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Qualitative and Quantitative Phytochemical Analysis, FTIR Profiling, and Antimicrobial Properties of *Andrograhis paniculata* Leaf Extract

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Abstract

Infectious diseases caused by bacteria, fungi, and other pathogens remain a major global health concern, prompting the search for safer, plant-based therapeutic agents. *Andrographis paniculata* is widely used in traditional medicine and is known for its diverse pharmacological properties, largely attributed to its rich phytochemical profile. This study investigated the qualitative and quantitative phytochemical composition, FTIR characteristics, and antimicrobial activity of the ethanolic leaf extract of *A. paniculata*. Fresh leaves were collected, authenticated, extracted with ethanol, and subjected to standard phytochemical assays. FTIR analysis was performed to identify major functional groups, while antimicrobial activity was evaluated using the agar well diffusion method against selected bacterial and fungal pathogens. Qualitative screening revealed the presence of carbohydrates, flavonoids, alkaloids, steroids, triterpenes, terpenoids, and cardiac glycosides, while saponins, tannins, and anthraquinones were absent. Quantitative analysis showed that flavonoids were the most abundant constituent (13%), followed by alkaloids (7%), cardiac glycosides (7%), terpenoids (5%), and steroids (3%). FTIR spectra indicated functional groups such as O–H, C–H, C=O or C=C, and C≡C or C≡N, confirming the presence of phenolics, alcohols, aromatics, and other bioactive compounds. The extract exhibited dose-dependent antimicrobial activity, with notable inhibition against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Tinea capitis*, while *Aspergillus* showed sensitivity only at higher concentrations. Overall, the findings demonstrate that *A. paniculata* contains significant bioactive compounds that contribute to its broad-spectrum antimicrobial potential. These results support its traditional medicinal use and highlight its promise as a natural source for developing new antimicrobial agents.

Keywords: Andrographis paniculata; phytochemical analysis; qualitative screening; quantitative analysis; FTIR; ethanolic extract; antimicrobial activity; secondary metabolites; medicinal plants.

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1. Introduction

Pathogenic bacteria, viruses, fungi, and protozoa continue to cause global health challenges. Diseases caused by organisms such as *Mycobacterium tuberculosis*, *Salmonella*, and emerging viruses remain major concerns (Morens & Fauci, 2020; Sabbahi *et al*., 2022). Foodborne pathogens like *E. coli*, *Listeria*, and *Salmonella* cause gastrointestinal infections and economic losses in the food industry (Havelaar *et al*., 2019).

Medicinal plants have been an essential component of traditional and modern health care systems worldwide. Their use spans centuries, contributing to the treatment and prevention of diseases through bioactive compounds such as alkaloids, flavonoids, terpenoids, and polyphenols. With the increasing focus on natural and sustainable healthcare solutions, medicinal plants are gaining renewed attention in both research and practice (Nabavi, 2023; Kadda *et al.,* 2021). Medicinal plants are the foundation of many traditional medical systems, such as Ayurveda, Traditional Chinese Medicine (TCM), and African traditional medicine. These plants are also a significant source for modern drug development (Smith and Khan, 2023; Bouammali *et al.,* 2023).

Phytochemical analysis is crucial for understanding the medicinal value of plant materials. Qualitative screening gives an initial idea of the secondary metabolites present in an extract by observing simple reactions such as color changes, precipitate formation, or frothing. These visual cues help identify groups of compounds like alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and phenolics. While these tests are quick and easy to perform, they mainly provide preliminary information and often need to be supported by more advanced analytical techniques (Nortjie *et al*., 2022; Ouahabi *et al.,* 2023; El Amri *et al.,* 2025; Kadda *et al*., 2026).

On the other hand, quantitative phytochemical analysis focuses on determining the actual amounts of specific compounds in a plant extract. This type of analysis generates more accurate data, making it easier to link phytochemical concentrations with their biological effects (Alshehri, 2022; Diass *et al.,* 2023).

