PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF 
*Cymbidium aloifolium* (L.) SW. LEAF

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Abstract

The aim of the present study was to determine the phytochemical screening and antimicrobial activity of *Cymbidium aloifolium* orchids in Kanyakumari district of South India. The phytochemical screening and antimicrobial activity were performed using different extracts (methanol, ethanol, chloroform, acetone and aqueous) of *Cymbidium aloifolium*. Four different bacteria (Gram positive: *Staphylococcus aureus* & *Bacillus subtilis*; Gram negative: *Escherichia coli* & *Klebsiella pneumoniae*) and two fungi (*Penicillium* sp. and *Aspergillus niger*) were used present study. The antimicrobial activity was assessed by using Disc diffusion method. A preliminary phytochemical screening was performed on the basis of detection of terpenoids, flavonoids, reducing sugars, phenols, alkaloids, saponins, tannins, steroids, amino acids and glycosides. All the extracts showed different degree of inhibitory action that potential against tested bacteria under this study. The antibacterial activity of *Cymbidium aloifolium* leaf revealed that the maximum inhibition zone of 34 mm recorded in aqueous extract against *Staphylococcus aureus*. Minimum inhibitory activity was recorded in acetone and chloroform extract. The aqueous extract had highest activity against *Aspergillus niger* and *Rhizopus* with inhibition zone of 45 mm and 42 mm respectively. Minimum inhibitory activity was recorded in acetone and chloroform extract.

Key words: *Cymbidium aloifolium*, Phytochemical, Antimicrobial activity and Disc diffusion method.

1. Introduction

Orchidaceae is one of the largest flowering plant families with cosmopolitan in distribution. Orchids are the most beautiful creation of the nature, consisting a group of flowering plants which are known from ancient times (Nagananda et al., 2013). Plants are the natural source of bioactive components. These bioactive components in plants are produced as secondary metabolites such as alkaloids, carbohydrates, flavonoids, tannins and phenols. Plants being the most reliable source of curatives are used as folk medicines for centuries. The presence of antibacterial substances in the higher plants was well established (Srinivasan et al., 2001).

*Cymbidium aloifolium* L. is an epiphytic herbaceous orchid belongs to Orchidaceae family (Nongdam and Chongtham, 2011). The indigenous people especially in hilly regions take immense pride in treasuring this plant because of its high utility in traditional healing and cure floriculture trade. It contains two substituted bibenzyls, dihydro phenanthrene and phenantraquinone (*Cymbinodin - A*), which are responsible for biological activity (Hossain et al., 2009). Traditionally, this plant was used in the treatment of anti-inflammatory, paralysis, joining fractured bones, fewer, weakness of eyes, chronic illness, burns, sores etc. Different types of extracts using the whole plants of *Cymbidium aloifolium* was prepared that

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subjected to sensitivity analyses against some bacterial and fungal agent. Therefore, the purpose of this paper is to investigate the phytochemical screening and antimicrobial activity of *Cymbidium aloifolium*.

2. Materials and Methods

Fresh plants of *Cymbidium aloifolium* was collected from Pechiparai in Kanyakumari District of South India. The plants were identified taxonomically. Fresh plants parts were washed thoroughly 2 - 3 times with running tap water and then with sterile water. Then, it was shade-dried, powdered and used for extraction.

**Figure - 1: Plant collected from natural habitat and maintained in the green house**

Plant blooms on a 75 cm pendant inflorescence with up to 75 flowers. Flowers are 4.25 cm wide with red stripes on petals and sepals. The plant produces four to five leaves per growth.

The aloe - leafed *Cymbidium* (*Cymbidium aloifolium*) is a species of orchid found in Asia, especially China and Southeast Asia from Burma to Sumatra. It can be found growing between rocks or on another plant. The word *Cymbidium* comes from the Greek kumbos meaning "hole, cavity" and the Latin specific name is just a translation of the English "aloe-leafed". The root is used to cure paralysis and joining fractured bones, leaf extract for treating boils and fever, whole plant used for treating weakness of eyes, chronic illness, vertigo, burns and sores [4]. An epiphyte with short pseudostem. Leaves 30 – 45 cm long, linear - oblong, curved, obtuse, fleshy, notched at the apex. Flowers yellowish red, in many flowered drooping long racemes, 23 - 38 cm long; petals as long as the sepals, oblanceolate - ovate; lip purplish, as long as the sepals, oblong, 3 lobed. Capsules are 5 - 6.3 cm long, elliptic and ribbed.

