Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **44** Issue **04 Year 2023** Page **788:793***

Analysis of Nutritional Components, Antioxidant Activity and Antimicrobial Activity of *Citrus Aurantiifolia* (Christm.) Swingle of Assam, India

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Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 27 Nov 2023

Abstract

Citrus aurantiifolia (Christm.) Swingle, mostly known as the Key lime or Mexican lime. In Assam, it is known as 'Gol Nemu', which is a small, evergreen citrus tree having significant place in the botanical geography of Assam, India. The present study is concentrated on the analysis of the nutritional components, antioxidant activity, and antimicrobial properties of Citrus aurantiifolia (Christm.) Swingle, cultivated in the region of Kamrup District, Assam, India. The results of this research provide scientific understanding of Citrus aurantiifolia as an abundant source of nutrients like soluble carbohydrate, soluble protein, free fatty acids, vitamin C, along with potent antioxidant and antimicrobial properties. The illumination of this study could have implications for its implementation in both nutraceutical and pharmaceutical industries, as well as in encouraging its consumption for enhanced well-being.

Keywords: *Citrus aurantiifolia (Christm.) Swingle, nutritional component, antioxidant, antimicrobial*

1. Introduction

Citrus aurantiifolia, generally known as the Key lime or Mexican lime, in Assam 'Gol Nemu' is a small, evergreen citrus tree that holds a noteworthy place in the miscellaneous botanical geography of Assam, India. Being a member to the Rutaceae family, this aromatic and tangy fruit-bearing tree has become an integral part of Assam's agrarian and culinary heritage. Its distinctive flavour and versatility have made it a cherished ingredient in local cookeries, while its hardy nature and adaptability have allowed it to thrive in the multiple ecological conditions of Assam. It is a small shrubby polyembryonic plant found in a lot of countries all over the world and grows in hot subtropical or tropical regions such as Southern Florida, India, Mexico, Egypt, and the West Indies. The plant belongs to the kingdom: Plantae; Phylum: Magnoliophyta; Class: Magnoliopsida; Order: Sapindales; Family: Rutaceae; Genus: Citrus and Species: *Citrus aurantifolia*. This plant is believed to have its origin from the south East Asia around 4000 BC and it is native to the Indo-Malayan region [1]. It is a predominant fruit crop in Asia, and India is the largest producer [2]. Collective names of this species are Key Lime, Lime tree, Bitter orange, Bigarade, Country lime, Mexican Lime, Seville orange, Sour lime, West Indian lime etc. Vernacular names in India are Nimbe (Kannada); Elumitcahi (Tamil); Limbu (Marathi); Nimma (Telugu); Erumichinarakam, Cherunaregam (Malayalam) [3].

In Assam, *Citrus aurantiifolia* is not only a fruit-bearing tree; it's a cultural emblem that has blended itself into the fabric of everyday life. The zingy flavour of Gol Nemu adds a distinctive twist to traditional Assamese cuisines and beverages. The fruit is used in various culinary delicacies, such as pickles, chutneys, and refreshing lime-based drinks that help combat the region's humid climate. Moreover, the tree's lush foliage and fragrant blossoms contribute to the aesthetic beauty of gardens and topography throughout the state. *Citrus aurantiifolia* is portrayed by its small, round to oval-shaped, bright green fruits with a smooth and thin skin. The aromatic leaves effuse a refreshing citrus fragrance when crushed. It is a perennial tree that can grow to a height of 3–5 m. Stem of this plant is

asymmetrically slender and branched and endowed with short and stiff sharp spines or thorns. Leaves are alternate, elliptical to oval, 4.5–6.5 cm long, and 2.5–4.5 cm wide with small rounded teeth all around the boundary. Petioles are 1–2 cm long and scarcely winged. It possesses white and fragrant flowers with short and axillary racemes. Petals are 5, oblong, and 10–12 mm long. Fruits are green, round, 3–5 cm in diameter, it is yellow when ripe. The fruit has the matching anatomical structure like other citrus fruits. Flavedo, the external part of the fruit has a lot of flavonoid compounds. The outer cell wall is comprised of wax and cutin, which prevents water loss from the fruit. Albedo is the white spongy portion, beneath the flavedo layer. Carpal membranes or septum extending around 8–11 glandular segments, usually aligned and located around the soft central core. Juice sacs are yellow-green pulp vesicles and seeds are small, plump, ovoid, pale, and smooth with white embryo [4]. The cross section of fresh *C. aurantifolia* is shown in fig 1.