FTIR is commonly used to evaluate the presence of antimicrobial, antioxidant, and therapeutic compounds. Studies show that intensities of functional groups such as –OH, C=O, and C–O–C correlate with antioxidant activity because these bonds belong to phenolics and flavonoids (Iqbal *et al*., 2020; Bouslamti *et al.,* 2023; Louafi *et al*., 2025;). It is also to detect vibrations of chemical bonds within plant compounds. This allows researchers to identify functional groups such as alcohols (–OH), phenols, amines, carbonyls (C=O), alkynes, and aromatics present in plant extracts. FTIR analysis helps confirm the presence of bioactive molecules like flavonoids, terpenoids, tannins, saponins, and alkaloids (Rohman & Che Man, 2019).

Andrographis paniculata, commonly known as the "king of bitterness," is a medicinal plant renowned for its diverse therapeutic properties, including anti-inflammatory, antiviral, and antioxidant activities (Nagajothi and Mekala, 2018). The plant is a native to South Asia, has long been used in traditional medicine due to its numerous therapeutic properties, including, antimicrobial, antioxidant, antidiabetic, and anticancer activities. The plant's pharmacological properties are largely attributed to its rich phytochemical profile, which includes diterpenoid lactones, flavonoids, phenolic compounds, and alkaloids. The Qualitative and quantitative phytochemical analyses of *Andrographis paniculata* have consistently identified a range of bioactive compounds, with andrographolide being particularly prominent. Recent studies continue to explore and confirm the plant's therapeutic potential, including its antioxidant, antibacterial, and hepatoprotective properties. These findings support the traditional use of *A. paniculata* in herbal medicine and suggest avenues for future research into its clinical applications (Roy *et al*., 2022). Comprehensive qualitative and quantitative phytochemical analyses are essential to elucidate the specific constituents responsible for its pharmacological actions.

2. Materials and methods

2.1. Sample Collection and Identification

Fresh *Andrographis paniculata* leaves were collected from the premises of Federal Polytechnic, Idah Local Government Area, Kogi State. The samples were authenticated by a botanist in the Department of Science Laboratory Technology, Federal Polytechnic Idah, Kogi State.

2.2. Preparation of the extracts

The collected leaves were thoroughly washed with distilled water to remove dirt and contaminants, air-dried at room temperature, and ground into fine powder using a mortar and pestle. The powdered samples were stored in airtight containers to prevent moisture absorption until further use (Kadda *et al.,* 2022; Festus *et al*., 2024).

2.3. Extraction Procedure

Two hundred grams (200 g) of the powdered leaves were soaked in 600 mL of ethanol (1:3 w/v) in a reagent bottle for seven days with continuous shaking. The mixture was then filtered through Whatman filter paper, and the filtrate was evaporated to dryness to obtain the crude extract (Festus *et al*., 2024).

2.4. Qualitative Phytochemical Screening

The crude extract was subjected to standard phytochemical tests to detect major secondary metabolites:

2.4.1. *Saponins (Frothing Test)*: 1 mL of extract was shaken with 5 mL distilled water. Persistent frothing for 10 minutes indicated saponins (Nnaebue *et al*., 2024).

2.4.2. *Carbohydrates (Molisch's Test)*: 2 mL of extract received 2 drops of Molisch's reagent and concentrated H_2SO_4 along the test tube side. A violet or reddish ring indicated carbohydrates (Okafor et al., 2024).

2.4.3. *Tannins (Ferric Chloride Test)*: 2 mL extract was treated with 5% ferric chloride; a blue-black or green-black color indicated tannins (Akinterinwa et al., 2024).

2.4.4. *Flavonoids (Alkaline Reagent Test)*: 2 mL extract was mixed with 10% NaOH. Yellow color that turned colorless with HCl confirmed flavonoids (Daniel *et al*., 2020).

2.4.5. *Alkaloids (Dragendorff's Test)*: 2 mL acidified extract was filtered and treated with Dragendorff's reagent; orange or reddish-brown precipitate indicated alkaloids (Sulaiman *et al*., 2024).

2.4.6. *Steroids (Libermann-Burchard Test)*: 2 mL extract with acetic anhydride and concentrated H_2SO_4 turned blue-green, indicating steroids (Nnaebue *et al*., 2024).

2.4.7. *Triterpenes (Libermann-Burchard Test)*: 2 mL extract with acetic anhydride and H_2SO_4 showed reddish-violet color, confirming triterpenes (Okafor *et al*., 2024).

2.4.8. *Terpenoids (Salkowski Test)*: 2 mL extract with chloroform and H_2SO_4 formed reddish-brown interface layer, indicating terpenoids (Akinterinwa *et al*., 2024).

