Int J Ayu Pharm Chem

RESEARCH ARTICLE

www.ijapc.com

e-ISSN 2350-0204

Chemical Characterization and Antibacterial activity of *Swarna Jibanti* (Coelogyne cristata Lindl.)

Achintya Mitra[1]*, Sreya Dutta[2], Dhirendra Nath Mandal[3] , Kumkum Bhattacharyya[4], Dipankar Bhattacharyya[5] and Jayram Hazra[6]

[1,2,3,6]National Research Institute of Ayurvedic Drug Development, CCRAS, Government of India, Kolkata, West Bengal, India

[4]Department of Microbiology, R.G.Kar Medical College & Hospital, Kolkata 700 004, West Bengal, India.

[5]College of Medicine and J.N.M. Hospital, West Bengal University of Health Sciences , West Bengal, India

Abstract

Ayurveda has a long therapeutic history since the time of immemorial which is still vulnerable as it is serving many of the ailments for large number of the population. *Swarna Jibanti* (*Coelogyne cristata* Lindl.) is being used as a single plant or as an ingredient for certain polyherbal formulations since the period of *Veda* for promoting the healthy life and rejuvenation. As it belongs to orchids and not widely available in the market, considering the huge demand in the industry, there is always chances of adulteration as no quality control profiles are ready in-hand. The standardization profiles and chemical characterization through pharmacognostic evaluation and chemical assays, viz. HPLC, LCMC, HPTLC profiles were carried out that can possibly help to differentiate the drug from its other species. *Swarna Jibanti* is anti-infective, potentiates immunity and antibacterial activities. The standardization of *Swarna Jibanti* was carried out pharmacognostically through cellular and chemo-taxonomic profiles. A module of standard operating procedure along with of pharmacognostical studies and chemical characterization has been developed with the help of modern sophisticated tools. The antimicrobial activity of the test drug in different solvent system has been carried out which reveals its significant activity against certain microorganisms. The standardized *Swarna Jibanti* was found to be potent antimicrobial against certain microorganisms.

Keywords

Coelogynae cristata, pharmacognosy, chemical assay, antimicrobial activity

Greentree Group
Received 12/08/15 Accepted 05/09/15 Published 10/09/15

INTRODUCTION

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [1]. The use of medicinal plant as a source for relief from illness can be traced back over five millennenia to written documents of the early civilization in China and India [2]. The potential of higher plants as source for new drugs is still largely unexplored. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and has an enormous therapeutic potential to heal many infectious diseases .Medicinal plants represent a rich source of antimicrobial agents [3].

Antimicrobial resistance in both medicine and agriculture is now recognized by the World Health Organization (WHO) along with other various national authorities, as a major emerging problem of public health importance. It represents a significant challenge of global dimensions to human and veterinary medicine with the prospect of therapeutic failure for life-saving treatments now a reality. In order to minimize the potential development of further antimicrobial resistance, "The Copenhagen Recommendations: Report from the Invitational EU Conference on The Microbial Threat" were published[4] which outlined the need for the development of "Novel principles for treating or preventing infections in humans and animals". Such an approach may thus be to examine the antimicrobial properties of native plants used in herbal medicine, as a novel source of such agents, as well as the employment of such novel compounds, and thus limit the use of conventional antibiotics to cases of severe and life-threatening infections, thus minimizing the development of resistance to such agents. Several traditional plant extracts have historically been known to have antimicrobial activity. Screening of medicinal plants for antimicrobial activities and photochemical is important for finding potential new compounds for therapeutic

use. Antibacterial activities of various plant extracts were documented in the various scientific reports. The plant extracts have been developed and proposed for use as antimicrobial substances [5]. Medicinal herbs practiced in traditional folk medicine in India were screened for the presence of antibacterial activity[6]. Inhibition on the growth of both was recorded from the leaf extracts of ethanol and methanol [7].The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential to evaluate plants of medicinal value systematically for various ailments that are used in traditional medicine. Hence, there is a need to screen medicinal plants for their promising biological activity.

Coelogynae cristata Lindl. *(Orchidaceae)*, is one of the plants, which is being used for therapy in ancient India as per the Vedas [8] . This plant popularly known as *Swarna Jivanti* for its golden colour on drying is distributed eastwards from about 75° E Longitude in the Garhwal region of northern India, through Nepal, Sikkim, Assam, Bhutan and into the Khasi hills of North Eastern India. Usually the habitat of the orchid is 1600-2600 m in moss-forests where they are found growing on both trees and rocks, often almost fully exposed to the sun[9] .This plant gained further importance for its inclusion in Ayurveda as one among ten plants defined as *Jeebaneeya verga* (life promoter and nutrient). It is used singly or in combination since long time in the treatment of asthma, cough, degenerative changes, fever, eye diseases, blood borne diseases, immune-suppression [10]. Despite the numerous medicinal uses attributed to this plant, there are no pharmacognostical reports on this plant. Hence, the present investigation deals with the morphological and anatomical evaluation, determination of physicochemical constants, phyto-chemical screening and HPLC, LCMC, HPTLC profiles of its hydro-alcoholic extract of Swarna Jivanti, *Coelogynae cristata. Lindl*. In search, may therefore consider as a virgin plant and to validate and to study the chemicals responsible for the observed therapeutic activity at the very outset the antibacterial activity of the plant in different solvents.

