Phytochemical screening of *Ormocarpum cochinchinense* Leaf extracts

M. Pazhanisamy¹* and G.A.I. Ebenezer²
¹Dept. of Botany, Government Arts College (Autonomous), Nandanam, Chennai-600035, TN, India
²Dept. of Botany, Madras Christian College (Autonomous), Tambaram, Chennai-600059, TN, India
m.pazhanisamy74@gmail.com*; +91 9176277127

**Abstract**

Phytochemical screening of *Ormocarpum cochinchinense* leaf extracts was carried out using different solvents. Leaf extracts were tested for the presence of bioactive compounds such as terpenoids, alkaloids, glycosides, steroids, phenols, tannins, Flavonoids and saponins by standard methods. Among different extracts analyzed, aqueous showed maximum number of phytochemicals followed by methanolic extract. Alkaloids were present in ethyl acetate, ethanol, methanol and aqueous extracts respectively. Quantification of alkaloids in the leaf sample was found to be 4.3 mg/g dry weight. Betacyanin was present in ethanol, aqueous, methanol and petroleum ether extracts. Cardiac glycosides was observed in methanol, petroleum ether and aqueous extracts whereas, Flavonoids was present only in two extracts namely methanol and aqueous. Saponins were found in methanol, ethanol, chloroform and aqueous extracts. Anthocyanins and glycosides were completely absent in all the solvent extracts.

**Keywords:** Solvents, bioactive compounds, methanol, phytochemicals, alkaloids, cardiac glycosides.

**Introduction**

Medicinal plants are the richest bio-resources in drugs of traditional medicinal systems, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals, intermediate chemical entitled for synthetic drugs. Natural remedies derived from herbs (Faodun, 2010), food or raw materials are used by pharmaceutical and food industries. It has been estimated that 14-28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered by following ethnomedicinal use of the plants (Nube et al., 2008). World Health organization estimated that 80% of the population of developing countries like India and China still relies on traditional medicines mostly plant drugs, for their primary healthcare needs (Mohanasundari et al., 2007). The medicinal value of these plants lies in the chemical substances that produce a definite physiological action in the human body. The most important of these phytochemicals are Flavonoids, alkaloids, phenolic groups, tannins etc. (Dhandapani and Sabna, 2008). India is one of the countries which possess lot of traditional knowledge in terms of medicinal plants and its effect against various diseases. *Ormocarpum cochinchinense* is one of the important and rare valuable medicinal shrubs belonging to family fabaceae. This plant was reported in Eastern Ghats by Pulliaiah and Sri Ramanurthi (2001). The roots of the plant are considered to be tonic and stimulant which is used in the treatment of lumbago and paralysis.

*Ormocarpum cochinchinense* is a shrub which is extremely efficacious in mending bone fractures, but at present its use is known only to handful of villagers in the tropical dry evergreen forest areas of Tamil Nadu for healing fractures (Maria John et al., 2011; Dinesh Kumar et al., 2013). The present study seeks the preliminary screening of various phytochemicals present in the different leaf extracts of *O. cochinchinense*.

**Materials and methods**

*Plant collection:* The disease free leaves of *Ormocarpum cochinchinense* were collected from Chengalpattu, Kancheepuram District of TN. The plant was identified using Gamble flora, herbarium specimen was prepared and deposited in the Dept. of Botany, MCC, Tambaram, Chennai. The leaves were air-dried under shade, after complete drying, the dried leaf material was ground into a coarse powder; the powder was kept in a small plastic paper bags with proper labeling and kept in an air tight container in refrigerator and used for the phytochemical analysis.

*Preparation of leaf extracts:* Preparation of leaf extracts was done according to methods of Pizzale et al. (2002) and Lu and Foo (2001). About 15 g of dried leaves from *O. cochinchinense* plant material were extracted with 150 mL of methanol (80%), ethanol (75%), ethyl acetate (Merck, extra pure), aqueous, chloroform and petroleum ether for 1 min using an ultra tura mixer (13,000 rpm) and soaked overnight at room temperature.
The sample was then filtered through Whatman No. 1 filter paper in a Buchner funnel. The filtered solution was evaporated under vacuum rotavapar at 40°C in order to get constant weight and dissolved in methanol, ethanol and water. The dissolving rate of the crude extracts was approximately 100% and the solution was stored at 4°C until use (Handa et al., 2008; Odey et al., 2012).

Phytochemical screening: The plant extracts were tested for the presence of bioactive compounds such as terpenoids, alkaloids, glycosides, steroids, phenols, tannins, flavonoids and saponins by standard methods (Yadav and Agarwala, 2011; Imron et al., 2012). Test for alkaloids: About 0.5 g of extract was dissolved in 5% HCl, filtered and tested with Dragendorff’s reagent and Mayer’s reagent separately. Any precipitate or turbidity with the reagents suggests the presence of alkaloids.

Test for anthocyanin and betacyanin: About 2 mL of leaf extract was added with 1 mL of 2 N NaOH and heated for 5 min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin and formation of yellow colour indicates the presence of betacyanin.

Test for cardiac glycosides (Keller-Killiani test): To 0.5 mL of the leaf extract, 2 mL of glacial acetic acid and few drops of 5% ferric chloride were added and 1 mL of concentrated sulphuric acid was added along the side of the test tube. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

Test for coumarins: Moistened plant extract (0.5 g) was shaken in a small test tube and covered with filter paper moistened with 1N NaOH. The test tube was kept in boiling water for few min. Then the filter paper was removed and examined in UV light for yellow fluorescence to indicate the presence of coumarins.

