DOI : 10.58993/ijh/2025.82.2.16

Indian J. Hortic. 82(2), June 2025: 231-239

Phytoconstituent analysis, secondary metabolite profiling, and antioxidant activities of immature dropped kinnow fruits: Unveiling nature's biochemical treasures

Manju Bala[1*], Mridula D.[2], Simran Arora[1], Sonmati R. Kumar[1], Shivani[1] and R.K. Vishwakarma[1]

[1]ICAR-Central Institute of Post-Harvest Engineering and Technology, Ludhiana 141004, Punjab, India.

ABSTRACT

The naturally dropped immature fruits of kinnow (mandarin) are usually considered as waste but they offer a great deal of potential for use as pharmaceuticals. The present study has been conducted to assess the status of polyphenols by LC-MS analysis of immature dropped kinnow fruits (IDKF) along with proximate composition, mineral analysis, and antioxidant capacity. The proximate analysis of IDKF revealed the presence of 10.70% protein, 7.40% sugars, 1.85% fat, 6.82% minerals, and 68.39% dietary fiber. Mineral analysis reported macronutrients namely Na, K, Ca, and Mg, and micronutrients namely Mn, Cu, Zn, and Fe. Vitamins like riboflavin and niacin were also reported in small quantities. Total phenolic and flavonoid contents were reported as 5.52 g GAE/100g and 8.80 g QE/100g. DPPH and FRAP activity of 364.04 mg AAE/100g and 8.61 g TE/100g, respectively were assessed to determine antioxidant potential. Amino acid profiling revealed the presence of arginine, aspartate, glycine, and serine, and essential amino acids such as threonine, and leucine. LC-MS analysis identified 55 metabolites, rich in specific flavonoids such as kaempferol, tangeretin, and nobiletin. GC-MS analysis of the hexane extract revealed the presence of 44 volatile compounds, including cis-Vaccenic acid, D-limonene, and 5, 7-dimethoxycoumarin. The goal of this compilation is to encourage the valorization of IDKF to enhance the production of value-added products like phytochemicals with various nutraceuticals properties.

Key words: Antioxidant, bioactive compounds, immature, metabolites, proximate.

INTRODUCTION

The agricultural ecosystems and industry generate vast amounts of waste, much of which is improperly disposed of, harming contributing to environmental degradation. Untreated agro-industrial waste is often burned, dumped, or sent to landfills, causing pollution and greenhouse gas emissions. Thus, sustainable approaches for utilizing agricultural waste are urgently needed. Two major strategies are the production of value-added by-products from agricultural and agro-processing wastes and the advancement of sustainable energy technologies. Many studies have explored using fruit waste and by-products in the food processing and pharmaceutical industries (Vilas-Boas *et al*., 22).

Citrus fruits, especially kinnow (*Citrus reticulata*), are widely produced and consumed due to their exceptional flavor and health benefits. Kinnow is rich in potassium, folic acid, pectin, and vitamin C. The fruit is not only valued for its edible components, such as juice rich in ascorbic acid and antioxidants, but also for its non-edible parts, including seeds and peels, which contain bioactive compounds like hesperidin and naringin with health-promoting properties, and essential oils used in preservatives, cosmetics, and pharmaceuticals (Manzoor *et al*., 14).

Kinnow's adaptability and high market demand make it a preferred crop for growers, particularly in states like Punjab, Rajasthan, and Haryana, with Punjab's agroecological conditions proving ideal for kinnow cultivation (Kaur *et al*., 10). However, a significant portion of kinnow fruits undergo physiological drop, particularly during the immature fruit stage, leading to the wastage of potentially valuable resources and contributing to environmental pollution. Immature fruits typically contain higher concentrations of bioactive compounds compared to mature fruits, making them beneficial for extracting value-added products. Immature droppings of kinnow fruits (IDKF) represent a major waste fraction, as only a small percentage of flowers develop into marketable fruit. Pre-harvest fruit drop results in considerable economic losses, with reports of a 10-20% drop during this stage (Kaur *et al*., 10).

Although IDKF was once considered waste, its high phytochemical content is now gaining attention for its potential to produce valuable compounds. However, no scientific literature is available regarding the nutritional and functional characteristics of these droppings. Metabolomics, using techniques like LC-MS and GC-MS, provides a powerful approach to study

*Corresponding author email: manju.bala1@icar.gov.in

[2]ICAR-Central Institute for Women in Agriculture, Bhubaneswar, Odisha, India

the changes in metabolites during plant growth and can be used to track biochemical and physiological parameters in plants (Saini *et al*., 18). Despite extensive metabolomics studies on citrus fruits, there is a lack of data on the profiling of secondary metabolites in immature kinnow fruit droppings.

This study aims to fill this gap by performing an in-depth analysis of the biochemical and physicochemical properties of immature kinnow fruit droppings. The goal is to provide insights into the valorization of this agricultural waste and develop new technologies to recover valuable bioactive compounds, thereby promoting the sustainable use of agro-waste.

MATERIALS AND METHODS

Immature dropped kinnow fruits (IDKF) were collected from Abohar, washed, and sliced. The average diameter and weight were 16.97±2.95 mm and 4.74±0.78 g, with moisture content of 68.91±0.04% and pH of 5.40±0.014. After blanching at 95°C for 3 minutes, the slices were tray-dried at 50°C, pulverized into fine powder, and stored at -4°C. The IDKF powder was analyzed for total protein, sugars, fat, dietary fibers, amino acids, minerals, vitamins, and bioactive compounds, with all analyses performed in triplicate (n=3). The colour properties of the IDKF sample were determined by using a Hunter lab colorimeter. The color coordinates such as 'L' for lightness (vary from 0 i.e., black to 100 i.e., pure white, 'a' for redness, and 'b' for yellowness were measured using 45/0 illuminant. The Chroma (C*) and Hue angle (h*) were calculated by using following equations.

$$\text{Chroma } (C^*) = (a^2 + b^2)^{1/2}$$

$$\text{Hue angle } (h^*) = \tan^{-1} (b/a), \text{ when } a > 0 \text{ and } b > 0$$

Water retention capacity (WRC) and oil retention capacity (ORC) were estimated and represented as g water retained by sample/g DW (dry weight) and g oil retained by sample/g DW. For the characterization of powder flowability, bulk and tapped density along with Carr index, Hausner ratio, and Angle of repose (AoR) were estimated (Kalsi *et al*., 9).

