PRELIMINARY PHYTOCHEMICAL SCREENING OF THE VARIOUS EXTRACTS OF ROTULA AQUATICA LOUR.

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ABSTRACT

Petroleum ether, chloroform, methanol and aqueous extracts of leaf, stem and root of Rotula aquatica were screened for the presence of phytochemical by standard procedures. Presence of alkaloids was observed in aqueous extracts of leaf, stem, root and methanol extract of root. Flavonoids were observed in methanol and aqueous extracts of leaf and root and petroleum ether and chloroform extracts of stem. Phenols and saponins were present in methanol and aqueous extracts of leaf and root and also aqueous extract of stem. Tannins and terpenoids were present in methanol and aqueous extracts of leaf and root and also aqueous extract of stem. Anthraquinones were found in all the extracts. Petroleum ether and chloroform extracts of leaf, stem and root were tested positive for anthocyanin. Chloroform, methanol and aqueous extracts of leaf, stem and root showed the presence of proteins and carbohydrates. The results obtained in the present study proved the efficacy of the plant Rotula aquatica.

KEYWORDS: Boraginaceae, Rotula aquatica, Phytochemical.

INTRODUCTION

Plants are known to be the source of many chemical compounds. Medicinal plants were used by people of ancient cultures without knowledge of their active ingredients. Metabolites are compounds synthesized by plants for essential functions, such as growth and development (primary metabolites), and specific functions, such as pollinators attraction or defence against...
herbivores (secondary metabolites). Thousands of secondary metabolites have been isolated from plants, and many of them have powerful physiological effects in humans and are used as medicines. It is only since the late twentieth century that secondary metabolites have been clearly recognized as having important functions in plants. Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits and seeds. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances.

Rotula aquatica belonging to the family Boraginaceae is represented by about 100 genera and 2000 species. R. aquatica is a mandatory compound of many ayurvedic drug preparations and is an important traditional medicine for kidney and bladder stones and also used for treating cough, cardiac disorders, dysuria, blood disorders, fever, ulcers and uterine diseases. In ayurveda, R. aquatica has been reported to be used for diabetes, cardiotonic activity and antiurolithiatic activity. A large number of research works on the phytochemistry, pharmacology and several other aspects have been conducted, but there is no report on phytochemical screening and in-vitro bioactivities of R. aquatica. So the present study focuses on the preliminary screening of the phytochemicals present in various extracts of Rotula aquatica.

MATERIALS AND METHODS

Plant collection and identification

The fresh plant parts of R. aquatica Lour. were collected from Kuttiyadi (Malapuram district) in Kerala state. The collected plant material was identified and their authenticity was confirmed by comparing the voucher specimen at the herbarium of Botanical Survey of India, Southern circle Coimbatore, Tamilnadu. India. The herbarium registered number in BSI/SRC/5/23/2012-13/Tech/415. Freshly collected plant materials were cleaned to remove adhering dust and then dried under shade. The dried samples were powdered and used for further studies.

Successive solvent extraction

The air dried, powdered plant material was extracted with petroleum ether, chloroform and methanol using soxhlet apparatus. Each time before extracting with the next solvent, the material was dried in hot air oven below 40 °C. Finally, the material was macerated using hot water with occasional stirring for 24 h. The different solvent was evaporated using a rotary
vacuum-evaporator (Yamato RE300, Japan) at 50 ºC and the remaining water was removed by lyophilisation (VirTis Benchtop K, USA).

**Phytochemical tests**

**Qualitative phytochemical analysis**

The extracts were tested for the presence of bioactive compounds by using following standard methods.

**Test for alkaloids**

**Mayer’s test**

A fraction of extract was treated with Mayer’s test reagent (1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml of water) and observed for the formation of cream colored precipitate.

**Wagner’s test**

A fraction of extract was treated with Wagner’s reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and observed for the formation of reddish brown colour precipitate.

**Dragendrof’s test**

To 1.0ml of the extract 1.0ml of Dragendorff’s reagent was added. Appearance of orange precipitate indicated the presence of alkaloids.

**Test for Flavonoids**

**NaoH test**

A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow orange colour.

