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Research article

Evaluating citrus juice: A comparative study of physicochemical, nutraceutical, antioxidant, and antimicrobial properties of citrus juices from Nepal

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ABSTRACT

Citrus fruit juice is highly beneficial to human health, providing essential nutrients like carbohydrates, vitamins, phytochemicals, and antioxidants. Juices from citrus fruit varieties grown in various regions of Nepal were analyzed for their physicochemical properties, nutraceutical content, antioxidant and antimicrobial properties. The pH of the juices ranged between 2.09 and 3.62, while total soluble solids (TSS) varied from 7° Brix to 12.3 °Brix. Among varieties, *C. aurantifolia* exhibited the highest titratable acidity at 7.39 g/100 mL. *C. limon* showed the highest moisture content (94.74 %), *C. reticulata* had the highest carbohydrate content (14.6 ± 0.4 g/100 mL, n = 3), and *C. aurantifolia* presented the highest protein content (34.1 ± 0.7 mg/100 mL). *C. sinensis* recorded the highest flavonoid content (91.4 ± 0.3 mg/100 mL), *C. reticulata* had the highest phenolic content (65.8 ± 0.6 mg/100 mL), and *C. limon* exhibited the highest ascorbic acid content (45.1 ± 0.4 mg/100 mL). The methanolic extracts of all citrus fruit juices demonstrated robust antioxidant properties, as determined by DPPH assay. Notably, *C. limon* and *C. aurantifolia* juice extracts demonstrated significant antimicrobial activity against a wide range of microbes. This study highlights the variation in nutrient and phytochemicals compositions among different citrus fruit juices, underscoring the nutritional and medicinal benefits of citrus species.

1. Introduction

Citrus fruits, also referred to as sour fruits, are widely consumed fruits in the world, and grown in more than 140 nations in which Oranges, lemons, limes, grapefruits, and tangerines are the most popular [1]. Citrus species originated in Southeast Asia and the Malay Archipelago and spread from Northern India to China, Malaysia, the East Indies, and the Philippines to the South [2]. Classification and evolutionary history of Citrus species are complex and contentious due to factors like the sexual compatibility among citrus and related genera, the frequent bud mutations, extensive cultivation history, and widespread dispersion [3,4].

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Recent years have seen growing interest in citrus fruits and juices with numerous studies showing that consuming citrus fruits and their edible parts benefits human health [5]. Citrus juices possess antioxidant, anti-inflammatory, antimicrobial, anti-diabetic, and anticancer characteristics as supported by several studies [6–14]. Citrus fruits and their value-added products have important contributions in food and beverage industries.

Consuming fruit juice contributes to overall health and aids in disease prevention, primarily due to their vitamin C content, also known as ascorbic acid [7]. The anthocyanin, carotene, pulp, and other chemical contents are the primary factors affecting the color and turbidity of the juices [15]. Bioactive substances such as minerals, vitamins, essential oils, phenolics, and flavonoids are also found in citrus juices and are considered to be responsible for several health benefits, including protection against oxidative stress, cancer, inflammation, and microbes [13]. The antioxidant and anti-inflammatory properties help body to eliminate uric acid and prevent diabetes [16]. Furthermore, the vitamin C aids in formation of collagen, cartilage, muscle, and blood vessels and iron absorption as well [17]. Citrus fruit is one of the horticultural crop that can support the country's food security, nutritional status, employment, income, and GDP growth [18].

Citrus fruits with diverse chemical compositions are cultivated across various regions worldwide. In Nepal, citrus species are predominantly cultivated in mid-hill regions at altitudes ranging from 800 to 1500 m above sea level. The nutritional and phytochemical composition of citrus fruits is influenced by various factors, such as species and varieties, growth conditions, maturity stage, and local climatic conditions [19]. To the date, no in-depth analysis has been conducted on the nutritional and phytochemical profile of citrus fruits cultivated in mid-hill regions of Nepal and their juice. Comprehensive analysis of the nutritional and phytochemical profiles of citrus fruits and their juices can reveal their health benefits, such as immune support, disease prevention, and antioxidant activity. This understanding can inform the development of targeted applications, including dietary supplements, functional foods, and therapeutic products.

To this end, this study investigates the physicochemical, nutritional, phytochemical, antioxidant, and antimicrobial properties of citrus fruit juices from seven different varieties commonly found in Nepal. Examining citrus fruits grown in the same environment ensures accurate comparison of properties across different citrus varieties. This approach not only highlights the unique attributes of each variety but also provides valuable insights into their potential benefits and applications.

