# Unveiling the Quantitative and Qualitative Traits of Solar-Dried Indian *Limonia acidissima* Powder and its Therapeutic Findings

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#### ABSTRACT

Background: Limonia acidissima is the only class of its type and an underused fruit in the Rutaceae family. Materials and Methods: The goal of the current study was to create powdered fresh and solar-dried wood apple and then analyze the physical, proximate, and trace mineral contents of each. The solar-dried powder had a relatively low moisture content (12.27%) and high amounts of fat (8.53%), protein (17.60%), and carbohydrates (54.62%). Results: The solar-dried powder showed noticeably greater trace mineral compositions. Nineteen bioactive compounds were identified using the GCMS examination; the most commonly discovered compounds were acetic acid, propanoic acid, octadecanoic acid, and N-hexadecanoic acid. The compounds exhibited strong antioxidant, antibacterial, and anti-inflammatory properties. The SEM-EDX analysis revealed seven elements, including C, O, Mg, P, Nb, Cl, and K, demonstrating the existence of significant compounds in the solar-dried powder. Carbonyl compounds, aldehydes, ketones, carboxylic acids, and esters, as well as aromatic compounds, methylene groups, methyl groups, esters, ethers, carboxylic acids, and alcohols, were found to have good biological qualities by FTIR analysis. Given that higher drying temperatures will hinder the components included in food, the study's conclusions indicate that solar drying is a fantastic technique to employ in the field of food technology today. Conclusion: Its many health advantages are a result of these ingredients. With its antibacterial qualities, the dried powder aids in the fight against bacterial and fungal illnesses. Additionally, it has hypoglycemic properties, which may help diabetics. Although further research is required in this area, the presence of bioactive chemicals in wood apple powder suggests possible anticancer effects. Gaining knowledge about the methods used to produce wood apple powder and its health-promoting qualities will help the food sector and help incorporate it into a balanced diet.

Keywords: EDX, FTIR spectra, GCMS, Proximate, Solar drying, Wood apple.

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Received: 05-11-2024; Revised: 14-11-2024; Accepted: 03-12-2024.

## **INTRODUCTION**

*Limonia acidissima* The Groff, also called the "Wood apple," is a tropical fruit belonging to the undervalued *Rutaceae* family of trees.<sup>[1]</sup> Penang Island, Sri Lanka, Southeast Asian countries, and arid and semi-arid regions of India are the primary growing regions for it. It is mostly found in the Western Himalayas, West Bengal, Uttar Pradesh, Maharashtra, Madhya Pradesh, and Chhattisgarh in India.<sup>[2]</sup> Among its well-known names are elephant apple, curd apple, monkey fruit, kothbel, koyito, pushpahala, and kaitha.<sup>[3]</sup>

The wood apple tree's fruit has a high content of flavonoids, tannins, glycosides, riboflavin, beta-carotene, ascorbic acid, and



Manuscript

DOI: 10.5530/pres.20252007

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saponins in addition to several vitamins and minerals. These phytochemical and nutritional elements, known as coumarins (which include osthenol, psoralen, demethylsuberosin, bergapten, and isopimpinellin), tri-terpenoids, amino acids, polyphenols, and saponins are often absent from a wide range of other fruits.<sup>[4]</sup> Considering all of this, research has demonstrated that wood apple fruits possess potent antioxidant properties and can scavenge free radicals. It is well-recognized that fruits, both ripe and unripe, have positive therapeutic potential. It has been demonstrated that the ripe fruit helps heal ailments of the liver.<sup>[5]</sup>

The most popular food processing technology in developing countries is drying, which comprises oven, vacuum, microwave, freeze-drying, and solar drying techniques.<sup>[6]</sup> This is because the drying process is inexpensive and requires less energy, making it suitable for farming areas with limited resources. Additionally, fresh produce is often dried out throughout the food drying process to safe storage moisture content levels, which increases shelf life.<sup>[7]</sup> Food weight and volume are also significantly lost

during the drying process, which lowers the cost of packaging, shipping, and storing the dried goods.<sup>[8]</sup> Food drying inhibits the formation of mold and fungus on the dried goods, employing well as other forms of microbial degradation.<sup>[9]</sup>

The least expensive processing method is sun drying, which also doesn't require special knowledge of drying methods. Conversely, open sun drying is more popular due to the long drying period, weather dependence, exposure to rain, dust, insects, and animals, color deterioration, insufficient rehydration, and lower-quality final product. However, solar dryers have been developed to preserve product quality while saving time. Additionally, solar dryers use an unconventional and clean energy source.<sup>[10]</sup> Enhancing the safety and quality concerns related to sun-dried food items can be achieved through the use of a solar dryer.<sup>[11]</sup> Furthermore, a solar dryer can match customer standards by drying products quickly, consistently, and hygienically.<sup>[12]</sup>

