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PRELIMINARY PHYTOCHEMICAL ANALYSIS & ANTIBACTERIAL ACTIVITY AGAINST CLINICAL PATHOGENS OF MEDICINALLY IMPORTANT ORCHID *CYMBIDIUM ALOIFOLIUM* (L.) SW.

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ABSTRACT: *Cymbidium aloifolium* is a potent medicinal epiphytic orchid plant in the Indian systems of medicine. Traditionally this plant was used in the treatment of Anti-inflammatory, Paralysis, joining fractured bones, fever, weakness of eyes, chronic illness, burns, sores etc. In the present study three different solvent (Hexane, Chloroform, Methanol) extracts of *Cymbidium aloifolium* plant was studied for Antibacterial activity by Agar Well Diffusion method against ten clinical pathogenic bacteria. It was observed that Chloroform extract showed significant activity than the Methanol and hexane extracts. Preliminary phytochemical screening of this plant revealed that the presences of Simple sugars, Alkaloids, Tannins, Flavonoids, Anthraquinones, Terpenoids etc. The presence of bioactive constituents is associated with the antimicrobial activity. Antibacterial activity of various solvent extracts of this plant was carried in attempt to support the use by medicinal practitioner for the treatment of various diseases. Medicinal importance of this orchid will target to meet the therapeutic demands.

INTRODUCTION: Infectious diseases are the number one among all cautions of death, accounting approximately one-half all death throughout the world. About 50-75% of hospital deaths are reported due to infectious diseases [1]. Scientists from divergent fields is investigating plants with a new eye for their antimicrobial usefulness and as an alternative source to chemical constituents offer a promising source of new antimicrobial agents with general as well as specific activity [2].

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There are several reports on the presence of antimicrobial compounds in various plants [3, 4, 5, 6, 7]. It is an evident that the use of plants for various diseases since from Vedic period. Documentation of the Ayurvedic system recorded by Susruta and Charaka dates from about 1000 BC [8]. Plants are a goldmine of novel chemicals; much impressive number of modern drugs has been developed from them [9].

Herbal medicines have received much attention as a source of new antibacterial drugs since they are considered as time-tested and comparatively safe both for human use and the environment [10-12]. Researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against multidrug resistant microbe strains [13].

In developing countries, due to the cost of efficient antimicrobials, a large proportion of the population utilizes medicinal plants for the treatment of infectious diseases. According to the World Health Organization's estimation, traditional healing provides the primary health care needs for a large majority (80%) of the population in Africa [14].

Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants. Still play a vital role to cover the basic health needs in the developing countries. The medicinal values of these lies in some chemical active substances that produce a definite physiological action on the human body. Plants are also rich in compounds which have pain relieving and healing abilities [15]. The most important of these bioactive constituents of plants are Alkaloids, Tannins, Flavonoids and Phenolic compounds [16].

Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action [17, 18]. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [19]. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. Systematic screening of them may result in the discovery of novel active compounds [20]. Nowadays the pharmaceutical industries are facing many challenges favouring the use of plant natural products for the treatment of different diseases [21].

Orchidaceae family is one of the largest, most diverse and most important categories of botanically and commercially significant flowering plants with 20,000-30,000 species [22]. Orchids are well known for their beauty and its medicinal use [23]. *Cymbidium aloifolium* L. is an epiphytic herbaceous orchid belongs to Orchidaceae family [24]. *Cymbidium aloifolium* occupies a significant position in the everyday life of tribal people of North-Eastern India due to its medicinal and ornamental values. 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.

Some tribal people used its seeds for healing wounds [25]. Plant leaves and seeds were also used to cure ear ache, cut and wounds [26]. *Cymbidium aloifolium* roots were used in the treatment of Paralysis, joining fractured bones, boils, fever. Whole plant was used for treating weakness of eyes, chronic illness, vertigo, burns, and sores etc [27]. This plant is emetic & purgative; salep used as a nutrient and deculcent. Whole plant was collected on large scale for commercial use, probably used by the Siddha and Ayurveda pharmaceutical industries [28].

This plant investigation was taken up with an objective to evaluate the antibacterial potential against ten clinical pathogenic microorganisms and also detection of various bioactive compounds present in the whole plant. In this paper we report the results of such studies in order to orient future investigations towards the finding of new, potent and safe bioactive and antimicrobial compounds.

MATERIALS & METHODS: The plant parts (leaves) and of *Cymbidium aloifolium* was collected from the forest of Chinthapalli. The specimen was identified with the help of regional floras [29, 30] and the voucher specimen was deposited at Andhra University Herbarium (AU), Visakhapatnam, Andhra Pradesh, India.

