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Research Paper

Comparative Phytochemical Analysis of Rutaceae Family (Citrus Species) Extracts

Engineering

KEYWORDS : Citrus aurantifolia, Citrus hystrix, Citrus maxima, Citrus reticulate, Murraya koenigii, Citrus medica, Phytochemicals, Solvent extraction.

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ABSTRACT

Citrus fruits, which belong to the family of Rutaceae, synthesize and accumulate in their cells a great variety of phytochemicals including low molecular phenolics, acetophenones, terpenoids, flavanoids, stilbenes and condensed tannins. Solvents are effectively used in extracting phytochemicals from plant samples. In the present investigation different citrus species namely, Citrus aurantifolia, Citrus hystrix, Citrus maxima, Citrus reticulata, Murraya koenigii and Citrus medica have been used and were subjected to phytochemical analysis. Solvents such as methanol, hexane and ethanol were utilized for phytochemical analysis. The present study showed that methanolic and hexane solvents had more phytonutrients being extracted when compared to ethanolic solvent. C.reticulata showed the least amount of the phytochemicals in all the solvents used. Alkaloid, Carbohydrate, Cardiac glycoside were present in all the samples for all the three solvents used for extraction in the present study.a

INTRODUCTION:

Citrus fruits, which belong to the family of Rutaceae, are one of the main fruit tree crops grown throughout the world. Although sweet orange (Citrus sinesis) is the major fruit in this group accounting for about 70% of citrus output. The group also encompasses small citrus fruits such as tangerine tree (Citrus reticulata), grape fruit (Citrus vitis), lime tree (Citrus aurantifolia) and lemon tree (Citrus limonum) (Okwu and Emenike, 2007). Citrus fruits are notable for their fragrance, partly due to Flavonoids and limonoids (which in turn are terpenes) contained in the rind, and most are juice laden. The juice contains a high quantity of citric acid giving them their characteristic sharp flavor. They are also good sources of vitamin C and Flavonoids, the Flavonoids include various flavanones and flavones. (Katrine Baghurst, 2003).

C.aurantifolia is commonly called as key lime or bitter orange and is known to exhibit bioactive activities for cold fevers, sore throats, sinusitis and bronchitis, as well as helping asthma. It can be helpful for rheumatism arthritis, obesity and cellulite and has an astringent and toning action to clear oily skin and acne, helps with herpes, cuts and insect bites (Joy et al.,).

Citrus maxima (Family-Rutaceae) are also known as pomelo (English). Its leaves are traditionally used to produce sedative effect in cases of epilepsy, cholera and convulsive coughing. The hot leaf decoction is applied on swellings and ulcers. Its leaves have antitumor activity (Anonymous, 1956; Khare, 2007; Kirtikar, 2005).

Phytonutrients are mainly natural bioactive compounds from plants with general benefits to human health (Zhao, 2007). Citrus plants synthesize and accumulate in their cells a great variety of phytochemicals including low molecular phenolic, acetophenones, terpenoids, flavanoids, stilbenes and condensed tannins (Craig, 2002).

The Flavonoids have strong inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They show antiallergic, anti-inflammatory, anti-microbial and anti-cancer activity (Okwu, 2004). Tangeritin is polymethoxyleted flavones that are found in tangerine and other citrus peels and shows potential as anticancer agent (Orlikwoski, 2001).

Limonoids possess the ability to inhibit tumor formation by stimulating the enzyme glutathione S-transferase, a detoxifying enzyme that catalyzes the reaction of glutathione to form less toxic and more importantly water-soluble compounds that can be easily excreted from the body (Hasegawa and Zhang Lamlkt, 1994).

MATERIALS AND METHODS:

Collection and processing of plant samples

The leaves of *C.aurantifolia, C.hystrix, C.maxima, C.reticulata, Murraya koengii, C.medica* were obtained from **University of Agricultural Sciences, Bangalore**. The leaves were taken in different trays and kept for drying under shade at room temperature for 3 weeks. The dried plant samples (leaves) were taken separately and ground to obtain a fine powder. The powdered samples were stored in a clean glassware container until needed for analysis.

Solvent extraction process

10gm of dry powder each was taken and dissolved in 100ml of methanol and incubated at room temperature for 48 hours. The extracts were filtered through a whatman filter paper. The extracts were concentrated using a rotary evaporator with the hot water bath set at 40°C. For extraction 10gm of dry powder is taken from each sample and dissolved in 100ml of the solvent each (Hexane, Ethanol, Methanol) and incubated at room temperature for 48 hours.

Phytochemical analysis

Qualitative analysis of Alkaloids

The samples were extracted using three different solvents like methanol, hexane and ethanol. A fraction of extract was treated with 3-5 drops of Wagner's reagent and observed for the formation of reddish brown precipitate. Wagner's Reagent [1.27gm of iodine and 2gm of potassium iodide in 100ml of water]

Qualitative analysis of Carbohydrates

Few drops of Molisch's reagent were added to 2ml portion of the extracted solvents. This was followed by addition of 2ml concentrated sulphuric acid down the side of the test tube. The mixture was then allowed to stand for 2-3 minutes. Formation of a red or dull violet color at the interphase of the two layers was a positive test.

