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A Review on Analytical Approaches for Identifying Citrus Phytochemicals

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Citrus species are well recognized for their wide range of biological activities, including antimicrobial, anticancer, cardiovascular, and renal-protective effects. The objective of this review is to systematically compile and demonstrate the various analytical approaches used to identify and quantify phytochemicals in citrus species. Modern analytical techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), liquid chromatography–mass spectrometry (LC-MS), and infrared spectroscopy have been explored, alongside classical extraction methods including Soxhlet, maceration, and steam distillation, as well as newer techniques like solvent-free microwave extraction and pressurized liquid extraction. Specific citrus species — Citrus hystrix, Citrus aurantifolia, and Citrus microcarpa and their bioactive constituents are discussed in relation to their pharmacological effects. This review aims to guide future research by providing insights into suitable analytical strategies and highlighting the need for standardization in citrus phytochemical analysis.

Keywords: citrus species, phytochemicals, flavonoids, extraction methods, analytical techniques

Introduction

In recent years, growing interest in the impact of food on human health and the environment has brought increased focus to nutrition, food processing, and waste reduction. Among these, citrus-based products have gained attention for their unique flavors and potential health benefits. Citrus fruits are rich in bioactive compounds like flavonoids, carotenoids, and limonoids, known for their antioxidant, anti-inflammatory, and anticancer properties. Moreover, citrus peels, flesh, leaves, and juice have long been used in traditional medicine to treat various ailments. The chemical composition of citrus-based products is complex and influenced by factors such as species, processing methods, and storage conditions. Both non-volatile compounds (sugars, organic acids, amino acids) and volatile compounds (terpenes, aldehydes, ketones) contribute significantly to their distinctive flavor and aroma. Investigating these chemical profiles is crucial for advancing our understanding of the intricate relationship between food and human health. By using a conscious process and utilizing all parts of the citrus fruit, we can create products that not only taste great but also contribute to a more sustainable and healthy food system [1].

Citrus is a genus in the Rutaceae family that includes widely consumed fruits like oranges, lemons, and limes [2]. Originating in Asia and the Pacific, citrus species spread globally through trade and migration routes [3]. Sweet varieties are eaten fresh, while sour ones are commonly used in cooking and beverages. Citrus fruits are also processed into juices, marmalades, and desserts, reflecting their nutritional and economic value. In order to meet the needs of the agro-food business and export, citrus types are usually chosen for production [4].

The phytochemical profile of herbal medicines, including citrus species, is influenced by species type, plant parts used, geographical origin, processing methods, and seasonal factors [1-3]. While earlier reviews have emphasized the analytical methods of citrus phenolic compounds, few have focused specifically on the advanced analytical techniques used to evaluate fruits [2, 5-6]. Modern tools like ambient ionization mass spectrometry and PDA-ESI-MS are underreported despite their growing relevance. This review bridges that gap by systematically exploring published studies on *Citrus aurantifolia*, *Citrus microcarpa*, and *Citrus hystrix*, sourced from PubMed and Google Scholar. Various techniques such as HPLC, GC-MS, GC-FID, LC-MS, UV, PDA, and DAD have been employed to analyze citrus phytochemicals in both raw and processed forms. The review emphasizes how different methods yield variable results depending on the sample and technique used. Its core objective is to provide a critical overview of these analytical tools, discussing their advantages and limitations, and offering guidance for future research in food science, pharmacognosy, and quality control.

PHYTOCHEMICALS OF CITRUS

Common citrus trees found in Malaysia include the kaffir lime (*Citrus hystrix*), key lime (*Citrus aurantifolia*),

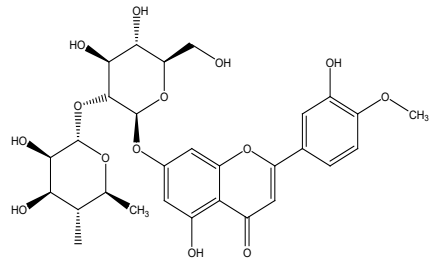
and calamansi lime (*Citrus microcarpa*). Flavanones, flavones, polymethoxyflavone aglycones, flavanone- and flavone-O-glycosides, and flavone-C-glycosides are among the flavonoids that have been found in citrus [7]. Citrus species contain limonene, terpinen-4-ol, terpineol, β-pinene, α-terpineol, citral, nerol acetate, polymethoxylated flavones, rutin, kaempferol, tangeretin, hesperidin, naringin, etc. [8].

The main chemical compounds isolated from citrus species are shown in Figures 1 and 2.