Prepation of plant extracts: The collected leaves were shade dried, powered and extracted with hexane, chloroform and methanol using soxhlet apparatus for 8 hours. The extracts were filtered and filtrates were concentrated under reduced pressure at 40^{o} C using a rotaflash evaporator. The crude sample was subjected to antibacterial screening against the pathogenic bacteria. Various concentrations of plant extracts (500, 250, 100 mg/ml) were dissolved in DMSO (Di methyl sulphoxide).

Collection of microorganisms: The microbial strains viz., *E. coli* (isolated), *Proteus vulgaris* (isolated), *Xanthomonas sps.* (isolated), *Pseudomonas mirabilis* (isolated), *Pseudomonas aerosinosa* (isolated), *Klebsella oxytoca* (isolated), *Staphylococcus aureus* (isolated), *Staphylococcus epidermidis* (isolated), *Staphylococcus mitis* (MTCC 2696), *Staphylococcus anginosus* (MTCC 1929) were used and these organisms obtained from Visakha Eye Hospital (Isolated strains),

Visakhapatnam, Andhra Pradesh, India and Microbial Type Culture Collection centre, Institution of Microbial Technology (IMTECH), Chandigarh, India.

Antibacterial Assay: Antibacterial activity of extracts was determined by well diffusion method on nutrient agar medium. Nutrient agar medium [31] was prepared, sterilized and 0.2 ml of 24 hrs broth culture was mixed in the nutrient agar medium and poured in petriplates. After solidifying wells (6mm diameter) are made in nutrient agar plates using cork borer [31, 33, 34]. Different concentrations (500, 250, 100 mg/ml) of different solvent extracts (hexane, chloroform and methanol) were poured in wells and incubated at 37^{o}C for 24 hrs. The antibacterial was evaluated by measuring the zone of inhibition around the well. The experiment was done in triplicate and the mean diameter of the inhibition zone was calculated.

Phytochemical screening: About 20g of whole plant powder was soaked in 100 ml methanol solvent and incubated for 48 hrs and then filtrated using Whattmann No.1 filter paper to obtained Methanol plant extraction. Phytochemical analysis was carried out using Methanol plant extract using standard methods[35-38].

Identification Tests for Phytochemical Constituents: The tests were performed to find out the presence of active chemical constituents such as alkaloids, terpenes, flavones, flavonoids, steroids, reducing sugars, proteins, aminoacids, carbohydrates, tannins, anthraquinones, glycosides, cardiac glycosides by the following procedure. Phytochemical analysis was carried out for all the extracts using standard methods.

1. **Alkaloids:** Extracts were dissolved individually in diluted hydrochloric acid. The resulting acidic solution was tested for alkaloids by adding Mayer's reagent, Wagner's reagent, Dragendroff reagent and Hager's reagent. The formation of a faint turbidity or precipitation on the addition of the above reagents indicates the presence of alkaloids.

2. **Flavonoids:** 2-3ml of plant extract was dissolved in 50% of methanol and warmed then add a piece of magnesium ribbon and 1ml of conc. Hcl. Red or yellow coloration of the solution indicates the presence of flavonoids.

3. **Steroids:** 1ml of extract dissolved in 1ml of acetic anhydride, 1ml of chloroform and 1ml of Conc. HCl separately. Formation of green color indicates the presence of steroids.

4. **Triterpenoids:** To 1 ml of extract tin bit and thionyl chloride were added. Appearance pink color indicates the presence triterpenoids.

5. **Coumarins:** To 1ml of plant extract, 1ml of 10%NaOH was added. The formation of yellow color indicates the presence of coumarins.

6. **Cardiac glycosides:** To the plant extract few ml of glacial acetic acid, ferric chloride and conc.H_2SO_4 were added. Green color indicates the presence of cardiac glycosides.

7. **Terpenes:** To the plant extract few ml of chloroform was added, filtered. To the filtrate few drops of acetic anhydride and H_2SO_4 were added. The color changing from blue to green indicates the presence of terpenes.

8. **Anthraquinones:** Benzene extract was taken to this 5ml of 10% ammonia was added. Pink, red or violet color indicates the presence of anthraquinones.

9. **Phlabotannins:** Plant extract was dissolved in distilled water. The filtrate was boiled with 2% HCl. Red precipitate indicates the presence of phlabotannins.

10. **Quinones:** To 1ml of extract 1ml of conc.H_2SO_4 were added, formation of red color indicates the presence of quinones.