The moisture content was determined by drying the sample at 105°C in the oven until a constant weight was attained. Total sugars, crude protein and the fat content were determined using standard methods (AOAC, 3). Mineral analysis of IDKF samples was performed using ICP-AES after di-acid digestion. A 500 mg sample was digested in a 3:1 nitric acid-perchloric acid mixture, then cooled, diluted to 25 mL, filtered, and stored. Elements (K, Mg, Na, Ca, P, Fe, Zn, Mn, Cu) were quantified. The instrument operated at 27.12 MHz and 2.5 kW with a CID detector, and mineral content was expressed as mg/100 g dry weight.

Amino acid analysis of IDKF was conducted using HPLC. Following derivatization, a 2.5 µL extract was injected into a 150 × 4.6 mm column at 40°C, with detection at 338 nm. The mobile phase included 40 mM NaH_2PO_4 (pH adjusted with NaOH) and a solvent mixture (Acetonitrile: Water: Methanol, 45:10:45). Separation followed a gradient program, starting at 0% B for 1.9 minutes, increasing to 53% B over 16.3 minutes, with a 2 mL/min flow rate. The process concluded with a 26-minute washing (100% B) and equilibration (0% B). Metabolite analysis was conducted using LC-MS with ESI. Gradient elution (water, methanol, acetonitrile) was followed by 15-minute degassing at 21°C. A 10 µL sample was injected at 0.350 mL/min onto a C18 column. The Orbitrap mass analyzer (30,000 resolution, m/z 50–1300) identified metabolites based on retention time and mass spectra comparison with standards. GC-MS analysis was performed for saturated alkanes in IDKF hexane extract using AI 3000 autosampler and DSC II mass spectrometric detector. An analytical column of length,

IDKF powder was extracted with 50% ethanol (1:10 w/v) using ultrasonication (750 W, 6.63 min, 45.82% amplitude, 4.24 sec pulse cycle). The extract was centrifuged (4000 rpm, 20 min), evaporated, and freeze-dried. Total phenolic acid content (TPC), total flavonoid content (TFC), ferric-reducing antioxidant power (FRAP), and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity were estimated by following the method given by Hayat *et al*. (6). Total alkaloid and saponins content were estimated by the method given in Okwu *et al*. (15).

RESULTS AND DISCUSSION

The L*, a*, and b* values of dried IDKF (in powder form) were 57.45, 0.55, and 20.45, respectively. The Hue angle and chroma of IDKF were reported as 88.46° and 20.46° respectively. Hue is generally considered as a qualitative color aspect i.e., red, yellow, green, and blue are represented by 0°, 90°, 180°, and 270° respectively. On the other hand, chroma is considered as quantitative aspect. The water and oil absorption capacities of IDKF were 3.31 g/g and 1.42 g/g samples, which are also important if they are used in product development formulations. The AoR of IDKF was measured as 26.93°. The powder with AoR less than 40° is regarded as a free-flowing material and is also influenced by its moisture content indicating an excellent flowability of IDKF. The AoR of powdered materials affects the flowability, packing, and handling of the powder, making it an important parameter in a variety of industries, including the food and agriculture sectors (Kalsi *et al*., 9). It is also crucial for process optimization and

quality control to comprehend and characterize the angle of repose for IDKF powder.

The bulk, tapped and true density of IDKF were reported as 0.724 g/ml, 0.7825 g/ml, and 1.63 g/ml respectively, while Carr index and Hausner ratio were observed as 7.407% and 1.08 respectively along with porosity of 55.6%. Bulk density is a pivotal determinant in establishing the packaging and transportation needs of a product. The higher porosity indicates that IDKF is more prone to oxidation and the Carr index from 5 to 10% represents its excellent flow properties (Anuar *et al*., 2). Bakshi and Ananthanarayan, (4) reported a Hausner ratio of 0.72 and 0.97 in lemon and coconut peel powder respectively suggesting that powder with a Hausner ratio of less than 1.4 is characterized by better flowability. Therefore, all these parameters are very crucial for characterizing the flow properties of powders in order to understand how it will be handled during processing. The crude protein and total sugars were reported as 10.70% and 7.40% in dried IDKF respectively (Fig. 1). The fat content and moisture content were found to be present as 1.85 and 9.34% respectively in dried IDKF. The total dietary fiber content was 68.3% and the insoluble fiber content was found to be 45.3% in dried IDKF. However, the soluble dietary fiber content was about 23% in dried IDKF (Fig. 1). Heena *et al*. (8) also reported moisture, fat, and protein content of immature dropped fruits of *citrus reticulata* as 9.5%, 7.0%, and 13.25% respectively.

