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Original Article

Pharmacognostic Evaluation & Antimicrobial Activity of Endangered Ethnomedicinal Plant *Crepidium acuminatum* (D. Don) Szlach

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ABSTRACT

Background: *Crepidium acuminatum* (D. Don) Szlach (family Orchidaceae) is an ethnomedicinal plant. It is used in breathing disorders, burning sensation, Cough, decrease in bone tissue, blood disorders, tuberculosis, as refrigerant, aphrodisiac, in insect bites, rheumatism, as tonic and in general debility. It is vital component of Ayurvedic formulation "Astavarga" with trade name "Jeevak means vitality of life. Despite the common utilization of this plant, no conclusive study has been reported so far regarding the pharmacognostic evaluation and antimicrobial activity. **Aim**: The present study was carried to evaluate pharmacognostic evaluation and the potential of *C. acuminatum* as antimicrobial. **Materials and Methods:** Organoleptic, histochemical, microscopic, physicochemical, extractive yield were studied to standardise pharmacognostic characters and well diffusion method were carried out for antimicrobial activity. Five extracts viz. Hexane, Chloroform, Ethanol, Ethyl acetate and aqueous were evaluated against 4 bacterial strains viz. *E. coli* (MTCC 40), *S. aureus* (MTCC 87), *P. aeruginosa* (MTCC 424), *B. subtilis* (MTCC 121). **Results:** The diagnostic characters were evaluated and documented. All the extracts showed good antimicrobial activity. **Conclusion:** Obtained standards will provide referential information for correct identification, purity, standardization and preparation of monograph. The work confirms that the studied plant has potent antimicrobial activity and has potential for antimicrobial drug. These results may constitute a basis for promising future applied research that could investigate the use of this plant as antimicrobial drug.
Key words: *Crepidium acuminatum* (D. Don) Szlach, Pharmacognostic Evaluation, Physicochemical, Histochemical, Zone of Inhibition, Antimicrobial.

INTRODUCTION

Natural products are utilized as therapeutics.[1] A large percentage of the world's population depends upon natural products for medicine. Folk medicine and ecological awareness suggest that natural products are harmless.[2] therefore trend is shifting from synthetic to herbal medicine, which has been called as 'Return to Nature'.[3] India, have a pluralistic healthcare system. Herbal drugs constitute a major share of all the formally recognised systems of health in India viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy, except Allopathy. Almost, 70% modern medicines in India are derived from natural products.[4] Natural products sustained to play a highly substantial role in the drug discovery and development process.[5] Medicinal plants play a crucial role not only as traditional medicines but also as trade commodities.[6] The role of information derived ethno medicine and its utility for drug discovery purposes is important.[7] A lot of work has been done on ethnomedicinal plants in India but still some important plants are still to be scrutinized. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found *in vitro* to have pharmacological properties.[8]

Crepidium acuminatum (D.Don) Szlach belonging to family Orchideaceae has been selected for the present study. It is an ethnomedicinal plant which is one of the eight drugs that comes under Astavarga.[9] In Indian system of medicine, its medicinal value is due to pseudobulbs and traded with name Jeevak.[10] This species grows in colonies in shady places, moist ground and in wet and mossy area. One colony contains 5-25 individuals, form mycorrhizal relation with a special fungus. The fungus attacks on the outer layer root cells which provide the nutrients to the plants that is essential for seed germination.[11,12] This species turned to be endangered due to several reasons like loss of forest due to animal grazing, anthropogenic activity and large collection of the rhizomes for drug preparation.[13]

Extensive literature survey has been done on this plant. This plant has been reported by other vernacular names as *Malaxis acuminata* D.Don, *Microstylis walichii* Lindl.[14] In Ayurveda *Crepidium*

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*acuminatum (*D.Don) Szlach has been stated in many Ayurvedic formulation such as Astavarga churna, chyavanprash Rasayan, Ghrita, Taila, Gutika, Agada etc.[14,15] As it is a ethnomedicinal plant, it used in Svasa (breathing disorders ,dyspnea), Daha (burning sensation), Kasa (Cough) , Ksaya(decrease in bone tissue), Raktapitta(bleeding disorder), Raktavikara(blood disorders), Sosa(tuberculosis). It is reported to be refrigerant therefore used to reduce fever (Febrifuge). It has been described as aphrodisiac and used in emaciation, seminal weakness. It is also used in insect bites and rheumatism. It is used as tonic and in general debility. [11-18] It has been found that in spite of its ethnomedicinal value, little work has been done on this plant.

