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Antimicrobial Activity of Chenopodium album Leaf Extract: An In Vitro Study

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Abstract- — Chenopodium album (Linn.), commonly known as lamb's quarters or bathua, is a fast-growing annual plant of the family Amaranthaceae with a long history of traditional medicinal use. This study investigates the antimicrobial potential of methanol and acetone leaf extracts of C. album against six pathogenic bacteria and six fungal strains using disc diffusion, well diffusion, and poisoned food techniques. Extraction was performed using the Soxhlet method with 25 g of powdered dried leaf material in 50 ml of methanol and acetone solvent mixture. Results demonstrated notable antibacterial activity against both Gram-positive and Gram-negative organisms, with acetone extract producing the largest inhibition zones against Escherichia coli (19.5 mm) by disc diffusion and 20.1 mm by well diffusion. Antifungal assays revealed that a mixture of methanolic and acetone extracts achieved up to 99% mycelial inhibition against Aspergillus niger at 7 days incubation. These findings suggest that C. album harbors broad-spectrum antimicrobial compounds with significant pharmaceutical potential.

Keywords— Chenopodium album, antimicrobial activity, Soxhlet extraction, disc diffusion, well diffusion, antifungal, bathua.

I. INTRODUCTION

Chenopodium album Linn. (family: Amaranthaceae) is a fast-growing annual plant widely distributed across temperate regions of Eurasia, the Indian subcontinent, North Africa, and the Americas. Known

by various vernacular names — bathua sag (Hindi), chandan betu (Bengali), and parupukkirai (Tamil)
— it occupies a distinctive position as both a cultivated leafy vegetable and an exploited weed in wheat, barley, mustard, and gram fields.

Green leafy vegetables are recognized worldwide for their health-promoting properties, serving as affordable and accessible sources of vitamins, minerals, dietary fiber, and essential amino acids. In developing nations where diets are predominantly starch-based, such vegetables become nutritionally indispensable. Despite this recognized importance, several locally occurring wild species including C. album remain under-exploited due to inadequate scientific documentation of their full nutritional and pharmacological profiles.

The genus Chenopodium encompasses approximately 250 species distributed globally (Risi and Galwey 1984), of which about 21 are represented in India alone. C. album has been traditionally employed in Ayurvedic and folk medicine as a blood purifier, diuretic, sedative, hepatoprotective agent, antiscorbutic laxative, and anthelmintic against roundworms and hookworms. Pharmacological investigations have further confirmed anthelmintic, sperm-immobilizing, contraceptive, antipruritic, and antinociceptive properties. The plant is also documented to possess antioxidant, anti-inflammatory, antidiabetic, and hepatoprotective activity.

Phytochemical profiling of C. album has identified a rich arsenal of bioactive compounds including flavonoids, polyphenols, saponins, cinnamic acid amides, the alkaloid chenoalbicin, apocarotenoids, xylosides, phenols, and lignans. Of particular interest is the growing body of evidence for the plant's antimicrobial activity — a property that could be harnessed to address the global challenge of drug-resistant pathogens.

The present study was conducted to systematically evaluate the antibacterial and antifungal activity of methanol and acetone leaf extracts of C. album collected from Jabalpur, Madhya Pradesh, using standardized in vitro methodologies including disc diffusion, well diffusion, and the poisoned food technique.

II. REVIEW OF LITERATURE

The pharmacological significance of C. album has attracted considerable research interest in recent decades, spanning its antioxidant, antimicrobial, anti-inflammatory, antidiabetic, anthelmintic, and hepatoprotective properties.

1. Antioxidant Potential

Naturally occurring antioxidants such as phenolic acids, polyphenols, and flavonoids play a critical role in quenching reactive oxygen species (ROS) and protecting cellular membranes from oxidative damage. Nowak et al. (2016) demonstrated that C. album extracts serve as an accessible natural source of antioxidants suitable for food supplementation. Lone et al. (2017) evaluated antioxidant activity via DPPH, riboflavin photo-oxidation, deoxyribose, and lipid peroxidation assays, confirming concentration-dependent scavenging of superoxide anion and hydroxyl radicals. Saini et al. (2019) corroborated these findings through DPPH free radical scavenging, total phenolics content estimation, and ascorbic acid analysis. Phytochemical screening by Arora et al. (2020) confirmed the presence of flavonoids, tannins, carbohydrates, saponins, proteins, and alkaloids.

