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Estimation of medico-potentiality of *Malaxis rheedei* Sw.- A promising orchid in Western Ghats

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Abstract: *Malaxis rheedei* Sw. (Orchidaceae) is a rare, terrestrial, medicinal orchid, which is commonly named as *Jeevakam*. The preliminary phytochemical analysis of whole plant extracts reveals the presence of flavanoids, tannins, phenols, glycosides, resins, steroids, terpenoids, cardiac glycosides and triterpenoids in different solvent system. Mostly the methanolic and ethyl acetate extract contained higher amount of secondary metabolites than the other solvent extracts.

Introduction

The drugs are derived either from the whole plant or from different plant parts, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from a excretory plant products such as gum, resins and latex (Patwardhan *et al.*, 2004). Phytochemicals are the chemicals produced by plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents includes the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. and these are directly involved in metabolic process. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from aminoacids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) these are not involved directly in metabolic pathways and they work as catalyst (Christy *et al*., 2015 and Parmar *et al.*, 2012).

The members of orchidaceae are phyto-chemically investigated, which are used traditionally for various ailments in globally, especially in hilly region because of itseasier availability (Jin-Ming *et al.*, 2003). Several orchids like *Orchis latifolia, Orchis mascula, Cymbidium aloifolium, Zeuxine strateumatica* and some species of *Dendrobium, Habenaria* (Maridass *et al.*, 2008 and Amirth, 2009), *Eulophia* (Kurapa *et al.*, 2012), *Nervilia plicata* (Renjini *et al.*, 2014) and *Geodorum densiflorum* (Theng *et al*., 2012) are used as a restorative in the treatment of various diseases. These observations shows that medicinal orchids have a promising future, because there medical activities have not investigated yet and it could be decisive in the treatment of present or future studies. (Immaculate *et al.*, 2015). Therefore knowledge on the chemical constituents of *Malaxis rheedei* Sw. is desirable for the discovery of some therapeutic agents to cure various ailments.

Materials and Methods

Plant Material

Vernacular name: *Jeevakam* and *Pachilaperumal*

Malaxis rheedei Sw., belonging to the family orchidaceae, it is an erect herb with swollen stem towards base to 15 cm long. Leaves broadly ovate or elliptic, to 12 x 6cm, with

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purple shades.Scape to 18 cm long.Bracts subulate, deflexed.Flowers orange yellow, 0.5 cm across. Sepals and petals linear, 3 mm long, tip reniform, margin pectinate. Habitat is in semi-evergreen and moist deciduous forests (Fig. 1).

Fig.1. Image of *Malaxis rheedei* (Orchidaceae)

Scientific name: ***Malaxis rheedei*** Sw.

Synonyms: *Seidenfia rheedei* (Sw.) Szlach. and *Microstylis rheedei* (Sw.) Lindl.

Preparation of plant extract

The freshly taken whole plant parts of *M. rheedei* were washed with tap water then it was shade dried and powdered coarsely. They were finely powdered by using pulverizer and passed through 40 mesh sieve and stored in airtight containers. About 250g of powdered aerial parts as well as roots were extracted in soxhlet apparatus with petroleum ether, chloroform, ethyl acetate and methanol. The extract was dried under reduced pressure at low temperature (40-50°C). The last traces of the solvent were removed under vacuum drier and the solid mass obtained was stored at 4°C for further analysis.

Quanlitative Analysis.

Alkaloids (Mayer's test)

1% HCl and 6 drops of Mayer's reagent were added to the extract. An organic yellow precipitate indicated the presence of alkaloids in the sample.

Flavonoids (Lead acetate test)

The aqueous extract was treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

Terpenoids (Salkowski test)

10mg of the extract was dissolved in 1ml of chloroform, followed by 1ml of acetic anhydride and 2ml of concentrated sulphuric acid was added to it. The formation of reddish violet colour indicates the presence of tri-terpenoides.

Cardiac glycosides (Keller-Killiani test)

0.5g of extract diluted with 5ml of water and then it isadded 2ml of glacial acetic acid containingone drop of ferric chloride solution. This wasunderlayed with 1ml of concentratedsulphuric acid. A violet ringmay appear below the brown ring, similarly greenish ring may form just above the brown ring and gradually spread throughout this layer.A brown ring at the interfaceindicates the presence of a deoxy sugar, which is the characteristic of cardenolides.

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.

Sterols (Liberman-Burchard's test)

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled followed by the addition of concentrated sulphuric acid to it. Formation of brown ring

at the junction indicates the presence of phytosterols.

Saponins (Froth Test)

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

Tannins (Lead acetate test)

In a test tube containing about 5ml of an aqueous extract, to which few drops of lead acetate was added. A yellow or red precipitate was formed. It indicates the presence of tannin.

Resins

5-10ml of acetic anhydride was added to 2ml of chloroform extract. It was dissolved by gently heating followed by cooling, then 0.5ml of sulphuric acid was added to it. Bright purple colour was produced. This indicates the presence of resins.

Glycosides

A small amount of alcohol extract samples was dissolved in 1ml water and then aqueous sodium hydroxide solution was also added to it. The formation of a yellow colour indicates the presence of glycosides.

Triterpenoids

10mg of the extract was dissolved in 1ml of chloroform then 1ml of acetic anhydride was added to it, followed by the addition of 2ml of concentrated sulphuric acid,this results in the formation of reddish violet colour, it indicates the presence of tri-terpenoid.

