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EVALUATION OF ANTIBACTERIAL ACTIVITY OF ETHONOLIC EXTRACT OF ANDROGRAPHIS PANICULATA LEAVES

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ABSTRACT

This study focused on the preparation and evaluation of a crude ethanolic extract from the air-dried leaves of *Andrographis paniculata*. The powdered leaves were extracted using methanol in a Soxhlet apparatus, followed by filtration and concentration under reduced pressure to yield a solid crude extract. Phytochemical analysis revealed the presence of various bioactive compounds including alkaloids, saponins, tannins, flavonoids, terpenoids, phenolic compounds, steroids, glycosides, and reducing sugars. Physicochemical parameters such as ash values, extractive values, crude fiber content, and foaming index were determined, indicating good quality and stability of the extract. The antimicrobial potential of the extract was assessed against multiple microbial strains. Results showed significant antibacterial activity against Gram-positive (*Staphylococcus aureus* and *Streptococcus mutans*) and Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria, as well as notable antifungal effects against *Candida albicans* and *Aspergillus niger*. These findings suggest that the ethanolic extract of *Andrographis paniculata* possesses promising antimicrobial properties and may serve as a natural source for developing alternative therapeutic agents.

KEYWORDS: The antimicrobial potential of the extract was assessed against multiple microbial strains.

1. INTRODUCTION

Andrographis paniculata, often known as "Kalmegh" or "King of Bitters," is a well-known medicinal plant in ancient systems of medicine including Ayurveda, Unani, and ancient Chinese Medicine. This herbaceous plant is native to South and Southeast Asia, especially India, Sri Lanka, and China. It has been used for a long time since it contains a wide range of healing characteristics. Andrographis paniculata has garnered considerable scientific interest in recent decades due to its antibacterial properties among its other pharmacological activity. The primary phytoconstituent of Andrographis paniculata, andrographolide, is a diterpenoid lactone that is renowned for its strong anti-inflammatory, antiviral, and antibacterial properties. Flavonoids, terpenoids, and phenolic chemicals are some of the other secondary metabolites that make it work as a medicine.[2] These substances are thought to work in several ways, including as breaking down bacterial cell walls, stopping the creation of nucleic acids, and blocking metabolic processes that bacteria need to stay alive. Many in vitro and in vivo investigations have shown that extracts of A. paniculata, especially methanolic and ethanolic extracts, have strong antibacterial effects against a wide spectrum of Gram-positive and Gram-negative bacteria.[3] Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus subtilis are some of the pathogens that have been affected by its bioactive extracts. This antibacterial action backs up the plant's long history of being used to treat illnesses including diarrhea, respiratory tract infections, and skin problems.[4] The rise of antibiotic-resistant bacteria has made the quest for other antimicrobial agents, especially those that come from nature, even more important. In this context, Andrographis paniculata emerges as a viable possibility for the creation of novel plant-derived antibacterial agents. It has a natural source, is safe, and works on several targets, which makes it better than regular synthetic antibiotics.[5]

2. MATERIAL AND METHODS

2.1 Plant Material Collection and Authentication

The plant material of *Andrographis paniculata* used in this study was collected from the Botanical Garden of Chaudhary Charan Singh (CCS) University, Meerut. The specimen was authenticated by Professor Vijay Malik, a distinguished botanist at CCS University. Authentication was performed through detailed morphological analysis and comparison with existing herbarium specimens to ensure accurate taxonomic identification. Professor Malik's expertise ensured the correct classification of the plant as *Andrographis paniculata*, belonging to the family Acanthaceae. The authenticated specimen was

used for all subsequent experimental procedures in this study.[6]

2.2 Extraction of plant

Dried and powdered plant material (approximately 100 grams) was subjected to continuous hot extraction using a Soxhlet apparatus. Ethanol (95%) was used as the solvent due to its efficiency in extracting both polar and moderately non-polar phytochemicals. The plant powder was placed inside a thimble made of filter paper and loaded into the Soxhlet extractor. A round-bottom flask containing 500 mL of ethanol was connected to the extractor and a reflux condenser was fitted on top. The setup was heated using a heating mantle, allowing the ethanol to vaporize, condense, and continuously siphon through the plant material for 6–8 hours or until the solvent in the siphon tube became colorless, indicating exhaustive extraction.[7]

