



PRELIMINARY PHYTOCHEMICAL ANALYSIS OF DRY LEAF POWDER EXTRACTS OF *Citrus aurantium*

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ABSTRACT

The plant *Citrus aurantium* is native of South Eastern Asia; grow in Tamil Nadu, Karnataka, Maharashtra, Punjab and Uttar Pradesh. It is an evergreen tree with sharp thorns. *C. aurantium* is commonly known as bitter orange. Leaf, fruit and fruit juice are used to make medicine. Bitter orange has many chemicals that protect the nervous system. The present study was carried out to evaluate the phytochemicals present in the leaf powder of bitter orange. The phytochemical study showed the presence of quinones, oil & fat, starch, carbohydrates and cellulose in all the four solvent extracts. Alkaloids and flavonoids were present in three of the four solvent extracts.

KEY WORDS: *Citrus aurantium*, leaf powder, phytochemical tests.

INTRODUCTION

Citrus aurantium plant belongs to the family Rutaceae. It is a small tree with a smooth greyish brown bark and branches that spread into a fairly regular hemisphere. In this plant, the parts used are fruit, flower and leaves. The bitter orange flower and bitter orange oil are used for gastrointestinal (GI) disorders including ulcers in the intestine, constipation, diarrhea, blood in feces, drooping anus or rectum and intestinal gas. In aromatherapy, the essential oil of bitter orange is applied to the skin and also inhaled as a painkiller. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal species for the treatment of various diseases (Azaizeh *et al.*, 2003). The plant and its products such as orange fruit peel, orange juice, sour orange fruits, sour orange leaves etc are beneficial for digestion, disinfectants and elimination of nausea and vomiting. Removal of digestive system parasites, treatment of gastric irritation can also be done. The citrus plants have been widely used as antioxidants. Citrus fruits are one of the important horticultural crops, with worldwide agricultural products of over 80 million tons per year (Marin *et al.*, 2007). In fact, citrus essential oils and their major components have gained acceptance in the food industry as they have been generally recognized as safe (Fisher and Phillips, 2008). In pharmaceutical industries, they are employed as flavouring agents to mask the unpleasant taste of drugs. The health benefits of citrus leaves have mainly been attributed to the presence of bioactive compounds, such as phenolics (*e.g.* flavanone glycosides, hydroxycinnamic acid) (Ross *et al.*, 2000), vitamin C (Knekt *et al.*, 2002) and carotenoids (Craig, 1997). The phytochemicals are also known as secondary metabolites that are derived from primary metabolites and are used as drugs (Akerle Heywood and Syngé, 1991). They also contribute to the flavor, colour and other characteristics of plant parts (Iniaghe *et al.*, 2009). Phytochemical analysis is very useful in the evaluation of some active biological components of some vegetables and medicinal plants

(Oloyed, 2005). Medically, the presence of phytochemicals explains the use of the vegetable in ethno medicine for the management of various ailments (Aja *et al.*, 2010). These secondary plant metabolites are extractable by various solvents that exhibit varied biochemical and pharmacological actions in animals (Trease and Evans, 1996). There are many families of phytochemicals that help the human body in a variety of ways. Phytochemicals may protect human beings from various diseases.

MATERIALS & METHODS

In the present study, dry leaves of *Citrus aurantium* were used. A study was carried out to analyze the phytochemical constituents present in the leaf powder using different solvent extracts.

Collection of plant samples

The fresh leaves of *Citrus aurantium* were obtained from Kengarai village in Nilgiri District of Tamil Nadu, India.

Medicinal Uses

Fruits are edible. The leaves are the source of an essential oil, which is used in confectionery, cosmetics and perfumery.

- Used in the preparation of confections, marmalades, liquors and other drinks.
- Rich source of provitamin A. Bergamot oil is extracted from the peel. Leaves and tender twigs yield petitgrain oil.
- Orange leaves are used as herbal medicine for digestion, as disinfectants, aromatic, elimination of headaches, nausea & vomiting, enhance memory, fortify heart & stomach, laxative, on broiler or heaters in room for aromatization, in the production of jam, pickle, to remove digestive system parasites, treatment of gastric irritation.

Preparation of Leaf Powder

The leaves of bitter orange were collected, cleaned and air dried under shade for about three weeks. After drying, the leaves were then blended using a household electric

blender. This fine powder was analyzed for phytochemical constituents present in it.

Preliminary Phytochemical Analysis

The leaf powder was dissolved in various solvents and the preliminary phytochemical tests were carried out using Harborne (1984).

Test for Alkaloids

Mayer's Reagent

To 1ml of the extract, 2ml of Mayer's reagent was added. Appearance of dull white precipitate indicated the presence of alkaloids.

Test for Flavonoids

To 1ml of extract, 1ml of neutral ferric chloride was added. The formation of brown colour confirmed the presence of flavonoids.