2.4.9. *Cardiac Glycosides (Keller-Killiani Test)*: 2 mL extract with glacial acetic acid and $FeCl_3$ followed by H_2SO_4 formed brown ring, indicating cardiac glycosides (Daniel *et al*., 2020).

2.4.10. *Anthraquinones* (Borntrager's Test): 3 mL extract shaken with benzene, filtered, and treated with 10% ammonia showed pink, red, or violet aqueous layer, confirming anthraquinones (Sulaiman *et al*., 2024).

2.5. Quantitative Analysis

2.5.1. *Flavonoids*

0.5 mL extract was mixed with 2 mL water, 0.15 mL 10% $AlCl_3$, and 0.15 mL NaOH, made up to 5 mL with methanol. After 10 minutes, absorbance was measured at 415 nm using quercetin standards. Results were expressed as mg quercetin equivalents per gram dry weight (mg QE/g DW) (Akinterinwa *et al*., 2025).

2.5.2. *Alkaloids*

5 g powdered sample was extracted with 10% acetic acid in ethanol, filtered, and concentrated.

Alkaloids were precipitated with ammonium hydroxide, filtered, washed, dried, and weighed. Total content was calculated and expressed as mg/g dry weight (Obioma *et al*., 2024).

2.5.3. *Steroids*

1 mL extract was evaporated, dissolved in chloroform, reacted with acetic anhydride and concentrated H_2SO_4, and absorbance measured at 640 nm. Content was expressed as mg cholesterol equivalents per gram dry weight (mg CE/g DW) (Nnaebue *et al*., 2024).

2.5.4. *Terpenoids*

1 mL extract was mixed with 1 mL phosphomolybdic acid and 1 mL concentrated H_2SO_4, left for 30 minutes, and absorbance measured at 540 nm. Results were expressed as mg linalool equivalents per gram dry weight (mg LE/g DW) (Daniel *et al*., 2020).

2.6. Antimicrobial Procedure

2.6.1. *Preparation of Culture Media*

Nutrient agar and Sabouraud dextrose agar were prepared following the manufacturer's instructions. The required amount of each medium was weighed, dissolved in distilled water, and sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the molten agar was allowed to cool to about 45–50°C and then poured into sterile Petri dishes. The plates were left at room temperature to solidify and dry for 15-30 min (CLSI, 2020).

2.6.2. *Preparation of Microbial Inoculum*

Fresh cultures of the test organisms were used. Bacterial cultures and fungal cultures were transferred with sterile inoculating loops into sterile normal saline. The turbidity of each suspension was adjusted to match the 0.5 McFarland standard to ensure a uniform microbial density (CLSI, 2020).

2.6.3. Inoculation *of Agar Plates*

Each solidified agar plate was inoculated using a sterile cotton swab dipped into the prepared microbial suspension. The surface of the agar was streaked evenly to ensure uniform growth. The plates were then left to dry for about 3-5 (Balouiri *et al*., 2016; CLSI, 2020).

2.6.4. *Well Formation and Application of Plant Extract and Controls*

Using a 6 mm sterile corkborer**,** wells were carefully created on the agar and evenly space to prevent overlapping of diffusion zone. The agar plugs were gently removed to avoid damaging the surrounding medium. Each well was labelled according to the extract concentration and control. Different

concentrations of the plant extract (1000, 500, 250, and 125 mg/mL) were prepared. Using a micropipette, 0.1 mL (100 µL) of each concentration was dispensed into the corresponding wells. Standard antibiotic (Amoxicillin for bacteria; Griseofulvin for fungi) The solvent used in preparing the extract was Dimethyl Sulfoxide (DMSO) (Balouiri *et al*., 2016; CLSI, 2020).

2.6.5. *Incubation of Plates*

The plates were incubated in an upright position to prevent the extract from spilling out of the wells at37°C for 24 hours and 28–30°C for 48–72 hours (Bacteria and Fungi respectively) (Valgas *et al*., 2007)

2.6.6. *Measurement of Zones of Inhibition*

After incubation, each plate was observed for clear zones around the wells, indicating antimicrobial activity. The diameter of the inhibition zones was measured using a transparent ruler and recorded in millimetres (mm) (Balouiri *et al*., 2016).