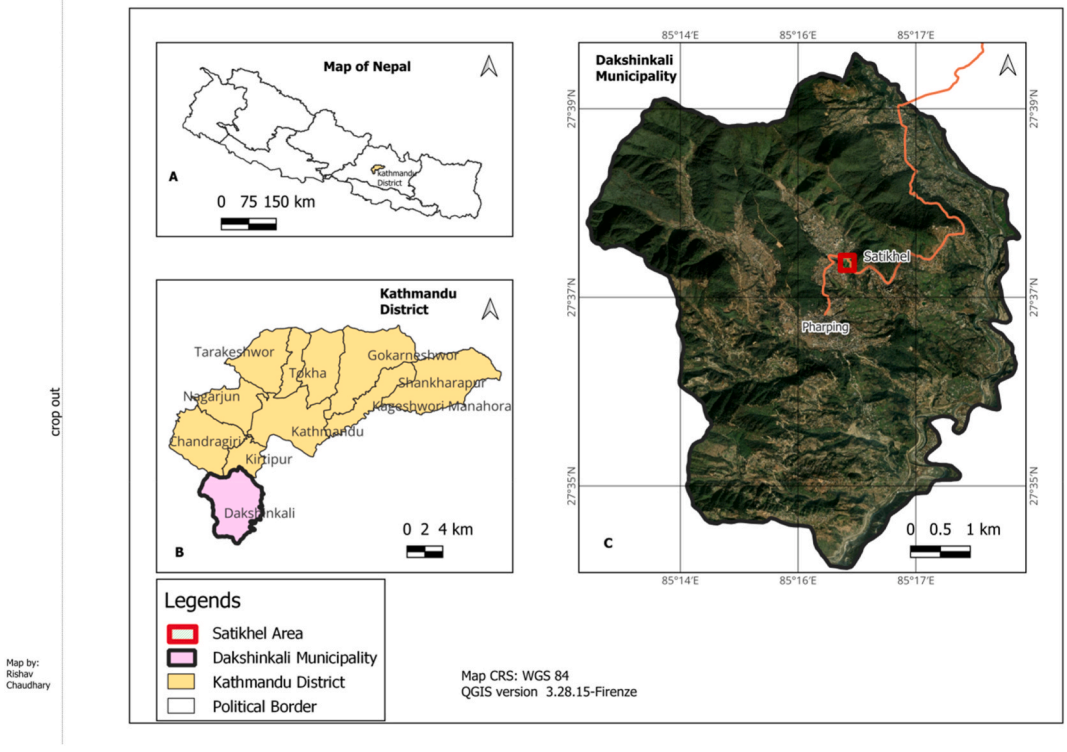

Fig. 1. Study area.

2. Materials and methodology

2.1. Collection and identification of citrus fruits

Seven species of Citrus fruits - *Citrus aurantifolia* (Christm.) Swingle, *Citrus* x *sinensis* (L.) Osbeck, *Citrus x jambhiri* Lush, *Citrus* ×*limon* (L.) Osbeck, *Citrus japonica* Thunb, *Citrus reticulata* Blanco and *Citrus maxima* (Burm.) Merr. were collected from Satikhel, Kathmandu. Fig. 1 represents the study area and Fig. 2 shows samples that were collected. The collected fruit species were identified using herbarium specimen from National Herbarium and Plant Laboratories, Godawari, Lalitpur, Nepal.

2.2. Juice extraction and physicochemical parameter determination

The collected samples were cleaned with distilled water, peeled, and cut into pieces. Juice was extracted using a hand juice

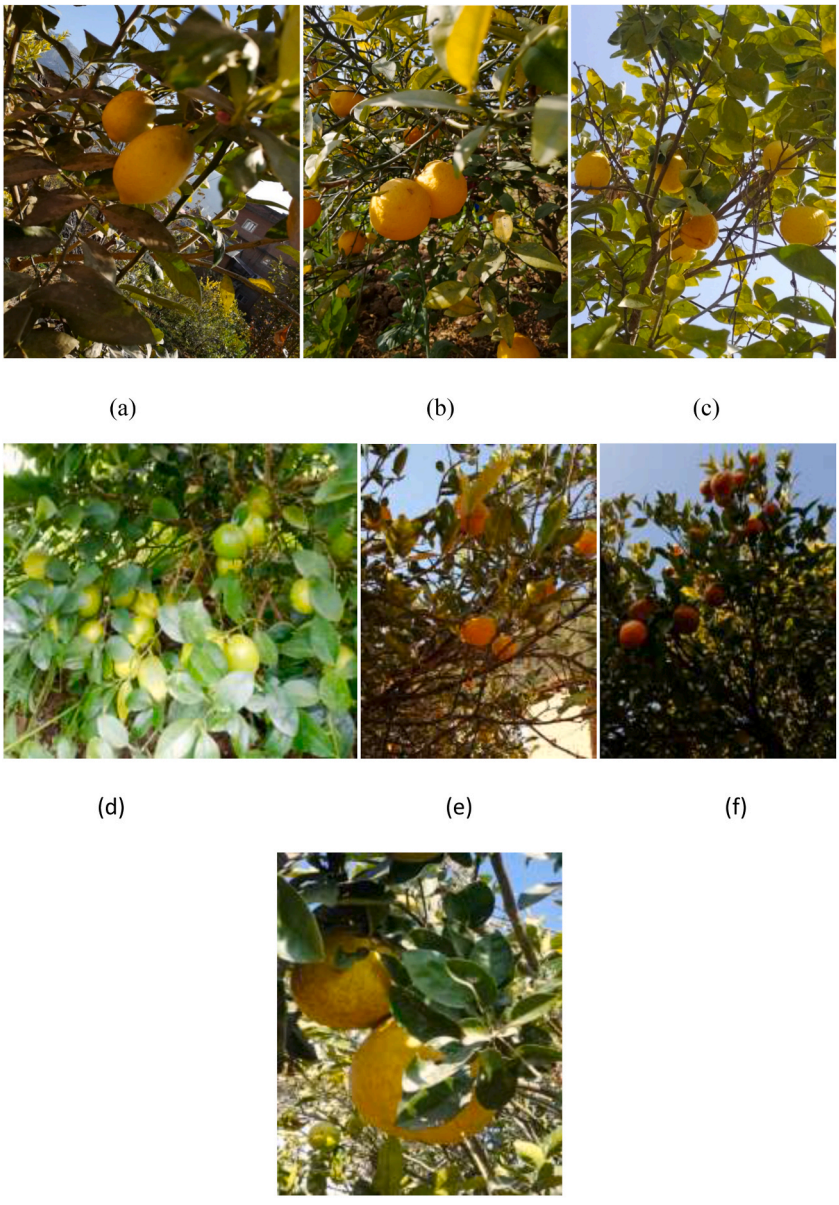

Fig. 2. Pictures of collected Citrus fruit species (a) *C. aurantifolia* (b) *C. sinensis* (c) *C. jambhiri* (d) *C. limon* (e) *C. japonica* (f) *C. reticulata* (g) *C. maxima*.

extractor (Stainless steel fruit juicer), with pieces of pulp covered in sterile muslin cloth, followed by centrifugation at 3000 rpm for 10 min. The extracted juice was stored in sterile, airtight glass bottles in a refrigerator at 4 °C for further analysis.