Several studies have looked at how drying especially, solar drying affects the chemical, physical, and nutritional characteristics of fruits and vegetables. Tropical fruits comprise papaya, bananas, and mangos. The author evaluated the effects of solar tunnel drying on these properties and discovered that the fruit's physicochemical and antioxidant properties arose although its vitamin C content marginally decreased.<sup>[13]</sup> Lycopene, phenolic compounds, and antioxidant activity were significantly increased in tomato slices that were vacuum-dried at various temperatures and periods, but flavonoids and  $\beta$ -carotene were significantly decreased.<sup>[14]</sup>

The naturally occurring chemical compounds in plants that are biologically active, protect plants, and provide health benefits to the consumer are known as phytochemicals and their secondary metabolites are antioxidants.<sup>[15]</sup> They have the potential to prevent chronic diseases.<sup>[16]</sup> The phytochemicals include sugars, amino acids, proteins, purines, pyrimidines alkaloids, terpenes, and phenolics. Antioxidant compounds of wood apple include carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, and tocopherols.<sup>[17]</sup> Wood apple has been used in traditional medicine for about 4000 years in various forms. Fruits, both ripe and unripe, are known to offer medical benefits. The fruit as a whole has made an immense contribution to the field of Ayurveda besides providing nutritional benefits.<sup>[18]</sup> It is used to treat cardiac debility, wounds, tumors, hepatitis, dysentery, diarrhea, and asthma in the traditional medical system. Additionally, ripe fruit can treat liver and cardiac conditions. It contributes to decreasing blood cholesterol levels.<sup>[19]</sup>

Even though the effects of solar energy drying on fruit quality have been the focus of numerous studies, more investigation into this field is necessary to progress the technology used in the food industry. There aren't many studies that evaluate the structural characterization of wood apple powder and even solar drying. As a consequence, this study aims to study the physiochemical, trace elemental analysis, and characterize the bioactive compounds, structural, and functional properties of solar-dried wood apple powder using various analytical techniques.

## **MATERIALS AND METHODS**

#### Ingredients

In the study, two kilograms of completely ripe wood apples (*Limonia acidissima* L.) were acquired from local markets in Tamil Nadu, India, districts of Salem, Dharmapuri, and Krishnagiri. The initial moisture level of the wood apple fruit is 74%.

## **Solar drying process**

The experiment was carried out at Periyar University in Salem, Tamil Nadu, India during the winter months of December and January (11.71833°N in latitude and 78.07806°E in longitude). The winter months had an average ambient temperature of 38.3°C and relative humidity of 30.8%, with 8 hr of sun radiation. The low-cost indirect solar drier that was created was used in one drying experiment. The black body radiation idea, which is dependent on solar radiation, ambient weather, and airflow settings, is used by the solar collector of the designed indirect solar dryer (Figure). Knowing the initial moisture level, the drying process was continued until the moisture content reached 10%, which Alharbi and Csala stated was the ideal value for the dried product. The dry material was packed hermetically in brown glass bottles to keep it out of the light and moisture.<sup>[20]</sup>

#### Preparation of wood apple fruit powder

Fruits were first washed to remove the dirt. The hard shell was cracked openScooping out the soft, yellowish-brown pulp is done. To produce a homogeneous mass, the pulp was physically mashed. In the Department of Environmental Science at Periyar University in Salem, Tamil Nadu, India, the solar drying process was used in agreement with Figure 1. The pulp was taken out following the Figure 2 schematic diagram. In the lab of Periyar University's Department of Food Science, Technology, and Nutrition in Salem, Tamil Nadu, India, the pulp was removed. Totally two kinds of wood apple powder such as fresh wood apple powder and solar-dried wood apple powder were developed using below Figure 2.

## Physical properties of wood apple powder pH

pH of wood apple powder was calculated with a digital pH meter that was standardized with buffer solutions of 4.0 and 7.0 according to the standard method. To determine the pH, a pestle motor was used to smash the powder, which was then utilized to measure the pH.<sup>[21]</sup>



Figure 1: Representation of the solar drier and its drying process.

Table 1: Physical properties of fresh and solar-dried wood apple
powder.

	•	
Physical properties	Fresh	Solar dried
pH (%)	$3.22 \pm 0.02^{a}$	$3.06 \pm 0.01^{b}$
Water activity $(a_w)$	$0.98 {\pm} 0.01^{\mathrm{b}}$	$0.40 \pm 0.02^{a}$
Colour profile		
L*	$49.64 \pm 0.58^{b}$	54.20±0.52ª
a *	10.95±0.35ª	$12.72 \pm 0.54^{b}$
b*	26.71±0.15ª	$24.49{\pm}0.26^{ab}$

<sup>a-b</sup>Each value represents the mean of three replicates. Mean values with the same superscript within columns are not significantly different (p<0.05).