Figure: 1, *Andrographis paniculata* plant

3. Results and Discussion

3.1. *Phytochemical screening*

The results of qualitative phytochemical screening presented in **Table 1,** showed that, carbohydrates, flavonoids, alkaloids, steroids, triterpenes, terpenoids, and cardiac glycosides were present, whereas saponins, tannins and anthraquinones were not detected. The frothing test gave no stable foam, indicating the absence of saponins in Andrographis paniculata. Saponins are glycosidic compounds with detergent-like properties, usually linked with antimicrobial, anti-inflammatory, hypocholesterolemic, and immune-boosting activities. Their absence suggests that the plant does not possess these typical saponin-related effects such as haemolysis or expectorant properties (Salehi *et al*., 2023). It also reduces the risk of saponin associated toxicity, which may occur at high concentrations in some plants.

Table 1: Qualitative phytochemical screening of the ethanolic extract of *Andrographis paniculata*

S/N	Phytochemical	Appearance
1	Saponin	-
2	Carbohydrates	+
3	Tannins	-
4	Flavonoids	+
5	Alkaloids	+
6	Steroids	+
7	Triterpenes	+
8	Terpenoids	+
9	Cardiac glycosides	+
10	Anthraquinones	_

+ = Present, - = Absent

Although saponins are absent, other bioactive groups like flavonoids and terpenoids may substitute their therapeutic role. The result therefore highlights that the pharmacological activities of A. paniculata are mainly derived from other classes of compounds.

The Molisch's test produced a violet ring, indicating that carbohydrates are present in the extract. These compounds are essential for energy and serve as fundamental structural components in the body. In plants, carbohydrates can appear as simple sugars or complex glycosides, which help improve the solubility and effectiveness of other metabolites. Their presence in A. paniculata not only contributes nutritional benefits but may also support the formation of key secondary metabolites such as flavonoids and cardiac glycosides (Silva *et al*., 2023). Carbohydrates also improve palatability of plant extracts.

Tannins are plant compounds with astringent taste and antimicrobial properties, capable of precipitating proteins. Since A. paniculata lacks tannins, it does not provide effects typically linked to them, such as controlling diarrhoea or aiding wound healing (Das *et al*., 2021). The absence of tannins suggests a lower risk of interference with dietary protein absorption, a common issue in tannin-rich plants. Although tannins often provide antioxidant benefits, in this case, flavonoids may fulfil that role. Therefore, tannins do not appear to play a significant part in the pharmacological properties of the plant.

A yellow colour observed in the alkaline test confirmed the presence of flavonoids in the extract. These polyphenolic compounds are well known for their antioxidant, anti-inflammatory, antimicrobial,

and liver-protective effects. By scavenging free radicals, flavonoids help prevent oxidative stress and related degenerative diseases. Their presence in *A. paniculata* supports its traditional use in managing fever, liver disorders, and infections (Gao & Zhang,2022). Flavonoids are also known to regulate enzyme activity and enhance immune defence. Their presence in the plant strongly supports its pharmacological significance, highlighting flavonoids as major contributors to its therapeutic potential.

The appearance of an orange-red precipitate in the Dragendorff's test confirmed the presence of alkaloids in the extract. Alkaloids, which contain nitrogen, are pharmacologically significant and are known for their antimicrobial, pain-relieving, anticancer, and central nervous system effects. Their presence in *A. paniculata* suggests they play a key role in the plant's wide-ranging medicinal properties. (Hassan & Abubakar, 2024). Alkaloids contribute to the plant's distinctive bitter taste, which has earned it the nickname "King of Bitters." While they enhance its medicinal value, high concentrations can be toxic, emphasizing the need for careful dosing. This finding underscore alkaloids as key bioactive compounds responsible for both the therapeutic effects and the characteristic sensory properties of the plant.

The green colour observed during the Liberman–Burchard test indicates that steroids are present in the sample. These steroidal compounds play key roles in stabilizing cell membranes and are known for their anti-inflammatory, pain-relieving, and liver-protective effects. Their presence in *A. paniculata* further supports the plant's traditional use in managing liver problems and inflammatory conditions (Salehi *et al*., 2023). Steroids act as precursors for essential hormones, highlighting their physiological importance. Their presence alongside flavonoids and terpenoids may produce synergistic effects that enhance the plant's medicinal activity. This finding provides a strong biochemical explanation for several of the therapeutic properties associated with the plant.

