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Evaluation of Preliminary Phytochemical Screening of *Citrus limon* (L) burm. f. Rutacea Peel Extract

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ABSTRACT

Medicinal plants are the important constituents of both traditional and conventional medicine preparations from ancient times. Majority of people prefer herbal-based medicines as compared to that of conventional medicine. Therefore, medicinal plants have become the essential part of human health care system. Moreover, medicinal plants have attained more attention because of their effectiveness, increased cost of current medicines and cultural preferences. *Citrus limon* (L) burm. f. (lemon) is known to cure several diseases and it has multiple medicinal, health and culinary advantages. In this study, phytochemical screening was carried out to identify various compounds present in the peels of *Citrus limon*.

KEY WORDS: *Citrus limon*, phytochemical analysis, Peel extract, Traditional plant.

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INTRODUCTION

Plants are used as medicinal sources for centuries as a most powerful remedy for treating many diseases because of presence of numerous bioactive constituents of immense therapeutic values. The world is now looking towards the production of new drugs for different diseases because of its undefinable medicinal properties¹. Plant based medicines are important therapeutic weapons to cure human diseases². In addition to providing the animal kingdom it's food, fuel and shelter, plants accumulate other phytochemical constituents - the secondary metabolites are produced as by-products and are sometimes not directly useful to them. These secondary metabolites give plants their medicinal value. Some of these include alkaloids, tannins, saponins, flavonoids, anthraquinones, glycosides, terpenines, essential oils, resins³. Phytochemicals are certain non-nutritive plant chemicals which have some disease preventive properties. They are not required by the human body for life sustenance, but they offer protection against pathogens⁴. Phytochemicals are already a part of our diet through vegetables and fruits. Citrus fruits are found to be rich in phytoconstituents⁵. Citrus peels, which comprise the dominant residue, exhibit potent antioxidant, antimicrobial and anti-inflammatory activities, and are considered potential sources of functional components^{6, 7, 8}. Except for ascorbic acid, citrus peels contain more bioactive compounds, such as phenolic acids, flavonoids, limonoids, and fiber than do juices^{9, 10}. Among the well-known citrus bioactive compounds, flavonoids, especially the citrus unique polymethoxy flavones and flavanone glycosides, attract considerable attention for their significant biological activities^{11, 12}. It is used mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (leaves, stem, root, juice, peel and flower) of lemon against various bacterial strains. Citrus fruits have a broad spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities due to alkaloid¹³.

Citrus limon (L) burm. f or Lemon is a small tree or shrub that reaches a height of 2 -5m with spiny shoots and alternately arranged evergreen leaves. It has irregular straggling branches and stiff twigs and long spines at the leaf axils. The leaves are green and lemon scented with slightly serrate edges, ovate lanceolate, about 3 - 5 inches long. Flowers are solitary or in small corymbs, about 2 - 4 cm in diameter, with five white petals and numerous stamens and are often very strongly scented. Fruit is usually globose to elongated about 10 cm long and 5.7 cm in diameter, with a leathery rind or peel^[14]. This fruit is widely used in the food, cosmetics and pharmaceutical industries mainly due to the essential oil present in its peel^{15, 16}. The main components of this fruit are limonene, p-cymene, terpenenol and citral, the first being the main constituent of its essential oil^{17, 18}. The aim of our study is to understand the secondary metabolites present in the *Citrus limon*(L) burm.f plant which is to

isolate and identify the structures of the active ingredients and to verify the pharmacological effects of these pure compounds.

Plant-based medicines have been used for decades especially in rural areas to prevent or even eliminate diseases worldwide and have proven to be promising in their actions. *Citrus* fruit is an important medicinal plant of the family Rutaceae. It is used mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (leaves, stem, root, juice, peel and flower) of lemon against various bacterial strains. *Citrus* fruits have a broad spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities due to alkaloid.

Taxonomy

Kingdom : Plantae
Subkingdom : Tracheobionta
Class : Magnoliopsida
Subclass : Rosidae
Order : Sapindales
Family : Rutaceae
Genus : *Citrus*
Species : *Citrus* × *Limon* (L.) Burm. f

Figure 1: The plant of *Citrus limon* (L) burm. f.



MATERIALS AND METHODS

Collection of Plant

Healthy fresh peels of *Citruslimon* are collected from my Grandmother's House, Coimbatore, Tamil Nadu. The peels are rinsed with tap water and also with distilled water. Dried at room temperature under well ventilated shade. The dried leaves are powdered and stored in air-tight container for further analysis.

Extraction of Plant material

100g of powdered peel extract is prepared in 200ml of ethanol for 48 hours by decoction method. After 48 hours, the solvent is allowed to evaporate at room temperature to obtain ethanolic extract of *Citrus limon*.