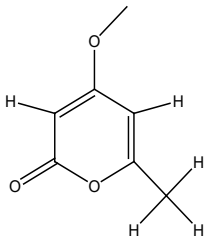
BOTANICAL DESCRIPTIONS

Citrus microcarpa, also called “limau kasturi” in Malaysia, is a small tree or bush that has a long root. It can grow as tall as a house, from 2 to 7.5 meters. People in places like Malaysia, Indonesia, Thailand, and the Philippines often see this tree [2]. Here’s an interesting tidbit: if a small part of this tree is planted, it will gradually develop into a sizable tree. When grown from seed, a patient wait of approximately 5 to 6 years is needed before the tree begins producing fruit. However, if a special technique is employed to generate new trees, the process can be expedited to only 3 years or even less. This tree is a real workaholic when it comes to making flowers and fruit. It produces them all year long, but it is busiest from August to October. The fruit looks like small, round balls. They start out greenish-yellow and become about 2 to 4.5 cm wide when fully grown (Figure 1). Interestingly, it only takes about five months from when the flowers appear to when the fruit becomes ready to eat [5]

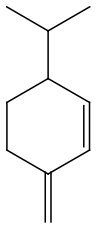
Citrus aurantifolia is a perennial evergreen tree that can grow to a height of 3–5 m (Figure 3A). Stem: irregularly slender, branched, and possessing short, stiff, sharp spines or thorns 1 cm or less. Leaves: alternate, elliptical to oval, 4.5–6.5 cm long, and 2.5–4.5 cm wide, with small, rounded teeth around the edge [6]. Flowers: a few short, axillary racemes with white, fragrant flowers are present. Five oblong petals, 10–12 mm long. Juice sacs are made up of yellow-green pulp vesicles, and seeds are tiny, plump, ovoid, light, smooth, and contain a white embryo [6]. All citrus fruits present the same anatomical structures.



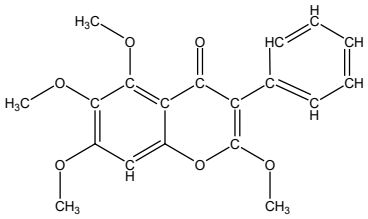
Diosmetin-7-O-neohesperidoside



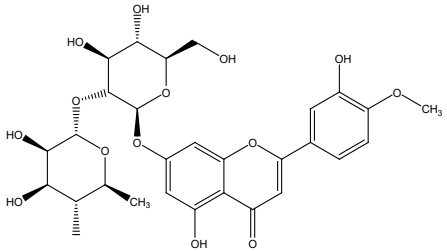
4-Hydroxy-6-methylpyran-2-one



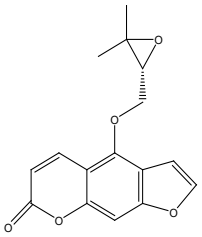
β- phellandrene



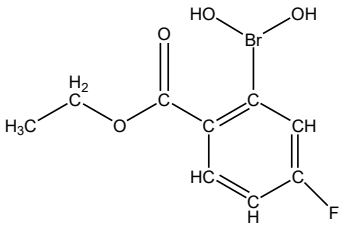
5’-Tetramethoxyisoflavone



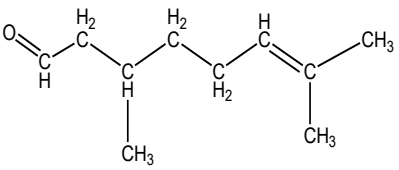
Diosmetin-7-O-neohesperidoside



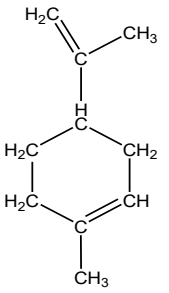
Oxypeucedanin



2-Ethoxycarbonyl-5



Citronellal



D-limonene

Figure 1. Chemical compounds of citrus species.