In the current investigation carried out, pharmacognostic evaluation and screening of different extracts of *C. acuminatum* pseudobulbs have been used against various types of bacteria in order to screen new sources of antimicrobial agents.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The pseudo bulbs of the plant *C. acuminatum* (family Orchidaceae) were purchased from Chandigarh in the month of February 2014 were air dried, powdered and stored in air tight container. Pseudo bulbs were authenticated and identified as *Crepidium acuminatum* (D.Don) Szlach from National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Pharmacognostic evaluation was done as per WHO Guidelines.[19]

Chemicals

All reagents and chemicals used for pharmacognostic evaluation and antimicrobial activity were of analytical grade.

Pharmacognostic Evalaution

The organoleptic studies were carried out by with sense organs using simple technique like shape, size, colour, odour, taste etc. Histochemical reactions were applied with concentrated hydrochloric acid and phloroglucinol for identification of lignified elements, iodine solution for starch grains, Sudan red-III for cuticle layer and oil globules, Ruthenium red for mucilage and acetic acid for calcium oxalate crystals.

Microsocopic study was done by taking transverse free hand sections of the fresh pseudobulbs. Physicochemical parameters such as loss on drying, ash values, pH value in 1% and 10% solution, aqueous, and alcoholic extractive values were carried out according to the methods recommended by the World Health Organization.[19]

Preparation of Plant Extract

After collection of pseudobulb samples, they were powdered. Powder material were passed through sieve no. 40 and used for extraction. Weighed powder was extracted using hexane, chloroform, ethyl acetate, ethanol and aqueous solution in Soxhlet apparatus till exhausted. The extract was evaporated at 40°C in rotary vacuum evaporator to dryness.[20] The extracts obtained from successive extraction *i.e.* Hexane extract (HE), Chloroform extract (CE), Ethyl acetate extract (EAE), Ethanol extract (EE) and residual Aqueous extract (AAE) were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, steroids, flavonoids, glycosides, tannins, phenolic compound, carbohydrates, proteins, amino acids and fats.[20,21]

Test Micro-organisms and growth Media

The antibacterial activity of different extracts were studied against four bacterial strains, two Gram-positive (*Staphylococcus aureus* MTCC 87, *Bacillus subtilis* MTCC 121) and two Gram-negative (*Escherichia coli* MTCC 40, *Pseudomonas aeruginosa* MTCC 424) based on their pharmacological importance. All the strains of micro-organism were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh.

The strains of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* and *Pseudomonas aeruginosa* were maintained on nutrient broth at 37°C and suspension were stored in refrigerator till used**.**

Commercially available Mueller-Hinton agar (MHA) (Hi-media, Mumbai) was prepared according to the instructions on the leaflet. Immediately after autoclaving the media, it was allowed to cool. Freshly prepared and cooled medium was poured into glass flat-bottomed petri-plates on a level, flat surface to give a uniform depth of approximately 4 mm. This corresponded to 30 ml for each plate with a diameter of 90 mm. The agar medium was allowed to cool at room temperature and unless the plates were used the same day otherwise these were stored in a refrigerator (2 to 8°C) for further use within seven days. Representative samples of each batch of plates were examined for sterility by incubating at 37°C for 24 hours or longer.[22]

Agar Well Diffusion Method for Determination of Zone of Inhibition (ZOI)

Antibacterial activity was carried out using well diffusion method. The test cultures were spread with the help of spreader on the top of the solidified media and allowed to dry. The tests were conducted with 100mg/ml concentrations of the crude extract per well with three replicates.

Dimethyl Sulphoxide (DMSO) (Himedia Mumbai) was used as negative control. Streptomycin discs (10**µg**/disc) of 6 mm were used as positive control. The plates were incubated for 24 h at 37 °C. Zone of inhibition (ZOI) was recorded in millimetres and the experiment was repeated thrice.

The inoculums were prepared by making a direct broth suspension of 24-hour agar plate. The suspension adjusted to match the 0.5 McFarland turbidity standards. Dried extracts were accurately weighed and dissolved in the DMSO to yield the 100mg/ml concentration, using sterile glassware. These were stored in refrigerator for further use. The wells were made in the incubated MHA media plates with the help of sterile cork borer (steel) of 4 mm and plates were labelled properly. 50 µl of the working solution of plant extract were loaded into the respective wells with the help of micropipette. The plates were incubated 24 h at 37C. The plates were then observed for the zone of inhibition (ZOI) produced by the anti- bacterial activity of different plant extracts. At the same time ZOI of different organism by different extracts were measured with the help of the ruler for the estimation of effectiveness of anti- bacterial substance and tabulated.

