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PHARMACOGNOSTICAL, PHYSIOCHEMICAL AND PHYTOCHEMICAL EVALUATION OF LEAF, STEM AND ROOT OF ORCHID DENDROBIUM OCHREATUM

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ABSTRACT
The present work was aimed to carry out pharmacognosical and phytochemical evaluation of individual root, stem, and leaves of orchid "*Dendrobium ochreatum.*" The plant was sun dried and was grounded to fine powder using mechanical grinder followed by sieving. The fine powder was collected and subjected to different pharmacognostical studies like fluorescence analysis under uv light at different wavelength. Physiochemical parameters were also evaluated of the dried plant parts like ash values, loss on drying. Each part of plant like root, stem and leaves were separated and subjected to extraction using soxhletion using different polarity solvents i,e hexane, chloroform, ethanol in gradient elution technique. All total nine plant extracts were obtained, phytochemical screening revealed the presence of important phytoconstituents like alkaloid, glycoside, saponins etc whereas only chloroform extracts of stem exhibited the presence of steroid/phytosterol.

INTRODUCTION

Orchids are one of the widely distributed plants which comprises of terrestrial, saprophytic and epiphytic species. 25,000-35,000 species with 800-1,000 genera has already been identified globally of this orchid out of which 1300 species and 140 genera of this orchid is only found in the lower Himalayan belt. For various bioactive profiles studying of plants and microorganism phytochemicals are key factors and phytochemicals including alkaloids, flavonoids, bibenzyl derivatives, phenanthrenes which are found to be the bioactive components in various orchids and already found to possess various biological properties like antimicrobial, antitumor, anti-inflammatory and antiviral etc.[1,2]. Pharmacognostical study involves standardization, authentication of plant"s crude drugs and generally this study is conducted in order to identify controversial species of plants, the pharmacognostical study

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includes authentication of plants through morphological, phytochemical and physicochemical analysis. Study includes parameters, which helps in identifying adulteration in dry powder form that is limited in taxonomical study, because the plant loses its morphology once it is powdered and chances of adulteration increases. Pharmacognostic studies helps to prevent plants from adulteration as it ensure plant identity and mention standardization parameters uniquely of a particular plant [3]. Here in this present study various plants like leaf, stem and root were subjected for various pharmacognostical and physiochemical studies to ensure their identity. Extractions using different solvents were done and all total nine different extracts were obtained which were further subjected to phytochemical and TLC studies.

MATERIALS & METHODS

Plant collection and authentication:

The plant were collected from Sarkar Nursery and the plant was authenticated by Professor Srinath Kumar,Assistant professor, Department of Botany, Thiru.A.Govindasamay Government Arts college, Tamil Nadu India.

Fluorescence behavior of powder

To study the fluorescence nature plant powder was treated with some chemical reagents *viz*.1N sodium hydroxide, 1N hydrochloric acid, 1N sodium hydroxide in methanol, picric acid, 1N nitric acid, acetic acid, acetone, 50% sulphuric acid, nitric acid in ammonia solution and observed under day light, long UV (365 nm) and short UV light (254 nm) [4]

Determination of various ash values [5]

Total ash

Total ash generally consists of phosphates, carbonates, silica and silicates which include both physiological ash and non-physiological ash, *e.g*, sand and soil. 2 gm of air-dried powdered drug was accurately weighed and taken in a silica crucible and incinerated at 450°C to free from carbon. Then the crucible was cooled and weighed. The percentage of total ash was evaluated with reference to the dried drug.

Water-soluble ash

Water-soluble ash is that part of the total ash portion which was soluble in water. Then total ash obtained was boiled for 5 minutes with 25 ml of water and insoluble material was collected in an ash less filter paper, incinerated at 450°C, subtracted the weight of the insoluble substance from the weight of the ash and computed the rate of water soluble ash amid reference to their dried drug.