Qualitative analysis of Cardiac glycosides

5ml of each solvent extract was treated with 2ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underplayed with 1ml of conc. sulphuric acid. A brown ring at the interphase indicated the presence of deoxy sugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

Qualitative analysis of Flavonoids

2ml of the sample extract was treated with few drops of 20% Sodium hydroxide solution. Formation of intense yellow color,

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which becomes colorless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Qualitative analysis of Phenols
A small volume of the sample extract was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black color.

Qualitative analysis of Saponins
To 2ml of the sample extract 6ml of water was added in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Qualitative analysis of Tannins
2ml of the plant extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Qualitative analysis of Terpenoids
1ml of chloroform was added to 2ml of each plant extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

Qualitative analysis of Quinones
A small amount of the sample extract was treated with Conc. HCl and observed for the formation of yellow precipitate (or coloration).

Qualitative analysis of Proteins
To 2ml of the plant extract 1ml of 40% NaOH solution and a few drops of 1% copper sulphate solution was added. A violet colour indicates the presence of peptide linkage molecules.

RESULTS
Screening of phytochemicals – Methanolic extract
The Methanolic extract of the plant samples were qualitativly screened for the presence of Phytochemicals. C.aurantifolia, C.hystrix, C.maxima, C.reticulata, Murraya koengii, showed positive for most of the phytochemicals wherein C.medica showed positive only for Cardiac glycosides and Taninn in the methanolic extract. Saponin protein was absent in all the samples except for Murraya koengii. Cardiac glycosides were present in all the samples. Phenol, Quinone was present in the three samples.

Table-1: Screening of phytochemicals from Methanolic extract.

Phytochemical	C.aurantifolia	C.hystrix	C.maxima	C.reticulata	Murraya koengii	C.medicaa
Alkaloid	+	+	+	-	+	-
Carbohydrate	+	+	+	+	+	-
Cardiac glycosides	+	+	+	+	+	+
Flavonoids	-	-	-	-	+	-
Phenol	+	+	+	-	-	-
Saponin	-	-	-	-	+	-
Tannin	-	-	+	-	+	+
Terpenoid	-	-	-	-	+	-
Quinone	+	+	+	-	-	-
Protein	-	-	-	-	+	-

Screening of phytochemicals - Hexane extract

The hexane extract of the plant samples were qualitativly screened for the presence of Phytochemicals. Alkaloid, Carbohydrate, Cardiac glycoside, Saponin was present in all the six samples, but Tannin and Protein was present only in *Murraya koengii* and *C.medicaa* respectively. Phenol was absent in all the six plant samples for the hexane solvent.

Table-2: Screening of phytochemicals from hexane extract.

Phytochemical	C.aurantifolia	C.hystrix	C.maxima	C.reticulata	Murraya koengii	C.medicaa
Alkaloid	+	+	+	+	+	+
Carbohydrate	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+
Flavonoids	+	-	+	-	-	+
Phenol	-	-	-	-	-	-
Saponin	+	+	+	-	-	-
Tannin	-	-	-	-	+	-
Terpenoid	-	-	-	+	-	+
Quinone	-	-	-	-	-	-
Protein	-	-	-	-	-	+

Screening of phytochemicals – Ethanolic extract
The ethanolic extract of the plant samples were qualitativly screened for the presence of Phytochemicals. Alkaloid, Carbohydrate, Cardiac glycoside was present in the six samples. *Murraya koengii* showed positive for most of the phytochemicals in the ethanolic extract where in *C.medicaa* was positive for Tannin and Protein was present only in *C.reticulata, Murraya koengii* and *C.medicaa.* All the other phytochemicals were absent in most of the samples.

Table-3: Screening of phytochemicals from ethanolic extract.

Phytochemical	C.aurantifolia	C.hystrix	C.maxima	C.reticulata	Murraya koengii	C.medicaa
Alkaloid	+	+	+	+	+	+
Carbohydrate	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+
Flavonoids	-	-	-	-	+	-
Phenol	-	-	-	-	-	-
Saponin	-	-	-	-	+	-
Tannin	-	-	-	-	+	+
Terpenoid	-	-	-	-	+	-
Quinone	-	-	-	-	-	-
Protein	-	-	-	+	+	+

DISCUSSION
From the above results, the phytochemical analysis of Methanolic and Hexane extracts showed more phyto-nutrients than Ethanolic extract. The variation in the expression of the phytonutrients could be due difference in the polarity of the solvents. (Devang Pandya, 2011) has reported that phytochemical analysis of Citrus maxima showed the presence of important classes of phyto-constituents like alkaloids, saponins and carbohydrates. This is similar to the results obtained in the present study. This indicates that this plant can be useful for treating different diseases because the therapeutic activity of a plant is due to the presence of particular class of compounds. Development of such a monograph would help in isolation of phytoconstituents, therapeutic investigations and standardization of formulations containing its leaf material.

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