2.3 Phytochemical analysis of *Andrographis paniculata* leaves extract

The phytochemical analysis of *Andrographis paniculata* leaf extract revealed the presence of several bioactive constituents known for their therapeutic potential. Qualitative screening showed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, and terpenoids. These compounds were identified using standard chemical tests specific to each class of phytochemicals. Flavonoids and phenols, in particular, were found in significant amounts, which supports the plant's known antioxidant and antimicrobial properties. The presence of saponins and tannins suggests potential astringent and antifungal effects, while alkaloids and glycosides contribute to its medicinal activities such as anti-inflammatory and antimicrobial actions. The diverse range of secondary metabolites detected in the ethanol extract of *Andrographis paniculata* supports its traditional use and provides a biochemical basis for its pharmacological activities.[8]

2.4 Physicochemical Constants of Asparagus racemosus Leave

2.4.1 Value of Ash

Determining the ash value is an important step in checking the purity and quality of crude pharmaceuticals by looking at how much inorganic material they include. The method includes burning a known amount of air-dried plant material in a silicon crucible at a temperature of roughly 500–600°C until the sample turns white or almost white, which means that there is no carbon in it. Then, the residue is chilled in a desiccator and weighed. Using certain techniques, this total ash may be split into acid-insoluble ash and water-soluble ash. These values assist find impurities, contaminants, or too much inorganic materials in herbal products.[9]

2.4.2 Total Ash

Total ash is the inorganic substance that is left over after plant material has been completely burned. It shows the overall quantity of minerals, which includes both physiological ash (from the plant itself) and non-physiological ash (from things like soil or sand that get into the plant). To find out how much ash is in something, a known amount of the air-dried sample is burned in a crucible at 500–600°C until it is carbon-free. The residue is weighed when it has cooled in a desiccator. This number is useful for checking the purity and quality of crude pharmaceuticals and finding out if they have been mixed with inorganic chemicals.[10]

2.4.3 Water-soluble ash

Water-soluble ash is the part of total ash that dissolves in water. It shows how much inorganic material is water-soluble. To find out, boil the complete ash with distilled water, filter the solution, and burn the rest of the insoluble materials. The water-soluble ash value is the difference in weight between the total ash and the residue after the water treatment. This test is crucial for checking the quality and purity of herbal materials since it can find water-soluble contaminants or additional chemicals in crude medications.[11]

2.4.4 Ash that dissolves in water

To find out how much water-soluble ash there is, first find out how much ash there is in the sample. After that, boil the complete ash with 25 mL of distilled water for 5 minutes. Use ashless filter paper to filter the mixture and catch the stuff that doesn't dissolve. Use hot water to wash away the residue, then burn it in a crucible that has already been weighed. Let it cool in a desiccator and then weigh it. Take the weight of the ash and subtract the weight of the residue from it. The difference shows how much water-soluble ash is in the sample. This helps find out whether there are any water-soluble inorganic components or adulterants in the sample.[12]

2.4.5 Ash with Sulphur

Sulphated ash is the inorganic residue that is left behind after organic content in a substance has been completely oxidized with the help of sulfuric acid. This is a frequent way to find out how much inorganic material is in a sample, especially in drugs. The technique involves moistening the sample with strong sulfuric acid and slowly burning it at 600 ± 25°C until all the carbon is gone and a white or almost white residue is left. After that, the residue is cooled and weighed. By assessing the non-volatile inorganic composition of pharmaceuticals, sulphated ash determination helps find adulterants and check their purity.[13]

2.4.6 The Value of Extraction

Extractive value tells you how much active ingredients were taken from a plant material using a certain solvent. This tells you about the plant's chemical composition and quality. To find out what it is, a known amount of the air-dried, powdered medicine is mixed with a determined amount of solvent (such water, alcohol, or a combination of the two) for a certain amount of time, generally 24 hours, with some shaking. After that, the mixture is filtered, and a certain amount of the filtrate is dried on a

water bath. Weigh the residue once it has dried. To find the extractive value, divide the weight of the residue by the weight of the original sample. This test helps figure out how many active components that can dissolve are in the crude medication.[14]

2.4.7 Value of Soluble Extracts

The soluble extractive value tells you how many active ingredients in a crude medication can be taken out using a certain solvent, such water, alcohol, or another solvent. To find out, you soak a known weight of the powdered medicine in the chosen solvent for a defined amount of time, filter the solution, and then let a measured amount of the filtrate dry. Weigh the residue that is left after evaporation and figure out the extractive value as a percentage of the weight of the original sample. This number helps figure out how strong and good the herbal material is by assessing the amount of soluble bioactive chemicals.[15]

