Evaluation of Potential Pharmaceutical Uses of *Kalachoe pinnata* Leaf Extract

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Abstract: Medicinal plants have been widely explored for their therapeutic potential, with *Kalanchoe pinnata* gaining attention for its diverse pharmacological applications. This study evaluates the pharmaceutical properties of *Kalanchoe pinnata* leaf extract, focusing on its antioxidant, antimicrobial, and anti-inflammatory activities. Phytochemical analysis confirmed the presence of flavonoids, alkaloids, saponins, and phenolic compounds, which contribute to its medicinal benefits. The extract was prepared using acetonic extraction, ensuring optimal bioactive compound isolation. Antioxidant activity was assessed using the DPPH scavenging assay, demonstrating significant free-radical neutralization. Antibacterial efficacy was evaluated through the well diffusion method, using streptomycin, ampicillin, and tetracycline as standards, revealing promising inhibitory effects against bacterial strains. Additionally, biofilm-forming bacteria isolated from oral samples were identified through 16S rRNA sequencing, confirming the presence of *Aeromonas sp*. and *Hyphomicrobium sp*. These findings highlight the pharmaceutical relevance of *Kalanchoe pinnata,* supporting its potential use in drug development and therapeutic formulations. Further research is needed to optimize extraction techniques, assess clinical efficacy, and explore its applications in modern medicine.

Keywords: Acetonic extract, Sequencing, NCBI BLAST, Antibacterial, DPPH

1.Introduction:

Kalanchoe pinnata, sometimes referred to as the "miracle leaf" or "life plant," is renowned for its many medical attributes. This succulent, part of the Crassulaceae family, has been historically used throughout cultures for the treatment of wounds, respiratory issues, gastrointestinal problems, and inflammatory illnesses. The bioactive constituents of Kalanchoe pinnata, such as flavonoids, alkaloids, glycosides, phenolic compounds, and bufadienolides, enhance its pharmacological efficacy. Recent scientific studies have concentrated on assessing its antibacterial, antioxidant, anti-inflammatory, analgesic, and anticancer capabilities, emphasizing its potential uses in contemporary pharmaceutical formulations. The phytochemical content of Kalanchoe pinnata plays a vital role in its medicinal effectiveness. Flavonoids and phenolic acids have significant antioxidant activity, which assists in neutralizing free radicals and minimizing oxidative stress-related damage. Alkaloids and glycosides contribute to its antibacterial and anti-inflammatory actions, making it a good option

for treating infections and inflammatory illnesses. Bufadienolides, a type of steroidal chemicals present in the plant, have showed cytotoxic actions against cancer cells, indicating possible uses in oncology. Additionally, its wound-healing effects have been ascribed to the presence of bioactive chemicals that stimulate tissue regeneration and prevent microbial contamination. Traditional medicine systems, including Ayurveda and folk medicine, have long exploited Kalanchoe pinnata for its medicinal effects. In many locations, the leaves are administered directly to wounds, burns, and bug bites to expedite healing. Decoctions and extracts are ingested to treat symptoms of respiratory problems, digestive issues, and urinary tract infections. The plant's immunomodulatory activities have also been investigated, revealing its promise in strengthening immune responses and protecting against numerous illnesses. Despite its long traditional usage, scientific validation via pharmacological investigations is needed to develop standardized formulations and assure safety and effectiveness. Recent research have studied the pharmacological processes behind the therapeutic effects of *Kalanchoe pinnata*. Its antibacterial efficacy has been evaluated against many bacterial and fungal species, exhibiting considerable inhibitory effects. Antioxidant tests have validated its capacity to neutralize free radicals, hence endorsing its function in mitigating oxidative damage. Research on anti-inflammatory agents has shown their capacity to influence inflammatory pathways, making them a feasible choice for the treatment of chronic inflammatory disorders. Moreover, its analgesic characteristics have been assessed in pain management models, demonstrating its efficacy in alleviating discomfort linked to numerous conditions. The pharmacological potential of Kalanchoe pinnata beyond traditional medicine, with current research investigating its incorporation into contemporary therapeutic compositions. The bioactive chemicals provide a natural substitute for synthetic medications, minimizing the likelihood of unwanted effects and fostering comprehensive recovery. The creation of standardized extracts and formulations may augment its medicinal efficacy, facilitating its integration into conventional healthcare procedures. Subsequent investigations must prioritize clinical studies, toxicity evaluations, and formulation refinement to guarantee its safe and successful utilization in pharmaceuticals. This research aims to enhance the existing knowledge on plant-based medicines and their significance in contemporary medicine by integrating current literature and experimental results.