The plates were then incubated in the inverted position at 37°C for 24 h The diameters of the zones of complete inhibition as observed by the unaided eye are measured, including the diameter of the disc/well. Zones were measured to the nearest whole millimetre, using a ruler, The petri plate is held in non-reflecting background and illuminated with reflected light. The zone margin were taken area showing no obvious, visible growth which can be detected with the unaided eye. The same procedure was followed for each strain and extract.[23]

RESULTS

Pharmacogostic evaluation

Pharamcognostic evaluation has been done with respect to organoleptic properties, histochemical evaluation, microscopy and physicochemical studies and successive extraction yield.

Organoleptic evaluation

Organoleptic evaluation which is done by sense organs is the simplest and quickest means to ascertain the identity and purity of a drug. Organoleptic characters as shape, size, colour, odour, taste etc. are evaluated. These features of the pseudo bulb powder of *C. acuminatum* sample were observed. The details of results are presented in Table1.

Histochemical Characters

Powder study by using particular chemicals has been done. The results are presented in Table 2.

Physicochemical Analysis

The parameters which have been studied are moisture content, loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values, swelling index, foreign matter, foaming index and pH analysis.

Ash values are useful to indicate presence of various impurities like carbonate, oxalate and silicate. The water soluble ash indicates amount of inorganic compound present in drugs whereas the acid insoluble ash indicate contamination with earthy material. Moisture content of drugs should be at minimal level to discourage the growth of microorganisms during storage. Extractive values establish the amount of the active constituents. The extractions of any crude drug with a particular solvent yield a solution containing altered phytoconstituents. The compositions of these phytochemicals depend upon the nature of the plant and the solvent used. Results of physicochemical analysis of pseudo bulbs of *C. acuminatum* has been presented in Table 3.

Table 1: Organoleptic evaluation of pseudobulbs of *Crepidium acuminatum* D. Don Szlach

S.No.	Parameters	Observation
1.	Colour	Green (fresh) and brown (dry form)
2.	Taste	Slightly bitter and astringent in taste
3.	Shape	Conical
4.	Odour	Characteristic
6.	Size	3-9 cm long and 1-3 cm in diameter
7.	Surface	Fleshy, smooth and shining. Covered with membranous sheath

Table 2: Histochemical study of pseudobulb powder

S.No.	Reagents	Test	Nature of colour change	Results
1.	Ruthenium red	Mucilage	Pink	+ve
2.	Weak Iodine solution	Starch	Blue	+ve
3.	Sudan III	Fixed oil and fats	Pink	+ve
4.	Sulphuric acid (60%)	Calcium oxalate	Soluble, on standing show needles of calcium sulphate	+ve
5.	Phloroglucinol-HCl	Lignins	Reddish brown to red rose	+ve
6.	Millon's reagent	Protein	Yellow to brown	-ve

(+): present; (-): absent

Microscopic study

Parenchyma tissue was irregular having large air spaces and vascular bundles were scattered throughout the ground tissue. Calcium oxalate crystals were seen which were confirmed by histochemistry. Conspicuous mucilaginous canals were present.

Successive solvent extraction with hexane, chloroform, ethyl acetate, ethanol and water

Percentage yield and physical characteristic of various extracts of *C. acuminatum* pseudobulb sample are shown in Table 4.

ANTIMICROBIAL ACTIVITY

Results obtained in the present study revealed that tested extracts possess potential antibacterial activity against *E. coli, S. aureus, P.aeruginosa, B.subtilis*. When tested by disc diffusion method the chloroform, Ethyl Acetate and Ethanol pseudobulbs extracts showed most promising results. The maximum ZOI has been observed with chloroform extract viz. 20 mm has been observed against *E. coli*, followed by *B. subtilis* (15.33 mm), *S. aureus* (14.33 mm) and least with aqueous extract. Ethyl Acetate pseudobulb extract of *C. acuminatum* exhibit highest activity against *E.coli* of 18.66 mm followed by *B. subtilis* 13.33 mm, *S. aureus* 10.33 mm and least against *P. aeruginosa* 8 mm. Ethanol extract showed maximum activity against *E. coli* 15.33 mm followed by *S. aureus* 12.33 mm, *B. subitilis* 11 mm and least in *P. aeruginosa* 8 mm. Hexane extract showed highest activity against *B.subtilis* as 9.33 mm almost same as for *S.aureus* 9.17 mm. hexane extract showed equal activity against *E.coli*

