5 McFarland standard. The fungal inoculum was prepared from the 48 h culture of C. albicans in Sabourauds Agar (SDA). The spectrophotometer (A595 nm) was used to adjust the spore density of fungus at a final concentration of 10 6 spores/ml [(Gonelimali et al., 2018)](https://paperpile.com/c/vUTkDU/v61d). Amoxicillin was considered as positive and ethanol as negative control.

## Antimicrobial Screening

Agar well diffusion method was performed to screen the antibacterial and antifungal activities of different solvent extracts [(Daoud et al., 2019)](https://paperpile.com/c/vUTkDU/TLZH). The concentration of extracts were kept to be 20% w/v. For antibacterial screening, 100 µl of the bacterial suspension with a concentration of 10 8 cells/ml were spread uniformly on the MHA plates and the inoculum were air dried. Around 8 mm diameter wells were created with the help of a sterilized forceps on the plates. The plant extract (0.1 mg/ml) were filled in the wells of the agar plates, followed by incubation for 2 hours at RT. The inoculated plates with bacteria were incubated at 37°C for 24 h in the inverted position and 48 h for the fungal strains. The MHA plate with L. acidophilus and S. mutans were incubated in anaerobic chamber for 24h at 37ºC. Antimicrobial activity was detected by measuring the zone of inhibition (including the wells diameter) appeared after the incubation period. DMSO at a concentration of 10% was employed as a negative control [(Nithya et al., 2021)](https://paperpile.com/c/vUTkDU/xcTe).

## Determination of Minimum Inhibitory (MIC) and Minimum Fungicidal Concentrations (MFC)

The MIC of A. paniculata was determined by the broth dilution method using 96- well titreplate according to the Clinical and Laboratory Standards Institute (CLSI). For MIC, each well consisted of 100 µl of Mueller Hinton Broth, 50 µl of overnight respective bacterial culture and 50 µl of stock of the plant material (0.1 mg/ml). The control well contained broth and plant extract. The microtitre plate were incubated at 37ºC for 24 h [(Ambulkar et al., 2021)](https://paperpile.com/c/vUTkDU/0Nxp). For L. acidophilus and S. mutans, the plates were incubated in an anaerobic chamber. For MFC, the well consisted of 100 µl of Sabouraud’s broth, 50 µl of C. albicans culture and plant extracts [(Meccatti et al., 2023)](https://paperpile.com/c/vUTkDU/g7Zd). The MIC value of the plant extract was evaluated as the lowest concentration that completely inhibited pathogenic growth after 24 h of incubation.

## Cytotoxic potential by MTT assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the inhibition of cancer cell proliferation by ethanolic extract of A. paniculata. Exponentially growing KB cells were seeded into 96-well plates (10 4 cells/well in 100 μL of media- DMEM contained 10% of fetal bovine serum and antibiotic) and allowed to attach for 24 h. After the incubation, cells were treated with different concentrations of extract (100 µg/mL to 500 µg/mL) and incubated for 24 and 48 h. Cells in the control group received only serum free media. The test compound containing media was removed and washed with 200 μL of PBS followed by addition of 20 μL of MTT reagent (5 mg/mL MTT in PBS) and incubated for 4 h at 37°C. The medium was removed and 100 μL DMSO was added and the absorbance measured using a micro plate reader at 540 nm followed by the calculation of percentage viability [(Valdiani et al., 2022)](https://paperpile.com/c/vUTkDU/S0fe).

# Results

Antioxidant activity quantification of different extracts of A. paniculata by DPPH and ABTS assay The antioxidant activity of the extracts was evaluated using the DPPH and ABTS assays, expressed as percentage scavenging (Table 1). Ascorbic acid in different concentrations were used as standard for both of the study. In the DPPH assay, the ethanolic extract of the plant exhibited 93.5% scavenging activity at a concentration of 100 µg/mL, followed by the ethyl acetate extract, which showed 81.4% inhibition at the same concentration. The IC50 of inhibition of the ethanol extract was estimated to be 52430 µg/ml. ABTS assay measures the relative strength of antioxidant to scavenge the ABTS+ radicals of A. paniculata. The ethanolic extract of the plant demonstrated the highest scavenging activity of 99.39% at a concentration of 100 µg/mL, while the ethyl acetate extract showed the lowest activity at 67.06% at the same concentration. The IC50 of inhibition of the ethanolic extract was estimated to be 58750 µg/ml.