11. **Flavanones:** To few ml plant extract, 10% of few drops of NaOH was added yellow color indicates the presence of flavonones.

12. **Anthocyanins:** To the plant extract 10% NaOH was added, blue colour indicates the presence of anthocyanins.

13. **Proteins (Biuret test):** To few ml of plant extract, 1ml of 40% NaOH solution and 2ml of

1%$CuSO_4$ were added. Violet colour indicates the presence of proteins.

14. **Xanthoprotiec test:** To few ml of plant extract, 1ml of conc. HNO_3 was added. White precipitate was observed boiled and cooled. Then 20% of NaOH or NH_3 was added. Presence of orange colour indicates the presence of aromatic amino acid.

15. **Tannic acid:** To few ml of plant extract, 10% of tannic acid was added. White precipitate indicates the presence of proteins.

16. **Carbohydrates:**

a) **Molisch's test:** To few ml of plant extract 1ml of alpha-Napthol solution and conc.H_2SO_4 was added along the walls of the test tube. Purple to reddish violet colour at the junction of the two layers indicates the presence of carbohydrates.

b) **Fehling's test:** Equal volumes of Fehling's-A & B were added. On heating the formation of brick red precipitate indicates the presence of carbohydrates.

c) **Benedict's test:** To 5 ml of Benedict's Reagent few ml of plant extract was added and boiled for 2min, cooled.The formation of red precipitate indicates the presence of carbohydrates.

17. **Amino Acids:** 2 drops of Ninhydrin Reagent was added to the plant extract. Purple colour indicates the presence of amino acids.

18. **Glycosides:** The extract was mixed with a little amount of Anthrone on watch glass and 1drop of conc. H_2SO_4 was added and made to fine paste and boiled gently on water bath. Presence of glycosides shows dark green coloration.

19. **19. Flavones:**

a. **Shinoda test:** Add a few magnesium turnings and 1-2 drops of conc. Hcl to the plant extract, formation of red color shows presence of flavones.

20. **Tannins:** A few ml of potassium dichromate added to the plant extract, formation of precipitate showed the presence of tannins and phenolics.

21. **Phenols:** Add a few drops of 10% aqueous $Fecl_3$ to the plant extract, appearance of blue or green color indicates presence of phenols.

22. **Saponins:** Frothing test: add 0.5 ml of plant filtrate to 5 ml of distilled water, frothing persistence means saponins were present.

RESULTS AND DISCUSSION: In the present study, the phytochemical screening and antibacterial activities were performed with Hexane, Chloroform, Methanol extracts of *Cymbidium aloifolium.* Screening of this plant, Methanolic extract revealed the presence of Tannins, Alkaloids, Flavonoids, Triterpenoids, Coumarins, Flavones, Flavonones and Carbohydrates (**Table 1**).

TABLE 1: PHYTOCHEMICAL SCREENING OF *CYMBIDIUM ALOIFOLIUM* (METHANOL EXTRACT)

S. No.	Phytoconstituents	Whole plant extract
1	Tannins	+
2	Alkaloids	+
3	Saponins	-
4	Cardiac glycosides	-
5	Steroids	-
6	Terpenes	-
7	Flavonoids	+
8	Phlabotannins	-
9	Anthraquinones	-
10	Triterpenoids	+
11	Coumarins	+
12	Flavones	+
13	Flavanones	+
14	Anthocyanins	-
15	Phenols	-
16	Glycoside	-
17	Quinones	-
18	Carbohydrates	+
19	Amino acids	+
20	Proteins	+

(+) – Positive, (-) – Negative

Phytochemical constituents such as Alkalloids, Flavonoids, Tannins and several other compounds are secondary metabolites of plants that serve as a defence mechanism against prediction by many microorganisms, insects and other herbivores[39]. Tannins bind to proline rich proteins and interfere with the protein synthesis [40].

Flavonoids are hydroxylated phenolic substance known to synthesized by plants in response to microbial infection and it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cellwalls [41]. Coumarins are also known act against Gram Positive bacterial and fungal infections which could be attributed to its antimicrobial activity [42].

Alkaloids are formed as metabolic byproducts and have been reported to be responsible for the antibacterial activity [43]. Triterpenoids are known for anti-inflammatory, lipolytic and anti-cholesteremic activities [44]. It has also been observed and accepted that the medicinal value of plant lies in the bioactive phytocomponents present in the plant [45].

In the present investigation, the bioactive phytocompounds of *Cymbidium aloifolium* was studied and further the antimicrobial activity of the plant extract was also tested against ten potential clinical pathogenic bacteria namely *E. coli, Proteus vulgaris, Xanthomonas sps., Pseudomonas mirabilis, Pseudomonas aerosinosa, Klebsella oxytoca, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus mitis, Staphylococcus anginosus* at different concentrations of the plant extracts to understand the most effective activity (**Table 2**).