2. Antimicrobial Activity

The antimicrobial potential of C. album has been documented against a range of clinically important bacterial and fungal pathogens. Kumar and Kumar (2017) reported significant antimicrobial activity of methanol extract against Propionibacterium acnes and Saccharomyces cerevisiae. Kaur et al. (2018) demonstrated maximum inhibition zones of 19 mm each against E. coli and Lactobacillus at 100% extract concentration using methanol, acetone, and chloroform leaf extracts. Saini et al. (2019) confirmed considerable inhibitory activity of methanolic leaf extract against E. coli, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans, and Candida glabrata. Notably, Javaid and Amin (2009) reported that methanolic inflorescence extract achieved up to 96% reduction in fungal biomass production against Macrophomina phaseolina. Earlier, Nayak et al. (2010) documented extensive inhibition zones including 17.3 mm against Staphylococcus aureus, 19.7 mm against Bacillus subtilis, and 18.3 mm against Candida albicans.

3. Anti-inflammatory and Antinociceptive Activity

Kim et al. (2017) investigated the anti-inflammatory properties of C. album extracts using DPPH scavenging and ELISA assays, reporting a 72.30% decrease in IL-6 and 77.85% decrease in TNF-alpha production in mouse spleen cells. Amodeo et al. (2019) confirmed significant inhibitory activity on nitric oxide production in lipopolysaccharide-stimulated cells. The ethanolic fruit extract of C. album has been shown to inhibit 5-HT-induced pruritic behavior at doses of 100-400 mg/kg, and to attenuate writhing responses in acetic acid and formalin-induced pain models (Dai et al. 2002).

4. Antidiabetic and Hepatoprotective Activity

Kant et al. (2018) demonstrated significant antidiabetic activity of methanolic root extract in STZ-induced rat models, showing normalization of insulin levels and plasma lipid profiles including reduced cholesterol, triglycerides, and LDL. Choudhary et al. (2021) evaluated the flavonoid fraction for dose-dependent antidiabetic activity in vitro and in vivo without notable toxicity. For hepatoprotection, Das and Borthakur (2020) reported significant alleviation of paracetamol-induced liver damage in albino rats, confirmed histopathologically.

III. MATERIALS AND METHODS

1. Collection and Preparation of Plant Material

Fresh leaves of Chenopodium album were collected from multiple locations across Jabalpur City, Madhya Pradesh, India. Leaves were washed thoroughly with distilled water to remove surface contaminants and shade-dried at room temperature until completely desiccated. The dried leaf material was then powdered using a mechanical blender and stored in airtight containers until further use.

2. Extraction (Soxhlet Method)

Plant compounds were extracted using the Soxhlet extraction method. Twenty-five grams of dried leaf powder were subjected to extraction with 50 ml of a methanol-acetone solvent mixture for 4-5 hours. The resultant extracts were collected and stored in sterile airtight containers at 4°C until use.

3. Culture Media

Nutrient Agar Medium (NAM)

Composition per 1000 ml distilled water: Beef Extract (3 g), Peptone (5 g), NaCl (5 g), Agar (20 g). The medium was sterilized by autoclaving at 121°C, 15 lbs pressure for 15 minutes.

Potato Dextrose Agar (PDA)

Composition per 1000 ml distilled water: Peeled Potato (200 g), Dextrose (20 g), Agar (20 g). The medium was boiled then sterilized by autoclaving at 121°C, 15 lbs pressure for 15 minutes.

4. Test Microorganisms

All bacterial and fungal cultures were procured from the Biodiversity Conservation and Rural Biotechnology Centre, Jabalpur, Madhya Pradesh.

Bacterial cultures

- Escherichia coli

- Bacillus subtilis
- Staphylococcus aureus
- Streptococcus sp.
- Salmonella typhi
- Pseudomonas aeruginosa

Fungal cultures

- Aspergillus niger
- Aspergillus flavus
- Penicillium spp.
- Fusarium oxysporum
- Alternaria alternata
- Curvularia lunata

5. Determination of Antimicrobial Activity

Disc Diffusion Method

Nutrient agar medium plates were prepared and sterilized. Fresh 18-hour bacterial suspensions were evenly spread over NAM plates using sterile cotton swabs. Sterile filter paper discs (5 mm diameter) were soaked in methanol or acetone extracts at various concentrations and placed at equidistant points on seeded plates. After a 30-minute diffusion period at room temperature, plates were incubated at 37°C for 24 hours. Zones of inhibition were measured in millimeters.

