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EXTRACTION AND PHYTOCHEMICAL SCREENING OF WHOLE PLANT OF *Peristylus goodyeroides* (D. DON) LINDL. FROM SOUTHERN WHESTERN GHATS OF INDIA

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ABSTRACT

Medicinal plants make many chemical compounds for biological function. The medicinally active compounds are called secondary metabolites. Extraction is used for separating medicinal active portions of plants using selective solvents through standard procedure. Phytochemical screening refers to the extraction, screening and identification of the medicinal active substances found in plants. These bioactive principles subsequently may lead to drug discovery and drug development. They are mainly used as traditional medicine in human diseases. **Aim and Objective:** It was to optimize the extraction method and preliminary phytochemical screening for phytoconstituents exist in the selected plant. **Method:** The extraction was carried out using cold maceration process. The experimental procedures were performed for identification of primary and secondary metabolites present in hydroalcoholic extract of *Peristylus goodyeroides*. **Results and Discussion:** The % yield of extract was found as 15.4%w/w. From the phytochemical tests, the results were obtained for the presence of active secondary metabolites like steroids, triterpenoids, alkaloids, glycosides and flavanoids. **Conclusion:** The detected class of active constituents will play major place in herbal drug development after characterization and pharmacological activity studies. Even rare species of plants also contain important active principles. From this, the care must be taken to protect exploitation of the species from the particular regions.

KEYWORDS: Hydroalcoholic, Lipophillic, Secondary metabolites, *Peristylus goodyeroides* (D. Don) Lindl.

INTRODUCTION

*Peristylus goodyeroides(D.Don) Lindl. b*elong to the family Orchidacaea. It is the second largest group of flowering plant. It is distributed in humid tropics and sub tropics. *Peristylus goodyeroides* are usually robust and the herbs are 70cm tall. In Indian traditional healing medicinal orchid *Peristylus Goodyeroides (D.Don) Lindl.* based herbal formulation are used by the traditional healers of different regions.[1-3]

Fig 1: Peristylus goodyeroides (D. Don) Lindl.

Extraction is the separation of medicinally active portions of plant using selective solvent through standard procedures. During extraction, solvents diffuse into the solid plant material and solubilise compounds with similar polarity. The quality of an extract is majorly depended on the solvent which is used for extraction procedure that is followed for extraction and which part of the plant is used as starting material.

Major extraction methods are percolation, digestion, infusion, decoction and maceration. It is a dilute solution of the readily soluble components of the crude drugs. Fresh infusions are prepared by macerating the solids for a short period of time with either cold or boiling water. This is a kind of maceration in which gentle heat is applied during the maceration extraction process. It is used when moderately elevated temperature is not objectionable and the solvent efficiency of the menstrum is increased thereby. This method is used for the extraction of the

water soluble and heat stable constituents from crude drug by boiling it in water for 15 minutes, cooling, straining and passing sufficient cold water through the drug to produce the required volume. This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an appropriate amount of the specified menstrum and allowed to stand for approximately 4 h in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstrum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h. The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstrum is added as required, until the percolate measures about three-quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient menstrum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting.

In maceration (for fluid extract), whole or coarsely powdered plant-drug is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermolabile drugs. The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of Rauwolfi a root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules.[4-7]

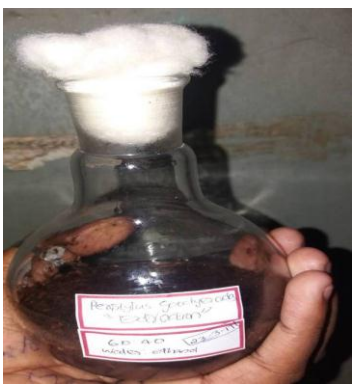

Fig 2: Extraction scheme

Fig 3: Drying of plant

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.

Detection of Alkaloids

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of Carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of Glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

General test

Test A: Dissolve 200mg of drug with sulphuric acid and then add 5%NaOH solution for neutralisation. Add fehling solution A and B to above mixture. Red colour is produced.

TEST B: Dissolve 200mg drug with sufficient amount of water. Add further water to dilute the solution. This solution is tested with fehling solution A and B. Red colour is produced from the reducing sugar present in the drug.

If the colour of test A is more intense than test B; glycosides present confirmed.

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of Saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of Phytosterols

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of Phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of Flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of Proteins and Aminoacids

Xanthoproteic Test: The extracts were treated with few drops of conc. nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin Test: To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Detection of Diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.[7-11]

Experimental Procedure

Materials

The intended plant contents are given in the table 01. The instruments, chemicals/reagents and glass wares/apparatus effective for the research are described in the table 02, table 03 and table 04 respectively.[2,11,14]

Table 1: Plant Details.

S. No.	Parameters	Subject
1.	Plant Name	Habenaria goodyeroides D.don
2.	Botanical Name	*Perystylus goodyeroides(D.Don)Lindl*
3.	Family	Orchidaceae
4.	Location	Southern western hills of India
5.	Part of the plant	Whole plant
6.	Authentication No.	BSI/SRC/5/23/2016/Tech/1915
7.	Place of Authentication	BSI, Coimbatore-641003, Tamil Nadu, India

Table 2: Instruments Used.

S. No.	Name of the Instrument	Model name
1.	Precision Balance	Wesnar
2.	Hot plate	Cintex

Table 3: Chemicals/Reagents Used.

