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Phytochemical screening of *Citrus aurantifolia* leaf extract

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Abstract

The phytochemical constituents of Citrus aurantifolia confer various health advantages, such as bolstering the immune system, facilitating collagen synthesis, augmenting iron absorption, mitigating inflammation (vitamin C), delivering anti-inflammatory and antioxidant properties, and exhibiting antiviral effects. Additionally, various phytochemicals such as limonoids, essential oils, alkaloids, coumarins, terpenes, and phenolics exhibit distinct health benefits. Citrus aurantifolia possesses antioxidant and anti-inflammatory properties that may mitigate illnesses such as arthritis. The essential oils derived from the fruit can suppress pathogenic microorganisms, but limonoids and coumarins exhibit anticancer properties. Extracts of Citrus aurantifolia may potentially be utilized to alleviate stress and regulate diabetes. They additionally serve as an analgesic, anti-cholinesterase agent, immunological modulator, anti-diarrheal, antiulcer, anthelmintic, and, unexpectedly, a contraceptive strategy for certain women. Citrus aurantifolia possesses inherent antibacterial capabilities that effectively combat foodborne infections, maintaining freshness and flavour while diminishing need on synthetic preservatives.

Key words : Citrus aurantifolia, Phytochemical, Essential oil, limonoids, Coumarins, Terpenes, Pharmaceuticals, Toxicology.

Herbal treatments are increasingly favoured for their effectiveness, few side effects, and affordability[3]. Plants are progressively utilized as components in the production of pharmaceuticals and food due to their rich content of secondary metabolites.[7] Phytochemicals are non-nutritive chemical constituents of plants that possess bioactive properties, including the prevention of disease pathogen growth and antioxidant capabilities that

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safeguard cells from oxidative stress and free radical damage.[8] Moreover, phytochemicals include flavonoids, phenolic compounds, and glycosides are mostly accountable for the efficacy of medicinal plants in practical domains such as herbal medicine and primary healthcare. *Citrus aurantifolia* (lime) is a little fruit belonging to the Citrus family, available in Sour limes contain higher levels of sugar and citric acid compared to lemons and exhibit an acidic and tangy flavour.[1] The nutritional profile encompasses a comprehensive range of nutrients, including carbohydrates, sugars, soluble and insoluble fibres, salt, vitamins, minerals, fatty acids, amino acids, and additional components. Limes possess distinctive flavonoid chemicals that exhibit antioxidant and anti-cancer activities. These flavonoids have demonstrated the ability to inhibit cell division in numerous cancer cell lines and are particularly noteworthy for their antibacterial properties.[6] *C. aurantifolia* demonstrates bioactive properties for the treatment of colds, fevers, sore throats, sinusitis, bronchitis, and asthma.[4]

Plant Description :

C. aurantifolia is a little shrubby tree, approximately 5 meters in height. It is a perennial, fruit-bearing tree characterized by thick, uneven branching with short, rigid spines (thorns). The leaves are arranged alternately; they are elliptical to oblong-ovate in shape, measuring 4-8 cm by 2-5 cm, and possess a crenulate margin. The flowers measure 1 inch in diameter and have a yellowish-white hue with a subtle purple tinge along the edges. The fruits are globose to ovoid berries measuring between 3 to 6 cm in diameter and occasionally possess an apical papilla. It is yellow upon ripeness but is typically harvested green for commercial purposes. Fruits and flowers are present year-round, but are most plentiful from May to September in the Northern Hemisphere. The fruit peels are exceedingly thin, featuring highly glandular segments containing yellow-green pulp vesicles. The fruit juice is acidic and aromatic, sour like lemon juice but more fragrant. It is typically praised for its distinctive flavour in comparison to other limes. The seeds are little, robust, ovoid, pallid, and sleek, featuring a white embryo[2,5].

Taxonomy

- **Domain**: Eukaryota
- **Kingdom**: Plantae
- **Phylum**: Spermatophyta
- **Subphylum**: Angiospermae
- **Class**: Dicotyledonae
- **Order**: Sapindales
- **Family**: Rutaceae
- **Genus**: Citrus
- **Species**: Citrus aurantifolia (Christm.) Swingle

Medicinal uses :

Citrus aurantifolia, commonly referred to as limes, possesses numerous therapeutic properties, Therapeutic applications including anti-inflammatory, antibacterial, antiulcer, anti-diarrheal. and antioxidant effects.

Fresh leaves of citrus aurantifolia were collected from the farms. Every reagent was bought from Molychem in Mumbai, India. Analytical grade compounds were all that were used.

***Citrus aurantifolia* tree**

***Citrus aurantifolia* leaf**

***Citrus aurantifolia* fruit**

Leaf extract preparation :

Citrus aurantifolia leaf were gathered and rinsed with water. After that, the leaves dried to get rid of any moisture left over. In a 250 mL glass beaker containing 100 mL of sterile distilled water, 50 g of cleaned, dried, and finely chopped *Citrus aurantifolia* leaves were added to prepare the extract. The combination was subsequently cooked for 60 minutes, or until the aqueous solution transitioned from a clear to a light-yellow colour. The extract was filtered using filter paper subsequent to cooling to room temperature.