2.2.1. Determination of percentage of juice yield

The percentage of juice yield was calculated by using equation (1).

$$\text{Juice Yield } (\%) = \frac{wt\ of\ juice\ obtained}{wt\ of\ whole\ fuit} \times 100\% \quad (1)$$

2.2.2. Determination of pH, total soluble solids (TSS) and total solids (TS)

The pH value, total soluble solids and total solids of each juice were determined following the methods outlined in the Association of Official Analytical Chemist [20]. Measurement of pH of each juice was performed using pH meter (Thermo-Scientific, ORION STAR A111) calibrated with phosphate buffer of pH 4.0 and pH 7.0. Total soluble solids of juice were measured by using digital refractometer (Milwaukee MA871 Digital Brix Refractometer) and total solids were determined from the moisture content of juice.

2.2.3. Determination of titratable acidity (TA)

The titratable acidity of each juice was determined following the method described by Rekha et al. [8]. The titratable acidity in juice samples was calculated in terms of citric acid by using equation (2),

$$\text{Titratable acidity } (g / 100\ mL) = Normality\ of\ juice\ X\ Equivalent\ weight\ of\ citric\ acid \quad (2)$$

2.3. Proximate composition analysis of juice

2.3.1. Moisture content

Moisture content of juice was determined by following the method described by Association of Official Analytical Chemist [20] with slight modifications. The moisture content was determined in percentage using equation (3);

$$\text{Moisture content } \% = \frac{(w2 - w1) - (w3 - w1)}{w2 - w1}\ X\ 100\% \quad (3)$$

Where w_1 is the weight of the empty crucible, w_2 is the weight of the crucible and sample, and w_3 is the weight of the crucible and dry sample.

2.3.2. Carbohydrate content

Carbohydrate content in citrus juices was determined by colorimetric anthrone method [21]. The juice extract was prepared by initially diluting 1 mL of the sample in 10 mL of distilled water, followed by a subsequent dilution of 1 mL of this solution in another 10 mL of distilled water, and finally, diluting 5 mL of the resulting solution to a total volume of 10 mL. The prepared juice sample was treated with anthrone reagent in 1:5 ratio and incubated in water bath for 12 min. Absorbance at 630 nm was measured using Agilent Technology Cary UV–Vis spectrophotometer taking 0.2 mL of each mixture in a 96-well plate, with a blank containing only water and anthrone and glucose solution (10–500 μg/mL) was used as a standard solution. The carbohydrates content of each juice was determined using the standard curve of glucose ($y = 0.0017x + 0.2068$; $R^2 = 0.9902$) and the results were expressed in g/100 mL of juice.

2.3.3. Protein content

Protein content in the sample was determined by Bradford reagent method with slight modifications [22]. Centrifuged juice (0.1 mL) was mixed with 1 mL of freshly prepared Bradford reagent in 2 mL microfuge tubes. After thorough vortexing, absorbance was measured at 595 nm in a spectrophotometer against a blank containing only water and Bradford reagent, following an incubation period of no more than 1 h. Bovine Serum Albumin (BSA) solution in water (10–500 μg/mL) was used as standard solution. The protein content of each juice was determined using the standard curve of BSA ($y = 0.0007x + 0.1948$; $R^2 = 0.9921$) and the results were expressed in mg/100 mL of juice.

2.3.4. Ash content

Ash content in juice was determined with the method described by Association of Official Analytical Chemist [20]. 5 mL of each juice was placed in clean, dried crucibles, and weighed. The juice was heated at 550 °C in a muffle furnace for 5 h until white to grey ash was obtained. After cooling, the crucible containing the ash was weighed, and the results were expressed as mg per 100 mL of juice. The ash content in the juice was calculated using equation (4).

$$\text{Total ash content} = W_1 - W_2 \quad (4)$$

Where W_1 is the weight of the crucible with ash and W_2 is the weight of empty crucible.

2.4. Phytochemical analysis of juice

2.4.1. Total phenolic content (TPC)

The total phenolic content was determined by Folin-Ciocalteu Colorimetric method [23,24]. 0.05 mL of sample was mixed with 0.15 mL of Folin-Ciocalteu (FC) reagent in 1.5 mL microfuge tubes and incubated for 3 min. Then, 0.15 mL of 35 % sodium carbonate solution was added, followed by the 0.65 mL of distilled water to make final volume of 1 mL. The mixture was kept in dark at room temperature for 90 min. Subsequently, 0.2 mL of each reaction mixtures was added to 96 well plates and absorbance was measured at 725 nm using spectrophotometer with blank containing mixture of methanol and FC reagent. Gallic acid solutions (10–500 μg/mL) in methanol served as a standard solution, and the blank consisting methanol and FC reagent. The phenolic content of samples were calculated using standard curve of gallic acid ($y = 0.0022x + 0.0558$; $R^2 = 0.9939$) and the results were expressed in gallic acid equivalent (GAE) per 100 mL of juice.

2.4.2. Total flavonoid content (TFC)

The total flavonoid content was determined by using the Aluminium chloride colorimetric method with slight modifications [25]. 0.5 mL of sample was mixed with 0.5 mL of 2 % $AlCl_3$ solution in 2 mL microfuge tubes. Absorbance was recorded after incubating the mixture in dark for 1 h. Quercetin solutions (10–500 μg/mL) in methanol were used as standard solution for calculating the total flavonoid content and was calculated using standard curve of quercetin ($y = 0.0004x + 0.0511$; $R^2 = 0.997$). Thes tests were performed in triplicates, and the results were expressed in quercetin equivalent (QE) per 100 mL of juice.