Studies have shown kinnow peel waste contains 52.04%–60.97% dietary fiber under ultrasound-assisted and enzymatic extraction (Kaur *et al*., 11). Other research reported dietary fiber levels of 60.4% in kinnow pulp and 45.11% in pomace, with protein content of 10.32% and 11.78%, respectively (Singla *et al*., 20). Kinnow waste is calcium-rich (2.4%) with 8.31% crude protein and 11.29% moisture (Chaudhary *et al*., 5). The pulp mainly consists of sugars and free amino acids, influencing taste and flavor. Kinnow fruits contain abundant fructose, with kinnow peels having sucrose, glucose, and fructose levels of 10.4, 10.0, and 11.2 g/100g dry weight, respectively (Hayat *et al*., 7). The total sugar content in kinnow fruit is about 5.28%, with reducing and non-reducing sugars at 2.51% and 2.90%, respectively indicating that sugar levels increase as the fruit matures, enhancing flavor (Ullah *et al*., 21).

The present study reported that dried IDKF constituted 1440, 981, 180, and 25.30 mg/100g of calcium, potassium, magnesium, and iron respectively along with 18.7, 2.29, 1.35, and 1.90 mg/100g of sodium, zinc, copper, and manganese respectively (Fig. 1). However, the total mineral content was about 6.82% in dried IDKF. This study

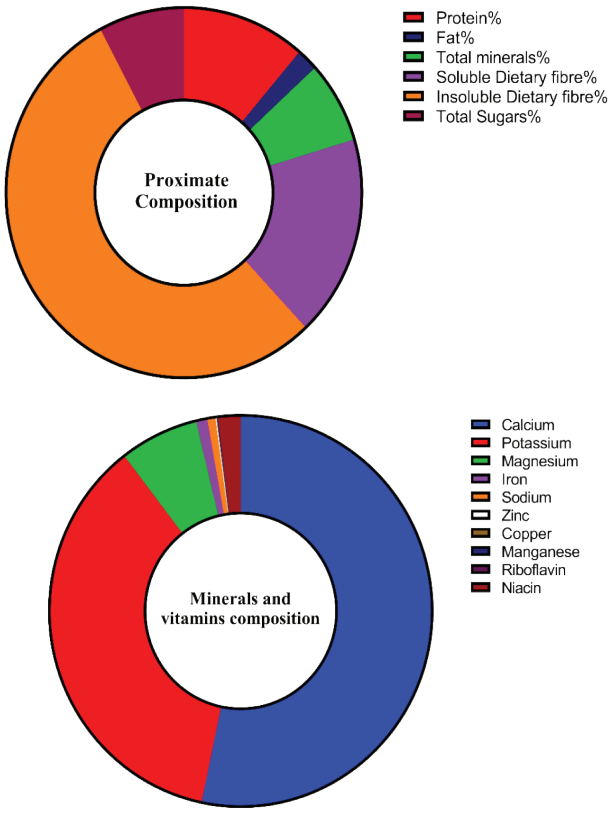

Fig. 1. Donut graphs showing proximate composition and minerals and vitamins content in dried immature dropped kinnow fruits (GraphPad Prism software version 8.4.3).

also reported 0.059 and 51.90 mg/100g of riboflavin and niacin respectively in dried IDKF (Fig. 1).

The investigation of multiple micro- and macro-elements revealed that they are found in many citrus fruits and are responsible for healing a variety of diseases. One of the studies has reported 152.2 µg of potassium, 324.67 µg of iron, and 85.3 µg of calcium per 100 g dried kinnow peels (Rafiq *et al*., 17). Minerals play important roles such as osmotic homeostasis and regulation of blood pressure. Purewal and Sandhu, (16) reported potassium, sodium, and calcium in the range of 10-30 µg/g, 160-250 µg/g, and 140-470 µg/g in kinnow fruits that are essential for maintaining bone health and activity of important mitochondrial enzymes. The potassium, calcium, sodium, and magnesium contents were investigated in the peel and pulp of kinnow fruit at different growth stages. It was found that about 6.84 mg/g of potassium, 2.36 mg/g of calcium, 3.32 mg/g of sodium, 0.26 mg/g of iron and 8.81 mg/g of magnesium in immature *citrus reticulata* fruits (Heena *et al*., 8). Our findings led to the conclusion that kinnow fruit with higher contents of calcium,

potassium, sodium, magnesium, and iron might be involved in food preparations to fulfill the routine mineral requirements to promote healthy nutrition by showing increased contents of major macronutrients i.e., potassium and sodium.

The amino acid profiling revealed that arginine (672 mg/100g) was the most abundant amino acid followed by leucine (543 mg/100g), aspartic acid (530 mg/100g), glycine (477 mg/100g), threonine (477 mg/100g), isoleucine (382 mg/100g), cysteine (370 mg/100g), proline (345 mg/100g), glutamic acid (321 mg/100g), phenylalanine (226 mg/100g), valine (226 mg/100g), tyrosine (216 mg/100g), histidine (215 mg/100g), lysine (180 mg/100g) and alanine (169 mg/100g) in dried IDKF. Methionine (58 mg/100g) was the least abundant amino acid in dried IDKF (58 mg/100g) (Fig. 2).

A study by Purewal and Sandhu (16) on kinnow juice reported arginine, aspartate, glutamine, and proline as the most abundant amino acids, aligning with our findings. LC-MS analysis of ethanolic extract from immature kinnow droppings (IDKF) identified 55 polyphenolics, including flavanones, flavonols, limonoids, flavones, and phenolics. Compounds were fragmented and separated based on their m/z values and retention times (Rt), influenced by structural properties such as methyl groups and sugar residues (Table 1). Peak identification and integration were manually reviewed for accuracy.

Kaempferol was detected at four Rt values (1.940, 2.210, 12.460, and 12.946) at m/z 287.2, indicating four isomeric forms. Epicatechin gallate appeared at m/z 441.1 with two Rt values (11.431, 12.101), showing distinct ion fragments. 7-Galloylcatechin was

Table 1. List of various metabolites analyzed by LC-MS.