The reddish-brown coloration confirmed the presence of triterpenes in the extract. Triterpenes are natural compounds known for their wide range of pharmacological effects, including hepatoprotective, antimicrobial, anti-inflammatory, and anticancer activities. Their presence in *A. paniculata* supports its traditional use in treating fever, infections, and liver-related conditions (Silva *et al*., 2023). Triterpenes frequently occur alongside steroids and can work synergistically to enhance biological activity. They also serve as valuable structural templates in drug discovery. Their presence in the extract underscores their role as important contributors to the plant's medicinal effects and overall pharmacological diversity.

The reddish-brown colour observed in the Salkowski test confirms the presence of terpenoids. These compounds are known for their antimicrobial, antioxidant, antimalarial, anticancer, and anti-inflammatory properties. Their presence in *A. paniculata* adds to the plant's medicinal value, since

terpenoids play important roles in boosting immunity and protecting the body from oxidative damage (Das *et al*., 2021). Terpenoids can also act as precursors for other bioactive compounds. Their presence supports the traditional use of the plant in treating infections and fever, highlighting terpenoids as key contributors to the extract's therapeutic potential.

The Keller–Killiani test produced a reddish-brown ring, confirming the presence of cardiac glycosides in the extract. These compounds are recognized for their action on the sodium–potassium ATPase pump, enabling them to regulate and enhance heart contractility. While beneficial at low doses for improving cardiac function and managing arrhythmias, they possess a narrow therapeutic window and may become toxic at higher concentrations (Hassan & Abubakar, 2024). The presence of cardiac glycosides in A. paniculata suggests potential cardiotonic effects, though careful dosage is essential for safety. This finding contributes to the plant's pharmacological diversity and emphasizes its broader physiological significance.

The absence of coloration in the Borntrager's test confirms that the extract does not contain anthraquinones. These phenolic compounds are typically associated with laxative and antimicrobial properties. Their absence indicates that *A. paniculata* does not depend on anthraquinone-related purgative effects for its therapeutic action. It also reduces the likelihood of gastrointestinal discomfort, which is a common drawback of plants rich in anthraquinones. (Gao & Zhang *et al*., 2022). The detection of cardiac glycosides in A. paniculata indicates potential cardiotonic effects, though proper dosing is crucial for safety. This highlights the plant's pharmacological diversity and underscores its broader physiological significance.

3.2. *Quantitative phytochemical*

The IR spectrum of the extract shown in **Figure 2**, revealed several characteristic absorption bands corresponding to distinct functional groups. A strong and broad peak observed at 3340–3350 cm^{-1} indicates the presence of O–H stretching, which is typical of alcohols and phenols. The broad nature of this peak suggests hydrogen bonding within the molecule, a feature commonly observed in hydroxyl-containing compounds.

A strong absorption at 2925.98 cm^{-1} corresponds to C–H stretching vibrations of sp^3 hybridized carbons, indicating the presence of alkyl groups within the molecular structure. The medium-intensity peak at 2091.58 cm^{-1} can be attributed to C≡C or C≡N stretching, suggesting the presence of a triple bond, either as an alkyne or nitrile functionality. The results of quantitative phytochemical analysis presented in **Table 2.** revealed that, Terpenoids were quantified at 5.00%, indicating a moderate but meaningful contribution to the chemical composition of *A. paniculata*. These compounds are well

known for their anti-inflammatory, antimicrobial, and anticancer properties, which support the traditional use of the plant in managing fever and infections (Singh & Singh, 2024).

