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## ANTIMICROBIAL POTENTIAL OF EXTRACT FROM LEAVES OF *KALANCHOE PINNATA*

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**ABSTRACT:** Herbal medicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. *Kalanchoe pinnata* is known as herbal medicine that having many special advantages. The objective of the study was to scientifically assess antimicrobial activity of 95% ethanolic, methanolic, 60% methanolic and aqueous extract of leaves from *Kalanchoe pinnata* on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*. It was found that 60% methanolic extract shows best result. The 60% methanolic extracts of the selected plants leaves were taken in different ratio randomly and antimicrobial tests were carried out. The most effective concentration was then determined by comparing the results of the zone of inhibition. Phytochemical screening of 60% methanolic extract was carried out and the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) was found significant activity against microorganisms. The literature survey show that this plant leaves has only antibacterial activity. But with this research it is conclude that the plant leaves not only have antibacterial activity but also antifungal activity.

**INTRODUCTION:** The occurrence of skin diseases in developing countries is highly correlated with poor hygiene, overcrowding, malnourishment, non-availability of potable water, high temperature and humidity.

Further, drugs used to treat them are antibiotics, steroids and sulfonamides, which are not only out of reach of local population in remote areas but also associated with adverse effects like atrophy, telangiectasia, hirsutism and sensitizing dermatitis which are far more troublesome.

Indigenous medicinal plants have been a readily available source of drugs since ancient times and even today almost 50% new drugs have been patterned after phytochemicals. Majority of the population in developing countries and approximately 25% people in developed regions use herbal medicine for prevention and treatment of diseases.

Recognizing the medicinal significance of indigenous plants, World Health Organization (WHO) in its 1997-guideline states that “effective locally available plants be used as substitutes for drugs.

Research work on medicinal plants and exchange of information obtained will go a long way in scientific exploration of medicinal plants for the benefit of man and is likely to decrease dependence on imported drugs”<sup>1</sup>.

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In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant<sup>2</sup>. Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs<sup>3,4</sup>.

*Kalanchoe pinnata* (*B. Pinnatum*) is rich in alkaloids, triterpenes, glycosides, flavonoids, cardenolides, steroids, bufadienolides and lipids. The leaves contain a group of chemicals called bufadienolides which are very active. Bufadienolides like bryotoxin A, B, C which are very similar in structure and activity as two other cardiac glycosides, digoxin and digitoxin and possesses antibacterial, antitumorous, cancer preventative and insecticidal actions<sup>5</sup>. The presence of phenolic compounds, Triterpenoids ( $\alpha$ -amyrin,  $\beta$ -amyrin) indicate that the plant possess anti-microbial activity<sup>6,7,8</sup>.

These findings supported its use in treating the placenta and navel of newborn baby, which not only heals fast but also prevent the formation of infections. The leaf extract at 5% v/v was found to bactericidal to a wide spectrum of gram-positive and gram negative bacteria such as *B. subtilis*, *S. aureus*, *S. pyogenes*, *S. faecalis*, *E. coli*; *Proteus* spp; *Klebsiella* spp; *Shigella* spp; *Salmonella* spp; *S. marcescens* and *P. aeruginosa* including the clinical isolates of these organisms possessing multiple antibiotic resistance<sup>5,9</sup>.

## MATERIAL AND METHOD:

**Plant Materials:** The leaves of *Kalanchoe pinnata* were collected from medicinal garden of Sanjivani Institute of Pharmacy and Research, Kopergaon.

The leaves were identified by Mr. P.S.N. Rao, Joint Director, Botanical survey of India, Koregaon road, Pune, by comparing morphological features (leaf arrangement, flower / inflorescence arrangement, fruit and seed morphology etc.). Fresh plant material were washed under running tap water and then with distilled water.

**Extract preparation:** Four different extract were prepared as follows

1. 95% Ethanolic Extract - Fresh leaves were coarsely grounded and was extracted with 95% ethanol using Soxhlet equipment and thereafter concentrated by evaporation<sup>10,11</sup>.
2. Methanolic Extract: Fresh leaves were coarsely grounded and was macerated with methanol for not less than 7 days in a close vessel and thereafter concentrated by evaporation at room temperature<sup>12,13,14,15</sup>.
3. 60 % Methanolic extract: Fresh leaves were coarsely grounded and was macerated with 60% methanol for not less than 7 days in a close vessel and thereafter concentrated by evaporation at room temperature<sup>16,17</sup>.
4. Aqueous Extract: Fresh leaves were coarsely grounded and was macerated with distilled water for not less than 7 days in a close vessel and thereafter concentrated by evaporation at room temperature<sup>16</sup>.

**Phytochemical screening:** Phytochemical screenings were performed using standard procedures for presence of Alkaloids, Glycosides, Carbohydrates, Flavonoids, Saponins, Steroids, Tannins, Terpenoids, and Phenols<sup>18</sup>.