2.4.3. Ascorbic acid content

1 mL of sample was dissolved in 9 mL of 1 % metaphosphoric acid and incubated in dark at room temperature for 45 min. The mixture was filtered through Whatman no. 1 filter paper. Then, 0.1 mL of filtrate was mixed with 0.9 mL of 2, 6-dichlorophenolindophenol (0.01 %, DCPIP) solution and kept in the dark at room temperature for 30 min. Subsequently, 0.2 mL of each mixture was added to 96 well plates, and absorbance was measured at 515 nm. Ascorbic acid solution (0–500 μg/mL) in methanol was used as the standard to calculate the ascorbic acid content in all samples [23] and was calculated with help of standard curve of ascorbic acid ($y = -0.0013x + 0.9761$; $R^2 = 0.9983$). The results were expressed in mg of ascorbic acid per 100 mL of juice.

2.5. Estimation of antioxidant activity

1 mL of juice was added to the 9 mL of methanol (80 %) and mixed for 3 h in shaking incubator at 100 rpm and room temperature. The solution was then centrifuged at 3500 rpm for 10 min, and the supernatant was stored at 4 °C until further analysis. The scavenging activity of juice extracts were studied by using the method of Tang et al. with slight modifications [26]. The 0.5 mL of as-prepared solutions of different concentrations (50 μL/mL, 100 μL/mL, 150 μL/mL, 200 μL/mL, and 250 μL/mL) in methanol was added to the 0.5 mL of 0.1 mM DPPH solution in 2 mL microfuge tubes. The mixtures were vortexed well and kept in dark at room temperature for 30 min. Subsequently, 200 μl of each mixture was added to 96 well plate and absorbance was measured at 517 nm. Mixture containing only methanol and DPPH was used as control to analyze the scavenging activity.

The scavenging activity (%) was calculated by using equation (5).

$$\text{Radical Scavenging activity } (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100\% \quad (5)$$

2.6. Study of antimicrobial activity

Juice samples were prepared by adding 5 mL of centrifuged juice to 5 mL of methanol (80 %) and the mixtures were incubated in shaking incubator (Innovative Life Science Tools, USA) for 24 h at 100 rpm. The solutions were filtered through the Whatman no. 1 filter paper and the filtrate was kept in refrigerator at 4 °C for further analysis in sterile air tight glass bottle. Muller Hinton agar (MHA) media was prepared by following the manufacturer's instructions and autoclaved at 121 °C for 15 min. The media was then poured into sterilized petri dishes and left to cool. All the selected organisms were grown MHA media. The bacteria and fungi chosen for antimicrobial study are listed in Table 1.

2.6.1. Antimicrobial activities by agar well diffusion method

Twenty milliter of MHA media was added to each Petri dish to prepare sterile MHA plates, achieving a thickness of about 4 mm. The

Table 1
List of microoganisms used in this study.

Gram positive bacterial strain	Gram negative bacterial strain	Fungi
Bacillus subtilis (ATCC 6051)	*Escherichia coli* (ATCC 25922)	*Candida albicans* (ATCC 10231)
Enterococcus faecalis (ATCC 29212)	*Salmonella typhi* (ATCC 19430)	
Staphylococcus aureus (ATCC 25923)	*Klebsiella pneumoniae* (ATCC 700603)	
	Pseudomonas aeurginosa (ATCC 39327)	

inoculated plates were covered and allowed to dry at room temperature. 6 mm wells were created in the inoculation medium using a sterile cork borer. 50 μl methanolic juice extracts and the same amount of negative control (80 % methanol) were added to each well. After a half hour of diffusion at room temperature, the plates were incubated for about 24 h at 37 °C. The zone of inhibition was measured in millimeters using a scale.

2.7. Data management and analysis

The data gathered from the experimental analysis were organized using Microsoft Office Excel 2016. All the determinations were carried out in triplicate (n = 3) and after statistical analysis; results were expressed in mean and standard deviation of the mean.

3. Results

3.1. Physicochemical properties

For physicochemical properties, Juice yield percentage, pH, total soluble solids, titratable acidity and total solids were measured. The results are presented in Table 2. Among the citrus varieties, *C. aurantifolia* exhibited the lowest juice yield (27.1 %), while C. *japonica* had the highest (38.9 %) using a hand juice extractor. Various factors, such as thickness of the peel of fruit and instrument like hand juice extractor used, can influence the juice yield percentage as the method based on extracting juice as much as possible and is expressed in terms of whole fruit. The pH values of juices ranged from 2.0 to 3.6, indicating their acidic nature. Among the varieties, *C. maxima* had the highest pH (3.6), while *C. limon* recorded the lowest pH value (2.0). The total soluble solids of centrifuged juice varied between 7 and 12.3 °Brix. High TSS values may indicate higher sugar content, but other factors like acidity also contribute to perceived sweetness. The titratable acidity of juice ranged from 1.4 g/100 mL to 7.3 g/100 mL of juice. Total solids content was determined by subtracting the moisture content from the total mass of the juice and it ranged from 5.2 % in *C. limon* to 10.9 % in *C. reticulata*.