2.4.8 Alcohol Extractive that dissolves

Alcohol soluble extractive is the quantity of active ingredients in a plant material that can be taken out using alcohol, generally 70% ethanol. It gives an idea of how many bioactive chemicals, such alkaloids, glycosides, and flavonoids, are soluble in alcohol. To find out, a known amount of powdered medicine is soaked in alcohol for a certain amount of time, filtered, and then a part of the filtrate is dried by evaporation. Weigh the residue and figure out the extractive value as a percentage of the original sample. This test is vital for figuring out how good herbal medications are and how much alcohol-soluble phytochemicals they contain.[16]

2.4.9 Steps for Finding the Fixed Oil Content

The Soxhlet extraction method is a standard way to find out how much fixed oil is in a plant sample. A known weight of the dried, powdered sample is put into a thimble and extracted with a non-polar solvent, such as petroleum ether or hexane, for a few hours. The solvent breaks down the oils and fats that are already in the sample. The solvent is evaporated after extraction, leaving behind the residue of fixed oil. The residue is weighed, and the amount of fixed oil is figured out as a percentage of the weight of the original sample. This approach gives a precise reading of the oil content in seeds, nuts, and other oily plant products.[17]

2.4.10 Ether-soluble volatile extract

Volatile ether soluble extract is the part of a plant that dissolves in volatile solvents like diethyl ether. The main things in this extract are non-polar substances such fixed oils, fats, waxes, and certain volatile oils. To find it, a known amount of the powdered material is taken out with diethyl ether using either maceration or Soxhlet extraction. Then, the solvent is allowed to evaporate, and the remaining material is weighed. The extractive value is shown as a percentage of the weight of the original sample. This test helps figure out how much ether-soluble stuff there is, which is helpful for figuring out how good plant materials with oils and other chemicals are.[18]

2.4.11 How to Find the Loss on Drying (LOD)

Loss on drying tells you how much moisture and volatile chemicals are in a sample by weighing it before and after it dries. You put a known weight of the material in a pre-weighed, dry container or crucible to perform the test. The sample is then put in an oven at a certain temperature (generally between 100°C and 105°C) for a certain amount of time, usually 2 to 5 hours, until its weight stays the same. After drying, the sample is put in a desiccator to cool down and then weighed again. The difference between the first and last weights shows the loss on drying as a percentage of the original sample weight. This test helps figure out how stable and how much moisture is in crude pharmaceuticals or pharmaceutical materials.[19]

2.4.12 Foaming Index

Foaming index is a qualitative test that looks for saponins in plant extracts and tries to figure out how much of them are there. When shaken with water, saponins can make stable foam. To find the foaming index, you make a series of dilutions of the water extract and shake them hard in test tubes. After a predetermined amount of time, generally 15 minutes, the height of the foam is measured. The foaming index is the maximum dilution that still makes the foam rise at least 1 cm. This test helps figure out how much saponin is in the sample. This is essential since saponins have therapeutic qualities, such as an expectorant and an antibiotic.[20]

2.4.13 Index of Swelling

The swelling index shows how efficiently a powdered medicine can absorb water and swell, which means it has hydrophilic components like polysaccharides and mucilage. To find it, a known amount of the powdered sample is put in a graduated cylinder, then distilled water is added. The combination is left alone for a set amount of time, generally between 1 and 24 hours, without being shaken. After settling, the swelling causes the volume to go up, and this is recorded. The swelling index is the percentage increase in volume compared to the powder's initial volume. This test helps figure out what herbal medications with mucilaginous qualities are and how good they are.[21]

2.5 Test organisms and inoculums

This study employed both Gram-positive and Gram-negative bacterial strains to evaluate antimicrobial activity. The Gram-positive strains included *Staphylococcus aureus* and *Streptococcus mutans*, while the Gram-negative strains comprised *Escherichia coli* and *Klebsiella pneumoniae*. Additionally, fungal strains such as *Candida albicans* and *Aspergillus niger* were also included. All microbial cultures were procured from the Department of Microbiology, Chaudhary Charan Singh (CCS) University, Meerut, Uttar Pradesh.