S. No.	Name of the Reagent	Company	Location
1	Petroleum Benzine boiling range 60.0^{0}C-80.0^{0}C GR (Petroleum ether)	Merck Specialities Private Limited	Mumbai – 400 018
2	Pyridine GR		
3	Ethanol AR 99.9%	Jiangsu Huaxi International Trade Co., Ltd.	China
4	Distilled water		
5	Chloroform	Reachem Laboratory Chemicals Private Limited	Chennai – 600 098
6	Formic acid LR		
7	Acetone LR		
8	Methanol LR	S d Fine Chemicals Limited	Mumbai – 400 030
9	Ethyl acetate LR		

Table 4: Glasswares and Apparatus used.

S. No.	Name of the Glassware	Capacity	Brand Name
1	Round bottomed flask	500.0ml	Riviera
2	Funnel	Medium Size	Sh Borosilicate Glass
3	Beaker	500.0ml	Borosilicate Glass
4	Measuring cylinder	10.0ml	Riviera
5	Measuring cylinder	50.0ml	Sh Borosilicate Glass
6	China dish	Big & Small size	Chinese Porcelain
7	Stirrer	Small size	Sh Borosilicate Glass
8	Conical flask	250.0ml	Borosilicate Glass
9	Test tubes	10.0ml	Borosilicate Glass
10	Pipettes	5.0ml	Borosilicate Glass
11	Beaker	250.0ml	Borosilicate Glass
12	Petridish lid	Medium size	Borosil S - Line

Miscellaneous

Non-absorbent cotton, Filter paper, Tripod stands, Test tube holders and test tube stands and butter paper.

METHODS

Plant Collection, drying and powdering

The plant was collected from the western hills region of India. The plant were dried and taken into laboratory for data analysis.Various morphological details of orchid species were noted along with flower colour, odour, habitat, host species etc. photographs of whole plant along with various plant parts were captured. Dried *Peristylus goodyeroides* whole plant was powdered and washed with Petroleum ether. It was extracted with the help of hydroalcoholic

solvents i.e water and ethanol (60:40%v/v). The reason for selecting the solvent was due to cost efficiency and higher alcohol produce high toxicity. 6.0g of powdered plant were subjected to hydroalcoholic extraction. The mixture of extraction solvent was prepared by water and ethanol in the ratio of 60:40 and kept in round bottom flask for 7 days with frequent shaking to achieve complete extraction. The round bottom flask was plunged with non-absorbing cotton.

The extraction mixture was taken and filtered by using muslin cloth. The filtered liquid was taken in a china dish, the retained volume after extraction was about 70.0ml. It was kept on hot plate at below 40^0C for evaporation. Finally semisolid extract was obtained and calculated the yield.[12-14]

- Weight of empty china dish= 95.0g
- Weight of china dish +liquid extract=161.3g
- Weight of china dish+ final extract after evaporation= 95.77g
- Weight of final extract=0.77g
- Percentage yield=15.40%

Preliminary Phytochemicals Evaluation

The metabolic products of the plant were detected by experimental procedures. From the analysis, primary metabolite was found to be proteins and amino acids and secondary metabolites were Alkaloids, Glycosides, Flavonoids, Triterpenoids and Steroids. [7-17]

Table 5: Preliminary Phytochemicals Evaluation.

S. No	Phytochemical	Name of test	Result
1	Alkaloid	Mayer's Test	+
		Hager's Test	+
		Dragendroff's Test	+
		Wagner's Test	+
2	Carbohydrates	Molisch's Test	-
		Benedict's Test	-
		Fehling's Test	-
3	Glycosides	General Test	+
4	Saponins	Foam Test	-
		Froth Test	-
5	Steroids and Triterpinoids	Libermann Burchard's Test	+
		Salkowski's Test	+
6	Phenols	Ferric Chloride Test	-
7	Flavonoids	Lead acetate Test	+

		Alkaline Reagent Test	+
8	Proteins and Aminoacids	Ninhydrin Test	+
9	Diterpenes	Copper acetate Test	-

Note: (+): Sign which indicates presences of phytochemical

(-): Sign which indicates absences of phytochemical

Fig. 4: Test for primary metabolite.

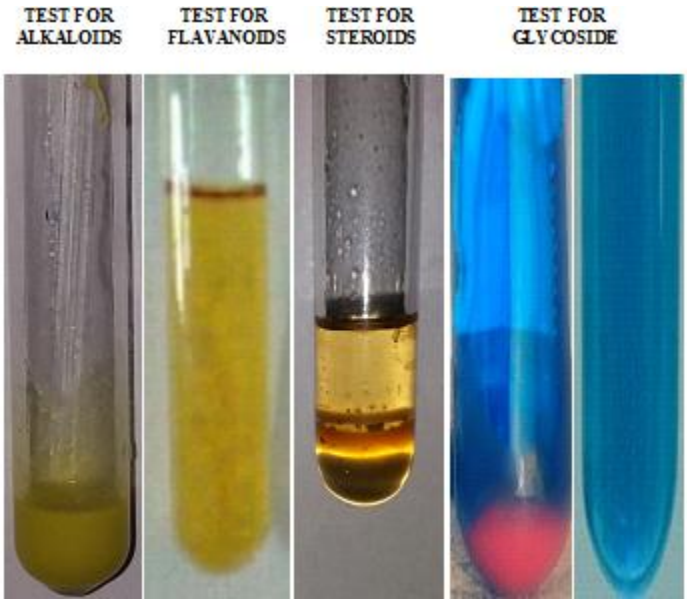

Fig. 5: Test for secondary metabolites.

CONCLUSION

The cold maceration process is an official and convenient method compared to other process and it is suitable for thermolabile substances. The biologicaly active components were detected through phytochemical screening are Alkaloids, Flavanoids, Glycosides, Steroids and Triterpenoids. The steroids may have antiallergic and gynaecological activities, Alkaloids have antituberculin and antibacterial activity, Glycosides have antioxidant activity and Flavanoids are used for skin allegies and sensitivities.

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