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Review Article

COMPARATIVE PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITIES OF TRADITIONALLY USED CITRUS SPECIES: *CITRUS LIMON, CITRUS AURANTIUM, CITRUS MEDICA*

Ashok Kumar Sharma[1], Mukesh Sharma[1], Shankar Lal Soni[1], Charanjeet Singh[2], Dilip Agarwal[3], Sanwar Mal Yadav[4]

[1]Associate Professor, Arya College of Pharmacy, Kukas, Jaipur
[2]Associate Professor, Biyani Institute of Pharmaceutical Sciences, Jaipur
[3]Principal, Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur
[4]Assistant Professor, Department of Pharmacology, Mahatma Jyoti Rao Phoole University, Jaipur

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Corresponding author: Ashok Kumar Sharma
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Abstract

Plants are very rich and important source of various therapeutic compounds which are tremendiously using in pharmaceutical industry. Several studies confirmed that the presence of phytochemicals contribute medicinal as well as physiological properties to the plants. Citrus fruits, which belong to Rutaceae Family, accumulate a great variety of phytochemicals including low molecular phenolics, acetophenones, terpenoids, flavanoids, stilbenes and tannins. Citrus Limon, Citrus Aurantium, Citrus Medica possess antimicrobial, antibacterial and antioxidant properties or agents which boost up the individuals immune system. The traditional medicine practice is recommended strongly for citrus plants/ fruits and it is suggested that further work should be carried out to isolate the various active constituents responsible for therapeutic activity of such plants which belongs to Rutaceae family. In the present review, various materials and methodologies are studied and comparative analysis of phytochemicals and phytoconstituents such as alkaloids, carbohydrates, flavoinds, tannins, proteins etc. are screened of *Citrus Limon, Citrus Aurantium, Citrus medica*.

Keywords: *Citrus Limon*, Aurantium, Medica, Phytochemical, Tannins, Flavonoids

INTRODUCTION

Phytochemicals are called as non-nutritive plant chemicals which possesses some disease preventive properties. They do not required by the human body for life, but they may offer protection against pathogens or disease causing microorganisms. There are different methodology by which a phytochemical can work. It also contain antioxidant property and act by protecting cells against free radical damage, eg. polyphenols, etc. It can also stimulate certain enzymes, thereby reducing risk for breast cancer, eg. terpenes. It also possess anti-bacterial and also hormonal-stimulant component. It can also even act as binders which prevents the adhesion of pathogens to the cell walls of humans. Phytochemicals or phytoconstituents are already a part of our daily diet through vegetables and fruits. Citrus fruits are major source of phytoconstituents. Most of the Citrus fruits belongs to the Rutaceae family, and this family is one of the major fruit tree crops

grown worldwide. The group encompasses small citrus fruits such as lime tree (Citrus aurantifolia), lemon tree (Citrus limonum), tangerine tree (Citrus reticulata), grape fruit (Citrus vitis) etc. Citrus fruits are recognised for their fragrance, partially due to Flavonoids and limonoids (which in turn are terpenes) present in the rind. The juice of these fruits contains a high quantity of citric acid which provide them their characteristic sharp flavor. These are very good sources of vitamin C and Flavonoids.

Citrus aurantifolia, a medicinal plant and commonly known as key lime or bitter orange, originated from Malaysia and commonly referred to Mexican lime or West-Indian lime. It is known to exhibit bioactive activities for cold cough and fevers, sinusitis, bronchitis, and sore throats, as well as helping asthma. It can also be helpful for rheumatism arthritis, obesity and cellulite.

Citrus medica, a medicinal plant and commonly known as Citron in English and bijapura in Ayurveda, it is shrub or a small tree. This plant is originated from apparently wild in Kumaon, Sikkim, Khasia Hills, Chittagong, the Western Ghats and Satpura range in Central India. Citrus limonum (lemon) is essential oils of lemon, which exhibit strong antioxidant Activitiesand also antiproliferative activity against HeLA cell line. The peel extracts of fruit were found to possess antibacterial and antifungal properties, and also found to reduce the permeability of blood vessels and in remedies for phlebitis. They can even prevent the development of abnormal growths on the skin and decreased the occurrence of squamous cell skin melanoma.

MATERIALS AND METHODOLOGY

Collection and processing of plant samples

The leaves and peel of fruits of *C. aurantifolia*, and *C. medica* were obtained. The leaves were taken in different petri dishes and kept for drying under shade at room temperature for 3-4 weeks. The peel/pulp of C. lemon were separated by cutting them into small pieces and then it was dried in shade for a period of 10-12 days, at room temperature. The dried plant samples were taken separately and triturated to obtain a fine powder. The powdered samples were stored in a clean air tight glassware container until needed for analysis.

Solvent extraction process

10gm of dry powder of each was taken and dissolved in 100ml of methanol and water separately and incubated at room temperature for 48 hours to achieve organic and aqueous extracts respectively. The extracts were filtered through whatmann filter paper. Both 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 (Methanol) and water followed by incubation at room temperature for 48 hours.