2.6 Standard Drugs

Standard antibacterial agents, specifically amoxicillin (30 µg/disc), and the antifungal agent ketoconazole (30 µg/disc), were provided as gift samples by Akums Drugs and Pharmaceuticals, Haridwar, Uttarakhand. These standard discs were employed as positive controls during the assessment of antimicrobial activity.[22]

2.7 Preparation of Nutrients Agar media

Nutrient agar was prepared by dissolving the required quantities of peptone (5 g), beef extract (3 g), sodium chloride (5 g), and agar-agar (15 g) in 1000 mL of distilled water. The mixture was heated gently while stirring continuously until the agar was fully dissolved and a clear solution was obtained. The pH of the solution was then adjusted to 7.2 ± 0.2 using either dilute hydrochloric acid or sodium hydroxide, depending on the requirement. Once properly adjusted, the medium was distributed into conical flasks or bottles and securely plugged with cotton or capped. Sterilization was carried out using an autoclave at 121°C under 15 pounds per square inch (psi) pressure for 15 minutes. After sterilization, the medium was allowed to cool to around 45–50°C before being poured into sterile Petri dishes for solidification. The prepared plates were stored in a refrigerator at 4°C until further use for microbiological analysis.[23]

2.8 Sterilization of Media

Sterilization of media is essential to eliminate any microbial contaminants before its use in microbiological experiments. In this study, the prepared nutrient agar medium was sterilized using an autoclave. The media, distributed into properly sealed containers, was subjected to moist heat at a temperature of 121°C and a pressure of 15 psi (pounds per square inch) for 15 minutes. This standard autoclaving process ensures the destruction of bacteria, spores, and fungi, making the medium suitable for accurate and contamination-free microbial analysis. After sterilization, the media was allowed to cool to approximately 45–50°C before being poured into sterile Petri dishes under aseptic conditions.[24]

2.9 Antimicrobial activity

The antimicrobial activity of the ethanolic extract of *Andrographis paniculata* leaves was assessed using the well diffusion method (Cup and Plate Method). Sterilized nutrient agar, previously liquefied and sterilized, was poured into sterile 100 mm Petri dishes. A total of ten plates were prepared and allowed to solidify completely. Using a sterile stainless steel borer, five wells of 6 mm diameter were carefully made in each plate. The ethanolic extract was prepared at a concentration of 10 mg/mL, using 1% Dimethyl Sulfoxide (DMSO) as the solvent. Standard antibacterial and antifungal agents, amoxicillin (30 µg/disc) and ketoconazole (30 µg/disc) respectively, were used as positive controls. Using a micropipette, 0.1 mL of the extract solution was carefully added to each well to ensure consistency. Additionally, discs impregnated with amoxicillin and ketoconazole were aseptically placed in the central well of each plate to serve as standards. The plates were then left at room temperature for one hour to allow the extract and standard drugs to diffuse properly into the agar medium. After diffusion, the plates were incubated at 37°C for 24 hours. Following incubation, the plates were examined for zones of inhibition around the wells, and the diameter of these zones was measured precisely to evaluate the antimicrobial potency of the extract.[25]

3. RESULTS AND DISCUSSION

3.1 Collection and authentication of *Andrographis paniculata* plant

The plant material of *Andrographis paniculata* used in this study was collected from the Botanical Garden of Chaudhary Charan Singh (CCS) University, Meerut. The specimen was authenticated by Professor Vijay Malik, a distinguished botanist at CCS University. Authentication was performed through detailed morphological analysis and comparison with existing herbarium specimens to ensure accurate taxonomic identification. Professor Malik's expertise ensured the correct classification of the plant as *Andrographis paniculata*, belonging to the family Acanthaceae. The authenticated specimen was used for all subsequent experimental procedures in this study.

3.2 Preparation of extract

The air-dried leaves of *Andrographis paniculata* were ground into a coarse powder. This powdered material was then extracted using methanol as the solvent in a Soxhlet apparatus. After continuous extraction, the mixture was filtered through muslin cloth to separate the solid residues. The resulting filtrate was concentrated by evaporating the solvent under reduced pressure using a rotary evaporator. Finally, the concentrated extract was vacuum dried to obtain a solid crude extract, which was then used for further analysis.

3.3 Phytochemical examination

Table 1: Results of phytochemical examination of Andrographis paniculata.