MATERIALS AND METHODS

Plant material

The pseudobulbs of *Coelogyne cristata* Lindl. were procured from the local drug market of Kolkata, West Bengal , India and

authenticated by Department of Botany, Burdwan University, West Bengal; voucher specimen was deposited in the museum of the Department of Pharmacognosy, National Research Institute of Ayurvedic Drug Development, Kolkata (NRIADD/ 2011/ 03). The photography of live plant was taken from its natural habitat at medicinal plant garden of Nagaland University, Lumani, Nagaland, India [**Fig1**].

Figure 1 *Coelogynae cristata* Lindl. in its natural habitat.

Procedure of Extraction

The dried pseudobulbs of *Coelogyne cristata* Lindl. were extracted with four different solvent systems, i.e., Ethanol, Petroleum Ether, Chloroform and Hydro-alcohol. The plant material was cleaned, and dried in shed and powdered in coarse with the help of pulverizer. The sieved coarse material was extracted successively and independently with ethanol (99.9%), petroleum ether (40^0-60^0C bp), chloroform (laboratory grade) and hydro-alcoholic (60%) for 72h. The solvent extracts were concentrated under reduced pressure and preserved at 4°C in airtight glass bottles till its further use.

Macroscopic and microscopic analysis

The pseudobulbs were examined macroscopically with reference to its colour, shape, size, odour, taste etc. For anatomical studies, microscopic examination of pseudobulb was carried out as per standard method. Powdered plant drug was treated [11] and the cell components were critically analyzed.

Physiochemical analysis

Physiochemical analysis such as the percentage of ash values and extractive values were carried out according to the official methods prescribed in the Ayurvedic Pharmacopeia of India[12] and WHO guidelines on quality control methods for medicinal plant materials. [13] The fluorescence and physico-chemical characters were determined. [14, 15.]

Chromatographic analysis through HPTLC profiles

Qualitative densitometric HPTLC analysis[16] was performed for development of characteristic fingerprint for hydro-alcoholic extract of *Coelogyne cristata* Lindl. 10µl of the sample solution was applied and the plates were developed. Developed plates were then scanned densitometrically at various wavelengths. Retention factor (R_f) values, peak area, peak height and spectrum of each peak were determined for the extracts.

Chromatographic analysis through HPLC profiles

Total alkaloids from the powdered collected of *Coelogyne cristata* were extracted and the extract was re-dissolved in 5mL absolute ethanol (analar grade) and filtered through Whatman filter paper No.1.The filtrate was used for high performance liquid chromatography study. An isocratic HPLC (Shimadzu HPLC class *VP* series) with two LC – 10 AT *VP* pumps (Shimadzu), variable wavelength programmable photodiode array detector SPD MIOA *VP* (Shimadzu), CTO-IOAS *VP* column oven (Shimadzu), SCL-10A *VP* system controller (Shimadzu) and a reverse phase Luna 5 mC18 (2) Phenomenex column (250 mm x 4.6 mm) was used. The HPLC system was equipped with software class *VP* series version 6.1 (Shimadzu). The mobile phase components acetonitrile: water (1:3) was filtered through 0.2 mm membrane filter before use and pumped from the solvent reservoir to the column at a flow rate of 1 mL/min which yielded a column backpressure of 16-165 Kgf/cm^2. The column temperature was maintained at 27^{0}C and 04 mL of sample was injected using Rheodyne injection (Model 7202, Hamilton). [17]

Chromatographic analysis and Mass Spectrum through LC-MS profiles

A chromatographic fingerprint of a medicinal plant is, in practice, a chromatographic pattern of pharmacologically active and or chemically characteristic constituents present in the extract. This chromatographic profile should be featured by the fundamental attributions of integrity and fuzziness or sameness and differences so as to chemically represent the medicinal plants investigated. The chromatographic fingerprint can successfully demonstrate both sameness and differences between various samples and the authentication and identification of Ayurvedic medicines can be accurately conducted even if the number and/or

concentration of chemically characteristic constituents are not very similar in different samples of formulations. Thus chromatographic fingerprint should be considered to evaluate the quality of Ayurvedic medicines all over the world considering multiple constituents present in the medicinal plant and its products[18].