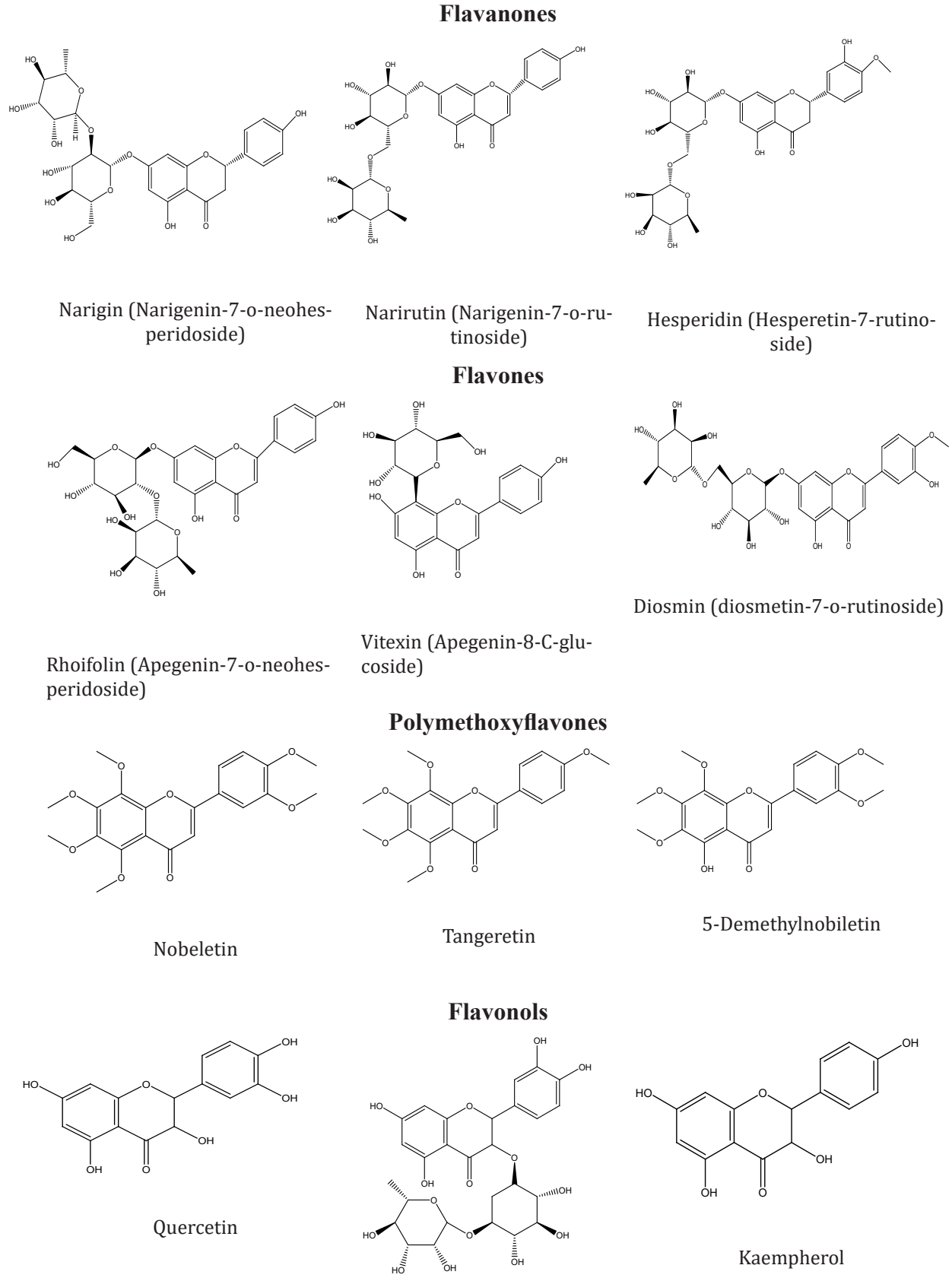


Figure 2. The major flavonoids of citrus fruits [7].

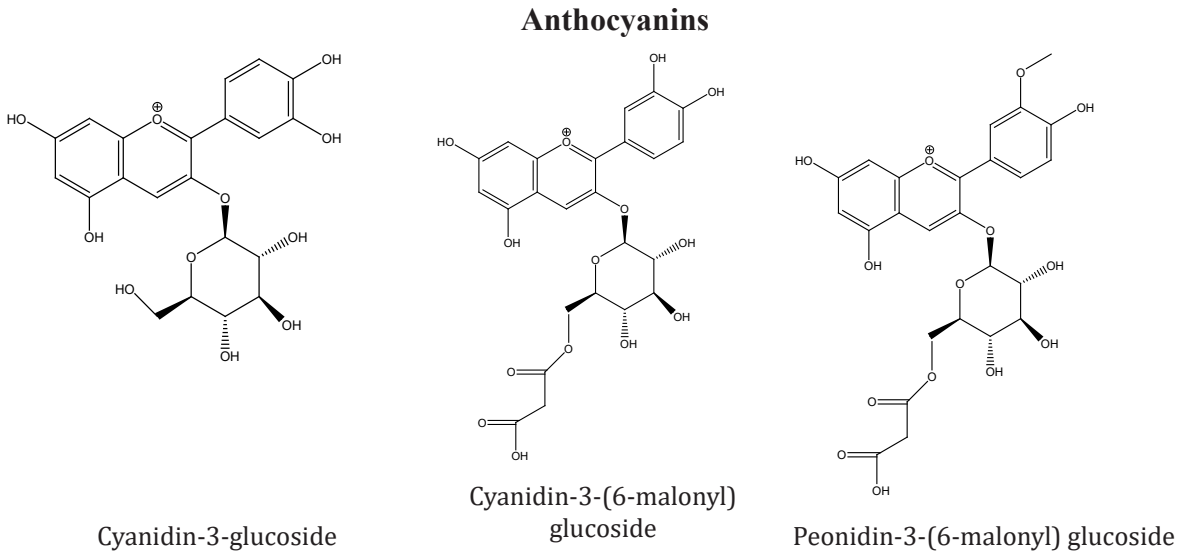


Figure 2 (continued). The major flavonoids of citrus fruits [7].

Figure 3. A - *Citrus microcarpa* mature fruits [5]. B - Gross morphology of *Citrus aurantifolia* (a) stems.(b) leaves; (c) white flowers in different stages; (d) ripe yellow and unripe green fruits; and (e) seeds [6].

The kaffir lime tree typically grows to a height of 3 to 5 metres and a width of 2.5–3 m. Small, dark green, warty fruits with a diameter of 4 cm accompany its tiny white blooms [9]. In some uses, the leaves of the kaffir lime are more significant than the fruits since they are different from those of other citrus species. Fruits are large, warty or bumpy, verrucose, globose, ovoid to elliptic, green turning yellowish-green when ripe, 5–7 cm in diameter (Figure 3B), with yellowish pulp, thick rind, and very acidic and bitter taste [9–10].

These leaves are lustrous and dark green in colour. Each leaf appears to be two leaves joined together. The top leaflet is joined to a lower leaflet that is wider on its upper border and has a bright point at the tip. The leaves can vary greatly in size, and the larger leaves often have deeper colours [10].