**Phytochemical Screening**

Phytochemical tests were carried out using various solvent extracts are described below. Methanol, ethanol, chloroform and hexane were subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents by standard procedures (Brindha, 1982; Harborne, 1998; Chandrashekar *et al*., 2013; Kalaiarasan *et al*., 2012).

**Antimicrobial activity**

**Bacterial and fungal strains and its culture**

Four different species of bacteria including both Gram positive (*Staphylococcus aureus* & *Bacillus subtilis*) and Gram negative (*Escherichia coli* & *Klebsiella pneumonia*) were evaluated. Cultures of these bacteria were grown in a nutrient broth (liquid media) at 37 °C for 24 hours. Pure culture of these bacteria was maintained at 4 °C on Nutrient agar medium. Two fungal isolates namely *Rhizopus* sp. and *Aspergillus niger* were used under this study.

**Antibacterial activity**

Antibacterial activity was carried out by Agar disc diffusion method (Bauer *et al*., 1966). A volume of 0.1 ml of 24 hrs old bacterial culture was spread in Muller Hinton agar plate by spread plate technique. The sterile disc containing respective solvent extract was placed on the surface of the medium and the antibiotic disc was used as positive control. The plates were incubated at 37 °C for 24 hrs. The plates were observed after 24 hrs for zone of inhibition.

**Antifungal activity**

Antifungal activity was determined using by Disc diffusion method (Bauer *et al*., 1966). Briefly, 100 μl of the test fungi was spread onto the Potato dextrose agar plate. The different test solvent extracts were loaded to the sterilized sterile 6 mm discs, allowed to dry and then the impregnated discs placed on the surface of medium. Plates were incubated at 37 °C at room temperature for 3 – 4 days. The diameters of the inhibition zones were measured in mm. Standard
antibiotic Fluconozole was served as positive control.

3. Results

The qualitative phytochemical analysis of the Cymbidium aloifolium leaf indicate the presence terpenoids, flavonoids, reducing sugars, phenols, alkaloids, saponins, tannins, steroids and aminoacids except glycosides. Cymbidium aloifolium leaf (control) showed the presence of terpenoids, flavonoids, alkaloids, tannins and steroids constituents besides it showed the absence of reducing sugars, phenols, saponins, amino acids and glycosides constituents. Ethanolic extracts of Cymbidium aloifolium leaf indicate the presence of flavonoids, alkaloids, saponins and steroids constituents and it showed absence of terpenoids, reducing sugars, phenols, tannins, aminoacids and glycosides constituents. Methanolic extracts showed the presence of terpenoids, flavonoids, phenols, alkaloids, tannins and steroids constituents besides it showed the absence of reducing sugars, saponins, aminoacids and glycosides constituents. Chloroform extracts revealed the presence of terpenoids, flavonoids, reducing sugars, phenols, alkaloids, tannins and steroids constituents. On the other hand chloroform extracts revealed the absence of saponins, aminoacids and glycosides constituents. Acetone extracts of Cymbidium aloifolium leaf indicate the presence of terpenoids, flavonoids, alkaloids, tannins, steroids and aminoacids constituents and it showed the absence of reducing sugars, phenols, saponins, and glycosides constituents. Meanwhile, Aqueous extracts revealed the presence of terpenoids, reducing sugars, phenols, saponins, steroids and aminoacids constituents besides it showed the absence of flavonoids, alkaloids, tannins and glycosides constituents.

In general, qualitative phytochemical analysis of the Cymbidium aloifolium leaf (control), ethanol, methanol, chloroform, and acetone extracts of Cymbidium aloifolium leaf indicate the presence of flavonoids, alkaloids and steroids constituents. Steroids were present in all the solvents. Chloroform and aqueous extracts showed the presence of reducing sugars all the other solvent extracts showed the absence of reducing sugars. Only acetone and aqueous extracts showed the presence of aminoacids all the other solvent extracts showed absence of aminoacids. Glycosides were absent in all the solvents.