**TABLE 1:** DPPH scavenging activity (%), ABTS scavenging activity (%), total phenolic (mg GAE/g) and flavonoid content (mg QE/g) and IC50 of DPPH Scavenging of various extracts of A. paniculata. All values are mean ± SD (n = 3).Mean ± SD, significantly different from their respective control at p< 0.01.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Samples (100µg/ml)** | **DPPH Scavenging (%)** | **ABTS Scavenging (%)** | **Total phenolic content (mg GAE/g)** | **Total flavonoid content (mg QE/g)** | **DPPH Scavenging (IC50 )** |
| Ethanolic extract | 93.50± 0.65 | 99.39± 0.13 | 190.07± 2.35 | 954.932± 0.89 | 52430± 0.93 |
| Ethyl acetate extract | 81.4± 0.34 | 67.06± 0.12 | 179.22± 3.33 | 911.295± 0.33 | 45579± 5.19 |
| Aqueous extract | 65.84± 0.22 | 69.16± 0.16 | 122.63± 2.29 | 610.812± 0.56 | 36891± 4.38 |

## Antioxidant activity quantification of reducing power potential of the plant extract

Ethanolic extract of A. paniculata illustrated the highest reducing power among the other extracts, with a concentration of 403.89 mg AAE/g extract, 283.54 AAE/g extract (ethyl acetate) and 248.22 AAE/g extract (Aqueous). The reducing power was found to be in order of ethanolic> ethyl acetate> aqueous extract (Table 1). The reducing power of all extracts increased sharply at lower concentrations but declined rapidly as the concentration increased.

## Total flavonoid and phenolic content

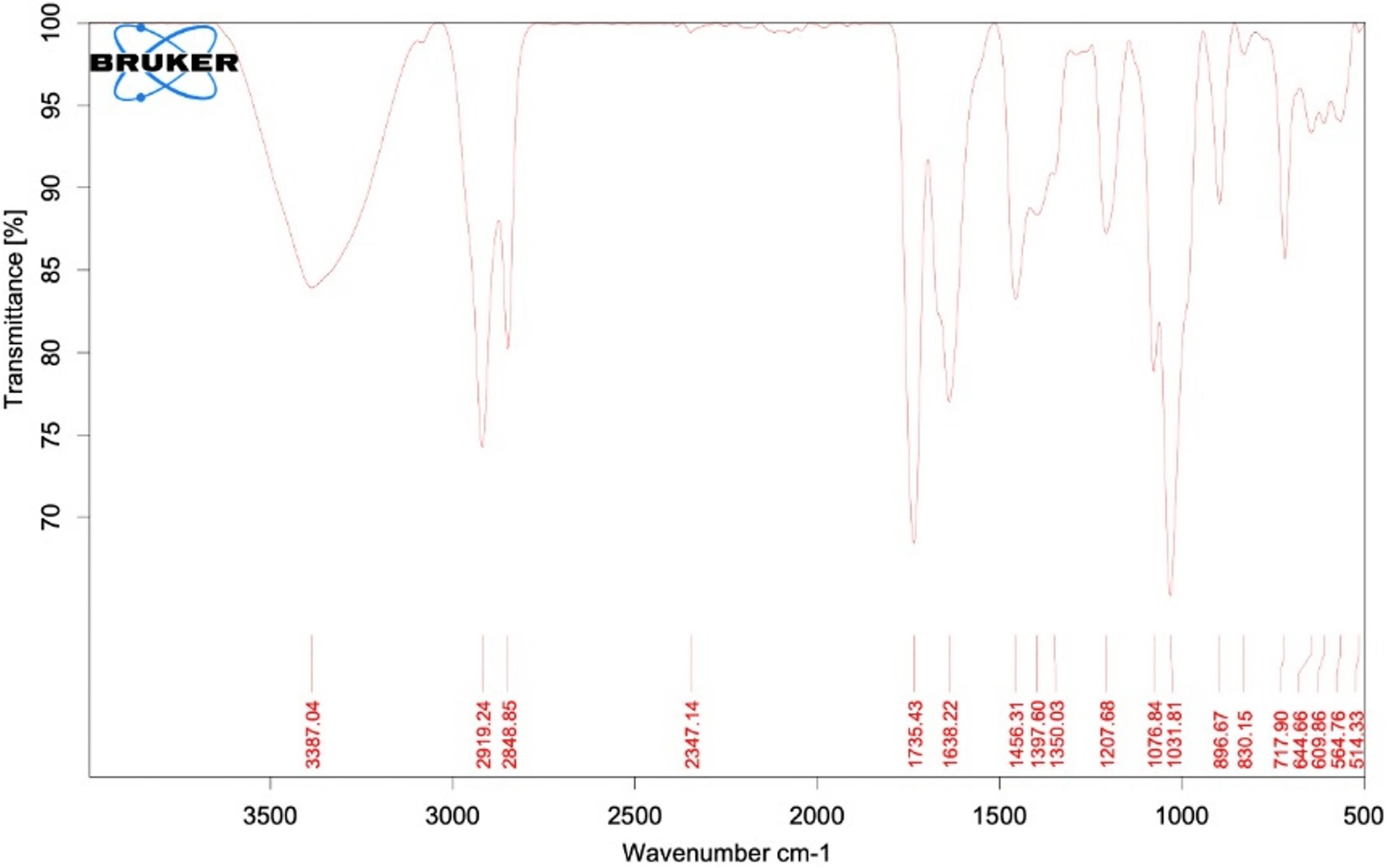
The total flavonoid content (TFC) of the plant extract was calculated using Quercetin as a standard. The highest flavonoid content was observed in ethanolic extract, with an estimate of 954.932 mg QE/g extract in 100 µg/ml sample, while the aqueous extract contained the lowest, with 610.812 mg QE/g extract in 0.1 mg/ml sample and in Ethyl acetate extract the TFC was estimated to be 911.295 mg QE/g (Table 1).Also, the total phenolic content (TPC) was estimated using a standard graph of gallic acid. The highest phenolic content was reported in the ethanolic extract, with an estimate of 190.07 mg GAE/g extract at a concentration of 100 µg/ml, while the lowest was found in the aqueous extract, with 122.63 mg GAE/g extract at the same concentration. In Ethyl acetate extract the TPC was estimated to be 179.22 mg GAE/g.