MEDICINAL VALUES OF CITRUS SPECIES

Numerous studies have been conducted to extract and identify the bioactive substances found in various citrus sections in an effort to better understand how these substances relate to nutrition, health benefits, or a decreased risk of disease. Sugars, pectin, carotenoids, flavonoids, vitamin C, folic acid, and essential oils are natural components found in citrus peel that have proven to be highly beneficial for both the food industry and the maintenance of human health. Citrus fruit has long been known to provide health benefits. James Lind, a British navy doctor, observed in 1747 that sailors with scurvy recovered completely after consuming oranges and lemons, which are today recognized as excellent sources of vitamin C [11].

Many pharmacological activities, including antibacterial, antifungal, antioxidant, anti-inflammatory, and anticancer activity, are exhibited by crude extracts and phytochemicals isolated from different sections of *C. hystrix* (Table 1) [12]. Citrus flavonoids, which prevent human platelet aggregation and lessen capillary fragility and permeability, have also been recommended for the treatment of vascular diseases [13]. Additionally, polyethoxylated citrus flavonoids improve lipid metabolism by reducing hepatic B

secretion, which in turn lowers blood cholesterol and triglyceride levels [13]. Citrus fruits include bioactive components such as ascorbic acid, flavonoids, phenolic compounds, and pectin, which have anti-proliferative, antibacterial, anti-aging, and antioxidant properties [14–17]. A previous review also discussed the antioxidant capacity of volatile compounds and the hypolipidemic effect of *Citrus aurantifolia* (Swingle) [18–19]. Table 3 summarizes some of the pharmacological activities of various citrus species, highlighting their antimicrobial, anticariogenic, and other beneficial effects. *C. aurantifolia*, for instance, shows antimicrobial activity against multiple pathogens, including S*taphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudo-monas spp.* [20]. Through in vivo investigations, the nutraceutical potential of dietary fibre made from the peel and pulp of citrus fruit may be further explored for broader use.

SAMPLE PREPARATION METHODS FOR CITRUS PHYTOCHEMICAL ANALYSIS

The initial phase in the analytical process, sampling, should be regarded as vital in the study because the outcomes rely largely on it. However, very few studies describe sample selection criteria in the case of citrus fruit, and samples might be either processed juice or fresh fruit without justification. Samples are often obtained from local markets or supplied by the manufacturer; in any event, the examination is typically confined to a single manufacturing batch. Extraction is the most important step in the separation and purification of bioactive components from natural materials [26]. A technique with a short extraction time, low solvent consumption, easy operation, low cost, and high extraction efficiency would be ideal. Because of the chemical composition of medicinal plants, numerous parameters are sometimes not satisfied. Heat reflux extraction is a traditional method for extracting citrus fruit, but it has the drawbacks of chemical transformation, solvent waste, and complicated operation. Some of the popular methods for the extraction of industrially important valuable compounds from citrus peels include reflux distillation, shaking, stirring, microwave extraction, ultrasonic extraction, and so on [27–32]. Modern and rapid techniques that use less solvent and energy include supercritical fluid extraction, ultrasonic extraction, the controlled pressure-drop procedure, and subcritical water extraction. Supercritical fluids provide a number of benefits, including nontoxicity, nonflammability, no chemical residue, and low/moderate working temperatures and pressures [33]. A study revealed that by employing advanced extraction techniques, researchers identified 17 volatile compounds in calamansi juice that had never been documented before [24]. Microwave hydro-diffusion and gravity (MHG) is superior to conventional techniques since it requires shorter extraction times and less solvent. It also operates at atmospheric pressure, utilizing microwaves and gravity without the use of any solvents. In the case of citrus species analysis, the hydrodistillation method is frequently employed. Essential oil is typically extracted from citrus peel, leaves, and flesh, and the active components are then isolated from this essential oil. The most common solvents include methanol, ethanol, n-hexane, and dichloroethane [13, 34–36].

Table 1. Pharmacological activity of various citrus species.

Name of Citrus species	Pharmacological activity	Type of extract	Reference
Citrus hystrix	Anti-inflammatory Antimicrobial	Oil extract	[12]
	Antioxidant Antifungal Hepatoprotective (liver-protective) Cardioprotective (heart-protective)		[21]
	Cytotoxicity against neuroblastoma and colon cancer cells		[22]
Citrus aurantifolia	Anticariogenic	Oil extract	[20]
	Cytotoxicity against colon cancer cells	Oil extract	[15]
Citrus microcarpa	Antimicrobial	Oil extract	[23]
	Rich in Vitamin C: Boosts immunity, enhances iron absorption, and provides antioxidant benefits. **Contains Phenolic Acids**: Offers antioxidant properties, reducing oxidative stress and supporting overall health.		[24]
	antimicrobial activity against various pathogenic bacteria		[25]

ANALYTICAL METHODS

Chromatographic methods including HPLC, GC-MS, GC-FID, LC-MS, near-infrared (NIR) spectroscopy, and NMR have been used to evaluate citrus species. Moreover, some advanced techniques such as HPLC-PDA are applied to citrus. It goes without saying that various strategies exhibit a variety of benefits and drawbacks.