The antimicrobial activity of Cymbidium aloifolium leaf extract revealed that the maximum inhibition zone of (43.5±0.84) recorded in aqueous extract against Rhizopus sp followed by Aspergillus niger (42±0.62). The aqueous extract showed inhibitory activity of (34.5±0.40) against Staphylococcus aureus. Methanol extract also exhibited some activity against Staphylococcus aureus (30±0.23) and Klebsiella pneumonia (25±0.47). Next activity was noticed in ethanol extract against Bacillus subtilis, Aspergillus niger, Klebsiella pneumonia and Staphylococcus aureus. No activity was noticed in ethanol extract against Escherichia coli and Rhizopus sp. The acetone extract showed significant activity against Bacillus subtilis, Aspergillus niger and Rhizopus sp. The pathogens such as Escherichia coli, Klebsiella pneumonia and Staphylococcus aureus exhibited no activity in acetone leaf extract of Cymbidium aloifolium. Chloroform leaf extract of Cymbidium aloifolium showed moderate antimicrobial activity against Aspergillus niger and Rhizopus sp. Among the different extracts aqueous extracts showed better results than the positive control. Minimum inhibitory activity was recorded in methanol extract and acetone extracts. Least activity was noticed in positive control against all the selected pathogens.
Table – 1: Qualitative analysis of *Cymbidium aloifolium* leaf extracts

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Solvent Extracts</th>
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<tbody>
<tr>
<td></td>
<td>C.a. Leaf</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Reducing Sugars</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
</tr>
<tr>
<td>Glycocides</td>
<td>-</td>
</tr>
</tbody>
</table>

Table – 2: Inhibition zone of five different solvent extracts of *Cymbidium aloifolium* against bacteria and fungi

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Zone of inhibition (in mm)</th>
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<tbody>
<tr>
<td></td>
<td>Ethanol</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>-</td>
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<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>16±0.81</td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td>13±0.00</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>28.5±0.25</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>25.5±0.40</td>
</tr>
<tr>
<td><em>Rhizopus sp.</em></td>
<td>-</td>
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</table>
4. Discussion

The present finding demonstrates that the leaf extracts of *Cymbidium aloifolium* provided a good zone of inhibition and phytochemical analysis. A good source of various phytochemicals such as terpenoids, flavonoids, reducing sugars, phenols, alkaloids, saponins, tannins, steroids, amino acids and glycosides. The previous observation of Bhattacharjee and Islam (2015) reported that the phytochemical analysis of *Rhynchostylis retusa* revealed the presence of alkaloids, terpenoids, flavonoids, tannins, steroids and amino acids. The ethanolic extract revealed the presence of flavonoids, alkaloids, saponins and steroids. Methanol extract revealed the presence of terpenoids flavonoids, phenols, alkaloids, tannins and steroids. The previous observation of Keerthiga and Anand (2014) reported the presence of terpenoids in chloroform and ethanol extracts *Geodorum densiflorum*. The present investigation chloroform extract revealed the presence of terpenoids flavonoids, reducing sugars, phenols, alkaloids, tannins and steroids. Acetone extract showed the presence of terpenoids flavonoids, alkaloids, tannins,
steroids and amino acids. The present study aqueous extract showed the presence of terpenoids, reducing sugars, phenols, saponins, steroids and amino acids. The earlier finding of *Vanda tessellata* showed the presence of methanol, ethanol and chloroform extracts Bhattacharjee et al. (2014). Terpenes play a role in and are under investigation for antibacterial, Antineoplastic and other pharmaceutical functions (Yamunadevi et al., 2011). Alkaloids are formed as metabolic byproducts and have been reported to be responsible for the antibacterial activity (Doughari, 2006). Steroids are known for anti-inflammatory, lipolytic and anti-cholesteremic activities (Chawal et al.,1987).

Hence, the presence of the secondary metabolites in the leaf extracts of *Cymbidium aloifolium* may be responsible for its potential use as a drug against pathogenic bacteria. The result of our antimicrobial study suggest that leaf extract showed maximum inhibition zone against the selected pathogen *Cymbidium aloifolium* methanol extract shows more effective on test organisms than the other solvents. Similar results were obtained by Radhika et al. (2013). The leaf extract of *Cymbidium aloifolium* was tested for its antimicrobial activity *Staphylococcus aureus* was found to be more susceptible towards the aqueous and methanol extract. Earlier findings of Uddin et al. (2015) are in coincident with our finding. Our study revealed that the solvent extracts exhibited better antimicrobial activity because of the extraction done by polar and non-polar solvents (Gupta and Katewa, 2014). Many of the previous finding reported the broad spectrum antimicrobial activity of *Cymbidium aloifolium* leaf extract and this may be attributed due to the presence of some phytochemical constituent. It was indicated that the leaf extract of *Cymbidium aloifolium* contains compounds that largely inhibit the growth of bacteria as well as fungal organisms.

5. Conclusion

The results showed the leaf extract of *Cymbidium aloifolium* have good antimicrobial properties. However, ethanol, acetone, chloroform, methanol and aqueous extracts show promising antimicrobial potential against the selected pathogens. The result of the study have justified the traditional use of the plants took cure various illness.

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6. Reference