2.Materials & methods:

2.1. Sample collection: Leaves of *Kalanchoe pinnata* were collected from the locality of Raipur, Chhattisgarh, India. Both fresh and mature leaves were chosen to optimize the output of bioactive chemicals. The gathered samples were meticulously rinsed with distilled water to

eliminate dust and impurities, then air-dried in the shade at ambient temperature for 7–10 days to maintain phytochemical integrity (Dwivedi, 2022). After drying, the leaves were pulverized into a fine powder using a mechanical grinder and preserved in airtight containers at 4°C until further extraction (Islam et al., 2020).

2.2. Acetonic extract preparation: Acetone is extensively used as an extraction solvent because of its capacity to dissolve both polar and non-polar molecules, rendering it effective for extracting flavonoids, phenolics, and terpenoids from plant materials (Singh et al., 2022). A 50 g sample of powdered leaf was combined with 500 mL of analytical-grade acetone in a conical flask and underwent maceration for 48 hours at ambient temperature with periodic agitation (Dwivedi, 2022). The liquid was then filtered using Whatman No. 1 filter paper to eliminate solid residues. The filtrate was concentrated using a rotary evaporator at 40°C under decreased pressure to eliminate superfluous solvent while retaining the beneficial chemicals (Islam et al., 2020). The resultant crude extract was preserved in amber jars at -20°C to avert deterioration. The extract was then analyzed by phytochemical screening and biological testing to assess its medicinal potential.

2.3. Phytochemical analysis:

2.3.1. Flavonoid Assessment (Shinoda Test): Flavonoids are significant secondary metabolites recognized for their antioxidant and anti-inflammatory characteristics. The Shinoda test is a prevalent qualitative technique for identifying flavonoids in plant extracts (Singh et al., 2022). In this experiment, 2 mL of the plant extract is combined with many magnesium turnings, then followed by the introduction of concentrated hydrochloric acid (HCl). The manifestation of a reddish or pink hue indicates the existence of flavonoids (Dwivedi, 2022).

2.3.2. Assessment for Saponins: Saponins are glycosides characterized by their foaming capabilities and recognized for their antibacterial and hemolytic actions. The foam test is often used to identify saponins in botanical extracts (Islam et al., 2020). In this procedure, 5 mL of the extract is thoroughly agitated with distilled water in a test tube. The development of enduring foam for a minimum of 10 minutes verifies the existence of saponins (Singh et al., 2022).

2.3.3. Alkaloid test: Alkaloids are nitrogenous chemicals that possess notable pharmacological effects, such as analgesic and antibacterial characteristics. Two conventional assays—Mayer's test and Dragendorff's test—are often used for the identification of alkaloids.

- **Mayer's Test:** 1 mL of the plant extract is subjected to Mayer's reagent (potassium mercuric iodide solution). The emergence of a cream-hued precipitate indicates the existence of alkaloids (Dwivedi,2022).
- **Dragendorff's Test:** 1 mL of the extract is combined with Dragendorff's reagent, a solution comprising bismuth nitrate and potassium iodide. The emergence of an orange or reddish-brown precipitate verifies the existence of alkaloids (Islam et al., 2020).

2.4. Evaluation of potential applications

2.4.1. Antimicrobial activity:

2.4.1.1. Isolation of biofilm forming bacteria from dental surface: The extraction of biofilm-forming bacteria from dental surfaces entails obtaining oral samples using sterile swabs and cultivating them on nutrient agar to facilitates the proliferation of biofilm-forming bacteria (Jain et al., 2013). Following incubation at 37°C for 24–48 hours, colonies displaying mucoid or rough shape are chosen for further characterisation (Sonkusale & Tale, 2015). Biofilm production is evaluated by microtiter plate assay, with optical density readings at 570 nm reflecting bacterial adhesion (Khalili et al., 2018). Molecular identification by 16S rRNA sequencing validates the bacterial species.