Phytochemical Analysis of Phyto-constituents

Qualitative analysis of Alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids. Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Qualitative analysis of Carbohydrates

Few drops of Molisch's reagent were added to 2ml portion of the extracted solvents. This was then followed by addition of 2ml concentrated sulphuric acid down from the sides of the test tube. Allow the mixture to stand for 2-3

minutes. Formation of a red or dull violet colour at the interphase of the two layers was considered to be positive test i.e. indicates the presence of carbohydrates. Qualitative analysis of Cardiac glycosides 5ml of each solvent extract was treated with 2ml of glacial acetic acid in a test tube and single drop of ferric chloride solution was added into it. Then carefully addition with 1ml of conc. sulphuric acid is done. Appearance of brown ring at interphase of two layers indicating the presence of deoxy sugar.

Qualitative analysis of Flavonoids

Alkaline reagent test: 2-3 drops of sodium hydroxide (NaOH) were added to 2 mL of drug extract. Initially, a intense deep yellow colour appeared but it gradually became colourless on addition few drops of dilute HCL, which indicates that flavonoids were present in extract.

Shinod's test: 2 ml of drug extract was treated with 10 drops of dilute HCL followed by addition of a piece of magnesium, this results in appearance of deep pink colour indicating the presence of flavonoids.

Qualitative analysis of Phenols

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

Qualitative analysis of Saponins

A few drops of Na2CO3 solution was added to 5 mL of drug extract in a test tube. Shake the mixture vigorouly, and keep it for rest for five minutes. Formation of foam confirms the presence of saponins.

Qualitative analysis of Tannins

2ml of the drug extract in test tube was reacted with 10% alcoholic FeCl2 solution and on vigorously shaking, formation of blue/greenish colour solution shows the presence of tannins.

Qualitative analysis of Terpenoids

2 ml of plant extract was dissolved in 2ml of chloroform and heated till evaporated to dryness. After this, 2ml of concentrated H2SO4 was added in test tube and again heated for about 2 minutes. A grayish/ reddish brown colour precipitate produced indicated the presence of terpenoids.

Qualitative analysis of Proteins

Biuret test: 2-3 drops of 3% copper sulphate (CuSO4) and a few drops of 10% NaOH solution were added to 1 mL of plant drug extract. A violet/red colour formation indicating the presence of proteins. Ninhydrin test: 2 drops of 0.2% freshly prepared Ninhydrin Reagent added to 1 mL of drug extract. Formation of purple colour shows the presence of proteins.

RESULT AND DISCUSSION

The organic extract of the plant samples were qualitatively screened for the presence of various Phytochemicals and constituents. C. aurantifolia, showed positive for many of the phytochemicals whereas C. medica showed positive result only for Cardiac glycosides and Taninn in the drug extract.

Table 1: Screening of phytochemicals

Phytochemical	C.aurantifolia	C.limon	C.medicaa
Alkaloid	+	+	+
Carbohydrate	+	+	+
Cardiac glycosides	+	+	+
Flavonoids	-	+	-
Phenol	-	+	-
Saponin	-	-	-

Tannin	-	+	+
Terpenoid	-		+
Protein	-	+	+

+ indicates: strong presence - indicates: strong absence blank indicates: no result shown

Table 2: Pharmacological activities of citrus plants

Species	Active Ingredients	Pharmacological Property
Citrus **Aurantifolia**	**Leaf:** essential oil **Peel:** essential oil sesquiterpene hydrocarbons, monoterpene hydrocarbons (sabinene, -pinene, limonene), monoterpene alcohols (linalool, terpinen-4-ol, -terpineol), -terpinene, esters, monoterpene aldehydes, aliphatic aldehydes, **Pulp:** sucrose, protein components Flavonoids and saponines	**Leaf:** antibacterial **Fruit:** antimicrobial **Pulp:** immunomodulatory effect Fruit pickle: antitoxic, appetizer, and stomachic.
Citrus medica	**Peel:** essential oil (monoterpene hydrocarbons [limonene], 5–6% citral + dipentene) **Pulp:** flavonoid glycosides (hesperidine), Vitamin **Leaf and Seed:** purine alkaloids	**Leaf:** Estrogenic, Anthelmintic **Peel:** aromatic and tonic, Anticholinesterase **Fruit: Analgesic, anticancer,** Insulin secretagogue, Antiulcer
Citrus limon	**Flower:** purine alkaloids (caffeine, theobromine, theophylline, paraxanthine) **Peel:** essential oil (2.5% maximum limonene, canfen, citral, citronelal, felandren, pinene, terpinol], coumarins citroflavonoids (neohesperidosides, rutinosides), Vitamin C, carotenoids, mucilage, calcium oxalate **Pulp:** pectin, sugars, organic acids (ascorbic, citric, malic), citroflavonoids	**Fruit:** analgesic, antianaemic, antiemetic, antiesclerotic, antipyretic, antiseptic, demulcent, moisturizing, remineraliser and Antitoxic **Pulp:** antidiarrhoeic, diuretic, intestinal mucosa protector, local haemostatic, vascular stimulant and protectant, vitaminic **Peel:** antiseptic, carminative, diuretic, eupeptic, vascular stimulant and protector, vitaminic

CONCLUSIONS

From the above results, the phytochemical analysis of C. aurantifolia, C.limon, C.medicaa shows the presence or absence of following chemicals. The variation in the expression of the phytonutrients could occur due to the variation in the polarity of the solvents. Several studies confirmed that the presence of such phytochemicals contribute medicinal as well as physiological properties to the plants. Therefore, extracts from these plants could be used as a good source for many useful drugs. The traditional medicine practice is recommended strongly for citus plants and it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of such plants which belongs to Rutaceae family.

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