RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING OF A THERAPEUTIC PLANT MUSSAENDA LUTEOLA DELILE (RUBIACEAE)

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ABSTRACT

The contribution of angiosperms to the biodiversity is extremely important since human life totally depends on it. They are of rich in secondary metabolites, vitamins and their derivatives. Mussaenda luteola Delile, is one such angiospermic plant in the family Rubiaceae. The present study emphasis on the preliminary phytochemical analysis of the leaf extracts of M. luteola under various solvent systems viz., petroleum ether, chloroform, ethanol and aqueous. The study validates the presence of steroids, triterpenoids, quinone, alkaloids, flavonoids, phenols, coumarines, glycosides, tannins in all the four solvent systems. The leaves of M. luteola were further investigated for their total flavonoid and steroid content and this plant records decent phenol content under studies. This reveals that the leaves have potential to serve in pharmaceutical industries as significant candidature. Since the leaf part of this plant shows the occurrence of various phytochemicals it could serve as an alternate drug in pharmaceutical industries and as an ideal model for the production and development of new novel herbal drugs as well as in the field of broad spectrum of research.

Keywords: Mussaenda luteola Delile, therapeutic plant, phytochemical analysis, novel herbal drugs.

1. INTRODUCTION

Plants have been an important source of medicine for thousands of years. The use of plant source as a medicine has gained more interest in the recent years. Various countries like Egypt, China, India and Greece use terrestrial plants as medicines and it has been proved to be an excellent reservoir of novel drug lead [1]. The medicinal activities of many herbal plants have been well documented in Ayurveda, Siddha, Unani and Homeopathy systems of medicine and this provides a good base for scientific exploration of new drugs. Today’s research is focused on characterization and evaluation of various plant and plant constituents against a number of diseases based on their traditional claims given in Ayurveda [2].

Rubiaceae is the largest family of class Magnoliopsida, and it possess genus of genetic lineage with several ethnomedicinal claims (3). This family has many pharmacological potential viz., antioxidant, antibacterial, anticancer properties (4). One among such genera is Mussaenda which has been studied extensively for its diverse phytochemicals. For chemical and biological studies, very few species of this genus have been explored. The species M. luteola shows bio potential activities towards various health issues. In tropical and sub-tropical areas of the world it is grown and adapts to cold climatic conditions. In the present investigation several phytochemical profiles were analysed in the leaf part extracts of M. luteola with sequential solvent extractions with various solvent systems viz., petroleum ether, chloroform, ethanol and aqueous solutions using standard procedures.

2. MATERIALS AND METHODS

The plant materials were collected during the month of November 2019 from Droog Fort also called Bakasuramalai is a historic fort located 15 kilometres from Coonoor, The Nilgiris, Tamil Nadu.

Freshly collected leaves of M. luteola was cleaned, shade dried and powdered. A 10 gram of the powdered sample was kept steeped for 72 hours in the solvents like petroleum ether, chloroform, ethanol and distilled waterand filtered through Whatmann No. 1 filter paper. The filtrate was then collected and concentrated by evaporation and used for qualitative and quantitative phytochemical studies.

2.1. Preliminary qualitative phytochemical analysis

In the present phytochemical study, the presence of alkaloids was tested by Dragendorff’s and Wagner’s method and flavonoids by Shinoda test. Similarly the presence of steroids and triterpenoids were estimated by Salkowski and Libermann-Burchard’s test and saponins, phenols, quinone, coumarins, terpenoids, carbohydrates, glycosides, proteins, oils and fat, gums and mucilages and tannins were studied by Froth, Lead acetate, Sulphuric acid, NaOH (5), Salkowski’s,
Molisch’s Keller- Kiliani, Ninhydrin, Saponification (6,7) and FeCl₃ (8) respectively. (Table1).

2.2. Quantitative phytochemical analysis
2.2.1. Determination of total flavonoid

The flavonoid content of the leaves of the plant was determined by the gravimetric method as was described by Harborne (9). 5g of the powdered sample was placed into a conical flask and 50ml of water and 2ml HCl solution was added. The solution was allowed to boil for 30 minutes. The boiled mixture was allowed to cool before it was filtered through Whatmann filter paper (No 42). 10ml of ethyl acetate extract which contained flavonoid was recovered, while the aqueous layer was discarded. A pre-weighed Whatmann filter paper was used to filter second (ethyl-acetate layer), the residue was then placed in an oven to dry at 60°C. It was cooled in a dessicator and weighed and the quantity was determined.

\[
\% \text{ Flavonoid} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times 100
\]

Where, \( W_1 \) = Weight of empty filter paper, \( W_2 \) = Weight of paper + Flavonoid extract.

2.2.2. Determination of total phenols

The concentration of phenols in the leaves of the plants was determined using the folin- cico Caltean colorimetric method described by Pearson (10). 0.2 g of the powdered sample was added into a test tube and 10ml of methanol was added to it and shaken thoroughly the mixture was left and to stand for 15 minutes before being filtered using Whatman (No 42) filter paper.