## FTIR profile of the extracts

The infrared spectroscopic analysis derives information about the possible functional groups present in the ethanolic dried extracts of the leaves of A. paniculata. Figure 2 shows the FTIR Spectrum of the extract. Absorption spectra of the ethanolic dried extract, obtained in the range of 1000- 500 cm-1 are illustrated in Table 1. The most intense bands were ranging from 1735.4 to 1031.8 cm-1. The peak of 3387.04 indicates a polymeric hydroxyl group (O-H), -H bond stretching, indicating presence of polyphenols. For the peak observed at 2919.24 and 2848.25, the -CH, -CH2 and -CH3, aliphatic group stretching can be seen, indicating presence of carbohydrates, proteins and lipids. The C=O vibrations at 1735.43 and C=C at 1638.22 shows the existence of ester fatty acids and unsaturated fatty acids, demonstrated the presence of lipid structures, respectively. In the range between 1000- 1500 cm-1, many bands were obtained. The bending vibrations at 1456.31 and 1397.60, confirms the presence of -CH group of alkyl structures. Peaks at 1350.03 demonstrates the presence of Amide III band of protein structure and at 1207.68, -CO group of ester fatty acids and fatty acids.

**TABLE 2:** Absorption spectra of the ethanolic dried extract of A. paniculata, obtained in the range of 1000- 500 cm-1

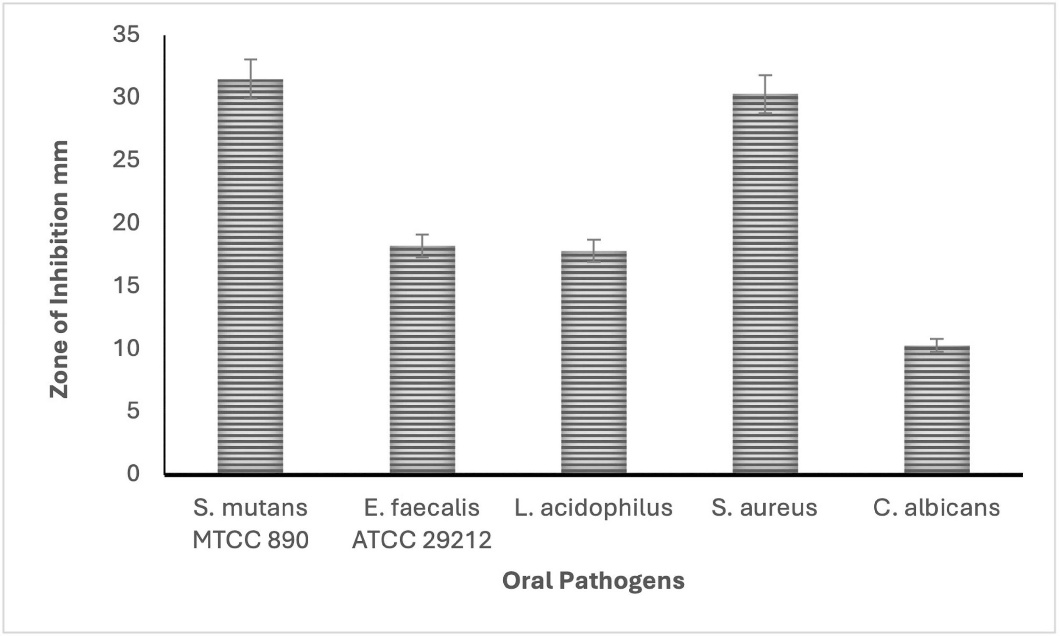
|  |  |  |  |
| --- | --- | --- | --- |
| Bond Type | Functional group | Wave numbers range | |
|  |  | σth | σexp |
| O-H | Carbohydrates, Proteins, Polyphenols | 3350-3450 | 3387.04 |
| C-H; -CH2 | Aliphatic group (Stretching) | 2850-2950 | 2919.24 |
| C-H; -CH2 | Aliphatic group (Stretching) | 2850-2950 | 2848.85 |
| C=O | Ester fatty acid | 1730-1740 | 1735.43 |
| C=C | Unsaturated fatty acid | 1610-1620 | 1638.22 |
| C-H | Aliphatic group (Bending group) | 1460-1370 | 1456.31 |
| C-H | Aliphatic group (Bending group) | 1450-1370 | 1397.60 |
| C-N | Amide III Band | 1240-1340 | 1350.03 |
| C-N | Amide III Band | 1240-1340 | 1207.68 |



**FIGURE 1:** FTIR Spectrum of ethanolic extract of ethanolic extract of A. paniculata leaves

# Antimicrobial screening

The antibacterial potential of the ethanolic extract of A. paniculata (100 µg/ml), were evaluated by measuring the zone of inhibition against 05 oral pathogenic strains. Significant antibacterial activity was observed against S. aureus (32 mm) and S. mutans (30 mm). Amoxicillin was taken as a standard which gave a zone of inhibition of about (40 mm) against S. aureus . The result of ZOI of the extract and its comparison with standard antibiotic Amoxicillin (100 μg/ml) is recorded in Table 2 and Figure 2.



**FIGURE 2:** Graph showing the mean inhibition zone diameter by ethanolic extracts of A. paniculata. The data are the means of

**TABLE 3:** Zone of inhibition produced by oral pathogenic strains against ethanolic extract of A. paniculata and standard Amoxicillin different concentrations ranging from 10-1000 µg/ml.

|  |  |  |
| --- | --- | --- |
| Strain | Ethanolic extract (100µg/ml) | Amoxicillin (100µg/ml) |
| S. mutans ATCC 890 | 30 | 37 |
| E. faecalis ATCC 29212 | 18 | 24 |
| L. acidophilus | 16 | 20 |
| S. aureus | 32 | 40 |
| C. albicans | 13 | 20 |

