Exploration of preliminary phytochemical studies of leaves of
*Murraya paniculata* (L.)

M.K. Gautam and R.K. Goel*
Department of Pharmacology, Institute of Medical Sciences,
Banaras Hindu University, Varanasi, (U. P.) - India

**Abstract**

*Murraya paniculata* Linn. (*Murraya exotica* L.) belongs to the family Rutaceae, and is commonly known as orange jasmine and Honeybush. It is distributed over the greater part of India and the Andaman Islands to an altitude of 1500 m. This study measure different phytochemical constituent in the *M. paniculata* leaves extract. The extract revealed the presence of alkaloids, flavonoids, phenolic compounds, carbohydrate, proteins & amino acids and; while fixed oil, saponins and mucilage were absent. The present study provides a scientific rationale for the traditional use of *M. paniculata* leaves and phytochemical exploration could be useful in future experimental studies.

**Key-Words:** *Murraya paniculata*, *Murraya exotica*, Phytochemical analysis, Hydro-alcoholic extract

**Introduction**

*Murraya paniculata* Linn. (Synonyms: Chalcas paniculata L., Chalcas exotica L. and *Murraya exotica* L.) belongs to the family Rutaceae, and is commonly known as orange jasmine. It is distributed over the greater part of India and the Andaman Islands to an altitude of 1500 m. Native to tropical Asia from India and Srilanka to Myanmar (Burma), southern China and Taiwan, Thailand, and east words throughout the Malesian region to northeastern Australia and Caledonia. It is a small tree with a spreading crown and short, often crooked, trunk; rather corky, fragrant. Leaves alternate, imperipinnate, 10-17 cm long; leaflets usually 3-5, mostly 3-7 cm long, ovate or elliptic-lanceolate or rhomboid, glossy and darker above, gland-doted, base cuneate or rounded. The leaves are stimulant and astringent; they are reportedly used in the form of an infusion to treat diarrhoea and dysentery in the Philippines. The powder leaves are applies to cuts to promote healing; there decoction is taken internally to treat dropsy. Among the Baigas of north eastern Madhya Pradesh, the crushed leaves are made in to a paste and mixed with molasses to make tablets that are taken orally to treat joint pain; the leaves, cooked with mustard or sesame oil along with dried ginger, are applied externally to relieve inflamed joints.

* Corresponding Author
E.mail: manishpharmacology@gmail.com
Mob.: +91-9793333909

The warm leaf paste is applied externally to promote the healing of broken bones among the Paudi Bhuinya in northern Orissa. In the Gandhamardan hills of Orissa, the leaves and twigs are boiled to make a bath that is used to relieve stomach-ache in children and rheumatic pains in adults. The leaves and root bark are sometime used to treat rheumatism, coughs and hysteria. Coumarins, murralongin, isomurralonginol isovalerate, murrangatin, minumicrolin (murpanidin), coumurrayin, toddalenone, aurapten, toddasin gardenin A, gardenin C, gardenin E and umhengerin was isolated from the leaves1,2. It is reported to have anti-diabetic and antioxidant3, anti-nociceptive and anti-inflammatory4, anti-diarrhoeal5, oxytocic6 and anti-fertility7 properties.

**Material and Methods**

**Collection of Plant**
The whole plant of *Murraya paniculata* (Family - Rutaceae) was collected from Botanical Garden of National Botanical Research Institute, Lucknow, India.

**Preparation of Hydro-alcoholic Extract**
The freshly collected plant materials (4 kg) of *Murraya paniculata* were washed with distilled water and air-dried at 30 ± 20C. Then dried it in tray drier under the control conditions and powderd. The powdered plant materials (1kg) was macerated with petroleum ether to remove fatty substances, the marc was further exhaustively extracted with of 50% ethanol for 3 days (3 X 3L) and centrifugation at 10,000 rev/min. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer.
(Labconco, USA) under reduced pressure obtain 95.0 g of solid residue.

**Preliminary Phytochemical Screening**

50% ethanolic extract of *Murraya paniculata* were subjected to qualitative tests for the identification of various active constituents viz., carbohydrate, glycoside, alkaloid, amino acids, flavonoids, fixed oil, tannins, gum and mucilage, phytosterols etc. according to \(^8\)Kokate CK 1990 and \(^9\)Khandelwal KR 2000.

**Test for carbohydrates and glycosides**

A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrate and glycosides.

**Molisch’s test**

The filtrate was treated with 2-3 drops of 1% alcoholic \(\alpha\)-napthol solution and 2 ml of concentrated H2SO4 was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

**Fehling’s test**

The filtrate was treated with 1 ml of Fehling’s solution A and B and heated on the water bath. A reddish precipitate was obtained shows the presence of carbohydrate.