Antimicrobial testing

Bacterial Strains

The following bacterial strains, *viz. Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 15380), *Pseudomonas aeruginosa* (ATCC 27853) were obtained from the R G Kar Medical College, Kolkata. These were sub-cultured on nutrient agar medium incubated at 37°C for 24 and stored at 4°C to maintain stock culture.

Preparation of inoculum

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria were incubation without agitation for 24 hrs at 37°C.The cultures were diluted with fresh Mueller-Hinton broth to achieve optical densities corresponding to $2.0 \cdot 10^6$ colony forming units (CFU/ml) for bacteria strains.

Antimicrobial susceptibility test

The disc diffusion method was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension of each bacterial strain was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The different concentrations of solvent extracts (100, 200, 400 and 600 mg/disc) were loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimetre. These studies were performed in triplicate.

RESULTS

Morphological / Macroscopic characters

Coelogyne cristata is an epiphytic orchid with creeping rhizome and pseudobulbs. The

pseudobulbs, are actually secondary stems, highly specialized and thickened consisting of one or more internodes acting as storing organs for moisture and food, arising from creeping rhizomes, ovoid or mostly elongated with distinct ridges and furrows, 2.8-8 cm long x 2-5.5cm broad; surface smooth, not punctuated; fractured longitudinal-fibrous; odour indistinguishable; mature pseudobulbs bright and shining golden-yellow to brown in appearance. Roots develop at union of rhizome and pseudobulb [**Fig 2**].

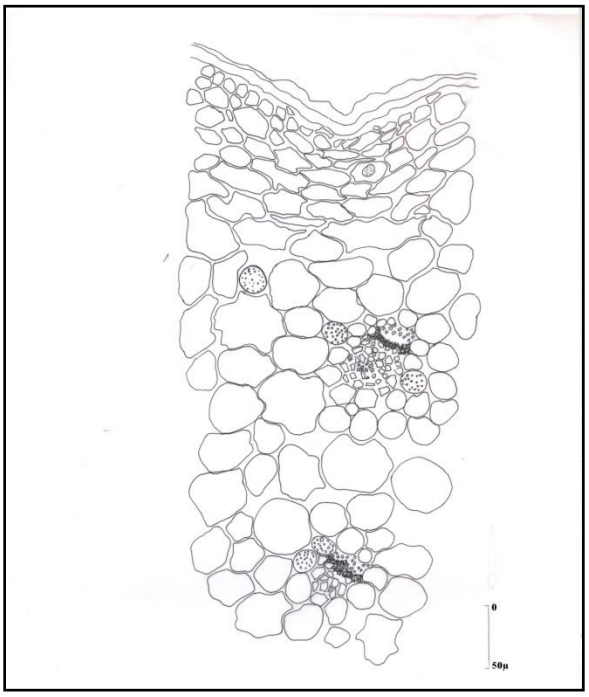


Figure 2 Transverse section through the pseudobulb of *Coelogynae cristata* Lindl.

Microscopic characters

Pseudobulbs- T.S. of dried pseudobulb showed thick cuticle with ridges and furrows sometimes loosened out of epidermis from periphery to centre. Epidermis single cell layered with barrel shaped cells and ground tissue made up of irregular parenchymatous cells of various shape and sizes extending unevenly with frequent air spaces; few starch and aleurone grains present within the parenchymatous cells. Distinct, oval shaped vascular bundles scattered throughout the ground tissue, each consisting of a cap of few sclerenchymatous cells, 1 to 3 central xylem vessels surrounded by thick walled parenchymatous cells and few phloem cells in between the cap and xylem region [Figure 2].

Powder drug analysis- Powder (# 60) is grayish-brown in colour with no distinguishable smell, slightly bitter and astringent in taste; shows groups of mesophyll cells, thin layer epidermal cells, parenchymatous cells, clusters of starch grains, aleurone grains, Ca-oxalate crystals, septate fibers, pollen grains and vessels with pitted and annular thickening[Figure 3]..

Physico-chemical/ Fluorescence studies

Physico-chemical values and fluorescence characters of the powder drug under ordinary light and ultra violet light (UV 254nm and 366nm) are presented in Table 1 and Table 2.