## Minimum Inhibitory Concentration and Minimum Fungicidal Concentration Estimation

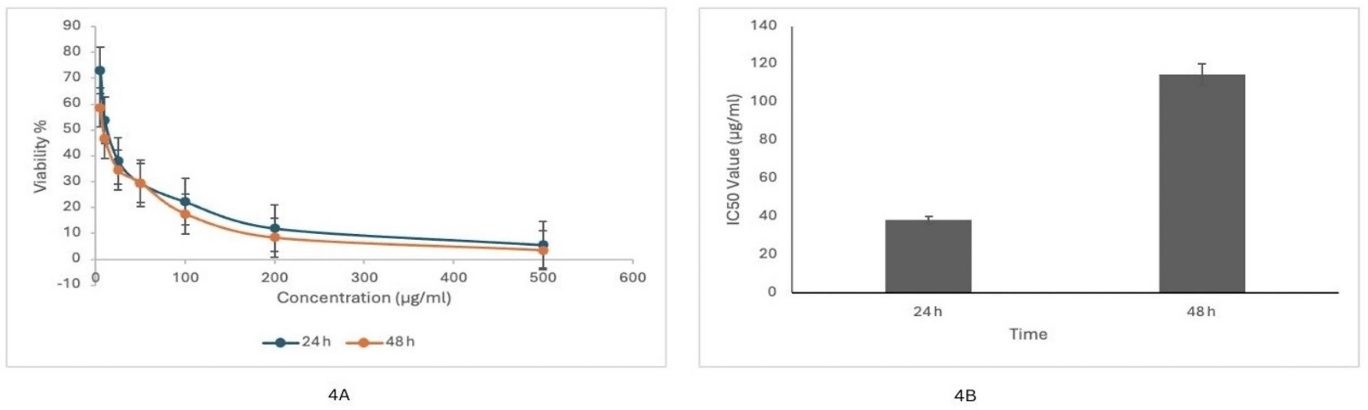
Minimum Inhibitory Concentration and Minimum Fungicidal Concentration Estimation The MIC and MFC were determined for the ethanolic extract of the plant, since they displayed significant antibacterial activity. The lowest MIC was calculated for S. aureus, at 1.12±1.2 µg/ml; at 7.99±0.0 µg/ml for E. faecalis; at 6.5±0.02 µg/ml for L. acidophilus; at 7.24±0.05 µg/ml for S. mutans; and at 4.4±0.22 µg/ml for C. albicans.

**TABLE 4:** MIC of ethanolic extract of A. paniculata against classified oral pathogens

|  |  |
| --- | --- |
| Strains | MIC (µg/ml) |
| S. mutans ATCC 890 | 7.24±0.05 |
| E. faecalis ATCC 29212 | 7.99±0.0 |
| L. acidophilus | 6.5±0.02 |
| S. aureus | 1.12±1.2 |
| C. albicans | 4.4±0.22 |

## Cytotoxicity studies of extracts of A. paniculata against KB cell lines using the MTT colorimetric method

The effects of ethanolic extracts of A. paniculata , on the viability of KB cells showed that the lowest IC50 dose was at 38.4 µg/mL at 24 h and 114.7 µg/mL at 48h (Fig 4A). This cytotoxic activity was compared to Cisplastin, which exhibited IC50 values of 7.28 µg/mL at 24 h and 10.11 µg/mL at 48h. Ethanol, used as negative control, showed higher IC50 values of 102.12 µg/mL at 24 h and 198.61 µg/mL at 48h. Higher concentrations of the extracts decreased the cell viability. Figure 4B represents the dose-response curves of KB cells viability against ethanolic extract, which implies the dose-dependent inhibition of A. paniculata.



**FIGURE 3:** A. Dose-dependent inhibition curve of KB cells treated with Andrographis paniculata extracts over 24 and 48 hours. Untreated KB cells were used as controls. The results demonstrate a significant decrease in cell viability with increasing concentrations of the plant extracts, indicating a lethal impact at higher doses. Data are presented as mean ± standard deviation; B. IC50 value in KB cells with respect to cellular viability.

