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

Renjini Haridas¹, Manorama S.¹ and Binu Thomas∗²

¹PG & Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore - 641 029, Tamil Nadu, India.
²PG & Research Department of Botany, St. Joseph’s College(Autonomous), Devagiri, Kozhikode - 673008, Kerala, India.

**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 phytochemically investigated, which are used traditionally for various ailments in globally, especially in hilly region because of its easier 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

*Corresponding author
E-mail: binuthomasat@rediffmail.com

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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-terpenoids.

**Cardiac glycosides (Keller-Killiani test)**

0.5g of extract diluted with 5ml of water and then it is added 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric acid. A violet ring may 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 interface indicates 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% NaNO₃ and 0.3ml of 10% AlCl₃ was added and it is followed by the addition of 2ml of 1M NaOH to it and then it was made 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 evaluate various phytochemicals of *Malaxis rheedei* Sw. were extracted with four solvents, viz; petroleuem ether (5.0g), chloroform (4.2g), Ethyl acetate (5.1g), methanol (10.1g) and water (10.1g). The extractive values were useful to assess the chemical constituents present in
the crude extract (drug) and also helps the
estimation of specific constituents,
which are soluble in a particular solvents. The
preliminary phytochemical evaluation on this
potential orchid reveals the presence of flavonoid, tannin, phenol, glycoside, resin,
steroids, terpanoids, cardiac glycosides and triterpanoids. Moreover 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

<table>
<thead>
<tr>
<th>Secondary Metabolites</th>
<th>Petroleuimether</th>
<th>Chloroform</th>
<th>Ethylacetate</th>
<th>Methanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>_</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>_</td>
</tr>
<tr>
<td>Phenol</td>
<td>_</td>
<td>+</td>
<td>_</td>
<td>++</td>
<td>_</td>
</tr>
<tr>
<td>Tannin</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>_</td>
</tr>
<tr>
<td>Glycoside</td>
<td>++</td>
<td>_</td>
<td>+++</td>
<td>+++</td>
<td>_</td>
</tr>
<tr>
<td>Saponin</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Resin</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>_</td>
</tr>
<tr>
<td>Steroids</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Terpanoids</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
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<tr>
<td>Cardiac glycosides</td>
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<td>+++</td>
<td>++</td>
<td>+++</td>
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</tr>
<tr>
<td>Triterpanoids</td>
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<td>+</td>
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<tr>
<td>Reducing sugar</td>
<td>_</td>
<td>_</td>
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<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Sample (µl)</th>
<th>Total Phenol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>20</td>
<td>2.22</td>
</tr>
<tr>
<td>Chloroform</td>
<td>20</td>
<td>0.09</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>20</td>
<td>0.12</td>
</tr>
<tr>
<td>Methanol</td>
<td>20</td>
<td>3.14</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Sample (µl)</th>
<th>Total Flavanoid Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>500</td>
<td>27.2</td>
</tr>
<tr>
<td>Chloroform</td>
<td>500</td>
<td>28.44</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>500</td>
<td>30.53</td>
</tr>
<tr>
<td>Methanol</td>
<td>500</td>
<td>40.53</td>
</tr>
</tbody>
</table>

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 valuable species in nearby future to cure some ailments.

References