Table 3: Physicochemical parameters of *C. acuminatum*

S.No.	Physicochemical Parameter	% Value (%w/w)
1.	Total ash value	1.49%
2.	Acid-insoluble ash value	0.49%
3.	Water soluble ash value	0.99%
4.	LOD	6.52%
5.	pH 1% Solution	7.5
6.	pH 10% Solution	6.8
7.	Moisture content	53%
8.	Foreign Matter	2%
9.	Alcohol soluble extractive value	4%
10.	Water soluble extractive value	16.5%
11.	80% ethanol soluble extractive	0.95%
12.	Alcohol soluble extractive value	4%
13.	Swelling Index	12.5%
14.	Foaming index	Less than 100

Table 4: Extractive yields of different extracts

Sr. No.	Extract	% Yield	Colour	Odour	Consistency
1.	HE	0.95%	Dark green	Characteristic	Sticky
2.	CE	0.8%	Brown	Characteristic	Sticky
3.	EAE	0.57%	Dark brown	Characteristic	Sticky
4.	EE	1.55%	Dark brown	Characteristic	Sticky
5.	AE	5.45%	Dark brown	Characteristic	Sticky

Table 5: zone of inhibition (in mm) of different extracts of pseudobulbs of *Crepidium acuminatum* (D.Don) Szlach against different microorganisms

ORGANISMS → EXTRACT ↓	*E. coli* MTCC 40 (gram -ve) Zone of Inhibition (diameter in mm)	*S. aureus* MTCC 87 (gram + ve) Zone of Inhibition (diameter in mm)	*P. aeruginosa* MTCC 424 (gram -ve) Zone of Inhibition (diameter in mm)	*B. subtilis* MTCC 121 (gram + ve) Zone of Inhibition (diameter in mm)
Aqueous	6.5 ±0.00	7.0±0.00	8.0±0.00	6..5 ±0.22
+ ve Control	19	7	24.5	24
-ve Control	0	0	0	0
Hexane	8.0±0.00	9.17±0.17	8.0±0.00	9.33±0.33
+ve Control	19	7	24.5	24
-ve Control	0	0	0	0
Chloroform	20.0±0.00	14.33 ±0.33	10.5±0.29	15.33±0.33
+ve Control	19	7	24.5	24
-ve Control	0	0	0	0
Ethanol	15.33±0.33	12.33±0.33	10.33±0.33	11.0±0.58
+ve Control	19	7	24.5	24
-ve Control	0	0	0	0
Ethyl acetate	18.66±0.33	10.33±0.33	8.0±0.00	13.33±0.33
+ve Control	19	7	24.5	24
-ve Control	0	0	0	0

and *P. aeruginosa.* Aqueous extract exhibited least activity among all the extracts tested. The data pertaining to the antimicrobial potential of the plant extracts are presented in Table 5 (results as mean ± standard error, standard error was calculated by on line statistical tool)

It is clear from the Table 5 that antibacterial activities of different extracts are showing promising results. *The growth inhibition zone measured ranged from 6.5 mm to 20 mm.* Trend of the activity of different extracts against *E.coli* and *S. aureus* is same i.e. Chloroform>Ethyl acetate>Ethanol>Hexane>Aqueous. Maximum ZOI has been observed for Chloroform extract *i.e.* 20 mm and least ZOI for aqueous i.e. 6.5 mm. Antibacterial activity against *P. aeruginosa* MTCC 424 has been observed in the pattern as Chloroform>Ethanol >Ethyl acetate=Hexane=Aqueous whereas antibacterial activity against *B.subtilis MTCC 121* has been observed as Chloroform>Ethyl acetate>Ethanol>Hexane>Aqueous.