Table 2: Quantitative phytochemical results of the ethanolic extract of *Andrographis paniculate.*

Phytochemical	Concentration (%)
Terpenoids	50.00
Steroids	30.00
Cardiac glycosides	70.00
Flavonoids	130.00
Alkaloids	70.00

Terpenoids also contribute to the characteristic bitter taste of *A. paniculata*, which has earned it the common name "King of Bitters." Additionally, their hepatoprotective effects underscore their relevance in liver-related therapies. Although not the predominant class of secondary metabolites, the moderate presence of terpenoids highlights their supportive role in enhancing the overall medicinal value of the plant. Steroids recorded the lowest concentration (3.00%) among the analysed phytochemicals, yet their presence remains valuable. Plant steroids stabilize cell membranes, serve as hormone precursors, and are reported to possess anti-inflammatory and analgesic actions (Ozioma & Okoye, 2024). Despite the small amount, steroids can act in synergy with other compounds such as flavonoids and terpenoids. Their supportive role may enhance the therapeutic diversity of *A. paniculata*. Thus, even at low levels, steroids contribute to the overall pharmacological strength of the plant. Cardiac glycosides were moderately abundant (7.00%), reflecting their pharmacological significance. These compounds are best known for their ability to enhance the contractile force of cardiac muscles, which is useful in managing heart conditions (Kumar & Rajan, 2024). Their presence may also explain the plant's traditional use in improving vitality and reducing fatigue. However, they can be toxic in excess, stressing the need for careful application. In addition to heart-related effects, they possess antimicrobial and anticancer properties, further increasing the medicinal value of *A. paniculata*. Flavonoids were the most abundant phytochemicals in *A. paniculata*, constituting 13.00% of the extract. This high concentration underscores their central role in the plant's bioactivity. Flavonoids are widely recognized for their antioxidant properties, mitigating oxidative stress and protecting cells from free radical damage. In addition, they contribute anti-inflammatory, anticancer, and hepatoprotective effects, which are consistent with the traditional therapeutic uses of the plant. The predominance of flavonoids suggests they are likely the primary contributors to the pharmacological

efficacy of *A. paniculata* (Zhang & Wang, 2025), corroborating previous studies that highlight their significance in this species.

Alkaloids were present at 7.00%, indicating a moderate but pharmacologically meaningful concentration. These compounds are well-known for their diverse bioactivities, including antimicrobial, analgesic, anticancer, and antihypertensive effects. Even at modest concentrations, alkaloids can exert pronounced physiological effects, reinforcing their relevance in the therapeutic profile of *A. paniculata* (Gupta & Sharma, 2024). Alongside flavonoids and terpenoids, alkaloids may act synergistically to enhance the medicinal properties of *A. paniculata*. The concentration observed in this study is consistent with values reported for other medicinal herbs, supporting their established role in traditional therapeutic application.

3.3. *FTIR*

Further, a medium-intensity band at 1635–1645 cm^{-1} is consistent with C=O stretching of carbonyl groups or C=C stretching in aromatic rings. This suggests the molecule may contain conjugated systems, which is typical in aromatic compounds or carbonyl-containing structures. Finally, the peak at 1407.68 cm^{-1} corresponds to C–H bending vibrations, indicative of methyl or methylene groups, further confirming the presence of alkyl frameworks (**Table 3 and Figure 2**).

Table 3: FTIR of the ethanolic extract of *Andrographis paniculata*

Peak (cm^{-1})	Intensity	Functional Group
3340-3350	Strong/Broad	O-H stretching (alcohols, phenols)
2925.98	Strong	C-H stretching (alkanes)
2091.58	Medium	C-C or C-N Triple bond stretching
1635-1645	Medium	C=O (carbonyl) or C=C (aromatic)
1407.68	Medium	C-H bending