S. No.	Name of phytochemicals	Outcomes
1.	Alkaloids	+
2.	Saponin	+
3.	Tannin	+
4.	Flavonoids	+
5.	Terpenoids	+
6.	Phenolic Compound	+

7.	Steroids	+
8.	Glycoside	+
9.	Carbonyl	-
10.	Reducing Sugar	+

3.4 Physiochemical constant examination

Table 2: Results of Physicochemical contents examination of *Andrographis paniculata.*

S. no.	Parameters	Percentage w/w
Ash value		
1	Total ash	11.23±0.23
2	Water soluble ash	6.01±1.24
3	Acid soluble ash	3.09±0.13
4	Sulphate ash	7.23±0.96
Extractive value		
5	Ethanol solubility extractive	85.5±1.32
6	Water soluble extractive	40.78±0.47
7	Ether insoluble non-volatile	23.90±0.30
8	Ether insoluble volatile	13.22±0.46
9	Loss on drying	5.24±0.22
10	Crude fiber content	17.26±0.21
11	Foaming index	Less than 100

3.5 Antibacterial activity

Table 3: Antibacterial activity gram positive of ethanolic extract of *Andrographis paniculata.*

S. No.	Drug	Concentration	Zone of inhibition value (mm) ± SD	
			Staphylococcusaureus	***Streptococcusmutans***
1	Ethanolic extract of *Andrographis paniculata*	10 (mg/ml)	16.07±0.06	15.24±0.78
2	Ethanolic extract of *Andrographis paniculata*	20 (mg/ml)	21.08±0.46	22.12±0.37
3	Amoxicillin	30 (µg/disc)	23.87±0.36	24.98±0.56

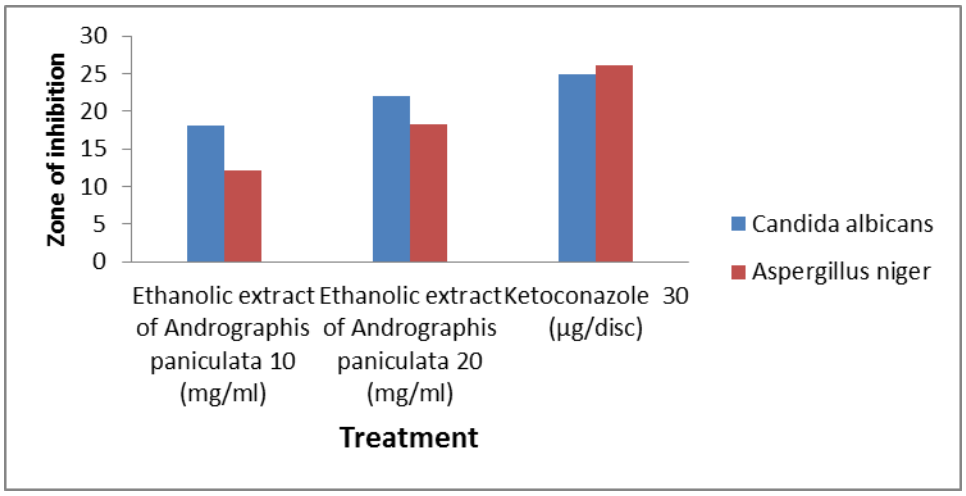

Figure 1: Antibacterial activity gram positive of ethanolic extract of *Andrographis paniculata.*

Table 4: Antibacterial activity gram negative of ethanolic extract of *Andrographis paniculata.*

S. No.	Drug	Concentration	Zone of inhibition value (mm) ± SD	
			Escherichia coli	***Klebsiella pneumoniae***
1	Ethanolic extract of *Andrographis paniculata*	10 (mg/ml)	13.04±0.03	14.25±0.23
2	Ethanolic extract of *Andrographis paniculata*	20 (mg/ml)	18.09±0.33	17.33±0.12
3	Amoxicillin	30 (µg/disc)	20.24±0.31	20.09±0.34