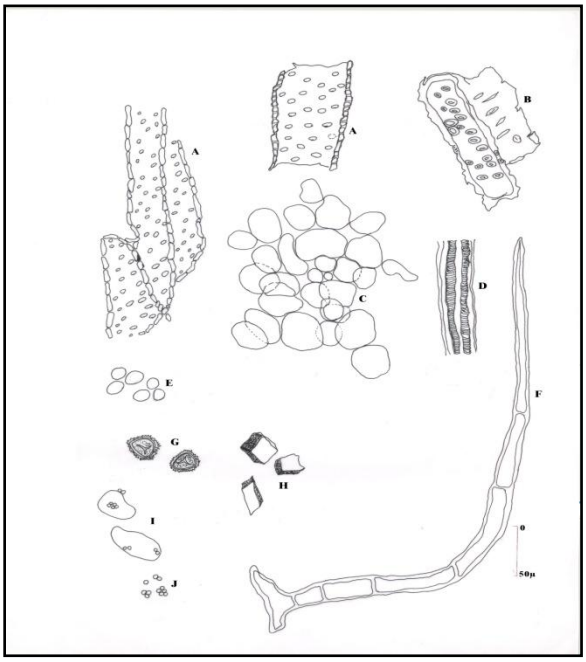


Figure 3 Powder drug analysis of pseudobulb of *Coelognae cristata* Lindl.

A & B= Pitted Xylem vessel, C = Group of Parenchymatous cells, D= Vessel with annular thickening, E= Starch grains, F= Septate Fibre, G= Pollen grains, H= Calcium oxalate crystals, I= Parenchymatous cells with aleurone grains, J = Aleurone grains

Table 1 Physico-chemical constants of *Coelognae cristata* Lindl.

S. No.	Parameters	Sample [values(%w/w)]			Values (%w/w)
		I	II	II	Mean
1.	**Total ash**	4.7	4.5	3.95	4.38
2.	**Acid insoluble ash**	0.65	0.67	0.65	0.656
3.	**Water soluble extractive**	6.9	7.0	6.8	6.9
4.	**Alcohol soluble extractive**	4.6	4.2	4.0	4.26
5.	**Foreign matter**	3.86	3.89	4.02	3.92

Chemical assay -Precoated (support on Aluminum sheets) Silica gel plates were used (TLC Silica Gel $60F_{254}$, Mfg. by Merck,26.09.2011, batch no. 1.05554.0007) . Ethyl Acetate: Methanol (90:10) [G R grade solvent used , mfg. by MERCK, India] was used for mobile phase. Applied volume 4 µL as 8 mm band and applied at 10 mm from the base of the plates and developed up to 80 mm in CAMAG Twin trough chamber. Plate preconditioning (temp 27°C and relative average humidity was 48%) was maintained, dipped in 20% aqueous Sulphuric acid and charred at 105°C for 10 minutes. R_f values were observed at 254 nm, 366 nm and white light after derivatisation [**Fig 4**] .

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. The successive extract of pseudobulb of *Coelogyne cristata* has revealed the presence of active biomarkers through HPLC profiles[**Fig 5**].

The qualitative HPLC alkaloid profiles of test drug were detected at a wavelength of 220 nm due to sharpness of the peaks, and proper baseline and the retention time (R*t* min), percent area and heights were recorded as seen in Figure 6. The HPLC chromatogram showed 12 peaks.

Table 2 Fluorescence analysis of *Coelogynae cristata* Lindl.

S. No.	Particulars of the treatment	Under ordinary light	Under UV light	
			Short UV (254 nm)	**Long UV (366 nm)**
1	Powder as such	Greyish-brown	No fluorescence	No fluorescence
2	Powder+1 N NaOH (aqueous)	Yellow with distinct brown boundary	Yellowish green with green border; no fluorescence	Fluorescence present
3	Powder+1N NaOH (ethanolic)	Straw yellow	Straw yellow; no fluorescence	Fluorescence present
4	Powder+1N Hcl	Yellow	No fluorescence	No fluorescence
5	Powder+H_2SO_4(1:1)	Dark slate	No fluorescence	No fluorescence
6	Powder+HNO_3(1:1)	Light slate	No fluorescence	Fluorescence present
7.	Extracts			
	a) Petroleum ether(40-60°C)	Faint yellow	Faint yellow; no fluorescence	Fluorescence present
	b) Benzene	Light yellow	No fluorescence	Fluorescent pink colour
	c) Chloroform	No colour	No fluorescence	No fluorescence
	d) Methanol	Straw yellow	No fluorescence	Fluorescence present
	e) Water	No colour	No fluorescence	No fluorescent

Table 3 Antibacterial activities of different solvent extracts of *Coelogynae cristata* Lindl.