High-Performance Liquid Chromatography (HPLC)

HPLC has been used in several studies to assess citrus species (Table 3). In *Citrus hystrix*, identification of phenolics and flavonoids was accomplished by comparing the retention times of peaks in samples to those of standards at 280 nm [37]. Eriodictyol-7-O-rutinoside, naringenin-7-O-rutinoside, isosakuranetin-7-O-rutinoside, diosmetin-7-O-rutinoside, hesperetin-7-O-rutinoside, and hesperetin-7-O-neohesperidoside were quantified from *Citrus microcarpa* by reference to standard calibration curves obtained with the PDA detector. In addition, *Citrus aurantifolia* was analysed using an HPLC system (HP 1100) equipped with a pump, UV–visible detector (280 nm), column oven, injector, and a C18 RP column (Phenomenex Luna 5 µm C18, 250 × 4.60 mm). As standards, naringin, hesperetin, hesperidin, rutin, nobiletin, tangeretin, quercetin, diosmin, and apigenin were used. Their content was determined using the integrated peak area of the sample and the associated standard [38]. An Agilent 1260 Infinity liquid chromatography system, equipped with a quaternary solvent delivery system, an autosampler, and a DAD detector, was used. A Zorbax SB-C18 column (4.6 × 150 mm, 3.5 µm) was utilized, and the detection wavelength was set at 310 nm to analyse *Citrus hystrix* [39].

Liquid chromatography-mass spectrometry (LC-MS)

Traditional methods for performing extensive chemical analyses on citrus species are inefficient and inadequate. Liquid chromatography with mass spectrometry has recently been employed to examine the complex bioactive components (Table 4). According to a study, the phytoconstituents of *Citrus microcarpa* root methanol extract (CRME) were discovered for the first time using LC-ESI-MS/MS. A total of 39 compounds were identified, including carboxylic acids, flavonoids, biflavonoids, polyflavonoids, catechins, and stilbene derivatives from calamansi fruit [40]. For analysing *Citrus hystrix*, HR-LC-MS was performed using a 1290 Infinity UHPLC system, a 1260 Infinity Nano HPLC with Chip Cube, and a TOF/Q-TOF Mass Spectrometer. Metabolites identified from the methanol extract of *Citrus hystrix* D.C. fruit are shown in Table 4 [39]. Five furanocoumarins were elucidated and identified by LC-MS and 1H NMR as follows: bergamottin, 6′,7′-dihydroxybergamottin, 6′,7′-epoxybergamottin, oxypeucedanin, and oxypeucedanin hydrate [41].

Table 3. Phytochemical analysis of citrus species by HPLC.

Species	HPLC Conditions	Part	Analytes	References
Citrus hystrix	**For Phenolics** Solvent system: Acetic acid solution (3%) and methanol Gradient/Isocratic: Likely isocratic **For Flavonoids** Solvent system: Acetonitrile, water, and acetic acid Gradient/Isocratic: Likely isocratic	Peel	Gallic acid, catechin, caffeic acid, epicatechin, p-coumaric acid, ferulic acid, rutin, and quercetin; naringin, hesperidin, naringenin, hesperetin, and nobiletin	[37]
Citrus aurantifolia	Solvent system: H_2O/formic acid (0.1%) (A) and methanol (B) Gradient: 2 min 100% A; 8 min 80% A; 55 min 100% B; 65 min 100% A Run time: 65 min	Peels, leaves	Rutin, apigenin, quercetin, kaempferol, nobiletin, tangeretin, hesperidin	[38]
Citrus microcarpa	Solvent system: Acetonitrile in water containing 0.1% formic acid Gradient: Yes Run time: Not specified (inferred from conditions) Flow rate: 1 mL/min Detection: UV at 280 nm (flavanones), 365 nm (flavonoids), and MS (negative ion mode, 100–2000 amu)	Peels, leaves	Apigenin-6,8-di-C-glucopyranoside, apigenin-8-C-glucoside-2′-rhamnoside, phloretin-3′,5′-di-C-glucopyranoside, diosmetin-7-O-neohesperidoside, hesperetin-7-O-rutinoside, hesperetin-7-O-neohesperidoside	[8]

Table 4. Phytochemical analysis of citrus species by LC-MS.

Species	LC-MS condition	Part	Analytes	References
Citrus hystrix	Solvent system: Solvent A: 0.1% formic acid Solvent B: 90% acetonitrile with 0.1% formic acid Column: Luna® 5 µm C18, 150 × 2 mm Gradient: Yes Flow rate: 0.2 mL/min Run time: Solvent A: 95% → 5% in 15 min, then 5% → 95% in 10 min until 15 min. Solvent B: 5% → 95% in 15 min, then 95% → 5% in 10 min until 15 min MS mode: Positive ionization MS range: 250–1000 m/z Scan rate: 1.00 spectra/sec	Pulp	4-Hydroxy-6-methylpyran-2-one, tranylcypromine glucuronide, 2H-indol-2-one, 1,3-dihydro-4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-, rhoifolin, diosmin, buddleoflavonoloside, 2-ethoxycarbonyl-5,7-dihydroxy-8,3′,4′,5′-tetramethoxyisoflavone, demethylmethoxsalen, phytosphingosine	[39]
Citrus microcarpa	Instrument: LC coupled with tandem MS/MS Ionization mode: Electrospray ionization (ESI), negative mode Column: Reverse-phase LC column (C18) Mobile phase: Solvent A: Water with 0.1% formic acid; Solvent B: Acetonitrile with 0.1% formic acid Flow rate: 0.3 mL/min Column temperature: 30 °C Injection volume: 10 µL MS settings: Scan range 100–1500 m/z; collision energy 20–40 eV (optimized) Detection mode: Full scan MS with MS/MS fragmentation Run time: ~30 min	Roots	A total of 39 compounds identified, including carboxylic acids, flavonoids, biflavonoids, polyflavonoids, catechins, and stilbene derivatives. In addition, phytochemical isolation of 2,3,2″,3″-tetrahydro-4′-O-methylamentoflavone and amentoflavone	[40]