It is clear from Figure 1A that Chloroform extract is showing more promising anti E. coli activity as compared to standard. Similarly ZOI is more against *S aureus* by Chloroform, Ethanol extract. Figure1B showed that ZOI in chloroform extracts of *C. acuminatum* is more as compared to positive control when tested against *E. coli.*

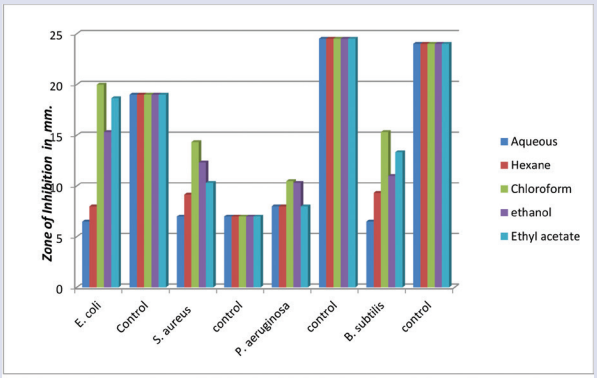
Figure 1C showed that tested extracts either exhibit equal or more activity as compared to standard when tested against *S. aureus*. Figure 1D showed that extracts are least effective against *P.aeruginosa* as compared to other strains tested. Figure 1E showed good activity of chloroform extract against *B.subtilis* as compared to other tested extracts. Figure 2 showed Zone of Inhibition against various microbes. Streptomycin was used as positive control and DMSO as negative control

DISCUSSION

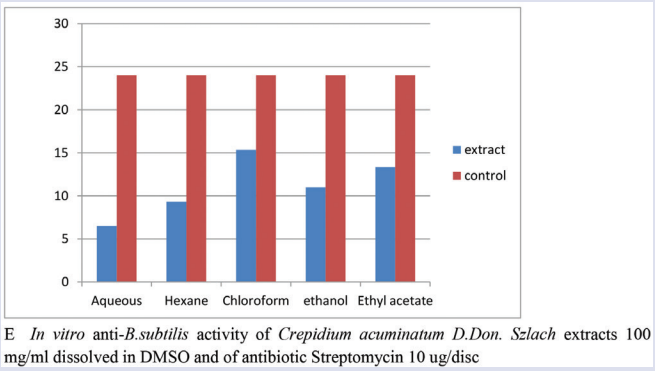
Ethno pharmacologists, Botanists, Microbiologists, Biotechnologists and natural-Products Chemists are exploring the mother Earth for phytochemicals and "leads" which could be developed into medicine. Plants are rich in secondary metabolites like tannins, terpenoids, alkaloids and flavonoids and these secondary metabolites are responsible for antibacterial properties. The use of plants and its preparations to treat diseases is an ancient practice in world especially in developing countries like India where there is dependence on traditional medicine. Interest in plants with antibacterial properties has revitalized as a result of current problems associated with the use of antibiotics. The present studies aimed at the investigation of *C. acuminatum* Endangered ethnomedicinal orchid plant *in vitro* antibacterial activity against Gram positive and Gram negative bacteria. The results presented here point out that this plant is a good choice for the development of new "leads".

Hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of *C. acuminatum* pseudobulb extracts showed significant zone of inhibition against "Gram-positive" bacteria, *Staphylococcus aureus* MTCC 87, *Bacillus subtilis* MTCC 121 and Gram-negative bacteria *Pseudomonas aeruginosa* MTCC424 *and Escherichia coli* MTCC 40.

This work shows that maximum ZOI has been observed in Chloroform extracts and least in aqueous extracts. This means active components showing better antibacterial property are more lipophilic as compared to hydrophilic. Phytochemicals such as alkaloid are generally reported in Chloroform extract.[20]One sterol namely β-Sitosterol has been reported along with two sugars (glucose and rhamnose) , Cetyl alcohol, Choline and diterpenes.[14-24,25] whereas in another study Piperitone, Citronellal, Eugenol, Limonene, 1,8-Cineole, p-Cymene, o-Methylbatatasin, has been reported by thin layer chromatography.[10-26] whereas in another study metal content and volatile constituents in *Microstylis wallichii* (*C. acuminatum*) which were analyzed by Atomic Absorption Spectrophotometer and GC and GC-MS. Not much work has been done on the phytoconstituents of this plant. Sharma, P and coworkers[27] studied The antimicrobial activity of pseudobulbs of *Malaxis acuminata* using the Butanol extract against some gram (+)ve, Gram (-)ve bacteria and fungi by agar cup diffusion method against *Escherichia coli, Klebsiella aerogenes, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus* and fungus, *Candida albicans*. The butanol extract showed better fungal inhibition than bacterial inhibition. They observed 19 mm and 18 mm ZOI with *E. coli* and *S. aureus* respectively at 50 mg/ml concentration.