Test for Steroids

Lieberman-Burchard's Test

The extracts were dissolved in 2ml of chloroform to which 10 drops of acetic acid and five drops of concentrated sulphuric acid were added and mixed. The change of red colour through blue to green indicated the presence of steroids.

Test for Terpenoids

Salkowski Test

Five ml of each extract was mixed in 2ml of chloroform and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown precipitate of the interface indicated the presence of terpenoids.

Test for Quinones

To 1ml of extract, a few drops of concentrated hydrochloric acid were added. A yellowish brown colour was observed that showed the presence of quinones

Test for Phenols

To 1ml of extract, lead acetate solution was added and the precipitate formation indicated the presence of phenolic compounds.

Test for Starch

To 1ml of extract, a few drops of iodine solution were added. Any characteristic colour change showed the presence of starch.

Test for Anthocyanin

NaOH Test

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

Test for Proteins

Ninhydrin Test (Acetone)

Ninhydrin was dissolved in acetone. The leaf extract was treated with ninhydrin and observed for the formation of purple colour.

Test for Carbohydrates

Molisch's Test

Two drops of Molisch reagent was added to an aqueous or hydrochloric acid solution of the extract and two ml of concentrated sulphuric acid was added by the side of the test tube. The formation of reddish violet ring at the junction of the liquids indicated the presence of carbohydrates.

Test for Cellulose

To 1ml of extract, a few drops of iodine solution were added followed by a few drops of sulphuric acid. Dark brown (or) red colour observed, showed the presence of cellulose.

Test for Fixed Oil and Fat

To 1ml of extract, a few drops of Sudan III solution were added. A shining orange colour obtained showed the presence of fixed oil and fat.

RESULTS & DISCUSSION

The tests conducted in *Citrus aurantium* for analysis of phytochemical constituents showed the following results.

Preliminary phytochemical analysis of dry leaf powder extracts of *C. aurantium*

In the present study, a preliminary phytochemical analysis was carried out to identify the active constituents such as alkaloids, flavonoids, sterols, terpenoids, quinones, oil & fat, phenol, starch, anthocyanin, protein, carbohydrate and cellulose present in the leaves of the medicinal plant. Preliminary phytochemical analysis of different extract (ethanol, chloroform, benzene and water) of *C. aurantium* showed the following results.

TABLE 1: Preliminary Phytochemical Analysis of *Citrus aurantium*

Test	H ₂ O	Ethanol	Chloroform	Benzene
Alkaloids	-	+	+	+
Flavonoids	+	-	+	+
Terpenoids	+	-	-	-
Quinones	+	+	+	+
Oil&fat	+	+	+	+
Steroids	+	+	-	-
Phenol	+	+	-	-
Starch	+	+	+	+
Anthocyanin	-	-	-	-
Protein	-	-	-	-
Carbohydrate	+	+	+	+
Cellulose	+	+	+	+

'+' - Present

'-' - Absent

The dried and powdered leaves of *C. aurantium* were dissolved in different solvents viz., water, ethanol, chloroform and benzene and the extracts thus obtained were analyzed for the presence or absence of secondary metabolites. In *C. aurantium*, the four different solvent

extracts showed varied results. Quinones, oil & fat, starch, carbohydrate and cellulose were present in all the four solvent extracts of the leaves of *C. aurantium*. Anthocyanin and protein were completely absent in the leaves of all the four extracts. The presence of alkaloids

was seen in ethanol, chloroform and benzene extracts, whereas, flavonoids were present in water, chloroform and benzene extracts. Terpenoids was observed only in water extract. Sterols and phenol were observed in water and ethanol extracts of the leaves of *C. aurantium* (Table-1). A phytochemical test carried out by Syed *et al.* (2013) on the leaf extracts of *Pisonea aculeata* revealed the presence of various phytoconstituents like alkaloids, carbohydrate, glycoside, steroid, protein tannins, terpenoids, flavonoids and phenol. The study carried out by Savithramma *et al.* (2011) on the preliminary phytochemical analysis of 54 Indian medicinal plants showed positive indication for alkaloids, flavonoids, steroids and cellulose. It should be noted that steroidal compounds are of importance and of interest in pharmacy due to their relationship with hormones. Steroidal structure could serve as potent starting material in the synthesis of hormones (Santhi *et al.*, 2011). Kantamreddi *et al.* (2010) has performed a qualitative preliminary phytochemical screening on 84 methanolic extracts prepared from 54 Indian plants belonging to 33 different families collected from the forest located in Eastern Ghat of India and showed the presence of alkaloids, flavonoids, steroids and terpenoids. Asquith and Butler (2012) showed that the ethanolic extract and aqueous extract of *Cnidioscolus aconitifolius* contain tannins that act as anti-diarrheal and antihemorrhagic agent. The presence of phytoconstituents goes a long way to show that the leaves of bitter orange have great potentials as suppliers of vital ingredients and could be used as starting material for drug synthesis or production. The presence of phytochemicals like flavonoids in the leaves of the medicinal plant studied indicates its medicinal action encountered in therapeutic uses.

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