**Test for fixed oils and fats**

**Spot test**

Small quantity of extract was pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

**Saponification test**

Few drops of 0.5% alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on the water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

**Test for proteins and free amino acid**

Small quantity of the extract was dissolved in few ml of distilled water and treated with following reagents.

**Millon’s test**

Appearance of red color shows the presence of proteins and free amino acids.

**Ninhydrin reagent**

Appearance of purple color shows the presence of proteins and free amino acids.

**Biuret test**

Equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution were added, appearance of pink or purple color shows the presence of proteins and free amino acids.

**Test for saponins**

**Foam test**

The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

**Test for phenolic compounds**

Small quantity of the extract was taken in distilled water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

- Dilute ferric chloride solution (5% w/v) - Violet color.
- 1% solution of gelatin containing 10% sodium chloride-White precipitate.
- 10% lead acetate solution-White precipitate.

**Test for phytosterols**

Small quantity of the extract was dissolved in 5 ml of chloroform separately. Then this chloroform solution was subjected to the following tests to detect the presence of phytosteroles.

**Libermann-Burchard’s test**

The above prepared chloroform solution was treated with few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3 ml of acetic anhydride. A bluish green color appeared indicates the presence of phytosterols.

**Salkowski reaction**

To 1 ml of the above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown color produced shows the presence of phytosterols.

**Test for Alkaloids**

Small quantity of the extract was treated with few drops of concentrated hydrochloric acid and filtered. The filtrate was used for the following tests.

- Mayer’s reagent – cream precipitate
- Dragendorff’s reagent – Orange brown precipitate
- Hager’s test – yellow precipitate
- Wagner’s test – Reddish brown precipitate

**Test for flavonoids**

**With aqueous NaOH solution**

Small quantity of the extract was dissolved in aqueous sodium hydroxide and appearance of yellow colour indicates the presence of flavonoids.

**With conc. sulphuric acid**

To a small portion of extract, concentrated sulphuric acid was added. Yellow orange color was obtained shows the presence of flavonoids.

**Shinoda’s test**

Small quantity of extract was dissolved in alcohol; to those pieces of magnesium followed by concentrated hydrochloric acid was added drop by wise and heated.
Appearance of magenta color shows the presence of flavonoids.

Results and Discussion
The extractive value was calculated and was found to be 9.5 % w/w. The extract was further examined for its physical characterization like color, odor and consistency. The color of the extract was dark green, with a semi-solid consistency. Extract had characteristic odor, showed the presence of desired phytochemicals. Phytochemical screening of M. paniculata extract revealed the presence of alkaloids, flavonoids, phenolic compounds, carbohydrate, proteins & amino acids and; while fixed oil, saponins and mucilage were absent (Table 1). Phytochemicals are bioactive, non-nutrient, naturally occurring plant compounds. Purified alkaloids, as well as their synthetic derivatives, are usually employed as medicinal agents for effects such as analgesic, antimalarial, anti-aseptic and antibacterial and Phenolic constituents may be responsible for immunomodulatory activity. Saponins are produced by plants to stop bacterial and fungal attacks, which makes them natural antibiotics. Therefore, the detection of saponins in the extracts could be a contributing factor for their antimicrobial properties. The presence of tannins in the extracts might be responsible for hastening the healing of wounds and inflamed mucous membranes. In general, the presence of these phytochemicals probably accounted for the much-touted medicinal efficacy of the extracts.

References
Table 1: Preliminary phytochemical screening of the 50% ethanolic extract of *M. paniculata*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Constituents</th>
<th>Tests</th>
<th>50% Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carbohydrate</td>
<td>Molish’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fehling’s test</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Fixed oil &amp; fats</td>
<td>Spot test</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saponification test</td>
<td>−</td>
</tr>
<tr>
<td>4.</td>
<td>Proteins &amp; amino acids</td>
<td>Million’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ninhydrin test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biuret test</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Saponins</td>
<td>Foam test</td>
<td>−</td>
</tr>
<tr>
<td>6.</td>
<td>Phenolic compounds</td>
<td>FeCl3 test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gelatin test</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lead acetate test</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Phytosterol</td>
<td>Salkowiski test</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Libermann burchard test</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Alkaloids</td>
<td>Dragendroff’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayer’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagner’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hager’s test</td>
<td>−</td>
</tr>
<tr>
<td>9.</td>
<td>Gum &amp; mucilage</td>
<td>Swelling test</td>
<td>−</td>
</tr>
<tr>
<td>10.</td>
<td>Flavonoids</td>
<td>Aqueous NaOH test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conc. H$_2$SO$_4$ test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shinoda’s test</td>
<td>+</td>
</tr>
</tbody>
</table>

Where, + = Presence, − = Absence