Antimicrobial and Antioxidant Activities of *Kalanchoe pinnata* Against Pathogens

Rashmi, Amanpreet Singh, Ashima Jain, Priyanka Kaushal, Sangeeta, Shruti, Diiva Bhatia and Deepak Kumar Malik*

Department of Biotechnology Engineering, University Institute of Engineering & Technology, Kurukshetra University Kurukshetra-136119, Haryana, India

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**ABSTRACT**

It was found that this plant showed various pharmacological activities such as anthelmentic, immunosuppressive, wound healing, hepatoprotective, antinociceptive, anti-inflammatory and antidiabetic, nephroprotective, antioxidant activity, antimicrobial activity, analgesic, anticonvulsant, neuropharmacological and antipyretic. The leaf organic extract of *Kalanchoe pinnata* (methanol, chloroform, petroleum ether, acetone, ethyl acetate) were prepared and antimicrobial activity were studied by agar gel diffusion method against human pathogen such as *E. coli*, *S. mutans*, *S. aureus*, *P. aeruginosa*. The methanol extract had wide range of activity on pathogen than other extract. The antioxidant assay showed the increase in reducing activity by increased concentration of organic extract of *Kalanchoe pinnata*.

**Key words:** *Kalanchoe pinnata*, Antimicrobial, Pathogens, Antioxidant.

**INTRODUCTION**

*Kalanchoe pinnata* is distinctive for the profusion of miniature plantlets that form on the margins of its leaves, a trait it has in common with the other members of the *Bryophyllum* section of the *Kalanchoe* genus. It is a popular houseplant and has become naturalized in temperate regions of Asia, the Pacific and Caribbean. *Kalanchoe pinnata* has become naturalized in temperate regions of Asia, Australia, New Zealand, West Indies, Macaronesia, Mascarenes, Galapagos, Melanesia, Polynesia, and Hawaii. *K. pinnata* is used in ethnomedicine for the treatment of earache, burns, abscesses, ulcers, insect bites, whitlow, diarrhoea and cithiasis. The plant is considered a sedative wound-healer, diuretic and cough suppressant. The plant is also employed for the treatment of kidney stones, gastric ulcer and edema of legs. The plant, *K. pinnata* is also widely used in ayurvedic system of medicine as astringent, analgesic, carminative and also useful in nausea and vomiting. It was found that this plant showed various pharmacological activities such as anthelmentic, immunosuppressive, wound healing, hepatoprotective, antinociceptive, anti-inflammatory and antidiabetic, nephroprotective, antioxidant activity, antimicrobial activity, analgesic, anticonvulsant, neuropharmacological and antipyretic. Anthelmentic activity was found due to the presence of tannins of the extract of *K. pinnata* and steroid glycosides such as bufadienolide showed wound healing activity. It was also found that the different flavonoids, polyphenols, triterpenoids and other chemical constituents of the plant were responsible for the antinociceptive, anti-inflammatory and antidiabetic properties. Quercetin had a marked protective effect on cadmiuminduced nephrotoxicity and possessed potent oral efficacy against cutaneous leishmaniasis. The aqueous extract of *K. pinnata* was evaluated for its protective effects on Gentamycin-induced nephrotoxicity in rats. It was observed that the aqueous extract of *K. pinnata* leaves significantly protects rat kidneys from Gentamycin-induced histopathological changes.

**MATERIAL AND METHODS**

**Collection and preparation of extract**

Leaves of *kalanchoe pinnata* were collected from University College, Kurukshetra University, Kurukshetra. Washed mature leaves were shaded dried and then powdered with the help of mortar and pestle. 10 grams of the powder was added in 100 ml of solvent and was soaked at room temperature for 48 hrs. The extract was filtered using whatman no.1 filter paper. The filtered extract was allowed to evaporate at 60°C for 2 hrs in water bath.

**Antibacterial activity**

Test pathogens (*E. coli*, *S. aureus*, *S. mutans* and *P. aeruginosa*) were obtained from IMTECH, Chandigarh. To maintain the cultures, Muller Hinton agar medium was used. The antibacterial activity against the pathogens was checked by agar well diffusion method. Cultures of pathogens were aseptically swabbed on Muller Hinton agar plates. Wells of 6 mm diameter were made aseptically by core borer in the inoculated plates and different dilutions of organic solvent extract were added into the labeled wells. The plates were incubated at 37°C for 24 hrs in upright position. The zone of inhibition in millimeter was recorded with the help of Hi Media Zone scale. The experiments were carried out in duplicates to minimize probability of error.

**Test for saponins and cardiac glycosides**

The 10 ml distilled water was added to 5 ml of extract. After 2 min., there was appearance of foam on vigorous shaking showing the presence of saponins. The 2 ml of extract was mixed with few drops of ferric chloride and 1ml of glacial acetic acid and conc. HCL. Appearance of green color revealed the presence of cardiac glycosides.