S. No.	Name of compound	RT	MS	Mass fragments
		Flavonols		
1.	Taxifolin (Dihydroquercetin)	1.635	305.0	305.0, 241.0, 71.9
2.	Kaempferol	1.940	287.2	287.2, 144.1
		2.210	287.2	87.2, 287.2, 144.1
		12.460	287.1	1035.2, 911.2, 675.1, 617.1, 433.1, 287. 1, 233.1
		12.946	287.1	750.3,617.1, 551.2, 441.1, 383.6, 287.1, 177.1
3.	Limocitrol	3.653	377.1	377.1,238.1,121.1
4.	7-Galloylcatechin	2.981	222.1	438.1, 284.1, 222.1, 121.1
5.	Kaempferol-3-o-rutinoside	8.564	595.2	595.2,442.2, 362.2, 150.1
6.	Epicatechin Gallate	11.431	441.1	661.1,513.1, 441.1, 261.1, 177.1
		12.101	441.1	593.2,441.1, 362.2, 249.2, 171.1
7.	Proanthocyanidin B1	23.096	579.3	801.4,579.3,358.3,261.1,71.9
1.	Chrysin 7-[rhamnosyl-(1->4)-glucoside]	5.047	563.1	563.1,337.1,150.1
2.	Diosmetin-8-C-glucoside	10.027	463.1	675.2,523.1, 463.1, 362.2, 177.1
		10.145	463.1	936.3,675.2,579.2, 463.1, 362.2, 177.1
3.	Apigenin	15.075	271.1	471.5, 359.1, 271.1,121.1
		15.137	271.1	477.1,359.1, 271.1
4.	Tangeretin	15.622	373.1	1139.3, 950.7, 767.2, 578.1, 373.1, 230.2
		20.062	373.1	767.2, 690.2, 578.1, 373.1, 272.2
5.	Nobiletin	18.173	403.1	827.2,767.2, 623.1, 403.1, 343.1, 244.2
		21.639	403.1	697.4,579.3, 478.2, 403.1, 244.2
6.	Vitexin	12.703	433.1	898.3,795.2, 617.1, 433.1, 261.1, 177.1
		19.267	433.1	887.2, 677.2, 433.1, 346.2, 258.2, 149.1
1.	Hesperidin	7.958	611.1	611.1,487.1,357.1, 249.2,150.1
2.	Naringenin	10.269	273.1	579.2,419.1, 273.1, 150.1
1.	Methyl 2-(2-methoxy-4-hydroxyphenyl)-6-methoxy-3-benzofurancarboxylate	13.493	329.1	725.2,329.1, 261.1

Contd...

Table 1 contd...

S. No.	Name of compound	RT	MS	Mass fragments
		Limonoids		
1.	Limonin	9.123	471.2	673.2,471.2, 336.1, 150.1
2.	Limonin glucoside	13.802	651.1	651.1,456.3, 359.1, 246.2, 177.1
3.	Diacetylnomilin	16.899	473.2	967.4,737.2, 473.2, 373.1, 244.2
4.	Nomilin	19.084	515.2	1051.4,515.2, 403.1, 346.2, 244.2
5.	Nomilinic acid 17-O-beta-D-glucoside	20.912	713.2	713.2, 579.3,455.2, 344.3, 244.2
1.	2,4,6-Phenanthrenetriol 2-O-b-D-glucoside	16.110 22.190	389.1 389.1	799.2,389.1, 274.2 799.2, 579.3, 389.1, 244.2
2.	Sinapic acid	17.514	225.1	771.2, 555.2, 397.1, 225.1
3.	trans-Resveratrol 3-O-glucuronide	14.288	405.1	689.1, 405.1, 216.2
4.	Dihydroferulic acid-3- Hydroxy -3-methyl glutaryl– glucoside	14.953	503.1	886.4,503.1, 359.1, 177.1
1.	6,8-Di-O-methylaverufin	17.812	397.1	963.3,725.2, 577.1, 471.1, 397.1, 316.2, 244.2
2.	Chryso-obtusin (2-hydroxy-1,6,7,8-tetramethoxy-3-methylanthracene-9,10-dione)	14.777	359.1	886.4,689.1,359.1, 261.1, 177.1
1.	3-Indoleacrylic acid	6.694	188.1	651.2,469.2,337.1,188.1
1.	Ginger glycolipid A	19.815	677.2	677.2,531.3, 333.1, 272.2
1.	Cytidine	16.716	244.2	550.2, 244.2
1.	Ascorbic acid	11.118	177.1	927.6,657.2, 590.2, 337.1, 254.1, 177.1
1.	UK	1.814	460.8	460.8, 324.9, 268.9, 104.1
2.	UK	2.240	208.1	208.1,150.1
3.	UK	4.319	379.1	379.1,236.1,120.1
4.	UK	5.528	337.1	337.1,268.1,136.1,71.9
5.	UK	5.962	614.2	614.2, 524.2, 468.2, 400.2, 252.1, 136.1, 71.9
6.	UK	6.444	491.1	667.1,491.1,337.1,282.1,150.1
7.	UK	7.113	409.1	409.1,227.2, 150.1
8.	UK	9.546	760.2	760.2,595.2, 475.3, 395.2, 246.1, 177.1
9.	UK	14.048	607	607.2, 681.1,525.2, 329.1, 202.2
10.	UK	15.379	477.1	771.2,693.3, 477.1, 375.1, 261.1
11.	UK	21.213	697.4	697.4,500.2, 359.1, 244.2
12.	UK	24.386	261.1	261.1, 71.9