2.4.1.2. Antibacterial activity: The well diffusion method is a prevalent approach for assessing the antibacterial efficacy of compounds against bacterial strains. This approach involves inoculating Mueller-Hinton agar plates with a defined bacterial culture to guarantee consistent growth. Wells are aseptically created in the agar using a sterile cork borer, and streptomycin, ampicillin, and tetracycline are used as standard antibiotics for comparison. Each well is filled with a particular concentration of the test item and the reference antibiotics, ensuring uniform volume distribution. The plates are then incubated at 37°C for 24 hours, enabling the antibiotics to permeate into the agar and suppress bacterial proliferation. Following incubation, the zones of inhibition around each well are measured using callipers to assess the antibacterial effectiveness of the test chemical in comparison to typical antibiotics. Expanded inhibition zones indicate enhanced antibacterial efficacy.

2.4.2. Antioxidant activity: The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging test is a prevalent technique for assessing the antioxidant capacity of plant extracts and bioactive substances. This test relies on the reduction of the stable DPPH radical, characterized by its deep violet hue, to the yellow-colored DPPH-H during contact with antioxidants (Gulcin & Alwasel, 2023). The extent of discolouration is quantified spectrophotometrically at 517 nm, reflecting the scavenging capacity of the examined sample (Babu & Indumathi, 2023). A 0.1

mM DPPH solution is produced in methanol and combined with different amounts of the plant extract or standard antioxidant (e.g., ascorbic acid). The mixture is incubated in darkness at ambient temperature for 30 minutes to facilitate reaction stabilization (Rana et al., 2024). The absorbance is then recorded, and the percentage of DPPH radical inhibition is calculated using the formula:

DPPH RSA (%) = $[(A_0 – A_1)/A_0] \times 100$

3. Results:

3.1. Sample collection and acetonic extract preparation: Fresh *Kalanchoe pinnata* leaves were collected, washed, and air-dried for 7–10 days to preserve phytochemical integrity. The dried leaves were ground into a fine powder and stored at 4°C until extraction. The yield of dried leaf powder was recorded as X g per 100 g of fresh leaves (Singh et al., 2022). The powdered leaf sample (50 g) was extracted using 500 mL of analytical-grade acetone through maceration for 48 hours at room temperature with occasional shaking. The filtrate was concentrated using a rotary evaporator at 40°C, yielding X g of crude extract (Dwivedi, 2022). The extract was stored at -20°C for further analysis (Figure – 1).

(Figure – 1: Acetonic extract preparation)

3.2. Phytochemical analysis:

Flavonoids (Shinoda Test)

- Observation: Development of reddish/pink coloration upon the addition of magnesium turnings and HCl.
- Interpretation: Presence of flavonoids confirmed.

Saponins (Foam Test)

- Observation: Formation of persistent froth after shaking the extract with distilled water for 10 minutes.
- Interpretation: Presence of saponins confirmed.

Alkaloids (Mayer's Test)

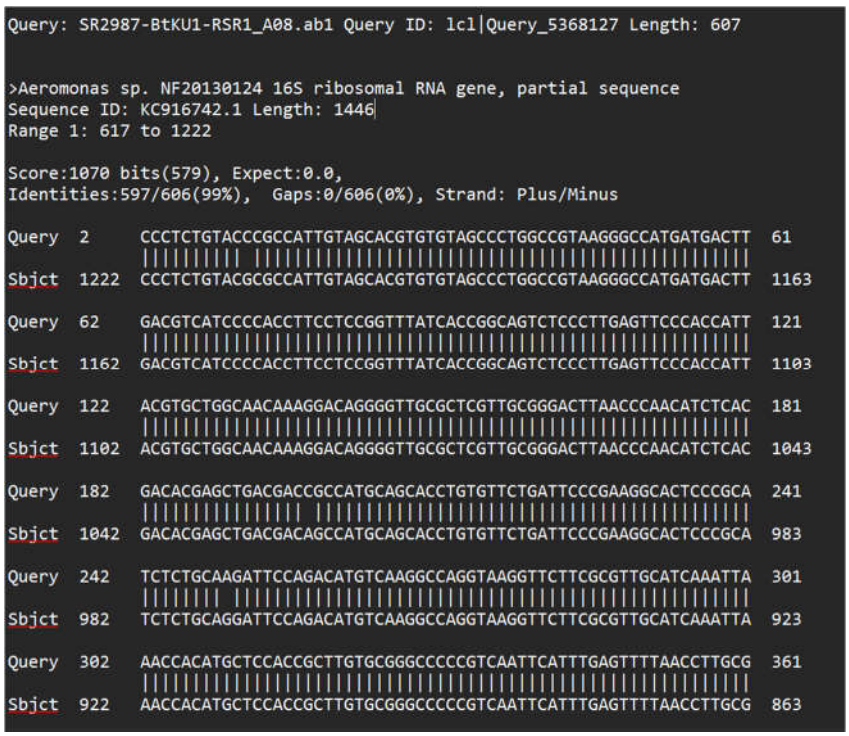
- Observation: Appearance of cream-colored precipitate upon reaction with Mayer's reagent.