Name of Bacteria with strain	Nature of Extract	Zone of inhibition (mm) at Concentration (mg/ml)		
		100 mg/ ml	200 mg/ ml	400 mg/ ml
Escherichia coli (ATCC 25922)	ET	17.50	18.75	22.25
	PT	12.00	13.00	15.25
	CF	13.16	14.25	15.25
Staphylococcus aureus (ATCC 25923)	ET	17.60	20.30	21.00
	PT	13.00	14.33	16.33
	CF	14.33	15.25	17.25
Klebsiella pneumonia (ATCC 15380)	ET	17.16	20.33	21.00
	PT	11.00	11.33	11.33
	CF	12.00	11.33	12.33
Pseudomonas aeruginosa (ATCC 27853)	ET	15.52	17.00	16.33
	PT	11.00	11.33	12.00
	CF	12.00	11.33	13.00
Enterococcus faecalis (ATCC 29212)	ET	17.16	20.33	21.00
	PT	15.00	14.33	14.33
	CF	11.66	11.66	16.66

ET- Ethanol Extract , PT- Petroleum Ether Extract; CF- Chloroform Extract;

One of the peak was prominent with significant percent area and height (>67.97%). The most abundant peak with area and height is observed at the retention time 10.69 (R*t*, min), which is probably diaboline, the prominent alkaloid of this plant.

Figure 4 Photography of HPTLC Plates at different Visualizations of *Coelogynae cristata* Lindl**.**

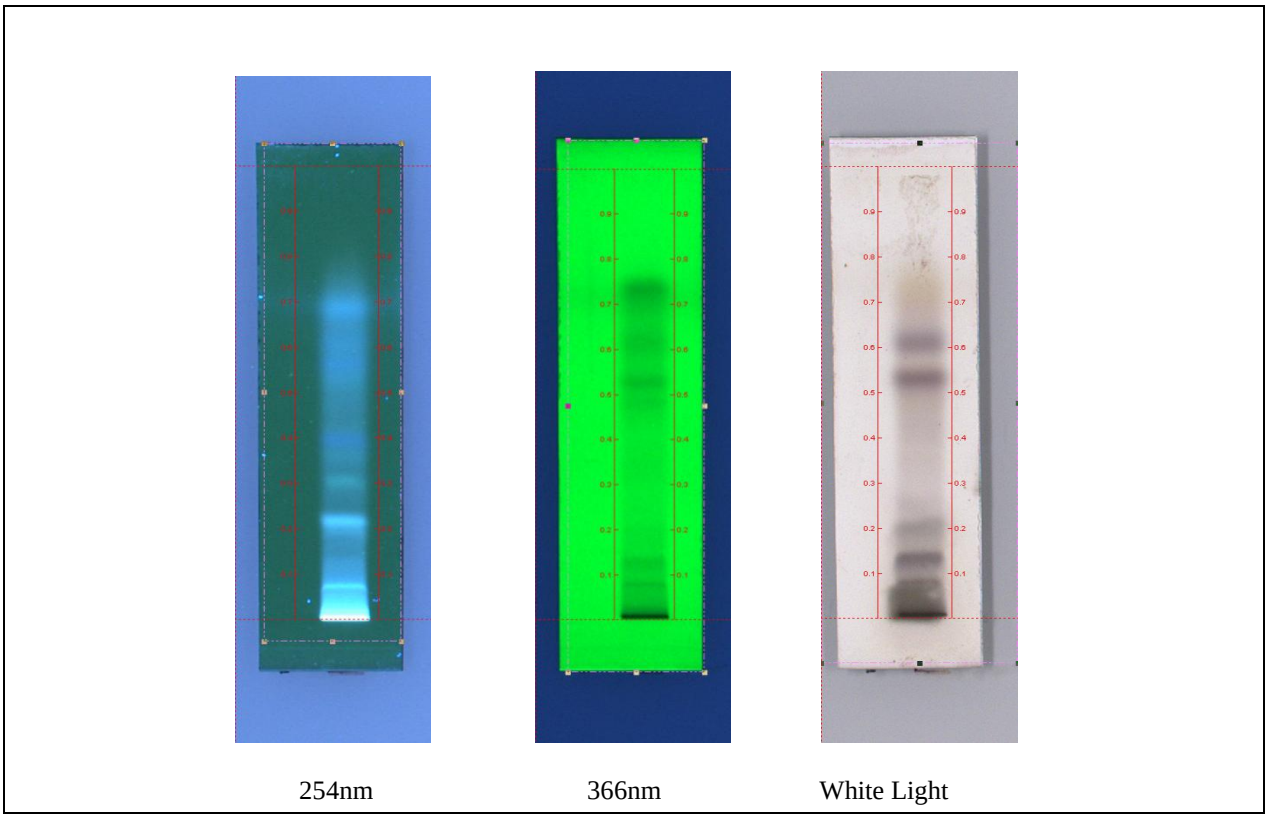


Figure 5 HPLC fingerprint of *Coelogynae cristata* Lindl.