#### Water activity

The initial pulp and final powder moisture contents were analyzed by the Hot air oven method at  $105^{\circ}C \pm 2^{\circ}C$  until constant weights were achieved. The water activity of the fruit powders was analyzed by Lab Swift portable-water activity meter.<sup>[22]</sup>

#### **Colour analysis**

The color of wood apple powder was measured using a digital chromometer (Konica Minolta R-400/410 digital chromometer). The color of the powder was measured in terms of L\*, a\*, and b\* values, in which the 'L\*' value represents lightness, the 'a\*' value indicates redness - greenness, and the 'b\*' value shows blueness - yellowness. The instrument was calibrated with a standard white base or pure black base. The chroma, C\*, and hue angle, h\*, of the sample were also calculated, where  $C^* = \sqrt{a^2 + b^2}$  and Ho = arctan (b\*/a\*). Hue shows color attributes by which red, yellow, green, and blue color spectrums are identified, while chroma or color intensity differentiates between dull and vivid colors.<sup>[23]</sup>

#### Proximate analysis of wood apple powder

The ash content (%) was determined by the AOAC method. The moisture (%) was estimated by the hot air oven method, protein (%) content by the Kjeldhal method and fat content (%) was estimated using the Soxhlet method; carbohydrate (%) was calculated by the difference method prescribed by Poongodi Vijayakumar *et al.*<sup>[24]</sup>

# Trace mineral analysis (ICP-MS) of wood apple powder

The analysis of mineral elements (Cd, Pb, Al, Cr, As, Ni, Cu, Zn, Fe, Mn, Se, Co, B, Ca, Mg, and Li (ppb)) in fresh and solar-dried wood apple powder was carried out using an Inductively Coupled Plasma/Mass Spectrometer (ICP-MS 7500, Agilent, US) by the method employed by Wati et al.[25] An Intermediate Solution (IS) was prepared using 5 mL of the stock solution in a 50 mL volumetric flask to make up a 100-ppm concentration of the stock solution. A multielement standard solution was used in the preparation of the calibration solution. The calibration curve was constructed from five concentrations (0-50 ppm) of the standard solution using 2% Nitric Acid (HNO<sub>2</sub>) as blank. The liquid sample was introduced into the ICP-MS nebulizer and spray chamber. The sample was dried, vaporized, atomized, and ionized inside the plasma chamber consisting of different heating zones. The elemental composition of the samples was obtained through the transformation of the liquid samples into excited atoms and positively charged ions.

Elements	Fresh (mg/kg)	Solar dried (mg/kg)
Cadmium (Cd)	$0.0008 \pm 0.0001$	$0.0006 \pm 0.0001$
Lead (Pb)	0.0163±0.0004	0.013±0.00
Aluminum (Al)	$1.302 \pm 0.05$	1.593±0.53
Chromium (Cr)	$0.652 \pm 0.02$	$1.087 \pm 0.04$
Ardennite (As)	$0.0004 \pm 0.0000$	0.001±0.00
Nickel (Ni)	0.161±0.01	$0.470 \pm 0.00$
Copper (Cu)	$0.425 \pm 0.02$	$1.149 \pm 0.04$
Zinc (Zn)	$1.144 \pm 0.04$	1.966±0.07
Iron (Fe)	2.22±0.06	5.16±0.19
Manganese (Mn)	0.821±0.02	2.421±0.06
Selenium (Se)	0.0026±0.0002	0.002±0.00
Cobalt (Co)	$0.003 \pm 0.00$	$0.008 \pm 0.00$
Boron (B)	$0.963 \pm 0.07$	2.271±0.03
Calcium (Ca)	499.86±13.58	1412.98±78.76
Magnesium (Mg)	117.892±11.39	333.671±18.24
Lithium (Li) (ppb)	$0.002 \pm 0.00$	0.002±0.00

#### Table 2: Trace minerals analysis of wood apple powder.

## **Structural characterization of wood apple powder** *GCMS analysis in wood apple powder Solvent Extraction*

A total of 5g of wood apple powder that had been solar-dried and powdered was then extracted thoroughly by macerating it in 200 mL of ethanolic solvents for a whole day at room temperature (28±20°C). In contrast, the extraction was reduced from 200 mL to 10 mL of concentrated crude ethanolic extracts, which were then dried at 500°C in an oven. Additionally, GC-MS analysis was performed on these extracts.

# Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The methanolic extract of solar-dried wood apple powder was subjected to GC-MS analysis. The analysis was carried out under Shimadzu QP 2010's GC-MS apparatus: RTX: Restek Corp.'s column type is 5 ms (30 m in length). The operating temperature ranged from 50 to 300 degrees Celsius, while the injector and detector were kept at 250 degrees. For 1 min, the temperature of the column was planned to rise by 40°C every minute, with a max temperature of 1200°C. After that, it was set to run at 120-3000°C for 5 min, increasing in temperature by 60°C per minute, for a total Retention time (Rt) of 60 min. A carrier gas of 50-500 Atomic Mass Units (AMU) was helium. Wiley's commercial libraries' computer searchers were used to identify the extract's constituents. Since the fresh powder was adopted as a reference after a thorough assessment of the literature, GC-MS analysis for the fresh wood apple powder was not performed.<sup>[26]</sup>

#### SEM-EDX analysis

The sample was thoroughly mixed, put on a glass coverslip, and allowed to air dry. Throughout the Scanning Electron Microscopy (SEM) investigation, the cover slip itself was utilized. A coater was then used to apply gold coating to the samples. Using a scanning electron microscope (JSM-6610LV, Jeol Asia PTE Ltd., Japan), the powdered sample pictures were captured. The images themselves were implanted with information on the size of the contents, the applied voltage, and the magnification. Using EDX, the powder's elemental content was verified. Dispersive energy analysis X-ray spectrometers make use of the light's photon character. A single photon's energy is just enough in the X-ray range to produce a quantifiable pulse X-ray; the corresponding quantum energy is statistically measured by the output of an ultra-low noise pre-amplifier coupled to the low noise. In conjunction with the process of physical science to analyze the spectrum, a semiconductor material is used to sight the X-rays. The EDX observations were performed using the JOEL Model JED-2300 at INCA Energy 250 in Oxford, Japan.

#### FTIR spectra analysis

The spectrophotometer UV-1800 from Shimadzu UV-vis was utilized to capture the extracted dye's absorption spectra in the 400-700 nm wavelength range. The Furrier transforms infrared spectra were recorded in the 4000 to 400cm<sup>-1</sup> wave number range using the Bruker ALPHAII FTIR spectrometer equipped with an ATR (Attenuated Total Reflection) sampling attachment.

#### **Statistical Analysis**

Data were subjected to mean, standard deviation, and one-way analysis of variance with a subsequent Duncan's test as post hoc comparison was applied for multiple sample comparison. All statistical analysis was done using SPSS 25.0 version software.

## RESULTS

#### Physical properties of wood apple powder

Table 1 above shows the physical properties of fresh wood apple powder and solar-dried wood apple powder. The pH of the powders was 3.22% and 3.06% respectively. The solar-dried powder had a lower pH, indicating its acidic nature, while the fresh powder had a higher pH. Color has a crucial role in assessing the quality of different foods. Non-enzymatic browning reactions can produce pigments and scents when food is dried. Table 1 presents the changes in color values of both fresh and solar-dried wood apple powder. The color values L\*, a\*, and b\* of fresh wood apple were 49.64, 10.95, and 26.71, respectively. The lightness (L value) of the wood apple significantly increased to 54.20 after solar drying (p<0.05). The redness (a-value) of the fresh powder changed significantly after solar drying, dropping to 12.72 (p<0.05). Similarly, there was a significant decrease in





yellowness (b\*value) in the solar-dried sample, which measured 24.49.

## Proximate analysis of wood apple powder

The ash content of solar-dried wood apple powder was 6.95%, which was higher compared to fresh wood apple powder at 1.60%. As shown in Figure 3, the average moisture content of fresh powder was 79.0%, while the solar-dried powder contained 12.27% moisture content on a wet basis. In Figure 3, it was evident that the carbohydrate content of fresh wood apple powder was 6.54%, while the solar-dried powder contained 54.62%. The protein content of the fresh wood apple powder was 7.0%, and the solar-dried wood apple powder contained 17.60% protein. Fresh wood apple powder exhibited 3.06% fat content, while solar-dried wood apple powder contained a relatively higher 8.53% fat content than the fresh variant.

## Trace minerals analysis of wood apple powder

The analysis of trace minerals in the solar-dried wood apple powders is presented in Table 2. Compared to fresh wood apple powder, the solar-dried powder exhibited higher concentrations of trace elements (Al, Cr, Ni, Cu, Zn, Fe, Mn, Co, B, Ca, and Mg), with only small amounts of certain trace elements (Cd, Pb, As, Se, and Li) present, all within safe consumption limits.