3.2. Proximate composition

The proximate composition was analyzed using centrifuged juices and results are displayed in Table 3. The moisture content varied from 89.0 to 94.7 %, *C. limon* showed the highest while *C. reticulata* had the least. The *C. reticulata* juice exhibited the highest carbohydrate content (14.6 ± 0.4 g/100 mL), while *C. limon* showed the lowest (3.0 ± 0.1 g/100 mL). The protein content ranged from 8.8 ± 1.0 mg/100 mL in *C. japonica* to 34.0 ± 0.7 mg/100 mL in *C. aurantifolia*. The ash content varied from 60.0 ± 0.6 mg/100 mL in *C. sinensis* juice to 472.5 ± 0.2 mg/100 mL in *C. reticulata* juice. The analysis revealed that citrus juices contained significant amounts of moisture and carbohydrates, but relatively lower amounts of protein and ash.

3.3. Phytochemical analysis

Phytochemical composition in citrus juices varied significantly. Table 4 displays the results of phytochemical analysis in citrus juices. Total phenolic content value ranged from 22.1 ± 0.8 to 65.8 ± 0.6 GA E mg/100 mL. *C. jyambhiri* juice had the lowest total flavonoid content (18.3 ± 0.3 QE mg/100 mL), while *C.sinensis* juice had the highest (91.4 ± 0.3 QE mg/100 mL). The ascorbic acid content showed the least variation across the results and it ranged from 37.5 ± 0.3 to 45.1 ± 0.4 mg/100 mL of juice.

3.4. Antioxidant activities

The antioxidant activity of the methanolic extracts of juice samples were accessed using DPPH assay and the results were expressed in terms of radical scavenging activity (RSA) %. The RSA % of all the juice extracts at various concentrations is shown in Table 5. The RSA % in our study indicates the effectiveness of citrus juices in neutralizing free radicals. It was found that, *C. sinensis* juice exhibited the highest RSA activity, showing 81.0 ± 0.9 % at a concentration 250 μl/mL. In contrast, *C. aurantifolia* showed least antioxidant property. The RSA % varied with increase in concentration of juice extracts. The data presented are based on a tenfold dilution performed during methanolic extract preparation. This result indicates that the citrus juices retained substantial antioxidant properties even after this dilution.

Table 2
Physicochemical analysis of juice including juice yield, pH, TSS, TA and TS.

Sample	Juice Yield (%)	pH	TSS (°Brix)	TA (g/100 mL)	TS (%)
C. aurantifolia	27.1	2.1	7.3	7.3	6.7
C. sinensis	29.4	3.0	10.9	2.5	8.8
C. jambhiri	27.8	2.2	8.8	4.9	6.8
C. limon	37.5	2.0	7.0	7.1	5.2
C. japonica	38.9	3.2	10.4	1.4	8.4
C. reticulata	27.9	3.4	12.3	2.2	10.9
C. maxima	35.2	3.6	8.9	1.9	5.7

Table 3
Proximate composition of juice.

Sample	Moisture Content (%)	Carbohydrate Content (g/100 mL)	Protein Content (mg/100 mL)	Ash Content (mg/100 mL)
C. aurantifolia	93.2 ± 0.5	4.0 ± 0.2	34.0 ± 0.7	414.0 ± 0.5
C. sinensis	91.1 ± 0.7	9.0 ± 0.1	16.2 ± 0.7	60.0 ± 0.6
C. jambhiri	93.1 ± 0.8	5.2 ± 0.2	26.3 ± 0.1	332.0 ± 0.2
C. limon	94.7 ± 0.5	3.0 ± 0.1	33.8 ± 1.2	233.3 ± 0.9
C. japonica	91.5 ± 0.6	11.2 ± 0.1	8.8 ± 1.0	386.6 ± 0.5
C. reticulata	89.0 ± 0.9	14.6 ± 0.4	23.0 ± 1.7	472.5 ± 0.2
C. maxima	94.2 ± 0.2	7.4 ± 0.0	14.4 ± 0.4	376.6 ± 0.4

Table 4
Phytochemical composition in Citrus juice.

Sample	Total Phenolic Content (TPC) (GAE mg/100 mL)	Total Flavonoid Content (TFC) (QE mg/100 mL)	Ascorbic Acid Content (mg/100 mL)
C. aurantifolia	22.1 ± 0.8	70.6 ± 1.8	44.6 ± 0.1
C. sinensis	38.0 ± 1.0	91.4 ± 0.3	40.4 ± 0.3
C. jambhiri	33.1 ± 0.8	18.3 ± 0.3	43.6 ± 0.1
C. limon	32.4 ± 0.4	50.3 ± 0.7	45.1 ± 0.4
C. japonica	54.0 ± 1.0	18.5 ± 1.8	40.1 ± 0.2
C. reticulata	65.8 ± 0.6	63.0 ± 1.1	37.5 ± 0.3
C. maxima	48.6 ± 0.5	46.3 ± 0.5	40.9 ± 0.8

Table 5
RSA % of methanolic extract of juice in different concentrations.

Sample	**RSA (%) in different concentration (v/v) of juice extracts**				
	50 μl/mL	100 μl/mL	150 μl/mL	200 μl/mL	250 μl/mL
C. aurantifolia	4.1 ± 1.3	9.8 ± 1.5	18.4 ± 1.3	23.1 ± 0.6	28.2 ± 1.8
C. sinensis	12.4 ± 0.4	25.0 ± 1.4	48.1 ± 1.2	63.3 ± 0.9	81.0 ± 0.9
C. jambhiri	9.8 ± 0.9	12.9 ± 0.5	27.1 ± 1.8	30.7 ± 2.9	36.5 ± 1.7
C. limon	14.8 ± 0.9	20.5 ± 1.8	25.0 ± 2.3	30.4 ± 0.7	35.5 ± 1.1
C. japonica	13.7 ± 2.1	29.1 ± 1.8	46.8 ± 0.7	60.3 ± 1.2	70.4 ± 1.1
C. reticulata	5.1 ± 1.1	21.3 ± 1.1	29.8 ± 1.4	41.2 ± 1.9	51.7 ± 0.6
C. maxima	11.5 ± 0.7	20.9 ± 0.9	31.1 ± 0.9	42.5 ± 1.1	54.9 ± 0.2

3.4.1. Antimicrobial activities

Antimicrobial activity of methanolic extracts of juices was tested against various bacterial and fungal strains. Table 6 lists the specific antibiotics as a reference standard used against each typical organism.