TABLE 2: ANTIMICROBIAL ACTIVITY OF LEAF EXTRACT OF *CYMBIDIUM ALOIFOLIUM*

S. No.	Name of organism	Zone of inhibition (in mm)								
		Hexane extract (mg/ml)			Chloroform extract(mg/ml)			Methanol extract (mg/ml)		
		500	250	100	500	250	100	500	250	100
1	*E.coli*	-	-	-	-	-	-	8	7	7
2	*Proteus vulgaris*	7	7	7	15	13	10	12	9	8
3	*Xanthomonas sps.*	8	7	7	13	11	10	11	9	9
4	*Pseudomonas mirabilis*	-	-	-	12	10	9	7	-	-
5	*Pseudomonas aerosinosa*	9	8	7	12	11	9	11	10	9
6	*Klebsella oxytoca*	8	7	7	11	10	8	10	8	7
7	*Staphylococcus aureus*	-	-	-	18	14	12	8	7	7
8	*Staphylococcus epidermidis*	8	7	7	10	9	8	9	8	7
9	*Staphylococcus mitis*	-	-	-	13	11	10	10	9	7
10	*Staphylococcus anginosus*	-	-	-	12	10	9	11	9	7

- No Activity

The maximum zone of inhibition was obtained for *Staphylococcus aureus* at a concentration of 500 mg/ml (**Fig. 1**). The chloroform extract was more effective on test pathogens showed significant inhibition zones ranged from 7-18 mm. Hexane extract of this plant shows least zone of inhibition (7-8 mm).

Hexane extract doesn't show any antibacterial activity against these test pathogens except *E. coli*, *Pseudomonas mirabilis, Staphylococcus aureus, Staphylococcus mitis* and *Staphylococcus anginosus*.

Cymbidium aloifolium chloroform extract shows more effective on test organisms than the methanol and hexane extracts. The results of antibacterial assay of three concentrations (100, 250,500 mg/ml) of different solvent plant extracts like chloroform and methanol exhibited most effective antibacterial activity in Table 2.

Cymbidium aloifolium contains several phytochemical compounds, which are very much necessary to control the growth of microorganisms.

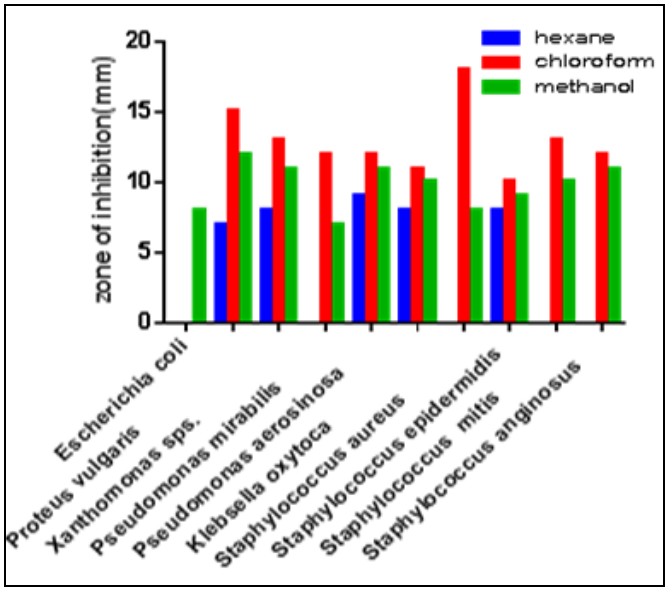

FIG. 1: Bar chart showing results of antibacterial activity against ten pathogenic bacteria of hexane, chloroform and methanol (500 mg/ml conc.) of *Cymbidium aliofolium*.

CONCLUSION: From the above results, it can be concluded that the *Cymbidium aloifolium* have great potential as antimicrobial compounds against clinical pathogens and that they can be used in the treatment of infectious diseases caused by pathogenic microorganisms. This plant showed maximum antibacterial activity and so it can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs.

However, further investigation on isolation and characterization of the active principle of this plant extract responsible for the antibacterial activity is necessary and it would give a comprehensive evidence of bioactive potential of medicinal plants. In conclusion, different solvent extracts of this plant showed promising antimicrobial activity against all the selected clinical pathogens. However, further experiments including detail evaluation of antimicrobial potentials are required to elucidate their mechanism of action at cellular and molecular level.

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