# Discussion

The current research investigates the antimicrobial activity of Andrographis paniculata leaves against clinically significant oral pathogens. Medicinal plants have been shown to influence the pharmaceutical industry by inhibiting the growth of pathogenic microbes . In the context of oral diseases, various plants including Abrus precatorius, Anacyclus pyrethrum, Calotropis gigantea, Cyperus rotundus etc. have historically been used as toothbrushes or dentifrices to treat dental issues. Different plant parts such as leaves, gum, bark, fruits, roots, and twigs have been utilized to extract active biomolecules using solvents like methanol, ethanol, and water [(Alara et al., 2019)](https://paperpile.com/c/vUTkDU/BX7K).A. paniculata leaves exhibit a range of medicinal properties, including choleretic, anti-diarrhoeal, immunestimulant, anti-inflammatory, hepatoprotective, anti-malarial, anti-hypertensive, antipyretic, antithrombotic, and antidote effects [(Chokkattu et al., 2023)](https://paperpile.com/c/vUTkDU/LHP33)[(Ramakrishnan et al., 2023a)](https://paperpile.com/c/vUTkDU/JPYA)[(Laghari et al., 2023; Ramakrishnan et al., 2023b)](https://paperpile.com/c/vUTkDU/pCi9k+ONgX). It is known for its rich phytochemical content, particularly diterpenoids and flavonoids. It is commonly referred to as Nilavembu in southern part of India and is an annual herb predominantly found in the southern regions of India. Major bioactive compound in A. paniculata are andrographolide, neoandrographolide, isoandrographolide, which demonstrates significant antibacterial activity against various gram-negative and gram-positive pathogens [(Hossain et al., 2021)](https://paperpile.com/c/vUTkDU/v7bM). Previous studies have shown that A. paniculata extracts possess antibacterial properties effective against bacterial strains responsible for diarrhoea and dysentery, such as Escherichia coli and Vibrio cholera [(Hossain et al., 2021; Oktavianawati et al., 2023)](https://paperpile.com/c/vUTkDU/v7bM+IiE4). The current study aims to evaluate the antioxidant activity, phenolic and flavonoid content of the extract, as well as its antibacterial and antifungal properties against oral pathogenic strains[(Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/vUTkDU/n12t) [(Sreevarun et al., 2023a)](https://paperpile.com/c/vUTkDU/I8fS)[(Sreevarun et al., 2023b)](https://paperpile.com/c/vUTkDU/OUMJo)The active compounds of A. paniculata, were extracted using water, ethanol and ethyl acetate, from the leaves of the plant[(Ganapathy et al., 2021)](https://paperpile.com/c/vUTkDU/0cjQ9). Significantly higher yields of polyphenols and effective antimicrobial activities were obtained in ethanolic extract, owing to the ability of ethanol to bind to both polar and non- polar compounds. It has been reported that it is easy to isolate terpenoids, saponin, steroids, alkaloids, tannins, and flavonoids through ethanol extraction [(Naomi et al., 2022)](https://paperpile.com/c/vUTkDU/4m9V). However, many previous reports have mentioned methanol as a better solvent for extraction of phytochemicals, and chloroform and aqueous fractions as the poorest yield solvents [(Sharma et al., 1992; Yusof et al., 2015)](https://paperpile.com/c/vUTkDU/brZG+i7JM). This discrepancy is believed due to variations in polarity and extractability of available bioactive compounds in the plant material. FTIR profile of the dried ethanolic extracts of leaves of A. paniculata, demonstrated presence of tannins, phenolics and flavonoids. Flavonoid are majorly responsible for antioxidant activity. A. paniculata has been reported to contain 02 phenolic compounds, and 01 flavonoid and rich in aliphatic amines, primary and secondary amines, or may be bend alkynes [(Abdul et al., 2021)](https://paperpile.com/c/vUTkDU/io8p). Majority of active compounds are extracted from leaves which includes andrographolide, neo- andrographolide, diterpenoids [(Rafi et al., 2020)](https://paperpile.com/c/vUTkDU/u8fB) and many flavones such as 5-hydroxy-7,8,2′,3′-tetramethoxy, andrographolide, andrographon, stigmasterol, andrographosterin 14-deoxy-11,12-didehydroandrographolide, andrographan, and homoandrographolide, as well as apigenin-7,4′-di-O-methyl ether [(Chauhan et al., 2019)](https://paperpile.com/c/vUTkDU/a5KI). The cytotoxic activity of andrographolide against various types of cancer cells is attributed to the presence