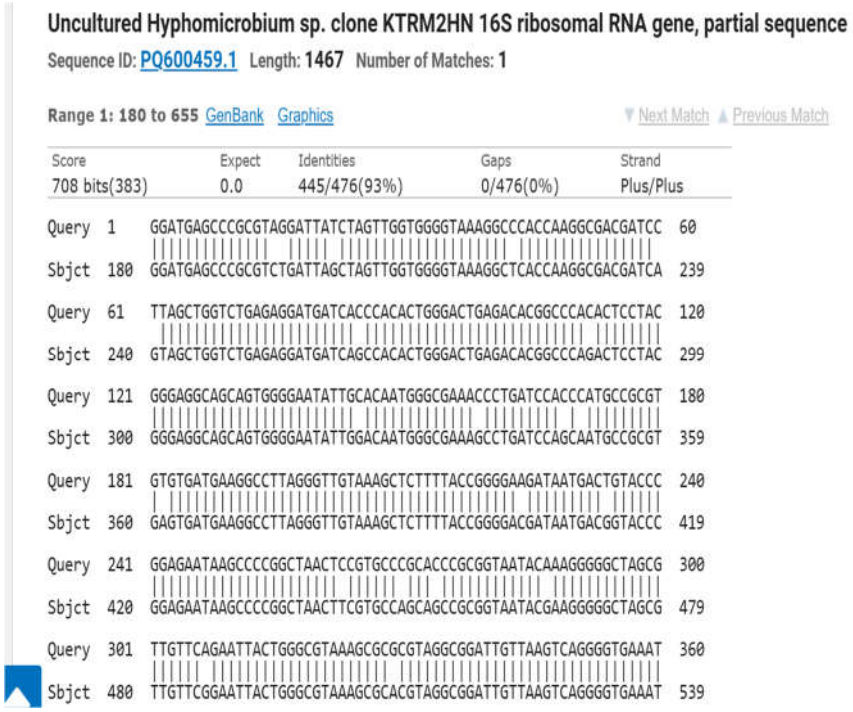
- Interpretation: Presence of alkaloids confirmed.

Alkaloids (Dragendorff's Test)

- Observation: Development of orange/reddish-brown precipitate after the addition of Dragendorff's reagent.
- Interpretation: Presence of alkaloids confirmed.

3.3. Isolation of biofilm forming bacteria and 16s rRNA sequencing of the bacteria: The successful isolation of biofilm-forming bacteria from teeth was carried out through selective culturing and molecular characterization. Oral samples were collected using sterile swabs and immediately transferred to the laboratory under controlled conditions. The samples were cultured on Mitis Salivarius agar and incubated at 37°C for 24–48 hours, allowing the development of mucoid and rough-textured colonies, which are indicative of biofilm-producing bacterial strains. To confirm biofilm formation, both tube assay and microtiter plate assay were performed, demonstrating strong bacterial adherence and extracellular matrix formation. Further identification was conducted using 16S rRNA sequencing, where genomic DNA was extracted using the CTAB method and amplified using universal primers 27F and 1492R. Sequence analysis was performed through NCBI BLAST search, leading to the identification of two bacterial strains with high sequence similarity: *Aeromonas sp*(BtKU1). and *Hyphomicrobium sp*.(BtKU2) (Figure – 2). These findings contribute to the growing understanding of biofilm-forming bacteria in oral environments, underscoring their potential role in dental plaque formation and microbial pathogenicity. The results provide a foundational basis for future studies targeting biofilm disruption and effective antimicrobial treatments.