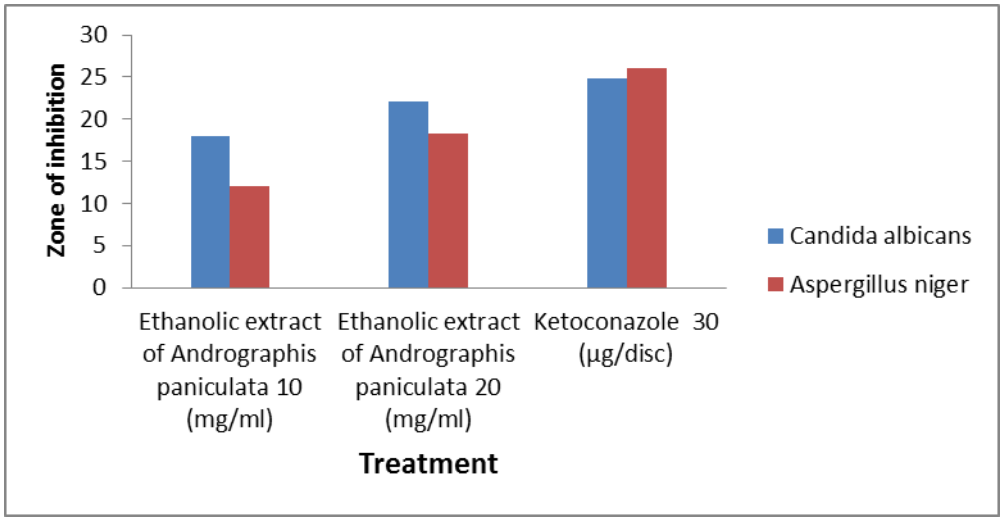

Figure 2: Antibacterial activity gram negative of ethanolic extract of *Andrographis paniculata.*

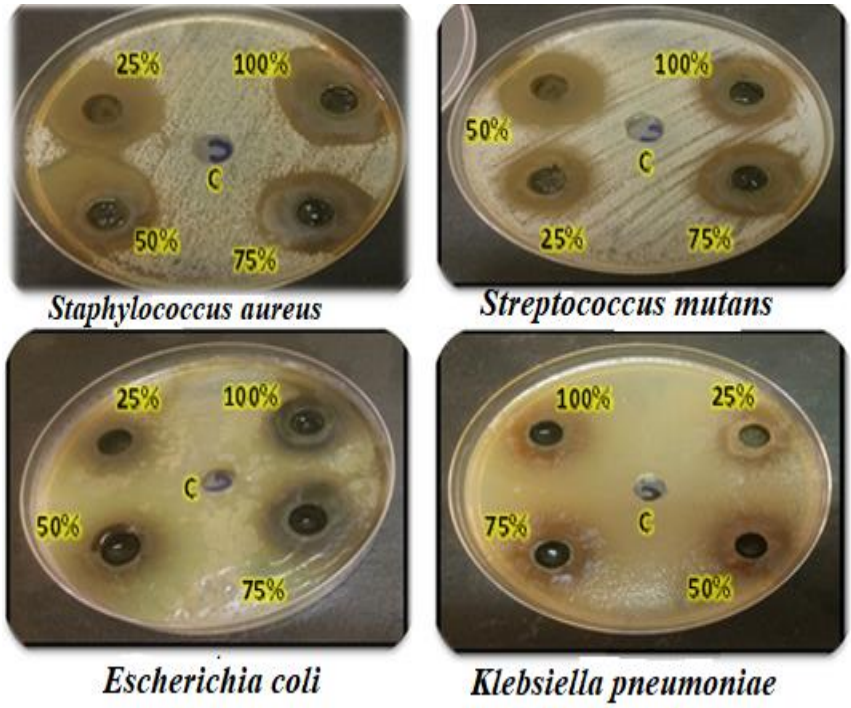

Figure 3: Antibacterial action of ethanolic extract of *Andrographis paniculata on Staphylococcusaureus, Streptococcus mutans, Escherichiacoli and Klebsiella pneumonia.*

Table 5: Antifungal activity of ethanolic extract of *Andrographis paniculata.*

S. No.	Drug	Concentration	Zone of inhibition value (mm) ± SD	
			Candidaalbicans	*Aspergillusniger*
1	Ethanolic extract of *Andrographis paniculata*	10 (mg/ml)	18.03±0.04	12.09±0.08
2	Ethanolic extract of *Andrographis paniculata*	20 (mg/ml)	22.03±0.12	18.22±0.14
3	Ketoconazole	30 (µg/disc)	24.87±0.15	26.02±0.88

