Antimicrobial Activity of Ethanol Leaf Extracts of *Catharanthus Roseus* From Saudi Arabia

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**Abstract.** The antimicrobial activity from ethanol leaf extract of *Catharanthus roseus* *from Saudi Arabia* was investigated against some human pathogenic microorganisms (*Staphylococcus aureus* and *E.coli*) as well as pathogenic fungi (*Candida albicans*). The tested extracts showed very strong antimicrobial activity against all organisms. The antimicrobial activity was evaluated by measuring the zone of inhibition using disc diffusion method. The strongest inhibition activity of the leaf extract was observed against *Staphylococcus aureus* (15 mm zone) at 100 mg/ml of leaf extract followed by *E.coli* which showed 11 mm inhibition zone at 100 mg/ml leaf extract. Ethanol leaf extract also demonstrates antifungal activity against the pathogenic fungi *Candida albicans* (12 mm zone of inhibition). This study also investigated the role of environmental factors on the antimicrobial activity of this plant.

**Keywords:** Saudi Arabia, *Catharanthus roseus*, Medicinal plant, Ethanol Leaf extract, Antibacterial, Antifungal

1. **Introduction**

Many products of Medicinal plants prove to be very useful in reducing the adverse effects of various chemotherapeutic agents as well as in prolonging longevity and achieving positive health care system (Kaushik *et al.*, 2002).

The research for new therapeutic treatments for various disease conditions is expanding. In many poor countries, plants have been looked at as a very promising source of new lead compounds for drug discovery and development (Kong *et al.*, 2003).

*Catharanthus roseus* (Fig. 1) is an important medicinal plant of family Apocynaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities (Jaleel *et al.*, 2008). Antibacterial potential in crude extracts of different parts of *C. roseus* against clinically significant bacterial strains has been reported (Muhammad *et al.* 2009).

In the last few decades bacterial resistance to antibiotics has become a serious therapeutic problem and the rate at which new antibiotics are being produced is slowing, (Russell *et al.*, and 2002). Thus, the search for novel antimicrobial agents is of the utmost importance (Gootz *et al.*, 1990). Worldwide attention has been shifted towards finding new herbal chemicals for the development of new drugs. These natural products can provide unique elements of molecular diversity and biological functionality, which is indispensable for novel drug discovery (Nisbet and Moore, 1997).

Several research groups have shown that *Catharanthus roseus* has a high potential for many varieties of medicinal properties, such as antibacterial (Carew and Patterson 1970), antifungal (Jaleel *et al.* 2007) and antiviral (Farnsworth et al. 1968).

More than 130 of different alkaloids are present in C. Roseu (Pereira *et al.* 2010). As an important medicinal plant, it has a good antioxidant potential throughout its parts under drought stress.

The present investigation was focused on screening of ethanol leaf extracts of *Catharanthus roseus from Saudi Arabia* for its antibacterial and antifungal activity against number of pathogenic microorganisms.
To our knowledge, this is the first report which investigates the antimicrobial activity of ethanol leaf extracts of Catharanthus roseus from Saudi Arabian environment. This study also investigates the role of environmental factors on the antimicrobial activity of this plant.

2. Material and Method

2.1. Plant Material

*Catharanthus roseus* were cultivated and collected at the flowering stage from university garden at King Fahd University of Petroleum and Minerals in the Eastern Province in Saudi Arabia. In this study, the leaves of *Catharanthus roseus* were used for testing their antimicrobial activity. The plant materials were dried in shade at room temperature (25°C).

2.2. Preparation of Catharanthus Plant Extracts

Leaves of *Catharanthus roseus* were subjected to mechanical grinding and powdered by electrical blender. Ten grams of this powder was soaked in 100 ml of ethanol for 48 Hrs. The contents were then filtered through whatman filter paper no.1. The filtrate was dried by using a rotary evaporator at 60°C. The dried extract was stored in sterile glass bottles at -20°C until use (Kandil et al., 1994). This dried extract was then dissolved in DMSO for testing its antimicrobial potential. Extracts were stored at 4°C until further use.

2.3. Cultures Used

*E.coli, Staphylococcus* and *Candida albican* were collected from the Department of Pathology – King Fahd University of Petroleum and Minerals Clinic.

2.4. Culture Medium

Nutrient agar medium (for bacteria) and a dextrose agar medium (for fungi) were used as growth media for these microorganisms in this study.

2.5. Antimicrobial Activity

The antibacterial and antifungal activity studies were carried out by disc diffusion technique (Newall et al., 1996). The nutrient agar media was sterilized at 121 °C under 15 psi pressures for 30 minutes. After cooling to about 65 °C, 25 ml of the medium was poured in Petri-dish. The plates kept at room temperature for solidification and stored at 4 °C until using. The same process was applied with dextrose agar plates which were used for the growth of *Candida albican*.