Tables 7 and 8 display the results of antimicrobial activity, where numbers indicate the zone of inhibition measurements (mm) and dashes indicate no inhibitory effect. Juice sample of *C. limon* and *C. aurantifolia* were the most effective against all tested microorganism. It was found that *C. jambhiri* was effective against *E. coli, B. subtilis, S. typhi, K. pneumoniae*, and *C. albicans*. The *C. maxima* found effective against *B. subtilis* and *K. pneumoniae*, while *C. sinensis* was found effective against *B. subtilis* and *E. faecalis*. However, *C. japonica* exhibited no inhibitory effect on any organism at the tested concentrations.

4. Discussions

Citrus juices supply a balance and combination of naturally occurring water, sugars, acids, vitamins, and minerals as well as they

Table 6
List of reference standards used for each microorganism.

S.N.	Name of microorganism	Name of reference standards used
1	*E.faecalis*	Amoxicilin (10 mcg)
2	*E.coli*	Amikacin (30 mcg)
3	*P.aeruginosa*	Ofloxacin (5 mcg)
4	*S. aureus*	Vancomycin (19 mcg)
5	*B. subtilis*	Erythromycin (15 mcg)
6	*S.typhi*	Ceftriaxone (30 mcg)
7	*K. pneumonia*	Norfloxacin (10 mcg)
8	*C. albicans*	Itraconazole (10 mcg)

Table 7
Zone of inhibition (mm) of juice samples against 4-g negative bacterial strains.

Sample/Organism	S.typhi	K. pneumonia	E.coli	P.aeruginosa
C. aurantifolia	15	15	18	19
C. sinensis	–	–	–	–
C. jambhiri	12	12	13	0
C. limon	11	13	15	11
C. japonica	–	–	–	–
C. reticulata	–	9	–	–
C. maxima	–	13	–	–
Positive control	28	34	25	24
Negative control	–	–	–	–

Table 8
Zone of inhibition (mm) of juice samples against 3-g positive bacterial strains and fungi.

Sample/organism	Gram negative			Fungi
	B. subtilis	E.faecalis	*S. aureus*	C. albicans
C. aurantifolia	16	18	12	14
C. sinensis	11	11	–	–
C. jambhiri	16	14	–	9
C. limon	16	12	9	11
C. japonica	–	–	–	–
C. reticulata	–	–	–	–
C. maxima	11	–	–	–
Positive control	21	19	19	26
Negative control	–	–	–	–

contain phytochemicals like polyphenols and other organic components [27]. Each citrus fruit species has its own unique set of characteristics and composition. Several factors, such as fruit variety, environmental conditions (such as climate, soil type, maturity at harvest, irrigation, fertilization, etc.), processing techniques, and analytical methods, significantly influence physicochemical characteristics [28]. The citrus fruits yielded satisfactory juice percentages, with *C. japonica* yielding the highest with 38.9 %. These results align with the findings of Tounsi et al. [29] for oranges and lemon. Juice yield is influenced by factors like fruit type; peel size, juice viscosity, and instruments used. The pH values ranged from 2.0 to 3.6. These values are influenced by fruit ripening stages and maturity [30]. These findings are consistent with previous studies on various citrus fruits [28,31–34]. The decreased pH value indicates the more acidic nature of juice [34]. TSS values represent the percentage (%) of dissolved solids in a solution [35] and are indicative of fruit sweetness [36]. TSS values in our study ranged from 7 °Brix in *C. limon* to 12.3 °brix in *C. reticulata*. These results align with those of Xu et al. [37], who reported similar TSS content for *C. reticulata* and *C. sinensis*, though our TSS for *C. limon* was lower. TSS values in our findings were comparable to those of Rehman et al. [32], who studied the juice of eight citrus varieties with TSS values ranging from 8.1 to 13.7 °Brix and those of Sicari et al. [38] who examined it in 15 citrus varieties. Additionally, the TSS of *C. aurantifolia* Juice was consistent with results of Shrestha et al. [39]. In comparison to sour juice, sweeter juice had a higher TSS and the juice with higher natural sugar can offer more flavor and energy, which increases consumer acceptability.

Titratable acidity expressed in terms of citric acid was the lowest in *C. japonica* (1.4 g/100 mL) and the highest in *C. aurantifolia* (7.2 g/100 mL). The findings align with Guo et al. [40] who examined ten citrus varieties but are marginally higher than those reported by Rekha et al. [8] who examined four different varieties of ripe and unripe citrus juices. Acidity which correlates with titratable acidity and pH in which pH determines whether microorganisms can grow in a particular food while titratable acidity is a more accurate indicator of how organic acids in food affect flavor [41]. The results of Total solids observed in this study were comparable to those reported by Ndife for various brands of oranges [42] and slightly lower than the findings of Rehman et al. [32]. Total solids increases with ripening of Citrus fruits [43].