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Phytochemical test :

Phytochemical test for the extract of leaves, were carried out. The extract was tested for alkaloids, flavonoids, carbohydrates, reducing sugar, steroids, tannins, phenolic compounds, amino acids etc.

Test for alkaloids :

Mayer's test: Sample (2-3ml) was treated with few drops of Mayer's reagent. Appearance of white precipitate obtained.

Test for flavonoids :

Alkaline test: Neutral $Fecl_3$ is added to the extract, a black precipitate is obtained.

Test for amino acids :

Ninhydrin test: Test sample (3ml) and 3 drops of 5% ninhydrin solution were heated in boiling water for 10 minutes. Purple colour appeared.

Test for steroids :

To the test sample add $CHCl_3$ and con. H_2SO_4, the solution changes from purple to blue or green in colour.

Test for Terpenoids :

To the test sample add 5 ml of $CHCl_3$ and 3 ml of con.H_2SO_4, a reddish-brown precipitate obtained.

Test for Phenol :

The sample solution was treated with lead acetate solution to get a precipitate.

Test for Saponins :

Foam test: To 1 ml of the extract 5 ml distilled water was added and shaken vigorously. A foamy lather obtained.

Tests for glycosides (Br_2 water test) :

On adding Br_2 water to the extract a pale-yellow colour appeared.

Anthocyanin (NaOH test) :

To the test sample add 2 ml of NaOH solution, blue green colour appeared.

Test for Tannins :

Add 5% $FeCl_3$, a black precipitate obtained.

Test for Reducing sugar :

Molisch's reagent is added to the extract, purple colour obtained.

Test for Xanthoproteins :

To the extract add con. HNO_3 and NH_3, reddish orange colour obtained.

Quantitative Analysis of Basic Radicals :

Test for Lead: To the leaf extract add KI, a yellow precipitate obtained.

Test for Bismuth: To the leaf extract add NH_4OH to excess, white or pale blue precipitate appears and dissolves to a deep blue solution.

Test for Copper: Cupron reagent and NH_4OH were added to the leaf extract, green colour appears.

Test for Zinc: Add potassium ferrocyanide to the leaf extract, white precipitate appears.

Test for Cadmium: To the leaf extract add dil. HCL, water and H_2S gas is passed. Yellow precipitate obtained.

Test for Iron : Add Potassium ferrocyanide to the leaf extract, Prussian blue colour appears.

Teat for Cobalt: To the leaf extract add potassium thiocyanate, blue colour appears.

Test for Aluminium: To the leaf extract add dil. HCL, aluminon reagent and ammonium carbonate is added. A bright red precipitate is obtained.

Test for Manganese: Conc. HNO_3, sodium bismuthate and water were added to the leaf extract a pink colour appears.

Test for Nickel: To the leaf extract add dimethyl glyoxime and NH_4OH, a scarlet red precipitate obtained.

Test for Barium : Acetic acid and sodium rhodizonate were added to the leaf extract, a brown spot is obtained.

Test for Calcium : To the leaf extract add NH_4OH and ammonium oxalate, a white precipitate is obtained.

Test for Strontium : To the leaf extract, add NH_4OH and sodium rhodizonate, a brown spot obtained.

Test for Magnesium : Magneson reagent and NaOH were added to the leaf extract, blue precipitate is obtained.

Test for Ammonium : To the test solution add NaOH and Nessler's reagent, reddish brown precipitate is obtained.

Phytochemical analysis :

The aqueous extract of *Citrus aurantifolia* were used to investigate the preliminary phytochemical screening. The table shows the results of preliminary phytochemical activity of citrus aurantifolia extract. The aqueous extract contains only few biologically active compounds like, alkaloids, flavonoids, phenol, terpenoids, steroids.

Table-1. (Phytochemical screening of *Citrus aurantifolia* leaf extract)

Phyto-chemicals	**Citrus aurantifolia leaf aqueous extract**
Anthocyanin	Absent
Glycosides	Absent
Terpenoids	Reddish brown precipitate
Phenols	Precipitate obtained
Tannins	Absent
Steroids	Green colour
Reducing sugars	Absent
Alkaloids	White precipitate
Phenolic compounds	Absent
Saponins	Absent
Flavonoids	black precipitate
Xanthoproteins	Absent

Basic Radicals :

The basic radicals present in citrus aurantifolia leaf extract are typically identified through quantitative chemical analysis based on its phytochemical composition, the following basic radicals may be present.

Table-2. (Basic radical analysis of *Citrus aurantifolia* leaf extract)

Basic radicals	Citrus aurantifolia leaf aqueous extract
Lead	Negative
Bismuth	Negative
Copper	Positive
Zinc	Positive
Cadmium	Negative
Cobalt	Negative
Aluminium	Negative
Manganese	Positive
Nickel	Negative
Barium	Negative
Calcium	Positive
Strontium	Negative
Magnesium	Positive
Ammonium	Negative
Iron	Positive

The leaves of *Citrus aurantifolia* contain many phytochemical constituents, including terpenoids, phenols, alkaloids, and flavonoids. These data suggest that *Citrus aurantifolia* leaves are a cost-effective dietary resource with potential therapeutic applications.

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