Bacterial culture was spread over the nutrient agar plates by using separate sterile spreader. Holes were made in the medium by using 7 mm cork borer. The dried plant extract was dissolved in dimethylsulfoxide (DMSO) to final extract of 100 mg/mL. Each hole in each plate was filled with 50 µl of plant extract. DMSO was used as a negative control in one of the plates. The plates were incubated for 24-48 hours at 37°C along with negative controls. The antibacterial activity of each extract was recorded based on the inhibition of bacterial growth by the extract at the end of incubation period. At the end of the incubation period the zones of inhibitions were measured to the nearest millimetre (Andrews et al., 2001). The inhibition zone is the area surrounded the hole and there is no growth of inoculated microorganism. For confirmation of the results each test was performed in duplicate.

3. Results and Discussion

Medicinal plants are very important and widely available resource for primary healthcare and complementary healthcare systems. The literature in this area of research showed that plant kingdom contain many species of plants harbouring substances of medicinal value that have yet to be discovered; though large numbers of plants are constantly being screened for their antimicrobial effects (Pankaj et al.2008). These plants show that they are very rich source of compounds with possible antimicrobial activities, but more pharmacological investigations are necessary.

The present study reveals the antimicrobial activity of ethanol leaf extracts of *Catharanthus roseus*. The antimicrobial activity of *Catharanthus roseus* leaf extract was tested against 2 pathogenic bacteria, (*Staphylococcus aureus* and *E.coli*) and one fungi (*Candida albican*) shown in table 1. The tested extract
showed very strong antimicrobial activity against these pathogenic microorganisms. The antimicrobial activity was evaluated by measuring the zone of inhibition. Figure 2 and figure 3 illustrate the zone of inhibition for *Staphylococcus aureus* and *E.coli* respectively. The strongest inhibition activity of the leaf extract was observed against *Staphylococcus aureus* (15 mm zone) at 100 mg/ml of leaf extract followed by *E.coli* which showed 11 mm inhibition zone at 100 mg/ml leaf extract (table 2).

The results of this work are in agreement with other studies that the leaf extracts of this plant was has significant antibacterial activity against *Xanthomonas campestris* (Satish et al 1999). This study illustrate that a Gram-positive bacteria were more susceptible to this extract as compared to Gram-negative bacteria species. This is probably due to the differences in chemical composition and structure of cell wall of both types of microorganisms (Pankaj et al.2008). Results of this work showed that the extraction of antimicrobial substances by organic solvents is better as compared to aqueous extracts (Thongson et al. 2004 The polarity of antibacterial compounds make them more readily extracted by organic solvents, and using organic solvents does not negatively affect their bioactivity against pathogenic bacteria species.

Figure 4 illustrate the Antimicrobial activity of *Catharanthus roseus* leaf extracts against *Candida albicans* giving 12 mm zone of inhibition. Despite what many researchers have reported that Candid albicans are very resistant fungi, this work demonstrate that ethanol leaf extract of *Catharanthus roseus* is effective against this pathogenic fungi. This result is in agreement with other studies showed that the leaf extract of *Catharanthus roseus is very effective on Candida albicans* (Uniyal et al. 2006, Bhadauria and Kumar 2011).

Since this is the first report about the antimicrobial activity of *Catharanthus roseus* from Saudi Arabia we believe that the harsh environment in the desert with very high temperature (reaches 45-50 0C in the summer time) affect the components of the plants and therefore the antimicrobial activity of their extract. Skula et al. 1999 reported that cultivating same plants from different geographic locations affect the essential oils of these plants and therefore the antimicrobial activity of their extracts, this could be due to the environmental as well as genetic variations of these plants.

The outcome of this work support the validity of the use of the leaf extract of *Catharanthus roseus* as medicine in ancient medicinal traditions as well as the traditional usage of the studied plants. It is very clear that these extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy against pathogenic microorganisms. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

<table>
<thead>
<tr>
<th>Microorganism Used</th>
<th>Concentration of Extract mg/ml</th>
<th>Average of Inhibition Zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>100</td>
<td>11</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>100</td>
<td>15</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>100</td>
<td>12</td>
</tr>
</tbody>
</table>

4. Conclusion

Based on our results, it can be concluded that ethanol leaf extract of *Catharanthus roseus* possess significant antibacterial and antifungal activity.

Results presented here may suggest that the *Catharanthus roseus* leaf extract possesses antibacterial properties, and is therefore a potential source of antibacterial ingredients for the food and pharmaceutical industry. The second stage of this work is to study the extract from flower, stem and roots of *Catharanthus roseus and screen it against large number of pathogenic microorganisms*. Further research is needed for the isolation and identification of active principles present in the extracts which could possibly be exploited for pharmaceutical use.

5. Acknowledgements
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6. References


Fig. 1: Catharanthus roseus "Rosea"

Fig. 2: Inhibitory effects of ethanol leaf extract of Catharanthus roseus against Staphylococcus areous on the left compare to the control on the right.

Fig. 3: Inhibitory effect of ethanol leaf extracts of Catharanthus roseus against E.coli on the left compare to the control on the right.)
Fig. 4: Inhibitory effect of ethanol leaf extract of *Catharanthus roseus* against *Candid albicans*.