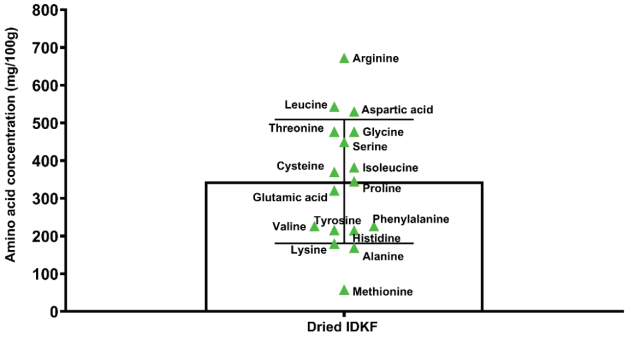

Fig. 2. Scattered plot showing the amino acids profiling in dried IDKF (GraphPad Prism software version 8.4.3).

identified at m/z 222.1 (Rt 2.981) with characteristic fragmentation. Toxifolin (m/z 305.0, Rt 1.635) displayed ion fragments at m/z 305.0, 241.0, and 71.9. Kaempferol-3-O-rutinoside (m/z 595.2, Rt 8.564) and Isorhamnetin-3-O-neohesperidoside (m/z 625.2, Rt 8.747) were also detected. Limocitrol, a hydroxyflavan, was found at m/z 377.1 (Rt 3.653). Chrysin 7-[rhamnosyl-(1→4)-glucoside], a flavone glycoside, was identified at m/z 563.1 with an Rt value of 5.047, showing characteristic ion fragments at m/z 563.1, 337.1, and 150.1. Diosmetin-8-C-glucoside appeared at m/z 463.1 with two Rt values

(10.027 and 10.145), suggesting its existence in two isomeric forms. Apigenin, a natural flavonoid, was detected at m/z 271.1 with Rt values of 15.075 and 15.137, also indicating two isomers. Nobiletin, a methoxyflavone, was found at m/z 403.1 with two Rt values (18.173 and 21.639), displaying characteristic ion fragments. Vitexin was observed at m/z 433.1 with two Rt values (12.703 and 19.267).

The LC-MS analysis identified several flavanones, including hesperidin at m/z 611.1 with an Rt value of 7.958, showing characteristic ion fragments at m/z 611.1, 487.21, 249.1, and 150.1. Naringenin was detected at m/z 273.1 with an Rt value of 10.269, exhibiting ion fragments at m/z 579.2, 419.1, 273.1, and 150.1. Among the limonoids, limonin was identified at m/z 471.2 with two Rt values (8.442 and 9.123), suggesting the presence of two isomeric forms. Its characteristic ion fragments included m/z 471.2, 362.2, 249.2, 150.1, and 673.2, 471.2, 336.1, 150.1. Limonin glucoside was detected at m/z 651.1 with an Rt value of 13.802. Nomilin, known for its antiviral and antiparasitic properties, was found at m/z 515.2 with an Rt value of 19.084, while diacetylnomilin was identified at m/z 473.2 with an Rt value of 16.899. Nomilinic acid 17-O-β-D-glucoside was detected at m/z 713.2 with an Rt value of 20.912, showing characteristic ion fragments at m/z 713.2, 579.3, 455.2, 344.3, and 244.2 (Table 3).

This study also identified various benzoic and cinnamic acid derivatives, including sinapic acid (m/z 225.1, Rt 17.514) and 2,4,6-Phenanthrenetriol 2-O-β-D-glucoside (m/z 389.1) with two peaks (Rt 16.110, 22.190). Other detected compounds included 3-Indoleacrylic acid (m/z 188.1, Rt 6.694), ascorbic acid (m/z 177.1, Rt 11.118), chryso-obtusin (m/z 359.1, Rt 14.777), cytidine (m/z 244.2, Rt 16.716), anthraquinone 6,8-Di-O-methylaverufin (m/z 397.1, Rt 17.812), and ginger glycolipid A (m/z 677.2, Rt 19.815), along with some unknown compounds.

This study highlights the rich flavonoid composition of immature kinnow droppings, with dominant flavonols (kaempferol, limocitrol, and kaempferol-3-O-rutinoside) and flavones (tangeretin, nobiletin). Prior research (Saini *et al*., 18) noted high hesperidin, naringenin, and sinapic acid levels in early fruit stages, with flavonoid content decreasing as fruit matures due to water dilution. Flavones like tangeretin and nobiletin have potential therapeutic benefits, including anti-cancer and neuroprotective properties. Kinnow peels contain hesperidin, catechin, and naringenin, while LC-MS analysis revealed quercetin, kaempferol, and hesperidin, supporting kinnow waste as a polyphenol source (Fig. 3). Limonin glucoside, responsible for bitterness, is abundant in immature fruits, with limonoid levels peaking early and declining due to glycosylation (Rafiq *et al*., 17). No prior studies have profiled metabolites in immature kinnow droppings.

Alkanes are simple hydrocarbons without functional groups, which limits their reactivity and they do not form hydrogen bonds and are not soluble in polar solvents like water. The sweet orange peel (*Citrus sinensis*) contains primarily very long chain fatty acids, alkanes, and primary alcohols in its wax. However, with maturity, the amount of very long chain alkanes increases while levels of fatty acids and aldehydes decrease in sweet oranges. Alkanes play significant roles such as regulating cuticle permeability, minimizing water loss after harvesting, and controlling the firmness and weight loss of fruits during post-harvest storage. The GC-MS analysis of volatile components in the IDKF extract revealed the presence of 39 distinct saturated alkanes, including compounds such as cis-Vaccenic acid, eicosane, n-hexadecanoic acid, octadecanoic acid, tetradecane, tetracosane, and 1-Hexadecanol. Sandoval-Montemayor *et al*., (19) documented the existence of 7-Methyl-Z-tetradecen-1-ol in *citrus aurantifolia* extract. Additionally, octadecanoic acid has been identified in waste orange seeds (*Citrus senesis*), which is utilized to enhance the lubricating characteristics of biofuels (Agu *et al*., 1). Hexadecanoic acid and octadecanoic acid were reported in the methanolic extract of *citrus aurantifolia* fruit. Zhang *et al*., (23) reported an abundant amount of D-limonene content in peels of *citrus reticulata* fruits. Among the octadecenoic acids identified in citrus fruit pulp is cis-Vaccenic acid, found in concentrations ranging from 1.9% to 95%. D-Limonene is the main bioactive food constituent of citrus peel oil with various chemopreventive effects, constituting 87% of mandarin peel oil, 95% of orange peel oil, and 75% of lemon peel oil.