## Secondary metabolite analysis using GC-MS

Bioactive substances were detected in the methanolic extracts of solar-dried wood apple powder by GCMS analysis. Table 3 lists the active ingredients together with their Retention Time (RT), molecular formula, Molecular Weight (MW), molecular structure, and peak area %. A methanolic extract of powdered solar-dried wood apple was found to have twenty different chemical components. The powder's recognized constituents

		-		-	-	
SI. No.	R. Time	Compound Name	Molecular formula	Mol. Wt	Peak Area %	Nature and its biological activity
1	3.021	<u> </u>	$C_4H_8O_2$	88	2.16	No activity
		Acetic acid ethyl ester				
2	3.529	, О	C <sub>c</sub> H <sub>10</sub> O <sub>2</sub>	102	32.51	Anticancer,
			5 10 2			Cardio-Protective and
		Propanoic acid, etnyl ester				Anti-Inflammatory
3	3.561		$C_5 H_{10} O_2$	102	10.22	No activity
		Acetic acid propyl ester				
4	1 391	Accele acia, propyresier	СНСО	116	1.46	Antiproliferative activity
4	4.374		$C_{12}\Pi_{14}C_{12}O_{3}$	110	4.40	antimicrobial activity
		Acetic acid, 2methylpropyl ester				,
5	6.129		СНО	130	6	Antimicrobial activity
C .	0.12)		0711402	100	U C	
		1-butanol, 3-methyl-, acetate	o o			
6	6.165		$C_7 H_{14} O_2$	130	1.54	Antimicrobial activity
		1-butanol, 2-methyl-, acetate				
7	11.379	au	$C_{11}H_{24}O$	172	1.39	No activity
		1-undecanol				
8	14.236		C <sub>10</sub> H <sub>26</sub>	252	2.42	No activity
		3-octadecene, (e)-	10 50			
9	15.756	н <sub>4</sub> Ссн <sub>3</sub>	C <sub>14</sub> H <sub>22</sub> O	206	0.91	Antioxidant activities,
		15				anti-inflammatory,
						cytotoxicity
10	16760	2,4-Di-tert-butyipnenoi	C U O	242	2 (1	A
10	16./68	1 h	C <sub>16</sub> H <sub>34</sub> O	242	3.61	Anti-inflammatory
11	10.020	1-nexadecanol	C II	252	2.02	N
11	19.039	-trate	$C_{18}\Pi_{36}$	252	2.93	No activity
		XXX				
		1-octadecene				
12	20.768	<b>\$</b>	$C_{16}H_{32}O_2$	256	6.6	Anti-inflammatory,
						Antioxidant, Pesticide,
		N-Hexadecanoic acid				
13	21.094		$C_{22}H_{46}O$	326	2.52	No activity
1.4	22.111	Behenic alcohol	0.11.0	220	0.01	NT // //
14	22.111		$C_{16}H_{30}O$	238	0.81	No activity
		7-hexadecenal. (z)-				
15	22.421	· · · · · · · · · · · · · · · · · · ·	C. H. O.	280	2.14	Anti-inflammatory
			18-32-2			hypocholesterolemic and
						antiarthritic activity
		9.12-octadecadienoic acid (z.z)-				
		, o controllollollollollollollollollollollolloll				

#### Table 3: GCMS analysis of the wood apple powder and its bioactive compounds.

SI. No.	R. Time	Compound Name	Molecular formula	Mol. Wt	Peak Area %	Nature and its biological activity
16	22.479		$C_{20}H_{38}O_2$	310	12.91	Used as plasticizers and lubricants, biological additives and hydraulic fluids
		Ethyl oleate				
17 22.676	OH (CH 2) 16 CH3	$C_{18}H_{36}O_2$	284	1.65	Anti-microbial properties	
	Octadecanoic acid					
18 22.969	22.969		$C_{26}H_{54}O$	382	1.77	Anti-inflammatory
		1-hexacosanol				
19 24.69	24.69		$C_{26}H_{54}O$	382	1.21	Anti-inflammatory
		1-hexacosanol				
20	25.666	Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	2.23	Anti-inflammatory, Antioxidant, Pesticide, Nematicide, Inhibitor

include the carboxylic ester acetic acid ethyl ester, which has no biological activity.