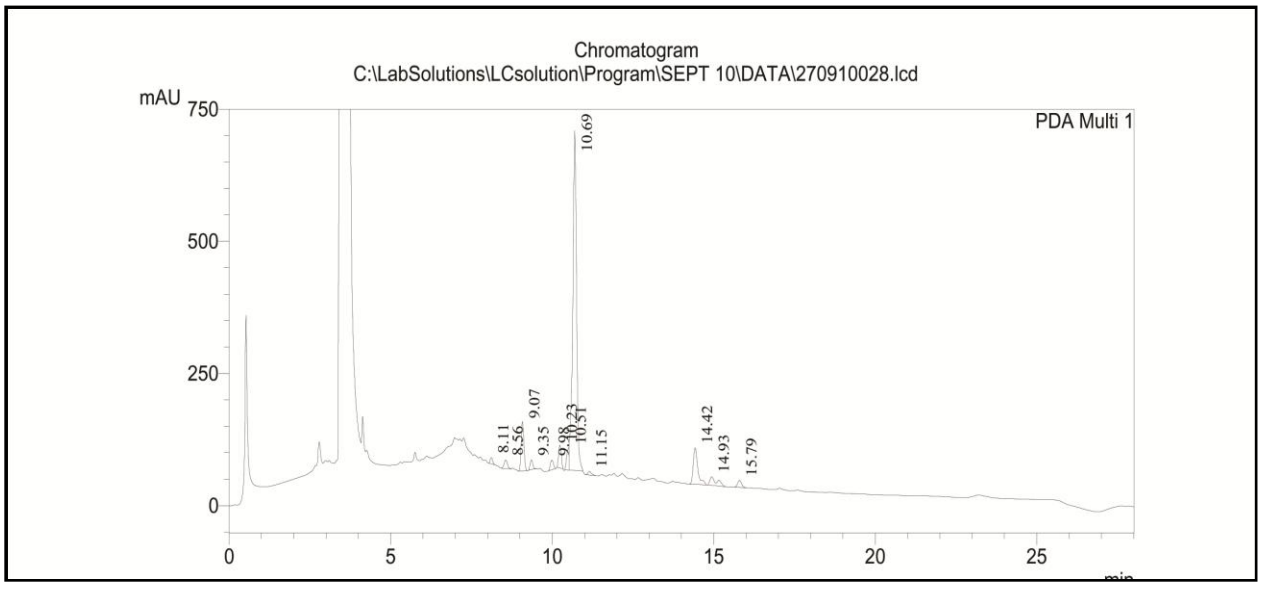
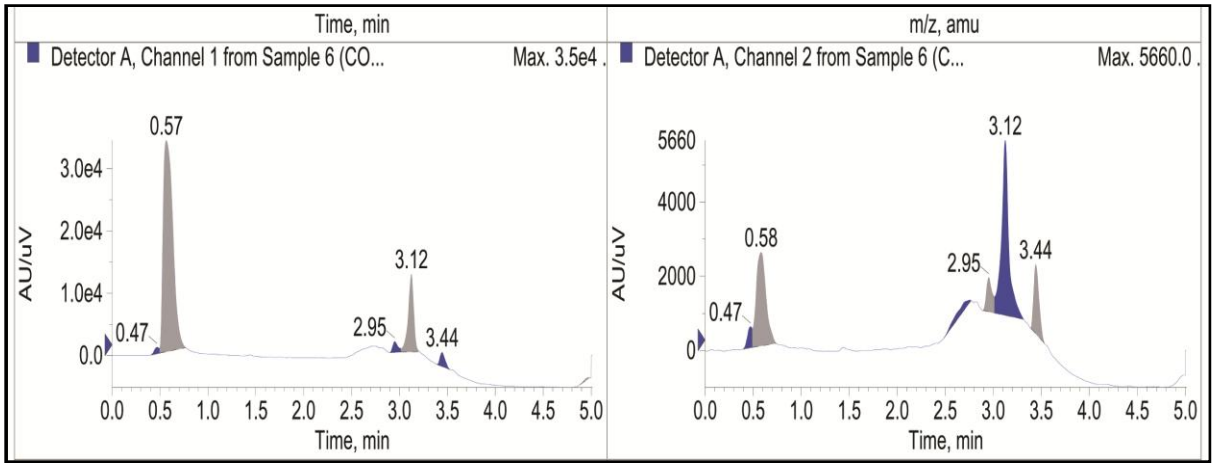


Figure 6 LCMS finger print of *Coelogynae cristata* Lindl**.**



Thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Therefore, the data generated from these experiments have provided the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

Principal phenolic compounds were detected by liquid chromatography–mass spectrometry (LC–MS). The mass spectrometric fingerprinting of complex botanical samples upon the examples of the pharmacologically important phenolic acids and flavonoids selectively extracted from *Swarna Jibanti*. In this study, we explore fingerprinting efficiency with a novel two-dimensional analytical system composed with mass spectrometric detection (LC-MS). The present study is basically focused on exploring the bioactive components, it was justified to carry out with the phenolic acid extracts selectively derived from the *Coelogynae cristata*, however further studies will be carried out.