Gas Chromatography–Flame Ionization Detector (GC-FID)

The key benefits of SPME-GC-FID (Solid Phase Microextraction Gas Chromatography with Flame Ionization Detection) are good selectivity, linearity, and higher upper values of the operating range [43]. This method is commonly used to evaluate citrus species (Table 5).

In kaffir lime (*Citrus hystrix*), GC–FID was performed using a fused-silica PB-WAX capillary column (60 m × 0.25 mm i.d., 0.32 µm film thickness), which identified 29 phytochemicals, with limonene, β-pinene, and terpinene-4-ol as the principal compounds [42].

Another study reported that GC-FID analyses were carried out on an Agilent 6890N gas chromatograph coupled to an FID detector. The chromatographic conditions and column (HP-5MS) were the same as for GC-MS. The FID detector was set at 300 °C. In the case of *Citrus aurantifolia* isolation, major volatile compounds were identified: α-phellandrene (48.5%), p-cymene (16.5%), α-pinene (12.6%), and (E,E)-α-farnesene (12.6%) [35]. A total of 60 volatile compounds in calamansi juice were detected using GC coupled with an FID and a 5975 inert MSD (Agilent Technologies, Santa Clara, CA), equipped with a fused-silica capillary column (60 m × 0.25 mm × 0.25 µm DB-FFAP, Agilent Technologies). The injector temperature was fixed at 250 °C. The GC oven program started at 50 °C for 5 minutes, then increased to 230 °C at 5 °C/min and held for 30 minutes. The FID temperature was 250 °C. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The ionization mode in MS was electron impact (EI) at an energy of 70 eV [24].

Although FID is a common detector used for GC in clinical laboratories, it responds poorly or not at all when exposed to substances such as H_2S, CCl_4, or NH_3. Because the detector is mass-sensitive rather than concentration-sensitive, variations in the carrier gas flow rate have little impact on detector response. With its wide detection range, FID is ideal for broad hydrocarbon gas analysis.

Gas Chromatography–Mass Spectrometry (GC-MS)

Volatile organics and phenolic acids from citrus species are determined using gas chromatography (Table 6). The GC-MS approach can identify bioactive components of citrus species, particularly volatile organics. It requires very high operating temperatures (typically 225–350 °C), but its accuracy level is much higher than that of other methods. A total of 30 chemical substances out of 33 were identified from *Citrus aurantifolia* [44]. A study showed that GC-MS revealed the oil of *Citrus microcarpa* comprised 30 chemical compounds in total, as listed in Table 7 [23]. While certain non-volatile components require more sophisticated procedures, GC-MS for volatile components offers simple, quick, and effective analysis. To examine volatile components, a 2D-GC system with high peak capacity and orthogonal characteristics can be employed, which should be considered for ongoing research.

UV/PDA Detector

The standard detector for qualitative and quantitative study of chemical components in citrus species is the UV detector [38]. Because of its low cost and ease of use, the detector has become one of the most commonly used analytical tools in laboratories. Consequently, it has been frequently employed to identify flavonoids and antioxidant compounds. Distinct types of biological substances have different detection wavelengths [40–45]. It has been reported that gallic acid, catechin, caffeic acid, epicatechin, p-coumaric acid, ferulic acid, rutin, quercetin, naringin, hesperidin, naringenin, hesperetin, and nobiletin are identified at 280 nm [37].

Table 5. Phytochemical analysis of citrus species by GC-FID.

Species	GC-FID condition	Part	Analytes	References
Citrus hystrix	Instrument: Perkin-Elmer AutoSystem Column: Fused-silica PB-WAX capillary (60 m × 0.25 mm i.d., 0.32 μm film thickness) Column temp: 90 °C for 4 min, ramp to 220 °C at 10 °C/min, hold for 3 min Injector temp: 190 °C Detector temp: 220 °C Carrier gas: Helium, 1.0 mL/min Injection volume: 1.5 μL Split ratio: 1:10	Peel	Limonene, β-pinene, ter-pinene-4-ol	[42]
Citrus aurantifolia	Instrument: Agilent 6890N Gas Chromatograph with FID detector Column: HP-5MS capillary (30 m × 0.25 mm × 0.25 μm) Carrier gas: Helium 5.0, 1.0 mL/min Temperature program: 60 °C to 150 °C at 3 °C/min; 150 °C to 280 °C at 5 °C/min; total run time 60 min Injector temp: 280 °C Injection volume: 1 μL (sample in pentane) Split ratio: 40.8:1 FID detector temp: 300 °C	Pericap	α-Phellandrene (48.5%), p-cymene (16.5%), α-pinene (12.6%), (E,E)-α-farnesene (12.6%)	[35]
Citrus microcarpa	Instrument: Agilent 6890N GC coupled with FID and MSD (5975 inert) Column: Fused-silica capillary DB-FFAP (60 m × 0.25 mm × 0.25 μm) Carrier gas: Helium, 1.2 mL/min Injection volume: 1 μL (HS-SPME, splitless) Injector temp: 250 °C Temperature program: 50 °C for 5 min; ramp to 230 °C at 5 °C/min; hold at 230 °C for 30 min FID detector temp: 250 °C	Flesh	Terpene hydrocarbons	[24]

Table 6. Phytochemical analysis of citrus species by GC-MS.