1 ml of the extract was placed in a test tube and 1 ml Folin-Cio Caltean reagent in 5ml of distilled water was added and color was allowed to develop for about 1 to 2 hours at room temperature. The absorbance of the developed colour was measured at 760 nm wave. The process was repeated two more times and an average data taken. The phenol content was calculated thus.

\[
\% \text{ Phenol} = \frac{100 \times \text{AU} / \text{AS} \times C / 100x \text{VF} / \text{VA} \times D}{\text{Where, W= weight of sample analyzed, A}= \text{Absorbance of test sample AS= Absorbance of standard solution, C = concentration of standard in mg/ml UF= total filtrate volume VA= Volume of filtrate analyzed D = Dilution factor}}
\]

were applicable.

2.2.3. Determination of total Steroid

The steroid content of the leaves of the plants was determined using the method described by Harborne (9). 5g of the powdered sample was hydrolysed by boiling in 50 ml hydrochloric acid solution for about 30 minutes. It was filtered using Whatman filter paper (NO42), the filtrate was transferred to a separating funnel. Equal volume of ethyl acetate was added to it, mixed well and allowed separate into two layers. The ethyl acetate layer (extract) recovered, while the aqueous layer was discarded. The extract was dried at 100°C for 5 minutes in a steam bath. It was then heated with concentrated amyl alcohol to extract the steroid. The mixture becomes turbid and a reweighed Whatmann filter paper (NO 42) was used to filter the mixture properly. The dry extract was then cooled in a desiccator and reweighed. The process was repeated two mere times and an average was obtained. The concentration of steroid was determined and expressed as a percentage thus

\[
\% \text{Steroid} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times 100
\]

Where, \( W_1 \) = weight of filter paper, \( W_2 \) = weight of filter paper + steroid.

3. RESULTS.

Phytochemical constituents that are found in plants are responsible for various medicinal properties of the plant. In the present investigation, the phytochemical constituents like alkaloids, flavonoids, phenols, steroids, quinone, terpenoids, oils and fats are found abundantly in the leaf part of M. luteola and its various solvent systems were analysed. However it is observed in the findings that saponin is completely absent in all four solvent extracts. The presence of these phytochemicals in all the solvent system could predict and reveals the fact that this plant can serve as a curative drug.

Quantitative determination of total flavonoids, steroids and phenols of M. luteola leaves.

In the present investigation the leaves of M. luteola showed the presence of steroid, flavonoid and phenols in a greater extent. The total flavonoid, phenol and steroid content estimated in the leaves of M. luteola were 0.550 mg/g, 0.644 mg/g and 0.510 mg/g respectively. So surely this plant could serve as a good source in the field of medicine (Fig 1).
Table 1. Qualitative phytochemical analysis of *Mussaenda luteola* leaf extracts

<table>
<thead>
<tr>
<th>S.No</th>
<th>Tests for the presence of secondary metabolites</th>
<th>Phytochemical solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Petroleum ether</td>
</tr>
<tr>
<td>1</td>
<td>Dragendorff’s test for alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Wagner’s test for alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Shinoda test for flavonoids</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Liebermann-Burchard test for steroids and triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Froth Test for Saponins</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Lead acetate test for phenols</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>Sulphuric test for quinone</td>
<td>++</td>
</tr>
<tr>
<td>8</td>
<td>NaOH test for coumarins</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Salkowski test for terpenoids</td>
<td>++</td>
</tr>
<tr>
<td>10</td>
<td>Molich test for carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Keller-kiliani test for glycosides</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Ninhydrin test for proteins</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Saponification test for oils and fat</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>Test for gums and mucilages</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>FeCl3 Test for tannins</td>
<td>-</td>
</tr>
</tbody>
</table>

(* = absent, + = present, ++ = moderately present, +++ = strongly present)

4. DISCUSSION

Herbal plants are the greatest possession from nature for the mankind and they are mainstay of a variety of phytochemicals which are utilized for human and animal diet. It is capable of synthesizing an overwhelming variety of low molecular weight organic components called secondary metabolites, usually with unique and complex structures (11). Since 1990s the phytochemistry of *Mussaenda* species has been studied extensively. The leaves of M. luteola plant that is taken under investigation showed the presence of alkaloids, quinone, terpenoids, oils and fats. Steroids, flavonoids and phenols are found abundantly in the current analysis and the total content of those were estimated in the leaves of M. luteola. Zhao et al., [12,13], made research on M. pubescens and reported the presence of monoterpenes and triterpenoid saponins namely mussaendosides U, V, M, O, P and Q. Kim et al. [14], investigated about the presence of Mussaendoside W7 in *M. macrophylla*. Xu and Xu [15] discovered the presence of saponins from M. pubescens but in case of M. luteola it is found to be completely absent in all the four solvent systems that is taken under study. This peculiar finding in the case plant made a remarkable observation that can be taken under consideration in future analysis. This kind of secondary metabolites found in the plant under study to a greater extent, shows that the plant could definitely serve as a drug in the recent medical field.

5. CONCLUSION

Medicinal plants are believed to be an important source of novel phytoconstituents with potential therapeutic effects. In the maintenance of health in humans and other animals many plant based substances are used. In this current investigation it is certainly found that substantial...
amount of phytochemicals were found in all the solvent extracts. This peculiar finding revealed that the leaves of this plant could serve as a remarkable rationale in the scientific environment. Thus M. luteola leaves could be a magnificent drug in various pharmaceutical fields and industries. Various novel drugs can be possibly produced from this plant. On further investigation of M. luteola plant pertaining under study on its pharmacological effects it will definitely play an active role in various broad spectrum of scientific research fields such as biotechnological, pharmaceutical and nutraceutical areas.

REFERENCES


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