Fig 1: Fruits of *C. aurantifolia*

2. Materials And Methods

The present study was conducted to study the nutritional constituents, antioxidant activity and antimicrobial activity of fresh fruits of *C. aurantifolia*, commonly known as acid lime. The investigation was carried collectively in University of Science and Technology, Meghalaya and Assam down town University during 2017-22. All the chemicals used in this study were of analytical grade and of standard companies.

Sample collection:

The fresh fruits of *C. aurantifolia* were collected from Kamrup district of Assam. The freshly collected fruits of *C. aurantifolia* were peeled off and juice was extracted with the help of juicer. The fresh juice was filtered to remove the pulp and the filtrate was used for the investigation.

Total soluble carbohydrate content:

The soluble carbohydrate was estimated by using modified Anthrone method [5]. 5 ml of juice sample was placed in 40 ml of 80% ethanol and boiled till 10 ml remained. The mixture was then centrifuged at 3000 rpm for 10 min. The supernatant was decanted and the residue was again dissolved in 20 ml ethanol and centrifuged at 3000 rpm for 5 min, then both the supernatants were combined. Simultaneously, the supernatant was dried over hot plate till about 6 ml remained and the final volume was made up to 20 ml with 20% ethanol. To 1 ml of the ethanolic extract 4 ml of Anthrone reagent was added and allowed to cool down at room temperature and absorbance was recorded at 630 nm in UV/VIS spectrophotometer (Systronics), against a blank in which the sample was replaced by 1 ml distilled water. D-Glucose was used as standard and the amount of sugar was expressed in mg/100 ml of fruit juice.

Total soluble protein content

The estimation of total soluble protein content was carried out by the method of Lowry et al. (1951) [6]. According to the method, 5 ml juice sample was taken and made the volume up to 50 ml with phosphate buffer. From the above solution 2 ml was taken and 2 ml of 20% trichloroacetic acid was added to it. After 30 min the solution was centrifuged at 3000 rpm for 25 min and washed with acetone twice and again centrifuged. The solid was dissolved in 5 ml NaOH (0.1N). 1 ml of above solution was mixed with freshly prepared 5 ml alkaline copper sulphate reagent. After 1 min, 0.5 ml Foline's reagent was added to it, mixed and allowed to stand for 30 min. The absorbance of the mixture was recorded at 660 nm with a blank which contained 1 ml NaOH (0.1N) instead of the sample aliquot. BSA (bovine serum albumin) was used as a standard.

Free fatty acid (acid value) content:

Free fatty acid was estimated as per the protocol of Sada sivam and Manickam, 2008 [7]. 1 ml of uncentrifuged juice was treated with 25 ml of neutral solvent (25 ml diethyl ether, 25 ml 95% alcohol, 1 ml 1% phenolphthalein solution; the mixture was added and the resultant solution was titrated against 0.01 N potassium hydroxide. Free fatty acid was estimated as per the following formula-

$$\text{Acid value (mg KOH/ ml)} = \frac{\text{(titre value} \times \text{Normality of KOH} \times 56.1)}{\text{Amount of sample (mg)}}$$

Total vitamin C (Ascorbic acid) content:

Determination of vitamin C was carried out as per the method of redox titration using iodine solution [8]. To 2 ml of the sample 15 ml of distilled water and 1ml of starch indicator was added and titrated against 0.005 mole/lit iodine solution.