Nutritional attributes such as moisture, carbohydrate, proteins, ash and lipid contents are correlated with the proximate composition of food items [44]. Moisture content in juices was found slightly higher as compared to report of 86.9–89.9 % by Rehman et al. [32]. This may be due to use of centrifuged juice for moisture determination in our study. Moisture plays a major role in food preservation, quality, and resistance to deterioration [45]. Carbohydrate content was found minimum in *C. limon* (3.0 ± 0.1 g/100 mL) and maximum in *C. reticulata* (14.6 ± 0.4 g/100 mL) (Table 3). Carbohydrate functions as a energy giving fuel in body which is required for physical activities and different body functions [46]. Samples with low carbohydrate may be the best option for patients with diabetes and hypertension who need low-sugar diets [47]. In the Bradford assay, protein is bound to the dye Coomassie Blue G250 and the amount of dye in the blue ionic form is measured for protein estimation which is achieved by measuring the absorbance of solution at 595 nm [48].The protein content in juice samples ranged from 8.8 ± 1.0 mg/100 mL in *C. japonica* and 34.0 ± 0.7 mg/100 mL in *C. aurantifolia.* These results align with previous study by Chuku et al [49], who reported low protein content in citrus juice. The ash content in juices was ranged from 60.0 ± 0.6 mg/100 mL in *C. sinensis* to 472.5 ± 0.2 mg/100 mL in *C. reticulata.* The ash content in food is related to the presence of macrominerals and microminerals which play crucial role in human health [50,51]. These minerals serve a wide range of functions including influencing muscle and nerve function, forming the structural components of our bones, and

regulating the water balance in our bodies [52].

Total phenolic content of juices ranged from 22.1 ± 0.8 mg GAE/100 mL in *C. aurantifolia* to 65.6 ± 0.6 GA E mg/100 mL in *C. reticulata.* These findings are in consistent with those of Fejzić, A. and Ćavar, S [53]. who reported the results in mg GAE/mL but they are lower than the results of Al-Juhaimi and Ghafoor [31] who found phenolic content between 79.2 and 107.3 mg GAE/100 mL. Variation in extraction techniques, solvents employed and origin of sample likely accounts for these differences [53]. Phenolic phytochemicals have a number of protective functions against pathogen, predator attacks, and UV light [54]. These compounds play a key role for human beings in defense mechanisms by acting as antioxidants, anti-aging, anti-proliferative agents, and anti-inflammatory agents [55]. Therefore, consuming the citrus fruits is beneficial for health.

Flavonoids have a potent antioxidant and radical scavenging activity in addition to influencing the appearance, taste, and nutritional value of fruit and juice [56]. The total flavonoid content in this study ranged from 18.5 ± 1.8 QE mg/100 mL in *C. japonica* to 91.4 ± 0.3 QE mg/100 mL in *C. sinensis*, higher than the data reported by Sicari et al. [38]. The variation of flavonoids in citrus fruits might be due to a variety of factors such as genetic makeup, environmental conditions, ripeness and harvesting and cultivation practices [19].The flavonoid concentration is higher in peel than in juice and pulp of citrus fruits [57], highlighting the health benefits of consuming whole citrus fruit. The flavonoids have been utilized as anti-wrinkle skin agents [58], natural dyes [59], and cosmetics and skin care products [60].

The phenolics, flavonoids and ascorbic acid in samples are compared in the form of bar graph as shown in Fig. 3. On comparing these constituents in each juice, flavonoid content was higher in *C. aurantifolia*, *C. sinensis* and *C. limon*, phenolic content was found higher in *C. japonica*, *C. reticulata* and *C. maxima* and ascorbic acid was found higher in *C. jambhiri.*

Vitamin C also known as ascorbic acid is not synthesized by the human body and it must obtained through diet [61]. Adequate vitamin C level is essential, as the poor vitamin C level is associated with to high blood pressure, increased risk of heart attack, cataract, and certain cancers. However, high levels of ascorbic acid in the human body can have negative effects, including gastric irritation and renal problems [62]. The ascorbic acid content ranged from 37.5 ± 0.3 mg/100 mL in *C. reticulata* to 45.1 ± 0.4 mg/100 mL in *C. limon*, consistent with previous studies [32,63]. Ascorbic acid content in citrus fruits and their products varies depending on the variety, cultural practices, maturity, climate, fruit quality, fresh fruit handling, processing factors, packaging, and storage conditions [64].

The line graphs in Fig. 4 compare the RSA% of different juice sample. It can be seen that the RSA% increases with increasing the concentration of juice in working samples which is due to the increase in quantity of antioxidants to scavenge DPPH radicals as concentration of juice increases. At lower concentration (50 μl/mL) the *C. limon* juice exhibited strong RSA% while at higher concentration (250 μl/mL) *C. sinensis* juice extract showed maximum RSA% among the tested samples. Notably, trend line for *C. sinensis* is more steeply than the others, indicating its superior antioxidant property. Phenolic compounds, flavonoids and ascorbic acids are the main antioxidant component in citrus fruits [65]. The study of antioxidant activity of different types of citrus fruit juice by DPPH assay was also studied by Refs. [6,8,31]. Results of analysis were different may be due to the citrus type, maturity of fruit and method used for estimation of antioxidant properties [66] but all of these studies including present findings revealed the good antioxidant properties of citrus juices. Antioxidants, secondary metabolites found in plants like citrus fruits, can protect against oxidative stress and cell damages. Consuming antioxidants-rich food may reduce the risk of diseases caused by free radicals [67].