Acid-insoluble ash

The acid insoluble ash is calculated by treating, the total ash with dilute HCl and weighing the residue. This limit indicates contamination with siliceous materials like sand sand by comparison with the total ash value for the same sample differentiation can be made between contaminating material and in the natural ash of the drug. The total ash was obtained by boiling with 25 ml of 2 N HCl for 5 min while the insoluble matter was collected in an ash less filter paper and washed with boiled water followed by ignition and cooling in dessicator and finally weighing. The proportion of acid-insoluble ash with reference to the dried drug was evaluated.

Sulphated ash

1 gm of air dried powder drug was treated with dilute sulphuric acid before ignition in a tared silica crucible to a constant weight. The ash obtained was weighed. The percentage of sulphated ash was intended with reference to the dried drug.

Loss on drying [6]

About 3gm of the powdered crude drug was accurately weighed in a tarred dish and dried in an oven at 100-105^{o}C.It was then cooled in a desiccators and weighed. The loss on drying was calculated with reference to the amount of the dried powder taken.

Loss on drying = (Weight of empty desiccators + Sample weight – Weight after drying)/ Sample weight

Determination of crude fiber content

2 gm of powdered drug extracted with diethyl ether and added 200 ml of boiling dilute sulphuric acid (1.25%) to the ether exhausted marc in a 500 ml flask. The mixture was refluxed for 30 min, filtered through filter paper and the residue was washed with boiling water until the effluent washing was acid free. The residue was rinsed in 200 ml of boiling sodium hydroxide solution (1.25%) and was further refluxed for 30 min finally it was filtered through ash less filter paper. The residue was washed with boiling water until the last washing was neutral. It was then dried at 110^{O}C to constant weight and then ignited to constant weight. The ash was cooled in dessicator, weighed and calculated as follows [7]

Percentage of Crude Fibre = weight of the ash obtained / weight of the drug sample ×100

Determination of swelling index

Swelling properties of medicinal plants shows specific therapeutic utility e.g. gums, pectin, or hemicellulose. 1gm of plant material was accurately weighed and placed into 25 ml glass stoppered measuring cylinder. 25 ml water was added and mixture was shakned thoroughly in every 10 min for 1 hr and finally allowed stand for 3 hrs at room temperature. Measured the volume in ml occupied by plant material and calculated the mean value of individual determination, related to one gm of crude plant material. [7]

Determination of Foaming index

The foam forming capability of plant material and their extract is measured in term of foaming index. 1gm of powdered root was accurately weighed and transferred in to a 500 ml conical flasks containing 100 ml water and boiled for 30 min, cooled and filtered into 100 ml volumetric flask and volume was made up with water. The decoction was poured into ten stoppered test tubes in consecutive part of 1 ml; 2 ml; etc up to ten ml and adjusted the volume of each test tube with water to 10 ml and shaken them in lengthwise motion for 15 sec. Allowed to stand for 15 min and measured the height of the foam. The results were assessed as follows:

1. If height of foam in every tube was less than 1 cm the foaming index was considered less than 100.
2. If height of the froth was higher than that of 1cm in every tube the foaming index was over than 1000. In such case repetitions was done by using a new series of dilutions of decoction in order to obtain the result.
3. If height of foam in any test tube was 1 cm, and volume of the crude plant material decoction in that tube (a) was used to determine the index.

Formula used for calculation of foaming index =a/1000

a = Volume of decoction that was used for preparing the dilution in tube where foaming height was 1cm measured [7]

Determination of bitterness value

Medicinal plant materials have a strong bitter taste and act as appetizing agents. The bitter properties of plant materials are calculated by comparing the highest bitter amount of a plant extract of the materials with that of a quinine hydrochloride dilute solution.

1. stock and diluted quinine sulphate solutions

0.1 gm quinine hydrochloride (R) was dissolved in safe drinking water to produce 100 ml. 5 ml of this solution was further diluted up to 500 ml by means of safe consumable water. This quinine hydrochloride (Sq) stock solution of contained 0.01 mg/ml. Nine serial dilutions were made each containing 0.042, 0.044,0.046, 0.048, 0.050, 0.052 0.054, 0.0.54 and 0.058 ml solution of Sqand volume made up to 10 ml with safe drinking water and obtaining a concentration of 0.1, 0.2, 0.3 up to 1 milligram/ml.