# Elemental analysis of wood apple powder using SEM-EDX

The elemental composition of the wood apple fruit powder was identified and quantified using EDX, which provided insight into the presence of different elements in the powder. It is evident from the SEM EDX observation result (Figure 4) that the wood apple powder that has been solar-dried has an uneven surface. The powder's uneven surface was predicted given its high fiber content of 0.86 to 1.14 mg/100g and its high amylopectin content, which was approximately 8.6%. Seven major elements were identified by SEM-EDX analysis of solar-dried wood apple powder: C, O, Mg, P, Nb, Cl, and K (Figure 4). The fraction of carbon (68.37%), oxygen (28.52%), magnesium (0.54%), phosphorus (0.52%), niobium (0.28%), chlorine (0.27%), and potassium (1.5%) that have these elements' atomic concentrations are shown in Figure 4.

### FTIR spectra Analysis of wood apple powder

One of the most significant analytical methods for virtually studying the physicochemical and structural features of any sample nowadays is FTIR spectroscopy. The presence of O-H and N-H stretching in the structure, which indicates the presence of alcohols, amines, and amides, was shown by the rice flour's FTIR spectra (Table 4) at the higher intense region 3413 cm<sup>-1</sup>. The following peak, which measured 2924 cm<sup>-1</sup>, was linked to alkanes, methylene groups, and the existence of C-H stretching and CH2

bending. Alkynes, nitriles, carbonyl compounds, aldehydes, ketones, carboxylic acids and esters, aromatic compounds, methylene group, methyl group, esters, ethers, carboxylic acids, alcohols, and ethers were among the different functional groups found in this solar-dried powder. Atomic groups known as functional groups join an organic molecule's carbon backbone and give it particular characteristics (Table 4).

## DISCUSSION

## Physical properties of wood apple powder

This acidity helps preserve the powder and inhibits the growth of microorganisms. Solar dryers are environmentally friendly and contribute to energy conservation. They serve as an alternative to open sun drying and are considered one of the most attractive and promising applications of solar energy systems. Solar drying is a renewable, environmentally friendly, and economically viable technology, especially in developing countries.<sup>[27]</sup> Wood apple powder's water activity varied between 0.98 and 0.40. Compared to the fresh powder (0.98), the water activity of the solar-dried wood apple powder (0.40) was noticeably reduced. The solar dryer's consistent heat distribution and transmission may have contributed to the samples' low water activity by effectively and rapidly removing water from the powder. Following drying and subsequent ambient storage, Mdziniso et al. showed similar results in green leafy and yellow succulent vegetables, suggesting that the solar drying approach is a viable, affordable vegetable drying technology linked to lower water activity.<sup>[28]</sup>

Drying processes often lead to changes in the color of the dried product. The color of dried products plays a significant role in



Figure 3: Proximate analysis of wood apple powder.

Table 4: FTIR spectra analysis of wood	apple powder and its compound.
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SI. No.	Frequency (cm <sup>-1</sup> )	Compound	Functional group
1	3413	O-H stretch, N-H stretching	Alcohols, amines, and amides
2	2924	C-H stretching, CH <sub>2</sub> bending	Alkanes. methylene groups
3	2012	C≡C stretching, C≡N stretching	Alkynes, nitriles
4	1727	C=O stretching	Carbonyl compounds, aldehydes, ketones, carboxylic acids and esters
5	1613	C=C stretching	Aromatic compounds
6	1441	CH2 bending	Methylene group
7	1396	CH₃ bending	Methyl group
8	1235	C-O stretching	Esters, ethers, carboxylic acids
9	1104	C-O stretching	Alcohols, ethers
10	1047	C-O stretching	Alcohols, ethers
11	893	C-H bending	Aromatic compound
12	872	C-H bending	Aromatic compound
13	831	C-H bending	Aromatic compound
14	819	C-H bending	Aromatic compound
15	803	C-H bending	Aromatic compound
16	781	C-H bending	Aromatic compound
17	731	C-H bending	Aromatic compound
18	605	C-Cl stretching	Alkyl halides
19	458	C-Br stretching	Alkyl halides





consumer acceptance.<sup>[29]</sup> Additionally, Ahmed et al. and Chong et al. have also noted a decrease in the L\* value with an increase in the darkness/brownness of food materials, and the destruction of pigments have been reported by various researchers.<sup>[30,31]</sup> According to the literature, color analysis of powders produced through various drying methods of different fruit products often shows significant changes in color coordinates. However, these changes can be advantageous for creating differently colored products using the fruit powders obtained in this research.<sup>[32]</sup> In a study of guava powder conducted by Verma et al., it was found that the L\* value was lower in the sun-dried sample (38.04) and higher in the freeze-dried sample (59.87), resulting in a brighter appearance.<sup>[33]</sup> The study also observed that the a\* value was higher in the sun-dried sample compared to the freeze-dried sample, indicating more redness in the sun-dried samples. These findings were consistent with the study based on the a\* value but inconsistent with the L\* value.