Antioxidants prevent radical-induced damage to vital biomolecules by acting as additives or

Table 2. Different bioactive compounds in IDKF extract after bioactive extraction with 50% ethanol by ultrasonication.

S. No.	Bioactive compounds	Content
1	Total phenolic content (g GAE/100g)	5.52
2	Total flavonoid content (g QE/100g)	8.80
3	DPPH (mg AAE/100g)	364.04
4	FRAP (g TE/100g)	8.61
5	Total alkaloids (mg Atropine equivalent/ 100g)	0.694
6	Total saponin (mg Diosgenin equivalent/ 100g)	4.97

Table 3. Various saturated alkanes identified by GC-MS analysis in immature dropped kinnow fruits (IDKF).

S. No.	RT values	Compound name	Molecular weight
1.	7.72	D-Limonene	136
2.	14.78	Tetradecane	198
3.	16.57	Pentadecane	212
4.	16.87	2,4-Di-tert-butylphenol	206
5.	17.79	Octadecane, 6-methyl-	268
6.	18.28	Hexadecane	226
7.	19.01	Tetradecane, 2,6,10-trimethyl-	240
8.	19.07	Disulphide, di-tert-dodecyl	402
9.	19.30	Ethanol, 2-(octadecyloxy)-	314
10.	19.43	Tetradecane, 2,6,10-trimethyl-	240
11.	19.89	Heptadecane	240
12.	19.99	Carbonic acid, eicosyl vinyl ester	368
13.	20.10	Ethanol, 2-(octadecyloxy)-	314
14.	20.57	tert-Hexadecanethiol	258
15.	20.77	Disulfide, di-tert-dodecyl	402
16.	20.87	1-Hexadecanol, 2-methyl-	256
17.	21.42	Eicosane	282
18.	21.79	i-Propyl 12-methyl-tridecanoate	270
19.	22.12	7-Methyl-Z-tetradecen-1-ol	268
20.	22.56	Dibutyl phthalate	278
21.	22.88	Heptadecane, 9-octyl-	352
22.	23.26	Hexadecanoic acid, methyl ester	270
23.	23.38	7,9-Di-tert-butyl-1-oxaspiro(4,5)de	276
24.	23.90	n-Hexadecanoic acid	256
25.	24.20	Hexadecanoic acid, ethyl ester	284
26.	24.61	Isopropyl palmitate	298
27.	25.61	12-Methyl-E,E-2,13-octadecadien -1-ol	280
28.	25.67	10-Octadecenoic acid, methyl ester	296
29.	26.43	cis-Vaccenic acid	282
30.	26.60	Octadecanoic acid	284
31.	26.90	17-Pentatriacontene	490
32.	28.12	Heptadecane, 9-octyl-	352
33.	29.29	Eicosane, 7-hexyl-	366
34.	30.48	Eicosane, 7-hexyl-	366
35.	31.24	Diisooctyl phthalate	390
36.	31.81	Tetracosane, 11-decyl-	478
37.	33.36	Tetracosane, 11-decyl-	478
38.	35.05	13-Docosenamide, (Z)-	337
39.	36.01	2,2,4-Trimethyl-3-(3,8,12,16-tetra methyl-heptadeca-3,7,11,15-tetrae nyl)-cyclohexanol	428

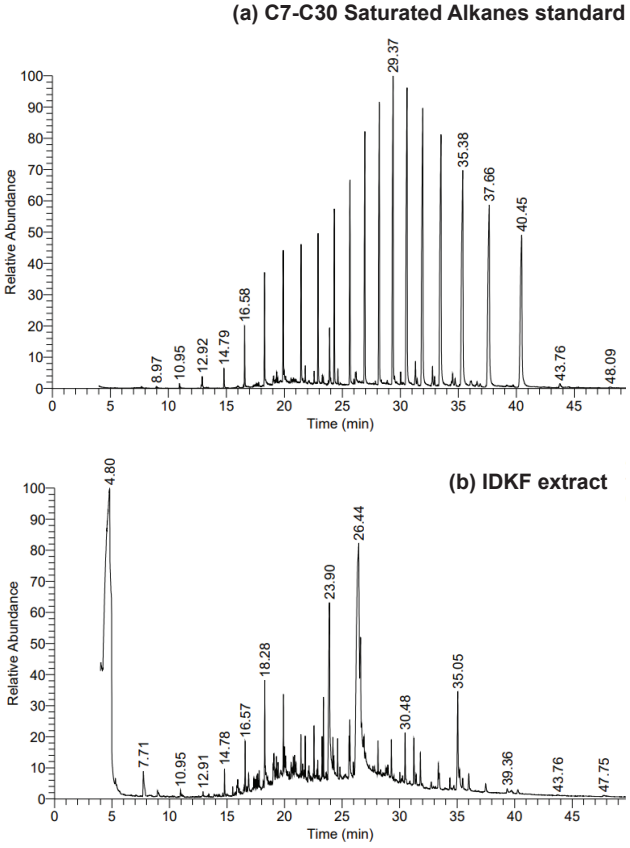

Fig. 3. Chromatograms for GC-MS analysis of (a) C7-C30 saturated alkanes standard Saturated Alkanes and (b) Immature dried kinnow fruit (IDKF) extract.

supplements. Phenolics, particularly flavonoids, exhibit strong antioxidant activity based on their structural properties. Hesperidin, a key flavonoid in immature citrus fruits, possesses anti-inflammatory, metal-chelating, and antioxidant properties (Kumar *et al*., 12). Phytochemical studies indicate the presence of saponins in both peel and pulp, while alkaloids are found only in the peel. Synephrine, the predominant alkaloid, makes up 85% of total alkaloids (Lv *et al*., 13). Citrus saponins, mainly triterpenes, constitute about 0.81% of *C. reticulata*.