**Microbial cultures:** The standard bacterial and fungal cultures were procured from IMTECH, Chandigarh, India and used in the present study (**Table 1**). The bacterial cultures were rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) and fungal culture were rejuvenated in Sabouraud dextrose at 37°C for 18h and then stocked at 4°C in Mueller-Hinton Agar and Sabouraud dextrose respectively. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards guideline. The bacterial culture was inoculated into sterile Nutrient broth and incubated at 37°C for 3h until the culture attained a turbidity of 0.5 McFarland units. For fungi, a loopful of culture was inoculated in 10 ml of sterile Dextrose broth and incubated at 37°C for 3h. Turbidity of the culture was standardized to 10<sup>5</sup>CFU with the help of SPC and turbidometer.

The final inoculum size was standardized to  $10^5$  CFU/ml with the help of SPC and Nephlo-turbidometer.

**TABLE 1: MICROORGANISM STRAINS**

Microorganisms	MTCC Code
<i>Staphylococcus aureus</i>	96
<i>Pseudomonas aeruginosa</i>	424
<i>Escherichia coli</i>	739
<i>Candida albicans</i>	227

**Agar well diffusion method:** 0.1ml bacterial suspension of  $10^5$ CFUml<sup>-1</sup> was uniformly spread on Muller-Hinton Agar plate to form lawn cultures. For well diffusion method, the punch having diameter of 10mm was used to prepare the well in agar plate. The well were filled with 0.08 ml of various extract and tested for antimicrobial activity against pathogens. After incubation of 24 hours at 37°C zone of inhibition was measured in mm<sup>19</sup>. For antifungal properties, 0.1 ml fungal suspension of  $10^5$ CFU ml<sup>-1</sup> was uniformly spread on SDA plate to form lawn cultures. The well (10mm diameter) was prepared and various diluted extract were tested for their antimicrobial activity against fungal pathogens by disc diffusion technique. After incubation of 24 h at 37°C, zone of inhibition of growth was measured in mm.

**Determination of Minimum Inhibitory Concentration (MIC):** To measure the MIC values, various concentrations of the stock, were assayed against the test bacteria. The minimum

inhibitory concentration was defined as the lowest concentration able to inhibit the growth of microorganism.

**Determination of Minimum Bactericidal Concentration (MBC):** Equal volume of the various concentration of each extract and Mueller Hinton broth were mixed in micro-tubes to make up 0.5ml of solution. 0.5ml of organism suspension was added to each tube. The tubes were incubated aerobically at 37°C for 24 h. Two control tubes were maintained for each test batch. These include tube-containing extract without inoculum and the tube containing the growth medium and inoculum. The MBC was determined by sub culturing the test dilution on Mueller Hinton Agar and further incubated for 24 h. The highest dilution that yielded no single bacterial colony was taken as the Minimum bactericidal Concentration<sup>20, 21</sup>.

**RESULTS AND DISCUSSION:** Herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way.

**Phytochemical screening:** Phytochemical screening of extracts indicated the presence of Alkaloids, Glycosides, Carbohydrates, Flavonoids, Saponins, Steroids, Tannins, Terpenoids and Phenols (**Table 2**).

**TABLE 2: PHYTOCHEMICAL SCREENING OF DIFFERENT EXTRACTS**

Test	95% Ethanolic extract	Methanolic extract	60% Methanolic extract	Aqueous extract
Alkaloids	+	+	+	-
Glycosides	+	+	+	+
Carbohydrates	+	+	+	+
Flavonoids	+	+	+	-
Saponins	+	+	+	+
Steroids	+	+	+	+
Tannins	+	+	+	+
Terpenoids	-	+	+	+
Phenols	+	+	+	-

Key: Present (+), Absent (-)

**Antimicrobial activity:** Antimicrobial activity of various extract of leaves of *Kalanchoe pinnata* (Diameter of zone of inhibition in mm) was shown in **Table 3**.

**TABLE 3: ANTIMICROBIAL ACTIVITY OF VARIOUS EXTRACT OF LEAVES OF KALANCHOE PINNATA**

Pathogens	Ethanolic extract	Methanolic extract	60% Methanolic extract	Aqueous extract
<i>Staphylococcus aureus</i>	15	21	21	18
<i>Pseudomonas aeruginosa</i>	18	21	21	18
<i>Escherichia coli</i>	18	25	27	20
<i>Candida albicans</i>	15	18	20	18

Among above four extract 60% methanolic extract shows better result.

*Staphylococcus aureus* is a bacterium that is a member of the Firmicutes, and is frequently found in the human respiratory tract and on the skin. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning. Disease-associated strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. If these bacteria can reach the bloodstream and end up in many different body sites, causes wound infections, abscesses, osteomyelitis, endocarditis, pneumonia that may severely harm or kill the infected person. So the prepared extract can act against such diseases, thus can save the life of person.

So MIC and MBC of 60% methanolic extract was found out against *Staphylococcus aureus*. It was found as 30 mg.

**CONCLUSION:** Phytochemical constituents might provide incentive for evaluation of the use of the plant in medicine and in agriculture. The literature survey show that this plant leaves has only antibacterial activity. But with this research it is conclude that the plant leaves not only have antibacterial activity but also antifungal activity. This data can be useful for the preparation of antibacterial and antifungal cream. This is very helpful to researcher to explore more about this valuable plant.

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