#### Proximate analysis of wood apple powder

According to Sharma *et al.*, the moisture content in wood apple fruit was 75.16% and increases as the fruit ripens.<sup>[34]</sup> Additionally, Vijayakumar *et al.* reported that when wood apples are dried in a hot air oven, the moisture content of the powder is 6.75%, which is lower than that of solar-dried wood apple powder.<sup>[24]</sup> Protein, a macronutrient comprising nitrogen, sulfur, carbon, oxygen, and sometimes phosphorus, iron, and copper, was found to have a content of 4.3% in wood apple fruit as per a recent study. This study

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also revealed that proteins can undergo hydrolysis, analogous to other polymerized substances or compounds like triglycerides.<sup>[35]</sup> Another study by Murthy and Paek (2021) reported a high protein content of 7.1% in wood apple fruits, consistent with this study's findings. Additional research by Thakur and Chugh demonstrated that matured fruits contain approximately 7.3g of protein, aligning with the present study.<sup>[36]</sup> Arya reported the fat content of 24.9% in wood apple flour, which also contains a high level of essential fatty acids and minerals. The study suggested the potential use of this seed powder in formulating food products to meet the increasing population's requirements, aligning with the current study's findings. Furthermore, the fat content in whole fruit powder was observed at 12±1.65% in the freeze-drying method. Meanwhile, during the oven-drying method, the fat content decreased to 9.07±0.46% in whole fruit powder.<sup>[37]</sup> Sharma et al. found that the average fat content of whole fruit was 3.55%,<sup>[34]</sup> while a report by Pal et al. indicated that fruit pulp contains nearly 0.6% fat.<sup>[38]</sup> In addition to being more energizing than protein and carbs, oil and fats are vital for human health.<sup>[35]</sup> A different study found that wood apple fruits have a high-fat content (3.7%).[39] According to a different study, the pulp of L. acidissima has a low-fat level (4.38%), which makes it a good dietary choice for overweight people.<sup>[40]</sup> When Abrol et al. examined how solar tunnel drying affected the physicochemical and antioxidant characteristics of tropical fruits, they found that it improved these characteristics while marginally lowering the vitamin C concentration.[41]

#### Trace minerals analysis of wood apple powder

According to Parvin et al., micronutrients are essential in trace amounts for the body's growth, development, and maintenance. Vitamins and minerals are crucial for releasing energy from carbohydrates, proteins, and lipids. A study using an atomic absorption spectrometer found that wood apples contain 16 essential minerals, including Na, K, Fe, and Zn. Specifically, the levels of Na, K, Zn, and Fe were determined to be 10.40, 58.24, 0.37, and 1.67 mg per 100 g of edible portion of fruits, respectively. Wood apples have comparatively high potassium levels, which are beneficial for maintaining a balanced neurological system and its function. The low Na: K ratio makes this fruit suitable for consumption by individuals with hypertension.<sup>[42]</sup> The wood apple, despite its low zinc content, possesses anticancer properties. It is considered a beneficial dietary supplement for treating anemia and iron deficiency. Micronutrients are chemicals that are necessary in small amounts but play a crucial role in the body's growth, development, and maintenance. A 100g serving of wood apple contains 1.67 mg of zinc. Zinc, an essential trace element, has diverse functions in the body. It is vital for a healthy metabolism and immune system, as well as for maintaining healthy skin and hair, promoting wound healing, and enhancing the senses of taste, smell, and fertility. Zinc is present in every cell in the body and enhances the immune system's ability to fight off germs and viruses. The body requires zinc for the production of proteins and DNA, the genetic material found in all cells. Additionally, zinc is essential for optimal growth and development during pregnancy, infancy, and childhood. In summary, zinc is crucial for good health.<sup>[43]</sup> A pleasant, fragrant pulp with 170 mg of riboflavin, 2 mg of vitamin C, and vital minerals like 0.17% calcium, 0.08% phosphorus, and 0.07 % iron is a characteristic of mature wood apple fruit.<sup>[44]</sup> According to a different study, wood apples have an average vitamin C content of 2.6 mg.[45] Mineral content in these fruits is high (1.9%), with calcium (130 mg), phosphorus (110 mg), and iron (0.48 mg) being the main constituents. Rodrigues et al.'s study found that wood apple pulp has a considerable amount of minerals, including calcium (15.9 mg), iron (3.5 mg), sodium (8.5 mg), phosphorus (46.5 mg), zinc (386.3 mg), copper (0.8 mg), and manganese (0.7).<sup>[46]</sup> Furthermore, wood apple fruits are a great source of vitamin C; their fresh pulp has 200-900 mg of this essential component.<sup>[47]</sup> These results are in line with those for powdered wood apples.