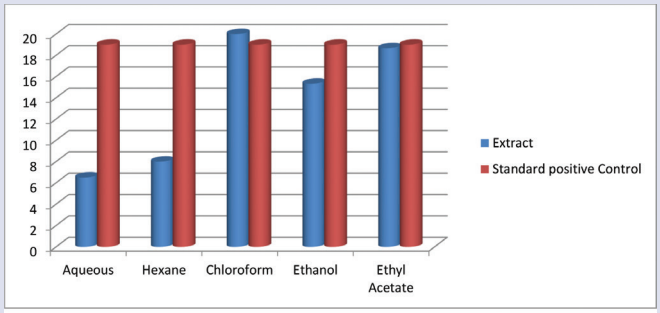


1A Showing antibacterial activity of different extracts against mentioned bacterial strains as compared to standard

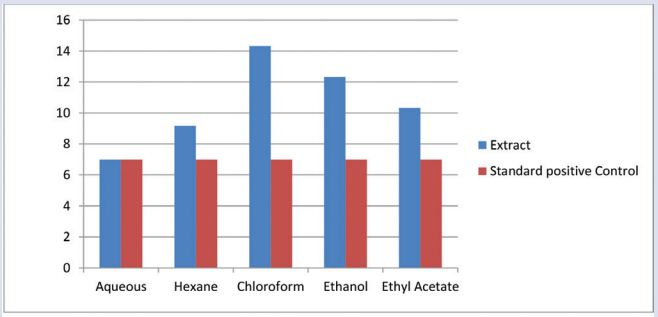


E *In vitro* anti-*B.subtilis* activity of *Crepidium acuminatum D.Don. Szlach* extracts 100 mg/ml dissolved in DMSO and of antibiotic Streptomycin 10 ug/disc

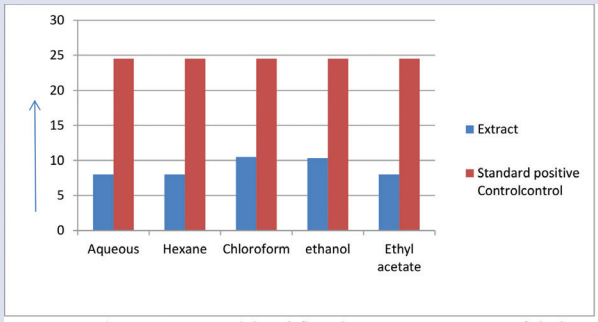
Figure 1: Antimicrobial activity response (ZOI) of extracts of different extracts of pseudobulbs of the plant. X-Axis –Types of extracts, Y-Axis-Zone of Inhibition in mm. DMSO is negative control and antibiotic Streptomycin 10 ug/disc as positive control



B. *In vitro* anti- *E.coli* activity of *Crepidium acuminatum D.Don. Szlach* extracts 100 mg/ml dissolved



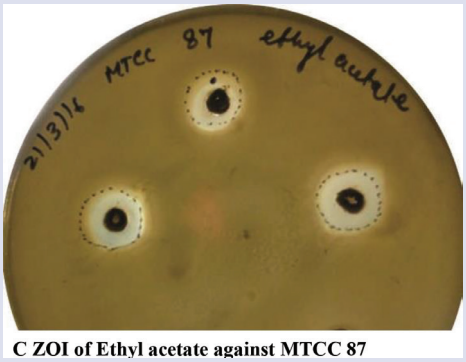
C. *In vitro* anti-*S.aureus* activity of *Crepidium acuminatum D.Don. Szlach* extracts 100 mg/ml dissolved in DMSO and of antibiotic Streptomycin 10 ug/disc



D. *In vitro* anti-*P. aeruginosa* activity of *Crepidium acuminatum D.Don. Szlach* extracts 100 mg/ml dissolved in DMSO and of antibiotic Streptomycin 10 ug/disc

2A ZOI of Chloroform extract against MTCC121

B ZOI of Ethyl acetate extract against MTCC 40



C ZOI of Ethyl acetate against MTCC 87

D ZOI of Hexane extract against MTCC 424

E ZOI of chloroform extract against MTCC 40

Figure 2: Antimicrobial activity of different extracts of pseudo bulbss against different microorganism *Staphylococcus aureus* MTCC 87, *Bacillus subtilis* MTCC 121 and Gram-negative bacteria *Pseudomonas aeruginosa* MTCC424 *and Escherichia coli* MTCC 40
(DMSO is negative control, antibiotic Streptomycin 10 ug/disc is positive control)