Test for flavonoids: Prepared plant extract (0.5 g) was shaken with petroleum ether to remove the fatty materials. The defatted residue was dissolved in 20 mL of 80% ethanol and filtered. The filtrate was used for the following test:

i. About 3 mL of filtrate was mixed with 4 mL of 1% AlCl$_3$ in MeOH in a test tube. Formation of yellow colour was observed to indicate the presence of flavonoids, flavones or chalcones.

ii. About 3 mL of filtrate was mixed with 4 mL of 1% KOH. A dark yellow colour was observed to indicate the presence of Flavonoids.

Test for glycosides: To 2 mL of the leaf extract, 3 mL of chloroform and 1 mL of 10% ammonium solution was added. Formation of pink colour indicates the presence of glycosides.

Test for phenol (Ferric chloride test): To 1 mL of the leaf extract, 2 mL of distilled water was added followed by few drops of 10% ferric chloride. Formation of blue or black colour indicates the presence of phenols.

Test for quinones: To 1 mL of the leaf extract, 1 mL of conc. sulphuric acid was added. Formation of red colour indicates the presence of quinones.

Test for saponins (Foam test): Plant extract (0.5 g) was dissolved in 2 mL of boiling water in a test tube, allowed to cool and shaken to mix thoroughly. Foam appears for 10 min indicating the presence of saponins.

Test for steroids: To 0.5 mL of leaf extract, 2 mL of chloroform and 1 mL of sulphuric acid were added. Formation of reddish brown ring at interface indicates the presence of steroids.

Test for tannins: About 0.5 g of plant leaf extract was boiled in 20 mL of distilled water in a test tube and then filtered, 1 mL of 0.1% FeCl$_3$ was added to the filtrate. Appearance of brownish green or blue black colour indicates the presence of tannins (Segelman et al., 1969).

Test for terpenoids (Salkowski test): To 0.5 mL of the leaf extract, 2 mL of chloroform was added and conc. sulphuric acid was added carefully. Formation of reddish brown colouration at the interface indicates the presence of terpenoids.

Determination of total alkaloids: Quantitative estimation of alkaloids was carried out by the standard methods (Okwu and Josiah, 2006). About 2.5 g of the fine powder was weighed and extracted using of 100 mL of 20% acetic acid in ethanol and covered to stand for 4 h, filtered and the extract was concentrated using water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed.

Results and discussion
Knowledge of phytochemical constituents of plant parts is required to understand the basis for any therapeutic effect. Generally the different solvent extracts of O. cochinchinense leaves indicated the presence or absence of different phytochemicals as shown in Table 1. Alkaloids were present in ethyl acetate, ethanol, methanol and aqueous. It plays a vital role in anti-inflammatory activity. The quantification of alkaloid was determined in the leaf sample which was found to be 4.3 mg/g dry weight. The alkaloid quantification was also analyzed in the same method as in Schotia latifolia (Mbaebie et al., 2012). Betacyanin was present in ethanol, aqueous, methanol and petroleum ether. Cardiac glycosides was observed in methanol, petroleum ether and aqueous. It may be involved in the various cardiac ailments and strengthening of heat muscles (Essielt et al., 2010). Coumarin was present in methanol, ethanol and aqueous extracts and could be used as an anticoagulant.
Flavonoids was present only in two extracts namely methanol and aqueous. Flavonoids are the potent water soluble antioxidants and free radicals scavengers, which prevent oxidative cell damage therefore, have strong anticancer activity (Salah et al., 1995; Okwu, 2004). Moreover, it greatly reduces mortality rates observed in people consuming high levels of plant based foods (Hertog et al., 1995). Phenols were almost present in all the six solvent extracts and the presence of phenolic compounds in the plant indicates that this plant may be used as an anti-microbial agent. This agreed with the findings of Ofokansi et al. (2005). Quinones were observed only in aqueous extract. Saponins were found in methanol, ethanol, chloroform and aqueous extracts. Saponins have the property of precipitating and coagulating red blood cells (Sodipo et al., 2000; Okwu, 2004). Steroids are observed in aqueous extract and it increases the nitrogen level in the body, thereby producing proteins that help in the production of muscles. It also plays a role in antibacterial activity and regulates the sex hormones. Tannins are present in methanol, ethanol and aqueous extracts. They are well known antimicrobial agents that could inhibit the growth of microorganisms and can be used against diarrhea (Trease and Evans, 2002). Presence of tannin in this plant could be useful in the treatment associated with heart, anti-inflammatory action, anticoagulant, diarrhea and dysentery (Bokhad and Rothe, 2012). Terpenoids were also present in methanol, ethanol and aqueous extracts and it is useful in treating cancer as it is an effective antioxidant (Ali et al., 2008). Anthocyanins and glycosides were completely absent in all the solvent extracts. Moreover, this study will promote a practical use of botanicals and must be continued focusing on its pharmacological validation. Further detailed exploration and collection of ethanobotanical information, chemical studies and screening for various medicinal properties will provide cost-effective and reliable source of medicine for the welfare of humanity.

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References


Table 1. Phytochemical constituents of Ormocarpum cochin chinense leaf extracts.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Aqueous</th>
<th>Ethyl acetate</th>
<th>Chloroform</th>
<th>Petroleum ether</th>
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<tbody>
<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Anthocyanin</td>
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<td>Betacyanin</td>
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<td>Cardiac glycosides</td>
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<td>Coumarins</td>
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<tr>
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</table>

Conclusion

As there was a lack in the scientific studies on O. cochin chinense, the present study fills up the lacuna and adds to it. Phytochemical screening is also used to isolate the pharmacologically active principles present in this plant. Preliminary phytochemical study provides useful information about its occurrence in the O. cochinchinense and its evaluation.