PRELIMINARY PHYTOCHEMICAL SCREENING

The phytochemical screening of the plant extract was carried out by the following methods of Trease and Evans (1978) and Harborne (1984). Ethanolic extract of *Citrus limon (L) burm. f.* was analyzed for the presence of phytochemical constituents.

PROCEDURE

i. Test for alkaloids

- a) 2mL of the extract was treated with Dragendroff's reagent and the formation of orange or red Precipitate shows the presence of alkaloids.
- b) 2mL of the extract was treated with Mayer's reagent and the formation of White precipitate or Turbidity Precipitate shows the presence of alkaloids.

ii. Test for Steroids and Sterols

a) Salkowski's Test

The extract was dissolved in 1 or 2mL of chloroform and equal volume of concentrated Sulphuric acid were added by the sides of the test tube. The upper layer turns red which reveals the presence of steroid and compounds in the extract.

iii. Test for flavonoids

- a) One mL of the extract was treated with magnesium turnings and 1-2 drops of concentrated Hydro Chloric acid.
- b) One mL of the extract was treated with 1 mL of ferric chloride. The formation of brown color confirms the presence of flavonoids.

iv. Test for Tannins and Phenolic compounds

- a) One mL of the extract was treated with few mL of 5% neutral ferric chloride. A dark blue or bluish black color product shows the presence of tannins.
- b) 1mL of the extract was treated with few mL of gelatin solution. A white precipitate reveals the presence of tannins and phenolic compounds.
- c) 1mL of the extract was treated with few mL of lead tetra acetate solution. The formation of precipitate production shows the presence of tannins and phenolic compounds.

v. Test for Carbohydrates

a) Fehling's test

The extract was treated with 5.0 mL of Fehling's solution and kept at boiling water bath. The formation of yellow or red color precipitate indicates the presence of reducing sugars.

b) Benedict's test

To 1 mL of the extract, 5 mL of Benedict's solution was added and kept at boiling waterbath. Red, yellow or green precipitate indicates the presence of reducing sugars.

vi. Test for saponins

- a) About 1 mL of alcoholic extract was diluted separately with 20mL of distilled water and shaken in a graduated cylinder for 15 minutes. A 1cm layer of foam indicated the presence of saponins.
- b) bTo 1 mL of the extract, alcoholic vanillin solution and a few drops of concentrated Sulphuric acid were added. A deep violet color confirms the presence of saponins.

vii. Test for fixed oils and fats

A small quantity of extract was processed between two-filter papers. Oil stains on the filter paper indicates the presence of fixed oil.

viii. Test for Terpenoids

a) Harizon test

To 1 mL of extract, 2 mL of trichloro acetic acid (TCA) was added and the formation of yellow to red precipitate shows the presence of terpenoids.

b) Lieberman test

To 1 mL of extract, 3 mL of acetic acid and few drops of concentrated sulphuric acid were added. Color change from red to blue indicates the presence of terpenoids.

ix. Test for Cardiac glycosides

Chloroform was added to the extract solution initially and Concentrated Sulphuric acid was added slowly through the sides of the test tube. The formation of a brown ring indicates the presence of cardiac glycosides.

RESULTS AND DISCUSSION

Preliminary Phytochemical screening of the peel extract reveals the presence of various bioactive components like alkaloids, Steroids and Sterols, Flavonoids, Fixed oil and fats. These properties are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

The results of phytochemical screening of ethanol extracts of *Citrus limon* (L) burm. f. is presented in the table 1.

Table 1: Phytochemical screening of ethanol extracts of *Citrus limon* (L) burm. f.

S.No	PHYTOCHEMICAL TESTS	RESULTS
1	Alkaloids	+++
2	Steroids and Sterols	++
3	Flavonoids	+++
4	Tannins and Phenolic compounds	–
5	Saponins	–
6	Carbohydrates	–
7	Fixed oils and fats	++
8	Terpenoids	–
9	Cardiac glycosides	–

CONCLUSION

Phytochemical analysis is intended to screen, identify, extract and isolate the phyto-constituents to evaluate the therapeutic potential of the plant and to develop phytochemical standards for the medicinal plant materials for quality control purpose. *Citrus limon* (L) burm f is extensively used in the traditional system of medicine for treatment and considered as an important medicinal plant. In the present study, most of the biologically active phytochemicals such as flavonoids, alkaloids, steroids and sterols, fixed oils and fats were found to be present in the ethanolic extract of *citrus limon* (L) burm f. plant.

Citrus limon(L) burm.f is extensively used in the traditional system of medicine for treatment of number of diseases but it is not known as an important medicinal plant. In the present study, biologically active phytochemicals such as flavonoids, alkaloids, steroids and sterols, fixed oils and fats were found to be present in the ethanolic extract of *citrus limon*.

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