In another study[28] antimicrobial properties against pathogenic microbial strains namely *Pseudomonas aeruginosa* (MTCC 4676) and *Staphylococcus aureus* (MTCC 7405) have been reported. The maximum activity out of the tested plants by them *C. acuminatum* was most active and out of various extracts studied by them ethanolic and methanolic extracts of the plant was found to be highly active against the test bacteria. The antioxidant activity of *Malaxis acuminata* was studied by Sharma, P.[29] by using methods:1, 1-diphenyl-2-picryl hydrazyl(DPPH) radical scavenging activity, Reduction capability by Fe3+-Fe2+ Transformation method and Hydrogen peroxide scavenging method. The results predicted that the extract showed a good antioxidant activity. Ethanolic (50% v/v) extracts of *Carissa carandas* (fruits) (Apocynaceae) and *Microstylis wallichii* (tubers) (Orchidaceae) were considered[30] for anti-inflammatory and analgesic activities in experimental animals. Extracts of both plants (50-200 mg/kg) caused a dose dependent inhibition of swelling caused by carrageenin significantly in cotton pellet induced granuloma in rats. A significant increase occurred in the analgesic meter induced pain in rats.

The studies commenced here also suggest that presence of good antibacterial potency of the extracts is due to active compounds in the extracts. The results indicate that the tested crude extracts are potential source to be explored to identify new compounds.

As this plant is used in Ayurvedic formulations the results also revealed the scientific basis of the traditional usage of *C. acuminatum* and therefore received attention.

This is supporting document to prove that this plant has therapeutic uses since ancient times. The use and exploration for drugs and dietary supplements derived from this plant have accelerated recently but much work has to be done.

CONCLUSION

Pharmacognostic evaluation play an important role in quality control of the crude drug. The different characters observed in the pseudobulbs of *C. acuminatum* serve as base for the identification of right sample of the plant as drug and other studies. Five extracts have been selected out of which Chloroform extract, Ethyl Acetate and ethanol extracts have shown more promising results as compared to hexane and Aqueous extracts. It can be concluded from this study that chloroform, ethyl acetate and ethanol are more suitable for further studies. Antibacterial leads seem to be more lipophilic in nature. The ZOI in chloroform extract is found to be even more as compared with standard drug Steptomycin against *E. coli* MTCC 40. The present study justified the claimed uses of pseudobulbs in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the prospective efficacy of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation for the potential discovery of new natural bioactive compounds.

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CONFLICTING INTEREST

We declare no conflict of interest

ABBREVIATIONS USED

ZOI: Zone of Inhibition; **MTCC:** Microbial type culture collection centre; **DMSO:** Dimethyl sulphoxide.

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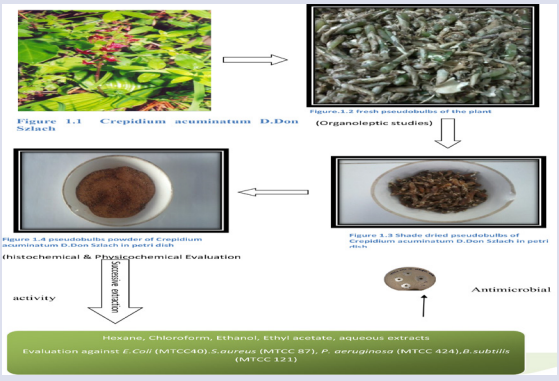
GRAPHICAL ABSTRACT



SUMMARY

- *Crepidium acuminatum* (syn. *Malaxis acuminata*) commonly known as Jeevak is used in breathing disorders, burning sensation, Cough, decrease in bone tissue, blood disorders, tuberculosis, as refrigerant, aphrodisiac, in insect bites, rheumatism, as tonic and in general debility. It is vital component of Ayurvedic formulation "Astavarga".
- Pharmacognostic evaluation of *Crepidium acuminatum* has been done w.r.t. parameters organoleptic properties, histochemical evaluation, microscopy and physicochemical studies and successive extraction yield.
- The studies commenced here also suggest that presence of good antibacterial potency of the extracts is due to active compounds in the extracts. The results indicate that the tested crude extracts are potential source to be explored to identify new compounds.
- The present study justified the claimed uses of pseudobulbs in the traditional system of medicine to treat various infectious disease caused by the microbes.

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