**Test for flavonoids, steroids and tannin**

The few drops of lead acetate were added to 2 ml of extract. Appearance of yellow colour revealed the presence of flavonoids. The 2 ml chloroform and 2 ml H₂SO₄ was added to 2 ml of extract. Shaking of mixtures results in red layer of acid followed by green colour revealed the presence of steroids. The 2 ml of extract was mixed with few drops of Ferric chloride, appearance of blue colour showed the presence of tannins.

**Antioxidant activity**

2.5 ml of phosphate buffer and 2.5 ml of potassium ferrocyanide were added to different concentration of extracts in 1 ml of water. The mixture was incubated at 50°C for 20 min. The 2.5 ml trichloroacetic acid was added to the mixture, centrifuged at 3000 rpm for 10 min. The upper layer solution was mixed with 2.5 ml distilled water and 0.5 ml of ferric chloride solution and the absorbance was measured at 700 nm.
RESULTS

Antimicrobial activity and test for saponins, cardiac glycosides, flavonoids, steroids and tannin

The diethyl ether, chloroform, methanol, ethyl acetate extract of *Kalanchoe pinnata* was not showing any inhibition of any pathogenic bacteria. The zones have been measured (mm) diameter of clearance. The dichloromethane extract exhibited maximum antimicrobial activity against *E. coli* followed by *C. galabrata* and *C. parapsilosis* on the basis of zone formation as shown in Table 1. Aqueous extract showed antimicrobial activity against *S. mutans* only. The presence of saponins, cardiac glycosides, flavonoids, steroids and tannin in different extract of *kalanchoe pinnata* was analysed as shown in Table 2. The butanol extract *kalanchoe pinnata* of was showing the presence of saponins, cardiac glycosides, flavonoids, steroids and tannin.

Table 1. Zone formation (mm) by different organic extracts of *kalanchoe pinnata* against pathogenic microbes.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Zone formation In mm</th>
<th>C. parapsilosis</th>
<th>C. glabrata</th>
<th>E. coli</th>
<th>S. mutans</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichloromethane</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>12</td>
<td>18</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aqueous</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Methanol</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Ethyl acetate</td>
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<td>-</td>
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</tr>
</tbody>
</table>

Table 2. Phytochemical analysis of different organic extracts of *kalanchoe pinnata*.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Saponins</th>
<th>Cardiac glycosides</th>
<th>Steroids</th>
<th>Tannins</th>
<th>Flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>+</td>
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<td>+</td>
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</tr>
<tr>
<td>Ethyl acetate</td>
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<td>+</td>
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<tr>
<td>Chloroform</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>Butanol</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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</tr>
</tbody>
</table>

Fig 1 The OD of ethyl acetate extract of *K. pinnata*.

Antioxidant assay

Antioxidant assay of various organic extracts of *kalanchoe pinnata* was tested. The increase in concentration of extracts was responsible for antioxidant properties. The reducing activity increased with respect to the concentration of dichloromethane extract, ethyl acetate and methanol extract. The increase in concentration of ethyl acetate extract of *K. pinnata*, OD was increasing which symbolized the better antioxidant at the higher concentration as shown in Fig 1.

DISCUSSION

The dichloromethane extract of *K. pinnata* exhibited maximum antimicrobial activity against *E. coli* followed by *C. galabrata* and *C. parapsilosis* on the basis of zone formation. Aqueous extract showed antimicrobial activity against *S. mutans* only. In a study found that 60% methanolic leaf extract inhibits the growth of five out of eight bacteria used at a concentration of 25 mg/ml [4]. *Bacillus subtilis*, *E. coli*, *Proteus vulgaris*, *Shigella dysenteriae*, *S. aureus* were found to be inhibited while *Klebsiella pneumoniae*, *P. aeruginosa* and *A. albicans* were found to resist the action of the extract. Methanolic extract of roots of *K. pinnata* was found to be most effective as antibacterial as compare to others while none of extract showed the activity against *C. albicans* [5]. In our study, the reducing activity increased with respect to the concentration of different organic extract (dichloromethane extract, ethyl acetate and methanol) of *K. pinnata*. *In-vitro* studies revealed that the *K. pinnata* leaf extract possesses significant antioxidant as well as oxidative radical scavenging activities. Quercetin and kaemferol have been detected in the leaves of *K. pinnata* [6]. The quercetin has a marked protective effect on cadmium-induced nephrotoxicity that results from an increase Metallothionein, a small cysteine-rich protein and endothelial nitric oxide synthase expression and the inhibition of cyclooxygenase-2 and inducible nitric oxide synthase expression [1].

REFERENCES


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