2. Plant materials stock and diluted solutions

The stock solution was prepared of the concentration of 10 mg/ml in distilled water (ST). 10 test tubes were used for serial dilution with 1 ml, 2 ml, 3 ml to 10 ml of (ST) and final volume made up with safe drinking water to 10 ml. First of all washing the mouth with safe drinking water, 10 ml of the most weaken arrangement was tasted while twirling it in the mouth fundamentally close to the base of tongue 30 sec. After 30 sec the solution was spit out and it was ascertained for 1 min whether a delayed sensation for bitterness existed. Then mouth was rinsed with water and after that highest concentration was not tasted until at least 10 min. The lowest concentration at which material continues to rouse a bitter feeling after 30 sec was referred the threshold bitter concentration. After a first series of test, rinsed the mouth systematically wash with safe consumption of water until no bitter feeling remains, wait at least 10 min before carrying out second test. [7]

Formula used for bitterness calculation = 2000 C/ A B

Where A = Concentration of stock solution (Sq) mg/ml, B = Volume of (ST) millilitre tube with bitter concentration, C = amount of quinine hydrochloride (in mg) tube with threshold bitter concentration.

Extraction of the plant materials

Leaves, stem and root was separated from plants and shadow dried for 7 days. Then these were crushed into fine powder using mill grinder. Each of leaves, stem and root powder were extracted separately with Hexane, Chloroform, and Ethanol using a Soxhlet extractor for 72 hrs. The solution of the extract was filtered through Whatman filter paper no.1and concentrated using rotary flash evaporator and stored in the refrigerator at 4^{o}C [8]

Thin layer chormatography

The plant extracts were spotted using capillary tube on TLC plates. The plates were developed using different solvent systems and then viewed under UV fluorescence light at wavelength 254 nm and 365 nm, and finally sprayed with the required detection reagent (Dragendoff, Ferrocynide, Vanillin, Liberman Burchard and Salkowski reagents) to determine the compounds present and the solvent system which gave the best observation and results is presented [8]

Phytochemical screening

Phytochemical screening of the extracts to determine different phytoconstituents were done using standard protocols. [9]

Table No- 1 Code no of different extracts

Sr. No.	Parts of *Dendrobium ochreatum*	Solvent used	Assigned code
1	Leaf	Hexane	LHDO
		Chloroform	LCDO
		Ethanol	LEDO
2	Stem	Hexane	SHDO
		Chloroform	SCDO
		Ethanol	SEDO
3	Root	Hexane	RHDO
		Chloroform	RCDO
		Ethanol	REDO

Table No- 2 Fluorescence natures of *Dendrobium ochreatum* leaf powder under ultra violet (UV) radiations at 365 nm (longer wavelength)

Sr. No	Powder of	Treatment with	Color observed	Remarks
1	Leaf	_	Light green	Untreated leaf powder
2		Powder + 1N HCl	Deep green	Glycoside absent
3		Powder + 50% HNO_3	Deep green	Phytosterol absent
4		Powder + Picric acid	Black	Alkaloids present
5		Powder + 1N NaOH in Methanol	Black	Flavanoids present
6		Powder + FeCl3	Black	Tannins present
7		Powder+ Chloroform	Black	Alkaloid present
8		Powder + 5% Iodine solution	Black	Carbohydrate present

Table No- 3 Fluorescence natures of *Dendrobium ochreatum* stem powder under ultra violet (UV) radiations at 365 nm (longer wavelength)