Antimicrobial activity

The results of investigation of antibacterial of pseudobulbs of Swarna Jibanti were studied in different solvent extracts (ethanol, petroleum ether and chloroform) and different concentrations (100mg/dl, 200mg/dl and 400mg/dl) against five pathogenic bacterial strains among them two gram positive, i.e. S.aureus ATCC 25923, E.faecalis ATCC 29212 and three gram negative, i.e. E.coli ATCC 25922,

P.aeruginosa ATCC 27853 , K. pneumonia ATCC 15380.

The antibacterial activities of different solvent extracts are presented in Table 3. The results indicated that all three solvent extracts, viz. those with petroleum ether, chloroform and aqueous. Ethanol showed varying degrees of antibacterial activities.

The minimum inhibitory concentrations (MIC) of the alcoholic extracts range from (100-400) µg/ml over the array of strains stored. Maximum activity was observed against *S.Aureus*, exact at a concentration of 100 µg/ml (Table 4).

Table 4 Minimum Inhibitory Concentration of *Coelogynae cristata* Lindl. with ethanolic extract.

Bacteria	Minimum Inhibitory Concentration (MIC) µg/ml
Bacteria	
Escherichia coli (ATCC 25922)	400
Staphylococcus aureus (ATCC 25923)	100
Klebsiella pneumonia (ATCC 15380)	300
Pseudomonas aeruginosa (ATCC 27853)	400
Enterococcus faecalis (ATCC 29212)	400

DISCUSSION

In the present study, the pharmacognostic characters along with physico-chemical The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular values reported in this article could be used as the diagnostic tool for the standardization of the medicinal plants. Adulteration, if any, can be easily identified using these parameters. The microscopic features could help in laying down microscopical standards as per WHO guidelines for authentication of the drug plant. Microscopical characters of the pseudobulbs i.e. presence of parenchymatous cells in irregular shapes with air spaces next to the epidermal layer and distinct vascular bundles scattered though out the ground tissues surrounded by thick sclerenchymatous cells are seen in the transverse section were the distinguishing features and can be used as anatomical markers.

Table 5 Drug sensitivity of *Coelogynae cristata* Lindl.with ethanol extract

Antibacterial Drugs	**Concentration (µg/ml)**	**Zone of Inhibition (mm)**
Ethanol Extract	500	22.00
Tetracycline	500	23.00
Erythromycin	500	18.00
Neomycin	1000	17.00
Norfloxacin	100	21.00
Gentamycine	1000	20.00

solvent. In this dimension pharmacognostic studies on *Swarna Jibanti (Coelogyne cristata* Lindl.) is a substantial step and it further requires a long term study to evaluate pharmacological action as well as therapeutic efficacy and toxicity to establish as the drug. Despite the numerous medicinal uses attributed to this plant, there are no pharmacognostical reports on this plant. The pharmacognostic study of the *Coelogyne cristata* Lindl. has been carried out for the first time. This could also serve in the identification and preparation of the drug for further study.

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols *etc*. The successive extract of pseudobulb of *Coelogyne cristata* has revealed the presence of active biomarkers through HPLC profiles. The recent study of chemical characterization shows that Coeloginanthridin, 9,10-dihydrophenanthrene derivatives, and coeloginanthrin, the corresponding phenanthrene analogue, were isolated from the orchid *Coelogyne cristata*, which earlier afforded coelogin and coeloginin . The structures of coeloginanthridin and coeloginanthrin were established as 3,5,7-trihydroxy-1, 2- dimethoxy -9, 10-dihydrophenanthrene and 3, 5 ,7 – trihydroxy-1, 2 -dimethoxyphenanthrene , respectively, from spectral and chemical evidence including the conversion of coeloginanthridin triacetate to coeloginanthrin triacetate.[19]

However, the major shortfall, which has hindered the acceptance of the Ayurvedic medicines in the developed countries, is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines. So, it becomes extremely important to make an effort towards chemical characterisation of the plant material to be used as medicine. The profile presented in this article may be established the authenticity of the drug from its adulteration. The HPLC chromatogram has shown one prominent peak with significant percent area and height (>67.97%) which may be considered as active biomarker.