Reducing sugar

The crude extract of each plant was shaken with 5ml of distilled water and filtered. The filtrate was boiled with drops Fehling's solution A & B for 2 minutes. An orange red precipitate was formed. It indicates the presence of reducing sugar.

Quantitative Analysis.

Determination of Flavonoids

Total flavonoid content was estimated by the aluminium chloride colorimetric assay (Zhishen*et al.,* 1999). An aliquot (1ml) of extract and standard solution of catechin (100mg/ml) was added to 10ml volumetric flask containing 4ml of distilled water. To this 0.3ml of 5% $NaNO_3$ and 0.3ml of 10% $AlCl_3$ was added and it is followed by the addition of 2ml of 1M NaOH to it and then it was make up to 10ml with distilled water. The solution was mixed well and the absorbance was measured against reagent blank at 510nm. The value of optical density was used to calculate the total flavonoid content present in the sample.The mean of the three values were expressed as milligrams of Rutin equivalents (mg RE/g) extract on a dry weight basis.

Determination of Total phenols

Total phenolics were quantified and expressed as gallic acid equivalents according to a method proposed by Singleton *et al.* (1999). About 3.9ml of distilled water and 0.5ml of Folin-ciocalteau reagent were added to 0.1ml of extract in a tube and incubated at room temperature for 3min after which 2ml of 20 % sodium carbonate was added and kept in a boiling water bath for 1min. Phenols react with phosphomolybdic acid in the folin-ciocalteau reagent in alkaline medium and produce a blue coloured complex (molybdenum blue) that can be estimated colorimetrically at 650nm. The total phenol content of the extract was calculated and expressed as gallic acid equivalent (GAE) mg/g extract.

Result and Discussion

The present study were carried out to evalutevariuos phytochemicals of ***Malaxis rheedei*** Sw. were extracted with four solvents, viz; peteroleum ether (5.0g), chloroform (4.2g), Ethyl acetate (5.1g), methanol (10.1g) and water(10.g). The extractive values were useful to assess the chemical constituents present in

the crude extract (drug) and also helps the estimation of specific constituents, whicharesoluble in a particular solvents. The preliminary phytochemical evaluation on this potential orchid reveals the presence offlavonoid, tannin, phenol, glycoside, resin, steroids,terpanoids, cardiac glycosides andtriterpanoids. More over this potential plant also reported to cure various ailments such as stomach pain, fever, burn and snake poison by *Katunayaka* tribes of Nilmbur forests of Kerala (Table 1).

Table 1. Qualitative phytochemical screening in various extracts of *M. rheedei*

Secondary Metabolites	Petroleumether	Chloroform	Ethylacetate	Methanol	Aqueous
Alkaloid	_	_	_	_	_
Flavonoid	_	++	+	++	_
Phenol	_	+	+	+	_
Tannin	+++	+++	+++	+++	_
Glycoside	++	+	+++	+++	++
Saponin	_	_	_	_	_
Resin	+++	+++	+++	+++	_
Steroids	+++	+++	++	++	++
Terpanoids	+++	+++	+++	+++	++
Cardiac glycosides	++	+++	++	+++	+++
Triterpanoids	++	++	+++	+++	+
Reducing sugar	_	_	_	_	_

Total phenolic and flavonoid content in different solvent extracts of *M. rheedei*.

In this study, the total phenolic content was determined and estimated using Folin-ciocalteu reagent. The total phenolic content in the methanolic extract of whole plant part of *M. rheedei* was measured as high (3.14µl) while petroleum ether extract of whole plant part *M. rheedei*was measured as moderate level (2.22µl). However ethyl acetate and chloroform extract showed very least value (0.12µl & 0.09µl) (Table-2). Whole plant part of *M. rheedei* measured highest content of total flavonoids in methanol extract followed by ethyl acetate extract and chloroform extract while petroleum ether showed very low flavonoid contents (Table-3). The present results revealed that *M. rheedei*(whole plant part) is a potential source of phenolic and flavonoid contents.

Phytochemical constituents are the basic source for the establishment of several pharmaceutical products. Such phytochemical constituents play a significant role in the identification of crude drugs (Wu, 2010 & Savithramma *et al*., 2012). Phytochemical screening of crude extracts of *M. rheedei* (whole plant parts) in different solvents and water reveals the presence of some important phytochemicals like, steroids, resins, tannins, glycosides, cardiac glycosides and phenols. This also indicates the value of this potential orchid in herbal medicine.

Table 2. Total phenol contents of whole plant of *M. rheedei* with different solvent extracts

Solvents	Sample (µl)	Total Phenol Content
Petroleum ether	20	2.22
Chloroform	20	0.09
Ethyl acetate	20	0.12
Methanol	20	3.14

Table 3. Total flavanoid contents of whole plant of *M. rheedei*.with different solvent extracts

Solvents	Sample (µl)	Total Flavanoid Content
Petroleum ether	500	27.2
Chloroform	500	28.44
Ethyl acetate	500	30.53
Methanol	500	40.53

Conclusion

The phytochemical analysis on potential medicinal orchid (*M. rheedei* Sw.)revealed that, this species is has reliable source of bioactive compounds. Hence the medicinal usage of this potential medicinal plant is highly remarkable among the tribes of study area. Further investigations on phytochemical discovery and subsequent screening are needed for opening new opportunities to develop pharmaceuticals based on family orchidaceae and specifically the genus *Malaxis.*, this may also provides some clues to develop new drugs from this valuble species in nearby future to cure some ailments.

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