Sr. No	Powder of	Treatment with	Color observed	Remarks
1	Stem	_	Deep green	Untreated leaf powder
2		Powder + 1N HCl	Black	Glycoside present
3		Powder + 50% HNO_3	Black	Phytosterol present
4		Powder + Picric acid	Black	Alkaloids present
5		Powder + 1N NaOH in Methanol	Black	Flavanoids present
6		Powder + FeCl3	Black	Tannins/ phenolic compound present
7		Powder + Chloroform	Black	Alkaloid present
8		Powder + 5% Iodine solution	Black	Carbohydrate present

Table No- 4 Fluorescence natures of *Dendrobium ochreatum* root powder under ultra violet (UV) radiations at 365 nm (longer wavelength)

Sr. No	Powder of	Treatment with	Color observed	Remarks
1	Root	–	Light brown	Untreated leaf powder
2		Powder + 1N HCl	Black	Glycoside present
3		Powder + 50% HNO_3	Deep green	Phytosterol absent
4		Powder + Picric acid	Black	Alkaloids present
5		Powder + 1N NaOH in Methanol	Black	Flavanoids present
6		Powder + FeCl3	Black	Tannins/phenolic compound present
7		Powder + Chloroform	Black	Alkaloid present
8		Powder + 5% Iodine solution	Black	Carbohydrate present

Table No- 5 Fluorescence nature of *Dendrobium ochreatum* leaf powder under ultra violet (UV) radiations at 252 nm (shorter wavelength)

Sr. No	Powder of	Treatment with	Color observed	Remarks
1	Leaf	-	Light green	Untreated leaf powder
2		Powder + 1N HCl	Deep green	Glycoside absent
3		Powder + 50% HNO_3	Deep green	Phytosterol absent
4		Powder + Picric acid	Dark brown	Alkaloids present
5		Powder + 1N NaOH in Methanol	Dark brown	Flavanoids present
6		Powder + FeCl3	Dark brown	Tannins/phenolic compound present
7		Powder + Chloroform	Dark brown	Alkaloid present
8		Powder + 5% Iodine solution	Dark brown	Carbohydrate present

Table No- 6 Fluorescence nature of *Dendrobium ochreatum* stem powder under ultra violet (UV) radiations at 252 nm (shorter wavelength)

Sr. No	Powder of	Treatment with	Color observed	Remarks
1	Stem	-	Deep green	Untreated leaf powder
2		Powder + 1N HCl	Deep brown	Glycoside prsent
3		Powder + 50% HNO_3	Dark brown	Phytosterol present
4		Powder + Picric acid	Dark brown	Alkaloids present
5		Powder + 1N NaOH in Methanol	Dark brown	Flavanoids present
6		Powder + FeCl3	Dark brown	Tannins/phenolic compound present
7		Powder + Chloroform	Dark brown	Alkaloid present
8		Powder + 5% Iodine solution	Dark brown	Carbohydrate present

Table No- 7 Fluorescence nature of *Dendrobium ochreatum* root powder under ultra violet (UV) radiations at 252 nm (shorter wavelength)

Sr. No	Powder of	Treatment with	Color observed	Remarks
1	Root	-	Light brown	Untreated leaf powder
2		Powder + 1N HCl	Deep brown	Glycoside present
3		Powder + 50% HNO_3	Dark green	Phytosterol absent
4		Powder + Picric acid	Dark brown	Alkaloids present
5		Powder + 1N NaOH in Methanol	Dark brown	Flavanoids present
6		Powder + FeCl3	Dark brown	Tannins/phenolic compound present
7		Powder + Chloroform	Dark brown	Alkaloid present
8		Powder + 5% Iodine solution	Dark brown	Carbohydrate present

Physiochemical study

Table No- 8 Physiochemical analysis of different solvent extracts of *Dendrobium ochreatum*

Sr. No	Extracts	Total ash value	Acid insoluble ash	Water insoluble ash	Sulphated ash
	Values (%) w/w				
1	Leaf powder	15.2	1.2	2.4	6.3
2	Stem powder	14.8	0.9	3.1	7.2
3	Root powder	21.2	0.7	2.6	6.8

Table No-9 Crude fiber content, loss on drying, swelling index, foaming index, tanins contents, bitterness value, for root, stem and leaf powder

Sr. No	Parameter	Observation in leaf	Observation in stem	Observation in root
1	Crude fiber content	8.26 %	7.32 %	7.88 %
2	Loss on drying	12%	16.23%	8.15%
3	Swelling index	No significant result	No significant result	No significant result
4	Foaming index	No significant result	No significant result	No significant result
5	Tannins	16	22	18
6	Bitterness value	1.6 unit / gm	2.5 unit / gm	2.7 unit / gm

N.B: No Swelling and Foaming index detected.