of α-alkylidene γ butyrolactone, the D12 (13) double bond, C-14 hydroxyl, and the D8 (17) double bond [(Sirion et al., 2012)](https://paperpile.com/c/vUTkDU/Fcdi).Ethanol is considered a high polarity solvent than water for appropriate for extracting polyphenols. Polyphenolic compounds have been reported to serve many functions in plants; some of which are cell wall strengthening, antibacterial and antifungal activities. The antioxidant potential of A. paniculata extracts was evaluated using DPPH and ABTS assays. Both the methods presented a significant correlation among themselves, suggesting that antioxidant compounds extracted by different methodologies respond in a different manner to different radical or oxidant compound. The ethanolic extract demonstrated higher IC50 values in both assays (52430 μg/ml for DPPH and 58750 μg/ml for ABTS) compared to the aqueous extracts [(Chaves et al., 2020)](https://paperpile.com/c/vUTkDU/OXvJ). Consequently, the ABTS assay proved to be a more accurate and sensitive method for estimating the antioxidant potential in A. paniculata. The superior antioxidant activity observed in the ABTS assay suggests a higher concentration of phenolics, flavonoids, alkaloids, and terpenoids. Antioxidant activity is conferred not only by Phenols and flavonoids, but by others phytochemicals such as carotenoids, tannins, α- tocopherols, ascorbic acid [(Javanmardi, 2003)](https://paperpile.com/c/vUTkDU/IBwN). Phenols are recognized as the primary active components responsible for antioxidant activity due to their aromatic ring structure, which allows for the stabilization and delocalization of unpaired electrons. This structural characteristic facilitates the donation of hydrogen atoms and electrons from their hydroxyl groups, enhancing their ability to neutralize free radicals [(Chaves et al., 2020)](https://paperpile.com/c/vUTkDU/OXvJ). Total phenolic content of the leaf extract of A. paniculata, was estimated highest in the ethyl acetate extract, as determined by the Folin- Ciocalteu (F-C) method using Gallic acid as a standard.The reducing power analyses the ability of antioxidant to donate electron. Antioxidant in the ethanolic extracts of the plant could reduce Fe 3+ to Fe 2+ [(Irshad et al., 2012)](https://paperpile.com/c/vUTkDU/PLyC). The reducing activity of A. paniculata was measured with reference to standard ascorbic acid, and higher degree of electron donation was estimated. Aqueous extract of the plant extract showed lesser degree of Fe 3+ reduction than ethyl acetate and ethanolic extracts. The reducing power of the plant extract was found to be in order of Ethanolic extract> Ethyl acetate extract> Aqueous extract. The rate of reducing power of ethanolic extracts were found to be increased rapidly at lower concentrations, i.e., 100 µg/ml but rapidly decreased at higher concentrations (1mg/ml). Ethanolic extracts of leaves of A. paniculata had relatively better amount of reductones, and their electron donors could react with free radicals to convert them into more stable products. Similar observations were made with Irshad et al., wherein the rate of reducing power of methanolic extracts of pulp and seeds of Cassia fistula, first increased rapidly with increasing concentrations, but later dropped down [(Irshad et al., 2012)](https://paperpile.com/c/vUTkDU/PLyC). In our studies as well as reported by Irshad et al. 2012, reducing power of ascorbic acid (standard) was found to be higher than the plant extract, conclusive of the fact that ascorbic acid had higher hydrogen donating ability than most of the plants [(Irshad et al., 2012; Shimada et al., 1992)](https://paperpile.com/c/vUTkDU/PLyC+Zdnv). The current study has revealed ethanolic leaf extract of A. paniculata demonstrating a significant antimicrobial activity on oral pathogenic strains as well as considerably efficient antioxidant properties, owing to the presence of relatively good numbers of polyphenols present in the ethanolic extracts of A. paniculata. Dental caries and periodontal diseases are among the most prevalent oral health problems worldwide, with bacteria playing a significant role in their etiology. Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis, S. aureus, Candida sp. etc. are common dental pathogens associated with these oral diseases [(Hossain et al., 2021; Oktavianawati et al., 2023; Yusof et al., 2015)](https://paperpile.com/c/vUTkDU/v7bM+IiE4+brZG). Candida sp. has been identified as a biofilm forming opportunistic dimorphic fungi, colonizing oral cavities and causing tooth destruction [(Doi et al., 2022)](https://paperpile.com/c/vUTkDU/dmMp). Ethanolic extract of A. paniculata has been known to reflect moderate inhibitory activity against E.coli USTCMS 1030, however, aqueous extract did not exhibit any inhibitory effect against E. coli ATCC 25922 at any concentrations [(Krithigaa et al., 2023; Zaidan et al., 2005)](https://paperpile.com/c/vUTkDU/l4n5+l6ko).Numerous scientific studies have established the significant antibacterial properties of A. paniculata against various human pathogens. However, research specifically focusing on its efficacy against oral pathogens remains limited. Our studies have demonstrated significant antibacterial efficiency of ethanolic leaf extracts of A. paniculata, against S. aureus and S. mutans. The MIC values of our studies have shown that gram positive bacteria such as S. aureus, S. mutans are more susceptible to the leaf extract than the gram negative such as E. faecalis. In other studies, the ethanolic extract of A. paniculata has been reported to exhibit highest zone of inhibition (7.5 ±0.3 mm) against S. aureus USTCMS 1097 [(Mejos et al., 2023; Mishra et al., 2009)](https://paperpile.com/c/vUTkDU/5ky0+Y2xr).Aqueous extract of A. paniculata has been shown to have good antibacterial activity as well, linking to presence of andrographolides and arabinogalactan proteins [(Aishvarya Rukmani et al., 2024; Hossain et al., 2021; Thiraviarajan et al., 2024)](https://paperpile.com/c/vUTkDU/v7bM+VVXg+arEh). Methanolic extract of leaves have shown remarkable antibacterial activity against S. aureus, E.coli, K. pneumoniae, B. subtilis, S. epidemidis (Rafi et al., 2024). Ethanolic extracts have found to be effective against Legionella pneumophila and Bordetella pertussis [(Hossain et al., 2021)](https://paperpile.com/c/vUTkDU/v7bM). Studies have indicated that A. paniculata exhibits concentration-dependent antibacterial activity, demonstrating greater efficacy against Gram-negative bacteria compared to Gram-positive bacteria [(Tan Lim et al., 2021)](https://paperpile.com/c/vUTkDU/eB6b). In the literature, methanolic and ethanolic extracts of A. paniculata have demonstrated lower minimum inhibitory concentration (MIC) values compared to aqueous extracts. This indicates that alcoholic extracts possess higher antibacterial activity than aqueous extracts, likely due to the more efficient extraction of phenolic compounds when alcohols such as ethanol are used in the extraction process [(Banerjee et al., 2016; Mejos et al., 2023; Thiraviarajan et al., 2024)](https://paperpile.com/c/vUTkDU/Y2xr+arEh+tGvV).The cytotoxic effect of ethanolic extract of A. paniculata on cancer cell lines such as KB [1]; MCF-7 (2,38); THP-1 and human myeloma cell line H929 [(Doi et al., 2022)](https://paperpile.com/c/vUTkDU/dmMp) has been previously assayed using MTT assay (Tuluwengjiang et al., 2024). Andrographolide, has been reported to exhibit anti- cancer properties, particularly in the lipid dependent cancer pathways [(Naomi et al., 2022)](https://paperpile.com/c/vUTkDU/4m9V). In another experiment, bioassay results showed that andrographolide and isoandrographolide possess cytotoxicity to the KB cell line with ED50 values of 6.5 and 5.1 μg/ml, respectively [(Cheung et al., 2005; Mustafa et al., 2024)](https://paperpile.com/c/vUTkDU/y6W8+TeDl). The present study confirms that A. paniculata extract can be toxic for tumor cell line, KB. The (24 h) IC50 concentrations of extract for reducing the cell viability of KB cells were determined at 38.4 µg/mL. This results are in coordination with previous findings by workers on human leukemia monocytic cell THP-1and human multiple myeloma H929 cell lines [(Doi et al., 2022)](https://paperpile.com/c/vUTkDU/dmMp). The concentration reported in the current study is lower than the other reported for breast epithelial cell line MCF-10A at 80 μM [(Li et al., 2007)](https://paperpile.com/c/vUTkDU/PDZK); 52 μM for colon cancer MDA-MB-231 cells [(Li et al., 2007)](https://paperpile.com/c/vUTkDU/PDZK); 40 μM for acute myeloid leukemic HL-60 cells [(Khan et al., 2018)](https://paperpile.com/c/vUTkDU/CnPn) and 60 μM for colon cancer HT-29 cells [(Aishvarya Rukmani et al., 2024)](https://paperpile.com/c/vUTkDU/VVXg).