Assessment of antioxidant properties:

DPPH free radical scavenging assay

The scavenging activity of fruit juice was determined by using the method of Blosis (1958) [9]. on the basis of scavenging ability of the fruit juices on the DPPH (1,1- diphenyl-2-picrylhydrazyl) free radical). 0.1mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml various quantities of sample juices (50-250 µg/ ml) in methanol. After 30 min absorbance was measured at 517 nm. BHA was used as reference compound. Percentage inhibition was calculated by comparing the absorbance of control and sample.

$$\% \text{ Inhibition} = (A_0\text{-}A_1)/A_0 \times 100$$

A_0=Absorbance of control, A_1=Absorbance of juice/ reference compound

Analysis of Antimicrobial activity:

Antimicrobial activity of the sample was assayed by agar well diffusion method. Microbial cultures including *Klebsiella, Escherichia coli*, *Pseodomonas aureus* and *Bacillus subtilis* were collected. Microbes were sub cultured on nutrient broth (pH 7.4) and stored in refrigerator at 4ºC for further study. Nutrient agar (NA) media was prepared and pH was adjusted up to 7.0. The prepared media was sterilized in autoclave (Yoroco, YSU-405) at 121ºC, 15 psi for 15 mins. After sterilization, media was poured to the four plates (15 cm diameter) aseptically inside Laminar air flow Chamber (Hitech, S.stable). After solidification and sterility testing of the agar, 100 µl of each microbial culture was added on the plates such as *Klebsiella, Escherichia coli*, *Pseodomonas aureus* and *Bacillus subtilis* accordingly and spread with a sterile glass spreader. By using a sterile cork borer, 1.0 cm well was punctured in agar medium plate previously. The wells were filled with 200 µl of the sample. Plates were sealed with paraffin and incubated in a BOD incubator (Yorco, YSI-440) at 37ºC for 24 hours. The diameter of the zone of inhibition was measured and recorded. Three replicate plates were used for each bacterium and data were subjected to statistical evaluation by one-way analysis of variance (ANOVA) [10,11].

3. Results and Discussion
Nutritional constituents:

The amount of different nutritional components present in the juice of fresh C. *aurantifolia* is given in the following table.

Table 1: Nutritional components of fresh C. *aurantifolia*

Serial no	Nutritional component	Concentration (mg/ml)
1	Total soluble protein	6.7
2	Total soluble carbohydrate	33.6
3	Free fatty acid	37.86
4	Vitamin C (Ascorbic acid)	0.047

Antioxidant activity:

DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants. The juice was able to reduce the stable radical DPPH to the yellow-coloured diphenylpicrylhydrazine at all concentrations [12]. The scavenging activity of fresh C. *aurantifolia* and ascorbic acid at different concentration is shown in the table 2.

Table 2: DPPH free radical scavenging activity (%) of fresh fruit and Ascorbic acid

Sl No	Sample concentration (µl)	DPPH scavenging activity of fresh fruit (%)	DPPH scavenging activity of Ascorbic acid (%)
1	50	81.32	81.59
2	100	82.44	94.30
3	150	84.74	94.87
4	200	89.93	95.25

The results from the table shows that DPPH scavenging activity of fruit juices increases with the increase in concentration of sample. These results are depicted in fig 2. Indriyani et al. also reported that ripe *C. aurantifolia* juice showed 91.02% scavenging against DPPH at a concentration of 100 µL.