Table No-10 Extractive values leaf, stem and root of *Dendrobium ochreatum*

Sr. No	Extracts	Type of extracts	Values (%w/w)
1	Leaf	Alcohol	35
		Water	38
2	Stem	Alcohol	46
		Water	38
3	Root	Alcohol	51
		Water	42

Table No -11 Nature and Yield values of different extracts of *Dendrobium ochreatum*

Sr. No	Plant extracts	Color	Physical appearance	Yield values (%w/w)
1	LHDO	Dark Brown	Sticky mass	2.0
2	LCDO	Greenish Brown	Semi solid	3.6
3	LEDO	Dark brown	Semi solid	2.5
4	SHDO	Blackish Brown	Sticky mass	1.0
5	SCDO	Black	Semi solid	6.5
6	SEDO	Dark Brown	Sticky mass	4.0
7	RHDO	Light Brown	Sticky mass	2.1
8	RCDO	Greenish Brown	Semi solid	3.7
9	REDO	Dark Brown	Semi solid	2.1

Table No- 12 R_f values of different extracts of *Dendrobium ochreatum* (Solvent system I-III)

Sr. No	Plant Extracts	Solvent system I		Solvent system II		Solvent system III	
		No. of spots detected	R_f Value	No. of spots detected	R_f Value	No. of spots detected	R_f Value
1	LHDO	2	0.38 0.57	2	0.10 0.40	1	0.43
2	LCDO	2	0.10 0.40	2	0.10 0.40	2	0.17 0.40
3	LEDO	3	0.18 0.34 0.10	4	0.22 0.47 0.10 0.32	3	0.14 0.42 0.10
4	SHDO	2	0.25 0.43	1	0.54	1	0.68
5	SCDO	2	0.14 0.87	2 1	0.10 0.40	3	0.24 0.56 0.10
6	SEDO	2	0.06 0.76	3	0.16 0.44 0.10	3	0.18 0.54 0.10
7	RHDO	1	0.43	2	0.10 0.40	2	0.32 0.47
8	RCDO	3	0.14 0.42 0.16	2	0.32 0.40	3	0.12 0.44 0.18
9	REDO	4	0.16 0.32 0.42 0.65	3	0.62 0.42 0.10	3	0.16 0.44 0.10

N.B:

Solvent system I:- methanol : acetic acid : chloroform (18:1:1 v/v/v). For alkaloids.

Solvent system II:- n-butanol : acetic acid: water (4:1:5 v/v/v). For flavanoids.

Solvent system III:- toluene : ethyl acetate (4:1 v/v). For terpinoids.

Table No - 13 R_f values of different extracts of *Dendrobium ochreatum* (Solvent system IV-VI)

Sr. No	Plant Extracts	Solvent system IV		Solvent system V		Solvent system VI	
		No. of spots detected	R_f Value	No. of spots detected	R_f Value	No. of spots detected	R_f Value
1	LHDO	2	0.38 0.57	2	0.48 0.62	2	0.42 0.53
2	LCDO	2	0.10 0.40	2	0.38 0.64	2	0.17 0.40
3	LEDO	2	0.16 0.44	3	0.18 0.40	2	0.16 0.44
4	SHDO	3	0.25 0.43 0.72	1	0.48	1	0.27
5	SCDO	2	0.14 0.87	2	0.38 0.64	1	0.42
6	SEDO	2	0.36 0.42	3	0.26 0.42 0.64	1	0.16
7	RHDO	1	0.76	2	0.10 0.40	2	0.44 0.61
8	RCDO	3	0.28 0.44 0.18	2	0.56 0.72	3	0.26 0.44 0.58
9	REDO	2	0.38 0.62	3	0.42 0.56 0.76	2	0.16 0.44 0.10

N.B:

Solvent system IV:- chloroform: ethyl acetate: acetic acid (50:50:1 v/v/v). For tannins and phenolic compounds.