The mass spectrometric fingerprinting of extracted from *Swarna Jibanti* with mass spectrometric detection, the phenolic acid extracts selectively derived from the *Coelogynae cristata*, however the further study will be carried out. The fingerprint of

hydroalcoholic extract of test drug in the HPTLC study showed identical features in respect of R_f values in 254nm, 366nm wave length and white light.

The antibacterial activity of pseudobulbs of *Swarna Jibanti* was carried by different extractions with ethanol, petroleum ether and chloroform in different concentrations against five important pathogenic bacterial strains which revealed the potency of the drug. This plant is being used for different ailments since Vedic period in India and probably it acts as the antibacterial for the certain conditions which was not explored.

CONCLUSION

The identification and authentication of *Swarna Jibanti* (*Coelogynae cristata* Lindl.) was carried out pharmacognostically through cellular and chemo-taxonomic profiles. The authenticated sample was being extracted for chemical analysis and proper characterization. *Swarna Jibanti* is being used incontinently; however there is paucity of literature concerning its characterization. In the present study, a module of standard operating procedure along with chemical finger prints has been developed with the help of modern sophisticated tools. The antimicrobial activity of the test drug in different solvent system have been carried out which revealed its significant activity in certain microorganisms.

ACKNOWLEDGEMENT

Authors are thankful to the Director General, Central Council for Research in Ayurvedic Sciences, New Delhi for providing the necessary facilities to carry out the study. Authors are also thankfully acknowledged the West Bengal University of Health Sciences, Kolkata for necessary approval. The initiation and kind guidance of Late Professor P.K.Debnath is also gratefully acknowledged.

REFERENCES

1. Duraipandiyan V, Ayyanar M and Ignacimuthu S ., 2006. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India . BMC Complementary and Alternative Medicine . 6,35.
2. Mahesh B and Satish S., 2008. Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens; World Journal of Agricultural Sciences. 4 (S), 839-843.
3. Sumathi P. and Parvathi A.,2010. Antimicrobial activity of some traditional medicinal plants. J.Med.Plant.Res. 4(4),316-321.
4. http://www.im.dk/publikationer/micro98/index.htm dated 30.01.2012.
5. Del Campo J, Amiot MJ, Nguyen C ., 2000. Antimicrobial effect of Rosemary extract. J. Food Protect. 63: 1359-1368.
6. Ghosh A , Das BK, Roy A, Mondal B, Chandra G., 2007. Antimicrobial activity of some medicinal plants extracts. Nat Med (Tokyo).62(2),259-262.
7. Panda SK, Thatoi HN, Dutta SK., 2009. Antimicrobial activity and phytochemical screening of leaf and bark extracts of *Vitex negundo L.*from simlipal biosphere reserve. Orissa.J.Med Plants Res.3(4),294-300.
8. Rig Veda. Verse no. 5/78/9,AV,e/14/6
9. Glossary of Indian Medicinal Plants. 1985. R.N. Chopra, S L Nayar and I C Chopra (Eds.), Publication and information Directorate, CSIR, New Delhi, pp.95.
10. Caraka Samhita.,1984. Nag B C. (Eds.), Navapatra Prakashan, Kolkata, India, p. 40.
11. Joshi G C, Tewari L M, Lohani N, Upreti K, Jalal J S and Tewari G., 2009. Diversity of Orchids in Uttarakhand and their conservation strategy with special reference to their medicinal importance. *Report and Opinion*. 1(3), 47-52.
12. Anonymous., 1996. The Ayurvedic Pharmacopoeia of India. 2nd ed. New Delhi; Ministry of Health and Family Welfare, the controller of Publication. pp. 947-948.
13. WHO / Pharm/92.559/rev.1., 1992. Quality Control Methods for Medicinal Plant Materials. Geneva: Organisation Mondiale De La Sante.pp.9-34.

14. Johansen D A., 1940. *Plant Microtechnique*. McGraw Hill Book Co., pp. 182-203.
15. Chase C R and Prett R J.,1949. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *J. Am. Pharm. Assoc*. 38, 324-31.
16. Chatwal G R and Anand S K., 2005. Industrial Methods of Chemical Analysis. 5th ed. New Delhi: Himalaya Publishing House. 2 ,272.
17. McPolin Oona., 2009 An Introduction to HPLC for Pharmaceutical analysis. Mourne Training Services, UK. pp.83-114.
18. Petsalo Aleksanteri., 2011. Development of LC/MS Techniques for Plant and Drug metabolism studies. University of Oulu. pp. 27-36.
19. Majumdar P.L. et al. 2001; Phenanthrene derivatives from the orchid *Coelogyne cristata*, Phytochemistry, Volume 58, Issue 4, pp. 581-586.