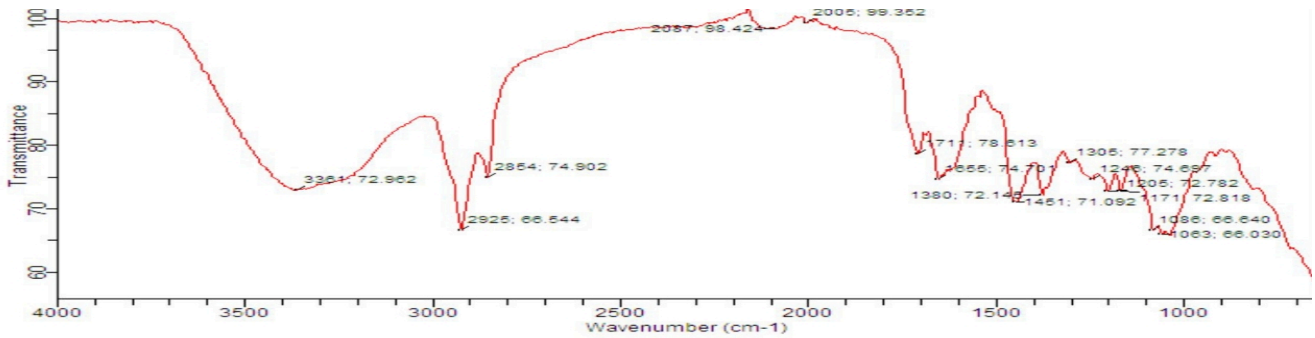

Figure: 2, IR spectrum

3.4. *Antimicrobial activity*

The antimicrobial activity of the extract presented in **Table 4**, was evaluated against a range of bacterial and fungal pathogens at varying concentrations (125–1000 mg/mL) and compared to a standard reference. The results showed a clear dose-dependent response, with higher concentrations generally producing larger zones of inhibition.

Table 4: Antimicrobial activity of the ethanol extract against Bacteria and Fungi

Concentration (mg/ml)	1000	500	250	125	Standard
Test organisms used					125
Staphylococcus aureus	15	13	12	10	28
E. coli	17	14	13	11	31
Salmonella typhi	13	10	8	7	22
Candida albicans	14	12	10	8	25
Aspergillus	12	10	00	00	27
Tinea captis	14	14	12	10	30

Among the bacterial isolates, *Staphylococcus aureus* and *Escherichia coli* were notably sensitive, exhibiting inhibition zones ranging from 10–15 mm and 11–17 mm, respectively, across the concentration gradient. *Salmonella typhi* showed moderate sensitivity, with activity decreasing from 13 mm at the highest concentration to 7 mm at the lowest, indicating that higher doses are necessary for effective inhibition.

For the fungal isolates, *Candida albicans* displayed moderate susceptibility, with zones of inhibition decreasing from 14 mm to 8 mm as concentration decreased. *Aspergillus* species showed strong inhibition at higher concentrations (12 mm at 1000 mg/mL and 10 mm at 500 mg/mL) but no detectable activity at 250 and 125 mg/mL, suggesting a concentration threshold for efficacy. *Tinea capitis* demonstrated relatively consistent inhibition (10–14 mm), indicating moderate and stable sensitivity across the tested concentrations.

Although the test sample generally showed lower activity compared to the standard, its highest concentrations produced appreciable antimicrobial effects. These findings suggest that the sample possesses broad-spectrum antimicrobial potential, particularly against Gram-positive bacteria and certain fungal species, warranting further investigation for pharmacological applications.

Conclusion

This study shows that the ethanolic leaf extract of *Andrographis paniculata* contains a rich profile of bioactive secondary metabolites, particularly flavonoids, alkaloids, cardiac glycosides, terpenoids, and steroids. FTIR analysis further confirmed the presence of important functional groups associated with therapeutic activity. The extract demonstrated notable antimicrobial effects against both bacterial and fungal pathogens, supporting its traditional use in managing infections. Overall, the findings highlight *A. paniculata* as a promising natural source of antimicrobial agents and provide a scientific basis for its continued application in herbal medicine. Further studies on compound isolation, mechanism of action, and in-vivo evaluations are recommended.

Suggestion

Based on the findings of this study, it is recommended that further research be conducted to isolate, purify, and characterize the specific bioactive compounds responsible for the antimicrobial activity of *Andrographis paniculata*. Advanced analytical techniques such as GC–MS, HPLC, and NMR should be employed to identify the active constituents, while in-vivo studies are needed to validate their therapeutic potential and safety. Additionally, exploring different extraction methods and solvent systems may enhance compound yield and potency. Collaboration between traditional medicine practitioners and scientific researchers is also encouraged to improve the development of standardized herbal formulations derived from *A. paniculata*.

Limitation

This study was limited by the use of only qualitative phytochemical screening and basic antimicrobial assays, which do not fully quantify or isolate the specific bioactive compounds responsible for the observed effects. The antimicrobial evaluation relied solely on in-vitro testing, which may not accurately reflect how the extract behaves in living organisms. Additionally, only a single extraction solvent and concentration range were used, potentially restricting the diversity and yield of detectable phytochemicals. The study also did not assess toxicity or safety profiles, which are essential for therapeutic application. These limitations highlight the need for more advanced analytical and in-vivo studies.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research.

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