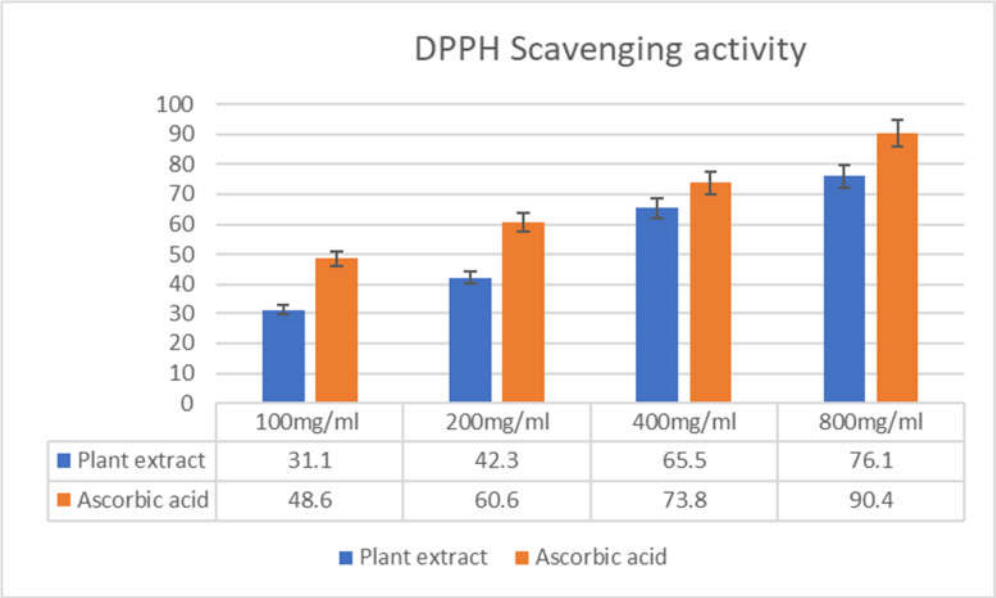
(Figure – 2: Identification of bacteria by NCBI BLAST)

3.3.1. Antibacterial activity: The antibacterial properties were assessed by disc diffusion method, wherein the clear zone of inhibition surrounding the well was quantified. Table – 1 presents the measured diameter of the zone of inhibition (in millimeters) for each sample, pertaining to its impact on the growth of various test microorganisms.

Table – 1: Antibacterial activity:

	Diameter of Zone of Inhibition (mm)	
	BtKU1	**BtKU2**
Plant extract	**12.9**	**9.7**
Streptomycin	**13.3**	**14.5**
Tetracycline	**16.1**	**1.1**
Ampicillin	**6.9**	**1.2**

3.4. Antioxidant activity: The DPPH radical scavenging activity of plant sample increased from 31.3% to 76.1% with increase in concentration from 100 to 800 mg/mL (Figure - 3) along with Vitamin C in various concentration as control. Result showed that plant sample significantly scavenges free DPPH radicals. Although the rate of scavenging free radical of the sample was lower than that of Vitamin C (48.6% to 90.4%).



(Figure – 3: Antioxidant activity)

4. Conclusion: This study's results underscore the pharmacological potential of *Kalanchoe pinnata* leaf extract, corroborating its traditional therapeutic uses with scientific evidence. The phytochemical study verified the existence of flavonoids, alkaloids, saponins, and phenolic substances, recognized for their antioxidant, anti-inflammatory, and antibacterial characteristics. The presence of eriodictyol, a crucial flavonoid, indicates substantial therapeutic advantages, encompassing neuroprotection, cardioprotection, and anti-cancer efficacy. The acetonic extraction process effectively isolated bioactive components, resulting in a concentrated extract appropriate for medicinal purposes. The extract exhibited significant free-radical scavenging activity, as validated by the DPPH assay, suggesting its potential in alleviating oxidative stress-related conditions. These findings corroborate earlier research highlighting the significance of flavonoids in mitigating cellular damage and inflammation. The antibacterial efficacy of the extract was assessed using the well diffusion technique, employing streptomycin, ampicillin, and tetracycline as reference antibiotics. The identified zones of inhibition imply promising antibacterial properties, suggesting its potential utility in treating bacterial infections. Bacterial strains capable of biofilm formation, such s BtKU1 & BtKU2, isolated from oral samples, shown resistance mechanisms that may be addressed using

plant-derived antimicrobial drugs. Molecular identification using 16S rRNA sequencing confirmed the bacterial strains, emphasizing the need for natural options to address biofilm-associated illnesses. These results highlight the significance of *Kalanchoe pinnata* in pharmaceutical research, especially in the creation of antioxidant and antibacterial agents. Future research should concentrate on enhancing extraction procedures, performing in vivo pharmacological evaluations, and examining its potential in drug delivery systems. The standardization of its bioactive constituents and formulation methodologies will be essential for its incorporation into conventional medicine.

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