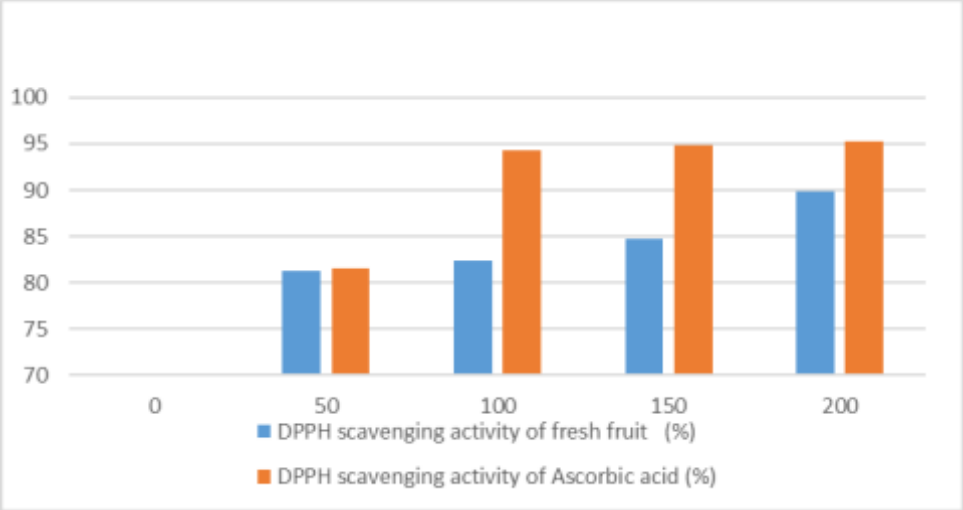
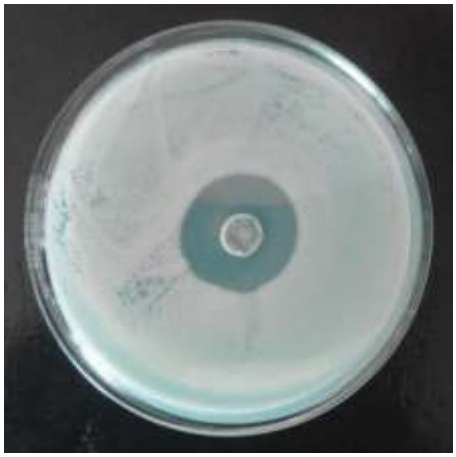


Fig 2: Graph showing scavenging activity (%) among fresh fruit, fermented fruit and ascorbic acid

Antimicrobial activity: Antimicrobial activity of fresh *C. aurantifolia* juice was assayed against *Klebsiella, E. coli, Pseudomonas aureus* and *Bacillus subtilis* by agar well diffusion method. This study showed that the juice has high antimicrobial activity against all the pathogenic strains. The zone of inhibition shown by different bacteria is shown in the table 3. The same results were also obtained by Aibinu I et al. [13].

Table 3: Inhibition zone of fresh *C. aurantifolia* against *Klebsiella, E.coli, Pseudomonas aureus* and *Bacillus subtilis*

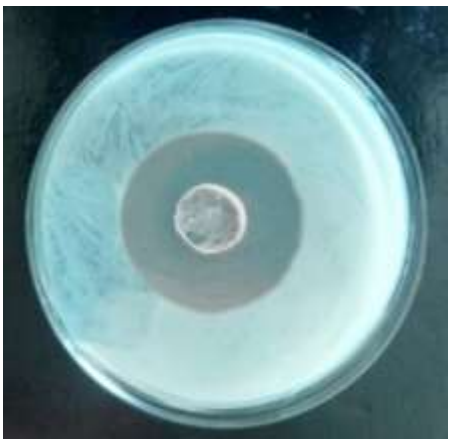
Serial No	Bacterial strain	Zone of inhibition (mm)
1	*Klebsiella*	33 ±0.352
2	*E.coli*	32 ±0.461
3	*Pseudomonas aureus*	34 ±0.231
4	*Bacillus subtilis*	35 ±0.705



Klebsiella

E.coli

Pseudomonas aureus



Bacillus subtilis

Fig 3: Bacterial culture plate showing zone of inhibition.

4. Conclusion

This study furnishes valuable perception into the potential health benefits of this citrus fruit. The analysis of its nutritional components reveals its richness in soluble carbohydrates, soluble proteins, free fatty acids and vitamin C and making it a valuable addition to a balanced diet. Over and above that, the antioxidant activity of *Citrus aurantiifolia* highlights its capability in combating oxidative stress and reducing the risk of chronic diseases. The ascertained antimicrobial activity against various pathogens suggests the power of *Citrus aurantiifolia* as a natural source of antimicrobial agents. This could have insinuation in the field of food preservation and possibly contribute to the development of novel therapeutic agents. However, there is a demand for further research to fully explicate the mechanisms behind the observed effects and to explore the potential applications of *Citrus aurantiifolia* in various industries. In conclusion, the study emphasizes the importance of *Citrus aurantiifolia* as a valuable dietary resource, providing a foundation for future studies and potential employment in promoting human health and well-being.

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