Well Diffusion Method

Sterile wells of uniform diameter were punched into solidified NAM plates seeded with bacterial suspensions. Different solvent extracts of C. album were pipetted into separate wells and plates were incubated at 37°C for 24 hours. Inhibition zone diameters were measured and recorded.

Poisoned Food Technique

The method of Grover and Moore (1962) was adopted for antifungal evaluation. Sterilized PDA with 10 mg streptomycin was poured into pre-sterilized Petri plates. Plant extract concentrations of 100, 300, 500, and 1000 mg were incorporated into the agar. After solidification, 7-day-old fungal inocula were placed centrally and plates were incubated at 26 ± 2°C for 6 days. Colony diameters of treated and control sets were measured and the percentage of mycelial inhibition was calculated using:

$$\% \text{ Mycelial Inhibition} = (dc - dt) / dc \times 100$$

where dc = average colony diameter in control; dt = average colony diameter in treated

IV. RESULTS AND DISCUSSION

1. Antibacterial Activity — Disc Diffusion Method

Both methanol and acetone extracts of C. album leaf demonstrated measurable antibacterial activity against all six test organisms. The acetone extract exhibited stronger overall antimicrobial efficacy than the methanol extract. Among Gram-negative bacteria, the largest inhibition zone was recorded against E. coli (acetone: 19.5 mm), followed by Streptococcus sp. (acetone: 15.2 mm) and Salmonella typhii (acetone: 13.7 mm). Pseudomonas aeruginosa showed the least susceptibility (acetone: 8.2 mm). For Gram-positive bacteria, the methanol extract produced good inhibition zones against Staphylococcus aureus (17.1 mm) and Bacillus subtilis (15.8 mm). All values were below the gentamycin control, which ranged from 32 mm (B. subtilis) to 44 mm (S. aureus).

Table 1: Antibacterial Activity of C. album Leaf Extract by Disc Diffusion Method

Test Organism	Methanol (mm)	Acetone (mm)	Gentamycin (mm)
Escherichia coli	19.2 mm	19.5 mm	37 mm
Bacillus subtilis	15.8 mm	15.4 mm	32 mm
Staphylococcus aureus	17.1 mm	11.9 mm	44 mm
Streptococcus sp.	12.1 mm	15.2 mm	40 mm
Salmonella typhii	12.9 mm	13.7 mm	38 mm
Pseudomonas aeruginosa	8.0 mm	8.2 mm	36 mm

2. Antibacterial Activity — Well Diffusion Method

The well diffusion method corroborated the disc diffusion findings. Acetone extract again demonstrated superior antimicrobial efficacy. E. coli showed maximum susceptibility (methanol: 20.4 mm; acetone: 20.1 mm). Notably, the well diffusion method produced larger inhibition zones for the methanol extract compared to disc diffusion, particularly against E. coli (20.4 mm vs. 19.2 mm) and Staphylococcus aureus (17.3 mm vs. 17.1 mm). Pseudomonas aeruginosa remained the most resistant organism in both assay formats. Compared to the gentamycin standard (25-31 mm), the plant extracts achieved approximately 65-80% of antibiotic efficacy for the most susceptible organisms.

Table 2: Antibacterial Activity of C. album Leaf Extract by Well Diffusion Method

Test Organism	Methanol (mm)	Acetone (mm)	Gentamycin (mm)
Escherichia coli	20.4 mm	20.1 mm	25 mm
Bacillus subtilis	16.2 mm	15.9 mm	28 mm
Staphylococcus aureus	17.3 mm	12.4 mm	31 mm
Streptococcus sp.	12.7 mm	15.8 mm	28 mm
Salmonella typhii	13.1 mm	13.9 mm	27 mm
Pseudomonas aeruginosa	8.9 mm	9.2 mm	29 mm

3. Antifungal Activity — Poisoned Food Technique

Antifungal assays revealed a clear dose- and time-dependent pattern of mycelial inhibition. The methanol extract showed maximum inhibition at 7 days against Fusarium oxysporum (69.2%) and Curvularia lunata (64.2%). Acetone extract achieved 80% inhibition of A. niger at 7 days, representing the highest activity in this group. Remarkably, the mixture of methanolic and acetone extracts produced substantially superior results — achieving 99% inhibition of A. niger at 7 days and 98% inhibition of F. oxysporum at the same timepoint. Mild inhibition was generally observed at the 5-day incubation period across all extracts and fungal strains.