Species	GC-MS condition	Part	Analytes	References
Citrus hystrix	Instrument: Agilent 6890N GC, 5973 inert MS Column: HP-5MS Carrier gas: Helium, 1.0 mL/min Injection volume: 1.0 µL Injector temp: 250 °C (split ratio 1:100) Oven program: 50 °C for 2 min → 300 °C for 5 min Head column pressure: 9.3 psi	Peel	β-Pinene (45.21%), α-pinene (2.37%), D-limonene (18.36%), citronellal (17.75%), terpinen-4-ol (5.00%), γ-terpinene (1.03%), α-terpineol (1.77%), citronellol (1.24%)	[25]
Citrus aurantifolia	Instrument: Perkin Elmer Clarus 600 GC-MS Column: Rtx-5MS (30 m × 0.25 mm i.d. × 0.25 µm, max temp 350 °C) Carrier gas: Ultra-high purity helium (99.9999%), 1.0 mL/min Injection volume: 1.0 µL Split ratio: 100:1 Injector temp: 280 °C Transfer line temp: 260 °C Ion source temp: 260 °C Ionization energy: 70 eV Mass range: 40–550 amu Oven program: 60 °C → 280 °C at 3 °C/min; hold 2 min	Leaves	D-limonene (63.35%), 3,7-dimethyl-2,6-octadien-1-ol (7.07%), geraniol (6.23%), E-citral (4.35%), Z-citral (3.29%), β-ocimene (2.25%)	[44]
Citrus microcarpa	Instrument: Agilent® 7890A GC, Agilent® 5975 MS detector Column: HP-5MS (30 m × 0.25 mm i.d. × 0.25 µm) Carrier gas: Helium Gas flow rate: 3 mL/min Column pressure: 70 kPa Injection volume: 2 µL Injector temp: 225 °C Detector temp: 270 °C Ionization energy: 1.25 kV Oven program: 50 °C for 6 min → 80 °C at 2 °C/min (hold 1 min) → 250 °C at 4 °C/min (hold 8 min)	Leaves, peels	D-limonene (29.52%), (R)-(+)-citronellal (13.76%), 3-isopropenyl-5,5-dimethylcyclopentene (8.88%), γ-terpinene (7.30%), citronellol (6.90%), terpineol (6.90%); calamansi peel also contained 4.61%	[23]

The highest concentration of hesperetin-7-O-neohesperidoside was found in *C. hystrix* leaf at 250–700 nm [46]. From *Citrus aurantifolia*, flavonoids such as vicenin 2; 5,7,4′-trihydroxyflavone 6,8-di-C-β-D-glucoside; lucenin-2 4′-methyl ether; 5,7,3′-trihydroxy-4′-methoxyflavone 6,8-di-C-β-D-glucoside; eriocitrin; 5,7,3′,4′-tetrahydroxyflavanone 7-β-rutinoside; isonaringenin; 5,7,4′-trihydroxyflavanone 7-β-rutinoside; hesperidin; and 5,7,3′-trihydroxy-4′-methoxyflavanone 7-β-rutinoside were identified at 365 nm using a UV detector [47].

However, DAD has better recognition capabilities than conventional UV detection [48]. It can be used to identify and quantify neutral and acidic compounds of citrus species. Nowadays, the PDA detector is commonly used for analyzing citrus species [13].

COMPARATIVE ANALYSIS OF ANALYTICAL TECHNIQUES FOR CITRUS PHYTOCHEMICAL PROFILING

The accurate identification and quantification of citrus phytochemicals require the selection of appropriate analytical techniques based on the chemical nature of the target compounds and the complexity of the sample matrix. Various methods, ranging from chromatography to spectrometry and UV-based detection, have been applied to investigate citrus bioactives. Each technique comes with distinct technical advantages and inherent limitations, influencing the depth, reliability, and applicability of the phytochemical data obtained.

High-Performance Liquid Chromatography

Table 7. The results of the chemical compound analysis of Calamansi peel (GC-MS) [23].