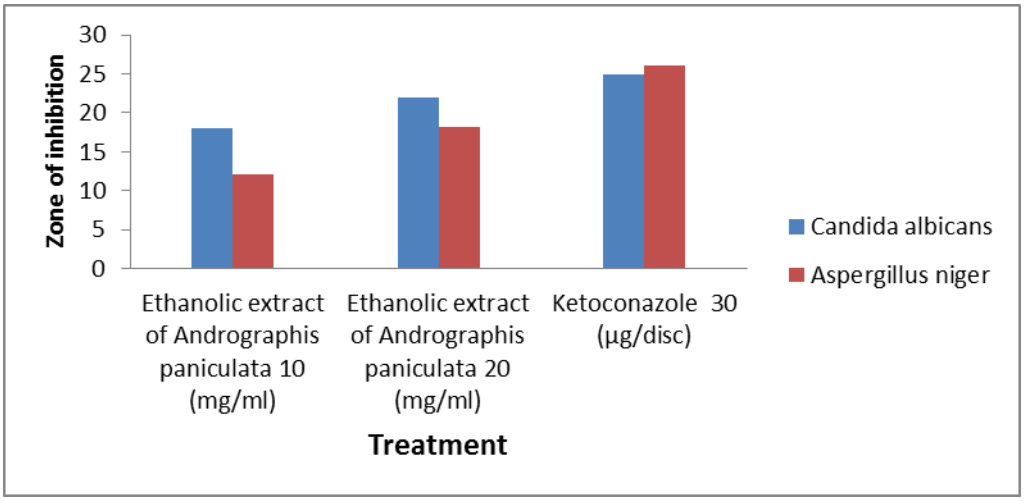

Figure 4.5: Antifungal activity of ethanolic extract of *Andrographis paniculata.*

3.6 DISCUSSION

The preparation of the ethanolic extract from the air-dried leaves of *Andrographis paniculata* using Soxhlet extraction with methanol as the solvent proved to be effective in isolating a wide range of phytochemicals. The use of methanol is advantageous due to its polarity, which facilitates the extraction of both polar and moderately non-polar compounds, contributing to a rich and diverse extract profile. The filtration and subsequent concentration steps ensured removal of solid residues and solvent, yielding a crude extract suitable for detailed phytochemical and biological assessments. Phytochemical analysis of the extract revealed the presence of several bioactive compounds, including alkaloids, saponins, tannins, flavonoids, terpenoids, phenolics, steroids, glycosides, and reducing sugars. These compounds are known for their therapeutic properties, particularly their roles in antimicrobial activity. For instance, alkaloids and flavonoids have been widely reported for their antibacterial and antifungal effects, while tannins and phenolic compounds contribute antioxidant and antimicrobial properties. The absence of carbonyl compounds suggests selective extraction, potentially enhancing the bioactive efficacy of the crude extract. Physicochemical evaluation showed appreciable ash values indicating the presence of both organic and inorganic constituents, with total ash at 11.23% w/w and varying amounts of water and acid-soluble ash, reflecting the mineral content and possible impurities. The extractive values suggested a high solubility of phytoconstituents in ethanol (85.5%), confirming the effectiveness of the solvent in extracting bioactive components. The lower water-soluble extractive value (40.78%) further emphasizes the selective solubility of compounds in ethanol, while the ether-insoluble fractions and loss on drying provide insight into the stability and moisture content of the extract. The antimicrobial studies demonstrated promising activity of the ethanolic extract against both Gram-positive and Gram-negative bacteria. The zones of inhibition increased with the concentration of the extract, showing dose-dependent efficacy. While the extract exhibited slightly lower activity compared to the standard antibiotic amoxicillin, the inhibition zones against *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, and *Klebsiella pneumoniae* were substantial, highlighting its potential as a natural antimicrobial agent. Similarly, the antifungal activity against *Candida albicans* and *Aspergillus niger* was notable, with inhibition zones approaching those of ketoconazole, a commonly used antifungal drug.

3.7 CONCLUSION

The study successfully prepared a crude ethanolic extract from the air-dried leaves of *Andrographis paniculata* using Soxhlet extraction with methanol. Phytochemical screening confirmed the presence of multiple bioactive compounds such as alkaloids, saponins, tannins, flavonoids, terpenoids, phenolics, steroids, glycosides, and reducing sugars, which are known for their therapeutic potential. The physicochemical analysis demonstrated the extract's stable nature and good extractive values, indicating the effectiveness of the extraction process. The antimicrobial assays revealed that the ethanolic extract exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacterial strains, as well as notable antifungal activity against *Candida albicans* and *Aspergillus niger*. Although the extract's efficacy was slightly lower compared to standard antibiotics and antifungal agents, the results highlight the potential of *Andrographis paniculata* as a natural source of antimicrobial compounds. These findings support its traditional medicinal use and warrant further research into its active constituents and possible pharmaceutical applications.

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