Table 3: Antifungal Activity (% Mycelial Inhibition) of C. album Leaf Extracts at Different Incubation Periods

Extract	Days	A. niger (%)	A. flavus (%)	Penicillium sp. (%)	F. oxysporum (%)	A. alternata (%)	C. lunata (%)
Methanolic	3	21	28	56	61.1	48.9	52
Methanolic	5	28.3	50.1	62	66.2	51.1	57.1
Methanolic	7	50.3	54	67.2	69.2	51.6	64.2
Acetone	3	67	56.8	62.3	62.8	52.8	66.1
Extract	Days	A. niger (%)	A. flavus (%)	Penicillium sp. (%)	F. oxysporum (%)	A. alternata (%)	C. lunata (%)
Acetone	5	70	64	67.2	67.6	67.3	73.1
Acetone	7	80	65.7	69.7	70.2	78.3	77.1
Mixture	3	90.5	82	80	80.1	76.9	90.1
Mixture	5	96.1	89	82.5	92.6	80.2	90.3
Mixture	7	99	90	90.6	98	95.1	95.4

4. Discussion

The observed broad-spectrum antimicrobial activity of C. album extracts is consistent with its established phytochemical profile. The plant contains bioactive compounds including phenolic acids (gallic, protocatechuric, caffeic, syringic, and vanillic acids), flavonoids, saponins, cinnamic acid amides, and the alkaloid chenoalbicin — all recognized for their antimicrobial mechanisms such as disruption of cell membrane integrity, inhibition of protein synthesis, and interference with bacterial cell wall synthesis.

The superior performance of the acetone extract over the methanol extract in most assays may be attributed to differential solubility of antimicrobial compounds in organic solvents. Non-polar to moderately polar compounds such as terpenoids and some flavonoid aglycones are more effectively extracted in acetone, whereas methanol preferentially dissolves polar glycosides. The synergistic effect observed in the mixed extract — achieving near-complete fungal inhibition — suggests complementary extraction of both polar and non-polar bioactive fractions.

The activity against both Gram-positive (Staphylococcus aureus, Bacillus subtilis) and Gram-negative (E. coli, Pseudomonas aeruginosa) organisms indicates the presence of broad-spectrum antibiotic compounds in the leaf. The relative resistance of Pseudomonas aeruginosa, a notoriously resistant Gram-negative pathogen with a highly impermeable outer membrane and efflux pump systems, is expected and consistent with published literature. These results strongly support the use of C. album as a potential source for novel natural antimicrobial agents, particularly at a time when antibiotic resistance poses a growing global health crisis.

V. CONCLUSION

This study provides systematic evidence for the antimicrobial potential of Chenopodium album leaf extracts using both methanol and acetone solvents. Acetone extracts demonstrated consistently stronger antibacterial activity, while the combination of both solvents produced the most potent antifungal effects — achieving up to 99% mycelial inhibition against Aspergillus niger. The plant extracts showed broad-spectrum activity against six bacterial and six fungal pathogens, including clinically significant organisms such as Staphylococcus aureus, Escherichia coli, and Candida species.

These findings confirm that C. album leaf harbors bioactive components with antimicrobial potency comparable to established antibiotics, making it a promising candidate for pharmaceutical development. Future studies should focus on bioassay-guided fractionation to isolate and characterize the specific compounds responsible for antimicrobial activity, minimum inhibitory concentration (MIC) determination, in vivo efficacy trials, and toxicity profiling to support safe clinical application.

Given its widespread availability, nutritional value, and demonstrated pharmacological versatility, Chenopodium album represents a valuable and underutilized botanical resource deserving greater scientific attention and systematic exploitation in the development of plant-based therapeutics.

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