No.	Content (%)	Retention time	Compound
1	29.52	14.88	D-Limonene
2	13.76	23.46	(R)-(+)-Citronellal
3	8.88	11.34	3-Isopropenyl-5,5-dimethylcyclopentene
4	7.30	24.86	γ-Terpinene
5	6.90	27.84	Citronellol
6	4.61	25.57	α-Terpineol
7	4.27	11.46	γ-Terpinene
8	2.58	19.71	L-β-Pinene
9	2.37	38.29	α-Farnesene
10	2.05	16.66	γ-Terpinene
11	1.82	8.82	(R)-α-Pinene
12	1.66	22.58	(+)-Neoisoisopulegol
13	1.60	28.02	Citronellol
14	1.50	13.74	α-Terpinene
15	1.46	12.36	β-Myrcene
16	1.37	45.33	α-Sinensal
17	1.18	28.05	Citronellol
18	1.17	17.60	trans-Linalool oxide (furanoid)
19	0.79	18.54	Terpinolene
20	0.72	13.07	Octanal
21	0.66	39.67	β-Germacrene
22	0.58	17.75	1-Octanol
23	0.58	18.64	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate
24	0.47	26.44	Decanal
25	0.45	28.95	3-Carene
26	0.39	32.96	2,6-Dimethyl-2,6-octadiene
27	0.38	20.75	γ-Terpinene
28	0.38	16.09	β-Ocimene
29	0.32	32.10	1-Terpinenol
30	0.31	37.26	Germacrene D

(HPLC) is widely utilized for detecting non-volatile and thermally unstable compounds such as flavonoids, phenolic acids, and glycosides. It offers excellent resolution and sensitivity and can be coupled with detectors like UV, PDA, or DAD for enhanced selectivity. However, HPLC systems are expensive to operate and may face challenges such as co-elution or compound adsorption onto columns, necessitating careful optimization and solvent selection [49].

Gas Chromatography–Mass Spectrometry (GC-MS) is the method of choice for volatile constituents like limonene, pinene, and citral found in citrus essential oils. It provides both separation and structural identification, making it highly effective for essential oil profiling. Nonetheless, it is not suitable for non-volatile or thermolabile compounds and may require prior derivatization for certain analytes.

Liquid Chromatography–Mass Spectrometry (LC-MS) has gained traction due to its ability to analyze a broad range of polar and heat-sensitive compounds without the need for derivatization. It is particularly useful in profiling complex matrices and enabling metabolomics studies [49]. Despite its high precision, LC-MS is prone to matrix effects such as ion suppression and is limited by its high cost and technical complexity [50].

Gas Chromatography with Flame Ionization Detection (GC-FID) is a simpler and more cost-effective tool for volatile compound analysis, offering high sensitivity and good reproducibility [51]. However, unlike MS, it provides no structural information, making it less informative for compound identification [52].

Spectrophotometric detectors such as UV, PDA, and DAD are commonly integrated with chromatographic techniques to detect citrus phytochemicals with UV-absorbing properties. These detectors are relatively affordable and allow multi-wavelength monitoring. However, they have limited sensitivity compared to MS and cannot distinguish closely related structural isomers without adequate chromatographic separation.

Given the diversity of phytochemicals present in citrus species, no single method can be universally applied. A combination of chromatographic separation and advanced detection technologies is often necessary to obtain comprehensive phytochemical profiles. The table below summarizes the key advantages and limitations of each analytical method.

CONCLUSIONS

This review has outlined a comprehensive range of analytical techniques, such as HPLC, GC-MS, LC-MS, and FTIR, used to extract, identify, and quantify phytochemicals in citrus species. These methods have proven effective in detecting flavonoids, essential oils, and other bioactive compounds across various citrus parts, including peel, leaves, pulp, and roots. Among them, LC-MS has emerged as a particularly powerful tool for profiling thermolabile and non-volatile constituents, while GC-MS remains valuable for volatile components.

Despite these advances, a major limitation is the lack of standardized analytical protocols, which hinders the reproducibility and comparability of data across studies. Variability in sample preparation, extraction methods, and detector types often leads to inconsistent outcomes. There is an urgent need to develop and adopt harmonized procedures, including validated extraction protocols, calibration techniques, and reporting formats, to ensure robust quality control and support regulatory acceptance in food, pharmaceutical, and nutraceutical applications.

Looking forward, future research should focus on:

Establishing certified reference materials and internal standards for key citrus phytochemicals;

Expanding studies to include underexplored plant parts such as roots, seeds, and pericarps;

Applying green and efficient extraction technologies such as pressurized liquid extraction and solvent-free microwave methods;

Leveraging data science tools, including chemometrics and machine learning, to interpret complex datasets;

Integrating multi-omics platforms (e.g., metabolomics and transcriptomics) to uncover novel bioactive compounds and metabolic pathways.

By advancing analytical precision and implementing standardized practices, future research can drive innovation and sustainability in citrus-based therapeutics, functional foods, and value-added products..

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Declarations Statement of Usage of AI

We confirm that artificial intelligence was not used in the preparation of this article.

Competing Interest

The authors declare no potential conflicts of interest.

Ethical Approval Not applicable
Informed Consent Not applicable

Table 8. The advantages and shortcomings of technique analysis for citrus species.

Technique	Advantages	Shortcomings	Reference
HPLC	Rapid analysis; convenient operation; high sensitivity and specificity	Requires volatile organic solvents; high cost	[53]
GC/GC-MS	Rapid analysis; low solvent consumption	Limited to volatile compounds; requires derivatization	[53-55]
GC-FID	Low cost; can detect low concentrations; rapid results	Can only detect organic substances; destructive method	[56-57]
LC-MS	High efficiency in separation; high resolution; applicable to a wide range of samples	Requires volatile buffer; expensive; requires specialized equipment and more space	[54]

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