Solvent system V:- ethyl acetate: methanol: water (100:13.5:10 v/v/v). For glycosides.

Solvent system VI:- chloroform: methanol (8:2 v/v/). For phytosterol.

Phytochemical study

Table No-14 Qualitative preliminary phytochemical analysis of different solvent extracts of *Dendrobium ochreatum*

Plant extracts	Alkaloids	Flavonoids	Glycosides	Carbohydrate	Protein/ Amino acids	Tannins/ Phenolic compounds	Steroids
LHDO	-	-	-	+	+	+	-
LCDO	+	+	-	+	-	+	-
LEDO	+	-	-	-	+	+	-
SHDO	-	+	-	-	-	+	-
SCDO	+	-	-	-	-	+	+
SEDO	+	+	+	+	+	+	-
RHDO	-	-	+	+	-	+	-
RCDO	+	+	-	-	-	+	-
REDO	+	+	-	-	+	+	-

N.B: 1.The sign indicates the following (+) Present, (-) Absent.

2**.** Steroid is only present in SCDO.

In this study, different pharmacognostical physiochemical and phytochemical parameters were evaluated for each root stem and leaf individually of *Dendrobium ochreatum*. The plants parts were dried and mechanically grinded to powder and after sieving the fine powder were analyzed under uv light at 252 nm (shorter wavelength) and 365 nm (longer wavelength) by applying different reagents. All the plant parts in maximum cases exhibited deep brown and black color in shorter and longer wavelength respectively. This study is done generally to assume the presence of different phytoconstituents in various plant parts. Pharmacognostical studies are important for crude drugs because when different parts of plant are powdered, the plant loses its morphology as it they are prone to adulterations, therefore pharmacognostical studies are helpul to identify those from its adulterations. Physiochemical studies like crude fiber content, loss on drying, swelling index, foaming index, tannins contents, bitterness value of each of leaf, stem and root of *D.ochreatum* were performed and the result signifies that the individual values are under the limits of WHO standardization of crude drugs. No swelling and foaming index were found from the results (Table No-9). Both alcoholic and aqueous extractive values were determined for individual plant parts along with different ash values. The reasons for reducing the crude drug to its ash are to remove all traces of organic matter, in order to avoid any interference during analytical determination. During incineration, crude drugs leave ash that may consists of carbonates, phosphates or silicates of sodium, potassium, calcium and magnesium. The ash value determination is useful for detecting low-grade products, exhausted drugs and excess of sandy or earthy matter and particularly applicable to powdered drugs.[3] Extraction was performed by using soxhelation for each individual plant parts (leaf, stem and root) using three solvents (hexane, chloroform and ethanol) in gradient elution techniques, all total nine extracts were obtained. TLC studies were carried out for all extracts by using six solvent systems (Table No-12,13). Phytochemical analysis was performed for each extract and result indicated that glycosides, flavonoids, carbohydrates, phenolic compounds/tannins are major phytoconstituents found generally among all extracts whereas phytosterol was found to be present only in chloroform extracts of stem (Table No-14).

CONCLUSION

The present study was focused on pharmacognostical, physicochemical and preliminary phytochemical screenings of different parts of *D. ochreatum* plant, various data obtained from the results are under limits of W.H.O. standardization of crude drugs. These studies provided useful in formations in authenticating the plant along with nature of phytoconstituents present in it. From the phytochemical study it was observed that the presence of phytosterol was only present in the chloroform extracts of stem, therefore if further isolation if carried out on this particular extract one can get the chance of obtaining a bioactive steroidal molecule

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

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