# Conclusion

The present study assesses the antimicrobial efficacy of Andrographis paniculata leaves against clinically relevant oral pathogens. The ethanolic extract of A. paniculata exhibited strong antioxidant activity, with an IC50 of 58750 μg/ml, surpassing the aqueous and ethyl acetate extracts, and contained higher quantities of phenolics and flavonoids. This study confirms that ethanol is as effective as methanol in extracting bioactive molecules from the leaves of the plant. The presence of alkanes, lactones, alkenes, methylene groups, aliphatic primary amines, secondary amines, conjugated alkenes, phenols, and carboxylic acids underscores the plant's bioactive pharmacological significance. The combined effect of these bioactive molecules contributes to the plant's antibacterial properties. Treatment with the ethanolic extract of A. paniculata induced cell death in tumor cells at a concentration of 38.4 µg/mL. The current study demonstrates that ethanolic extracts possess significant antioxidant and anticancer activities. The demonstrated antimicrobial efficacy of A. paniculata leaves against clinically relevant oral pathogens suggests potential use in the development of natural oral care products like mouthwashes, toothpastes, and antiseptic gels. The ethanolic extract’s significant antioxidant and anticancer activity highlights its potential as a complementary or alternative therapy in cancer treatments, particularly in targeting oral cancers. The bioactive compounds could be further explored for formulation in anti-cancer drugs or nutraceuticals. While in vitro studies demonstrate promising results, clinical trials are needed to evaluate the safety, efficacy, and dosage of A. paniculata extracts in humans, particularly in the context of oral health and cancer therapy.

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