**H₂SO₄ test**

A fraction of the extract was treated with concentrated H₂SO₄ and observed for the formation of orange colour.

**Aluminium chloride test**

2 drops of 1% aluminium chloride was added to 1 ml of the aqueous extract. Yellow colorations indicated the presence of flavonoids.
Test for Phenols

Ferric chloride test
The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour.

Liebermann’s test
The extract was heated with sodium nitrite, added H\textsubscript{2}SO\textsubscript{4} solution diluted with water and excess of dilute NaOH was added and observed for the formation of deep red or green or blue colour.

Test for Saponins

Sodium bicarbonate test
To few ml of the extract a few drops of sodium bicarbonate was added and shaken well. Formation of honey comb like structure indicates the presence of saponins.

Test for Sterols

Liebermann-Burchard test
Extract (1ml) was treated with chloroform, acetic anhydride and drops of H\textsubscript{2}SO\textsubscript{4} was added and observed for the formation of dark pink or red colour.

Test for Terpenoids

Liebermann-Burchard test
Extract (1ml) was treated with chloroform, acetic anhydride and drops of H\textsubscript{2}SO\textsubscript{4} was added and observed for the formation of dark green colour.

Test for Tannin

Braemer’s test
Added 2 ml of water to 1 ml of extract boiled it and then filtered. Added few drops of 5% ferric chloride to the filtrate. A dark green, blue or brown color indicated the presence of tannin.

Test for Anthraquinones

Borntrager’s test
About 50 ml of the extract was heated with 10% ferric chloride solution and 1 ml of concentrated Hcl. Cooled the extract, filtered and shaken the filtrate with diethyl ether. The
ether extract was further extracted with strong ammonia. Pink or deep red colorations of aqueous layer indicates the presence of anthraquinones.

**Test for Anthocyanin**

**NaOH test**
A small amount of extract was treated with 2M NaOH and observed for the formation of blue green colour.

**Test for Quinones**
A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

**Test for Volatile oils**
2.0 ml of extract solution was shaken with 0.1 ml of dilute sodium hydroxide and a small quantity of dilute HCl. Formation of white precipitate indicated the presence of volatile oils.

**Test for Proteins**

**Millon’s test**
To 2 ml of the extract 5-6 drops of Millon’s reagent was added. Formation of red precipitate indicated the presence of proteins and amino acids.

**Biuret test**
The extract was heated in distilled water and filtered. The filtrate is treated with 2% copper sulphate solution, to this added 95% ethanol and potassium hydroxide and observed for the formation of pink ethanolic layer.

**Bradford’s test**
To 1 ml of the extract add few drops of Bradford’s reagent (Coomassie Brilliant Blue G 250) and formation of blue color product indicates the presence of proteins.

**Test for Carbohydrates**

**Molisch’s test for Carbohydrates**
Few drops of Molisch’s reagent were added to each of the portion dissolved in distilled water, this was then followed by addition of 1 ml of conc. H₂SO₄ by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled
water. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

Fehling’s test for free reducing sugar
About 0.5 g of each extract was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling’s solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

RESULTS AND DISCUSSION
The results obtained in the present investigation of the extracts viz. Petroleum ether, chloroform, methanol and aqueous extracts and the different plant parts i.e., leaf, stem and root are summarized in table 1. The preliminary phytochemical tests result indicates the presence of alkaloids, flavonoids, phenols, saponins, steroids, terpenoids, tannins, anthraquinones, anthocyanins, quinones, volatile oils, proteins and carbohydrates in different extracts of various plants parts. The presence of wide range of phytochemical constituents indicates that the plant could be used in a multitude of ways which may be beneficiary to the population. Phytochemical may protect humans from a host of diseases. Phytochemical analysis conducted on the plant extracts revealed that the presence of constituents which are known to exhibit medicinal as well as physiological activities 7.

Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds 8. Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids, tannins, tocopherols 9. Alkaloids, flavonoids, tannins and anthraquinones could participate for its clot lysis activity 10. The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation 11. Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness 12. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity 13. Anthocyanins exhibit important anti-oxidant and anti-inflammatory actions as well as chemotherapeutic effects 14. Glycosides are known to lower the blood pressure according to many reports 15. Triterpenoids are a large class of natural isoprenoids present in higher plants, which exhibit a wide range of biological activities 16.
Table 1. Preliminary phytochemical screening of the different extracts and different plant parts of *Rotula aquatica*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phytochemicals</th>
<th>LEAF</th>
<th>STEM</th>
<th>ROOT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PEE</td>
<td>ChL</td>
<td>MeH</td>
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<tr>
<td>1</td>
<td><strong>Alkaloids</strong></td>
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<tr>
<td></td>
<td>a. Mayer’s test</td>
<td>+</td>
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<td></td>
<td>b. Wagner’s test</td>
<td>+</td>
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<td></td>
<td>c. Dragendrof’s test</td>
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<td>-</td>
<td>+</td>
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<td>2</td>
<td><strong>Flavonoids</strong></td>
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<td></td>
<td>a. NaOH test</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td></td>
<td>b. H₂SO₄ test</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td></td>
<td>c. Aluminium chloride test</td>
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<td>+</td>
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<tr>
<td>3</td>
<td><strong>Phenols</strong></td>
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<tr>
<td></td>
<td>a. Ferric Chloride test</td>
<td>-</td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td>b. Libermann’s test</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>4</td>
<td><strong>Saponins</strong></td>
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<tr>
<td></td>
<td>a. Sodium bicarbonate test</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>5</td>
<td><strong>Steroids</strong></td>
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<td></td>
<td>a. Libermann-Burchard test</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>6</td>
<td><strong>Terpenoids</strong></td>
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<tr>
<td></td>
<td>a. Libermann-Burchard test</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>7</td>
<td><strong>Tannins</strong></td>
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<tr>
<td>8</td>
<td><strong>Anthraquinones</strong></td>
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</tr>
<tr>
<td></td>
<td>a. Borntrager’s test</td>
<td>+</td>
<td>+</td>
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<tr>
<td>9</td>
<td><strong>Anthocyanin</strong></td>
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<tr>
<td></td>
<td>a. NaOH Test</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
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<td><strong>Quinones</strong></td>
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<td></td>
<td>a. Hcl test</td>
<td>+</td>
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<td>+</td>
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<td>11</td>
<td><strong>Volatile oils</strong></td>
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<tr>
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<td>a. NaOH test</td>
<td>-</td>
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<td><strong>Proteins</strong></td>
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<td></td>
<td>a. Bradford’s test</td>
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<td>+</td>
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<td></td>
<td>b. Millon’s test</td>
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<td>+</td>
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<td></td>
<td>c. Biuret test</td>
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<td><strong>Carbohydrates</strong></td>
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<td></td>
<td>a. Molish’s test</td>
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<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>b. Fehling’s test</td>
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<td>+</td>
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</tbody>
</table>

Key words: + indicates presence; - indicates absence, PEE- Petroleum ether, ChL – Chloroform, MeH – Methanol, AqE – Aqueous
Steroids (anabolic steroids) have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illness and they are very important compounds especially due to their relationship with compounds such as sex hormones. Anthraquinone and its derivatives were reported to have antifungal activity. Carbohydrate is reported to have numerous roles in living things, such as the storage and transport of energy (starch, Glycogen) and structural components (cellulose in plants, chitin in animals). Additionally carbohydrates and their derivatives play major roles in the working process of the immune system, fertilization, pathogenesis, blood clotting and development. The clear separation between these three types (eugenol, geraniol, and thymol), as shown in this study using aromatic volatile oil, flavonoid, and RAPDs supports and extends the earlier botanical taxonomic work by. The results revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which confirmed the identified photochemical to be bioactive. Several studies confirmed the presence of these photochemical contribute medicinal as well as physiological properties to the plants studied in the treatment of different ailments. Therefore, extracts from these plants could be seen as a good source for useful drugs. The traditional medicine practice is recommended strongly for this plant as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activities. Also additional work is encouraged to elucidate the possible mechanism of action of this plant extracts.

CONCLUSION

Our result suggests that the preliminary screening tests may be useful in the detection of the bioactive compound present in the plant. Further studies are progress to find out the active constituents is responsible for antiurolithiatic activity.

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REFERENCES