TPC in dried IDKF was 5.52 g/100g (as gallic acid equivalent), while the TFC was 8.80 g/100g. Alkaloids and saponins were present in minor amounts at 0.694 mg/100g and 4.92 mg/100g, respectively. The DPPH radical scavenging activity was 364.04 mg AAE/100g, and FRAP activity measured 8.61 g TE/100g. Heena *et al*. (8) reported similar findings, with 5.13 g GAE/100g TPC and 5.87 g QE/100g TFC in ethanolic extracts of dropped kinnow fruits, highlighting ethanol's efficiency in extracting phenolics. Both TPC and TFC decline with fruit maturation.

The alkaloid content of *C. reticulata* peel is 1.20%, aiding seed protection due to its antimicrobial properties, while saponins (0.56%) contribute to antifungal activity and bitterness (Okwu *et al*., 15). Kinnow peels extracted with 70% ethanol yielded 32.9 mg GAE/g TPC and 11.78 mg RE/g TFC (Hayat *et al*., 6), with FRAP and DPPH activities ranging from 2.23–4.85 mg/mL and 0.33–1.18 mg/mL, respectively. Other studies reported TPC (28.20–66.10 mg GAE/g) and TFC (17.65–28.91 mg CE/g), confirming kinnow peels as a rich bioactive source (Table 2). Antioxidant activity was highest in immature citrus fruits and declined with ripening. Freeze-dried immature *C. reticulata* extracts showed higher TPC (50–54 mg GAE/L) and antioxidant activity than oven-dried extracts (34–39.9 mg GAE/L) (Kumar *et al*., 12). Ultrasound-assisted extraction yielded 36.17 mg GAE/100g TPC, 64.70% DPPH scavenging activity, and 28.17 mM/100g FRAP activity (Kaur *et al*., 10).

This study highlights the nutritional, antioxidant, and biochemical properties of IDKF, emphasizing its agro-industrial and pharmacological potential. Its rich bioactive and nutrient profile enhances its value in various applications. Furthermore, it elucidates the nutritional, biochemical, and functional properties of immature dropped kinnow fruits (IDKF), highlighting their high antioxidant capacity, favorable flow properties, and rich metabolomic profile. A total of 55 secondary metabolites and 44 volatile compounds were identified in the IDKF through LC-MS and GC-MS analysis, respectively, including kaempferol, limocitrol, tangeretin, nobiletin, limonin, sinapic acid, cis-Vaccenic acid and D-limonene. With the rising global demand for nutrient-rich and antioxidant-packed foods, further research on the valorization of IDKF could support their application in pharmaceutical, nutraceutical, and agro-industrial sectors.

AUTHORS' CONTRIBUTION

Conceptualization of research (M.D. & M.B.); Designing of the experiments (M.D. & M.B.); Contribution of experimental materials (M.D., M.B., R.K.V. & D.G.); Execution of lab experiments and data collection (S.A., S.R.K. & S.); Analysis of data and interpretation (S.A., S. & S.); Preparation of the manuscript (M.B. & S.).

DECLARATION

The authors report no declarations of interest.

ACKNOWLEDGMENT

Authors thankfully acknowledge the funds received from ICAR under CRP on secondary agriculture and the facilities provided by the Director ICAR-CIPHET, Ludhiana.

REFERENCES

1. Agu, C. M., Ani, K. A., Ani, O. N., Chinedu, M. P., Kadurumba, C. H. and Ahaneku, I. E. 2024. Waste orange seeds (*Citrus sinensis* seed) transformation, a viable industrial bio-oil: Oil extraction, physicochemical characterization and vital instrumental analyses studies. *Waste Manag. Bull.* **1**(4): 134-142. http://dx.doi.org/10.1016/j.wmb.2023.10.008

2. Anuar, M.S., Tahir, S.M., Najeeb, M.I. and Ahmad, S.B. 2019. Banana (*Musa acuminata*) peel drying and powder characteristics obtained through shade and microwave drying processes. *Adv. Mater. Process. Technol.* **5**(2): 181-190. https://doi.org/10.1080/2374068X.2018.1545201

3. AOAC, 2000. Official method of analysis of AOAC International (17th ed., Vol. II) Association of Analytical Chemists, Washington.

4. Bakshi, G. and Ananthanarayan, L. 2022. Characterization of lemon peel powder and its application as a source of pectin degrading enzyme in clarification of cloudy apple juice. *J. Food Sci. Technol.* **59**(7): 2535-544.