Fig. 5 shows the antibacterial activity of juice samples. Notably, *C. aurantifolia and C. limon* effectively inhibited the growth of all microorganisms under study, consistent with findings by Shakya et al. [68] who studied *C. aurantifolia* juice and *C. limon* juice against *S. aureus, E. coli, S. typhi, P. aeruginosa* and *K. pneumoniae*. The *C. japonica* demonstrated no inhibitory effect against any of the microorganisms under investigation, while *C. aurantifolia and C. limon* each inhibited growth of all eight microorganisms' types. Adam et al. also reported no inhibitory effect of *C. reticulata* against *S. aureus* [69]. However, Shakya et al. reported antimicrobial activity against *S. aureus* [67]. The highest zone of inhibition was 19 mm for *C. aurantifolia* against *P. aeruginosa*. Oikeh et al. also reported the inhibitory effect of *C. limon* juice extract against *E.coli*, *P. aeruginosa, E. faecalis* and *S. aureus* and *C. albicans* [7]. Given the antimicrobial properties of citrus juices and the challenges posed by antibiotic-resistant pathogens, a diet rich in citrus fruit is anticipated to provide preventative medicine against these infectious agents [70].

5. Conclusion

The consumption of plant-based foods, particularly fruits, benefits human health due to their nutritional and therapeutic qualities. Citrus fruits are consumed in large quantities globally, and Nepal is an ideal country for growing citrus species. The purpose of this study was to examine the variations in physiocochemical, nutraceutical, antioxidant and antimicrobial properties amongst seven distinct citrus juices and their comparative analysis highlights their significance for human health. Consumption of citrus fruits can mitigate the risk of vitamin C deficiency-related illnesses like scurvy. Despite the close biological relationships between citrus fruit species, this study concludes that the proximate and phytochemical composition of these citrus species varies. This variation may result from differences in the species, soil composition, and maturity stage of fruits. Understanding the chemical composition, antimicrobial properties, and antioxidant qualities of the juice from varieties of citrus fruits aid in identifying their most appropriate application. For example, juices with higher sugar content, like *C. reticulata,* are ideal for energy drinks, while *C. limon* juice and *C. aurantifolia* juice have significant potential in the pharmaceutical industry due their antimicrobial activity against a broad range of microorganisms.

CRediT authorship contribution statement

Nirmal Karki: Validation, Methodology, Investigation, Formal analysis, Writing – original draft. **Hari Achhami:** Investigation,

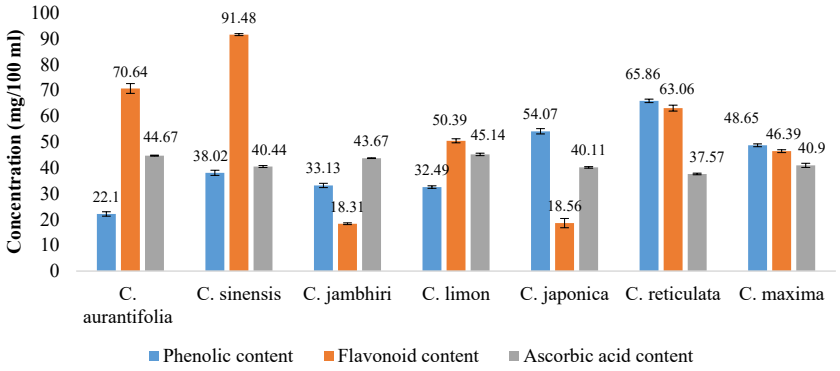

Fig. 3. Bar graph for comparison of Phytochemical composition of juices.

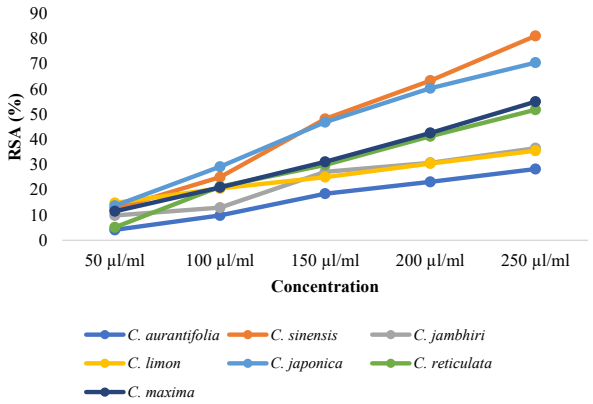

Fig. 4. Line chart for comparison of RSA % of juice extracts.

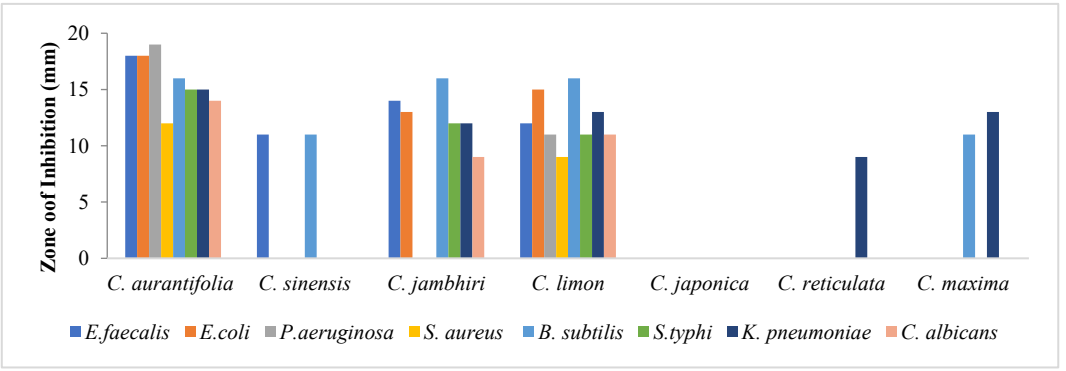

Fig. 5. Bar graph for comparison of antimicrobial activities of juice extracts in terms of zone of inhibition.

Formal analysis. **Bishwa Bandhu Pachhai:** Methodology, Investigation. **Susmita Bhattarai:** Methodology, Investigation. **Dikpal Kumar Shahi:** Methodology, Investigation. **Lok Ranjan Bhatt:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization, Funding acquisition. **Mahesh Kumar Joshi:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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