5. Chaudhary, S., Rastogi, A., Sharma, R.K., Raghuwanshi, P. and Khan, N. 2017. Formulation of kin now mandarin (*Citrus Nobilis* Lour × *Citrus Deliciosa* Tenora) waste and paddy straw based complete feed blocks and its utilization by goats. *Indian J. Anim. Res.* **51**(1): 105-10. https://doi.org/10.18805/ijar.5669

6. Hayat, K., Abbas, S., Jia, C., Xia, S. and Zhang, X. 2011. Comparative study on phenolic compounds and antioxidant activity of Feutrell's Early and Kinnow peel extracts. *J. Food Biochem.* **35**(2): 454-71. http://dx.doi.org/10.1111/j.1745-4514.2010.00395.x

7. Hayat, K., Zhang, X., Abbas, S., Hussain, S., Hussain, A. and Tahir, M.U. 2020. Effect of microwave treatment on the nutritional profile of the citrus mandarin cultivars peels. *J. Food Process. Preserv.* **44**(10). http://dx.doi.org/10.1111/jfpp.14791

8. Heena, Kaushal, S., Kalia, A. and Kaur, V. 2022. Proximate, mineral, chemical composition, antioxidant and antimicrobial potential of dropped fruits of *Citrus reticulata* Blanco. *J. Food Meas. Charact.* **16**(6): 4303-317. https://doi.org/10.1007/s11694-022-01532-w

9. Kalsi, B.S., Singh, S., Alam, M.S. and Sidhu, G.K. 2023. Comparison of ANN and ANFIS modeling for predicting drying kinetics of *Stevia rebaudiana* leaves in a hot-air dryer and characterization of dried powder. *Int. J. Food Prop.* **26**(2): 3356-75. https://doi.org/10.1080/10942912.2023.2283380

10. Kaur, A., Sran, A.S., Rani, A. and Singh, B. 2021. Studies on pre-harvest fruit drop of Kinnow with special reference to disease management through integrated approaches under Punjab Region. *J. Pharma Innov.* **10**(7): 1350-356.

11. Kaur, S., Panesar, P.S. and Chopra, H.K. 2023. Extraction of dietary fiber from kinnow (*Citrus reticulata*) peels using sequential ultrasonic and enzymatic treatments and its application in development of cookies. *Food Biosci.* 102891. https://doi.org/10.1016/j.fbio.2023.102891

12. Kumar, D., Ladaniya, M.S., Gurjar, M., Kumar, S. and Mendke, S. 2021. Quantification of flavonoids, phenols and antioxidant potential from dropped *Citrus reticulata* Blanco fruits influenced by drying techniques. *Mol.* **26**(14): 4159. https://doi.org/10.3390/molecules26144159

13. Lv, X., Zhao, S., Ning, Z., Zeng, H., Shu, Y., Tao, O., Xiao, C., Lu, C. and Liu, Y. 2015. Citrus fruits as a treasure trove of active natural metabolites that potentially provide benefits for human health. *Chemi. Cent.* J. **9**: 1-14. https://doi.org/10.1186/s13065-015-0145-9

14. Manzoor, M., Hussain, S.B., Anjum, M.A., Naseer, M., Ahmad, R. and Ziogas, V. 2023. Effects of harvest time on the fruit quality of Kinnow and Feutrell's early mandarins (*Citrus reticulata* Blanco). *Agron.* **13**(3): 802.

15. Okwu, D.E., Awurum, A.N. and Okoronkwo, J.I. 2007. Phytochemical composition and in vitro antifungal activity screening of extracts from citrus plants against *Fusarium oxysporum* of okra plant (*Hibiscus esculentus*). *Summa. Phytopathol.* **30**: 145-148.

16. Purewal, S.S. and Sandhu, K.S. 2020. Nutritional profile and health benefits of kinnow: an updated review. *Int. J. Fruit Sci.* **20**(sup3): S1385-S1405. https://doi.org/10.1080/15538362.2020.1792390

17. Rafiq, S., Singh, B. and Gat, Y. 2019. Effect of different drying techniques on chemical composition, color and antioxidant properties of kinnow (*Citrus reticulata*) peel. *J. Food Sci. Technol.* **56**: 2458-466. https://doi.org/10.1007/s13197-019-03722-9

18. Saini, M.K., Capalash, N., Varghese, E., Kaur, C. and Singh, S.P. 2022. A targeted metabolomics approach to study secondary metabolites and antioxidant activity in 'kinnow mandarin' during advanced fruit maturity. *Foods* **11**(10): 1410. https://doi.org/10.3390/foods11101410

19. Sandoval-Montemayor, N. E., García, A., Elizondo-Treviño, E., Garza-González, E., Alvarez, L. and Del Rayo Camacho-Corona, M. 2012. Chemical composition of hexane extract of *Citrus aurantifolia* and anti-Mycobacterium tuberculosis activity of some of its constituents. *Mol.* **17**(9): 11173-11184. https://doi.org/10.3390/molecules170911173

20. Singla, G., Krishania, M., Sandhu, P.P., Sangwan, R.S. and Panesar, P.S. 2019. Value addition of kinnow industry byproducts for the preparation of fiber enriched extruded products. *J. Food Sci. Technol.* **56**: 1575-1582. https://doi.org/10.1007/s13197-019-03670-4

21. Ullah, S., Khan, A.S., Malik, A.U., Afzal, I., Shahid, M. and Razzaq, K. 2012. Foliar application of boron influences the leaf mineral status, vegetative and reproductive growth, yield and fruit quality of "kinnow" mandarin (*Citrus reticulata* blanco.). *J. Plant Nutr.* **35**(13): 2067-79. https://doi.org/10.1080/01904167.2012.717661

22. Vilas-Boas, A.A., Pintado, M. and Oliveira, A.L. 2021. Natural bioactive compounds from food waste: Toxicity and safety concerns. *Foods* **10**(7): 1564.

23. Zhang, H., Xie, Y., Liu, C., Chen, S., Hu, S., Xie, Z., Deng, X. and Xu, J. 2017. Comprehensive comparative analysis of volatile compounds in citrus fruits of different species. *Food Chem.* **230**: 316-326. https://doi.org/10.1016/j.foodchem.2017.03.040

(Received : February, 2025; Revised : June, 2025; Accepted : June, 2025)