## Secondary metabolite analysis using GC-MS

Propanoic acid ethyl ester has been shown to suppress the immune system, decrease the quantity of fatty acids in the liver and plasma, decrease food intake, and maybe increase tissue insulin sensitivity. Therefore, in the context of preventing obesity and type 2 diabetes, greater PA synthesis by the microbiota may be advantageous.<sup>[48]</sup> According to reports given by Jisha *et al.*, 2-methyl propyl ester, 1-butanol, 3-methyl-acetate, and 1-butanol,

2-methyl acetate, have a high affinity for the plasma membrane and are therefore more toxic than less lipophilic compounds like ethanol, which only becomes toxic at high concentrations.<sup>[49]</sup> The present study showed the presence of octadecanoic acid ( $\omega 6$ fatty acid) and hexadecanoic acid (63 fatty acid). Omega-3 and omega-6 fatty acids are polyunsaturated fatty acids synthesized by plants and reported to influence the metabolic, immunological, and cardiovascular functions of the host.<sup>[50]</sup> According to Scorletti and Byrne, omega-3 fatty acids enhance lipid metabolism in the liver and have positive effects on fatty liver disease. N-hexadecanoic acid is a pesticide and nematicide that also reportedly has anti-inflammatory and antioxidant properties.<sup>[51]</sup> Anti-cancer activities have been reported for behenic alcohol, 7, -hexadecenal, and 9,12-octadecadienoic acid (Z, Z). According to past research, octadecadienoic acid (Z, Z) is well-known for its anti-inflammatory, hypo-cholesterolemia, and antiarthritic properties. An essential chemical substance with numerous economic uses is ethyl oleate. It is frequently utilized in detergents, tastes, scents, cosmetics, and medications. In addition, ethyl oleate finds application as a hydraulic fluid, biological additive, lubricant, and plasticizer. However, because natural sources only yield small amounts, their commercial application has been restricted.<sup>[52]</sup> Octadecanoic acid exhibits antimicrobial properties. 1-hexacosanol, a very long-chain primary fatty alcohol, has roles as a plant metabolite and insecticide. Octacosanol is used as a dietary and pharmaceutical supplement as well as an additive to animal feed. It has been studied for its anti-Parkinsonian effects, regulation of lipid and glucose metabolism, fatigue reduction, promotion of cardiovascular wellness, liver protection, relief of constipation, and anti-inflammatory and anti-nociceptive properties. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, is identified to have anti-inflammatory and antioxidant properties and is also used as a pesticide and nematicide.[53]

## Elemental analysis of wood apple powder using SEM-EDX

The production of distinctive X-rays that indicate the presence of elements in the specimens is facilitated by the Energy-Dispersive X-ray (EDX) microanalysis technique, which may be an elemental analysis method related to electron microscopy. The presence of the elements C, O, Mg, P, Nb, Cl, and K was verified by this study's investigation. These components perform a variety of roles in physiological processes. An essential mineral for the body, potassium is necessary for both electrical and cellular processes. A potassium-rich, low-sodium diet abundant in fruits, vegetables, and whole grains helps to maintain normal blood pressure and, in most cases, lowers increased blood pressure.<sup>[54,55]</sup>

### FTIR spectra Analysis of wood apple powder

Every class of organic molecule has a particular kind of functional group. In biological molecules, functional groups are essential to the production of molecules such as proteins, lipids, carbohydrates, and DNA. When Raharja et al. examined the rice flour's FTIR spectra, they found that the flour exhibited five spectrum peaks, each of which may have represented a functional group in the sample.<sup>[56]</sup> The spectrum indicated the existence of the following wavelength ranges: O-H groups: 3385.64-3393.27 cm<sup>-1</sup>; C-H groups: 2929.29-2930.50 cm<sup>-1</sup>; N-H groups: 2150.71-2154.20 cm<sup>-1</sup>; C=O groups: 1650.01-1655.52 cm<sup>-1</sup>; and C-N groups: 1155.48-1155.22 cm<sup>-1</sup>. Additionally, Sevenou et al. stated that the crystalline and amorphous starch indices in the sample were defined by bands at 1047 and 1022 cm<sup>-1</sup>, respectively. On the other hand, the absorption peaks at wave numbers 1400 and 1800 cm<sup>-1</sup> were indicative of amide bands, while the band 3400 cm<sup>-1</sup> represented the sample's H<sub>2</sub>O molecules' deformation vibrations.[57] This study corroborated with Sandhya et al. who studied the functional group analysis of ethno-medicinal plants for their medicinal properties. Further, the extract was found to contain compounds with amides, carboxylic acid, phenols, alcohol, and alkanes which serve as a significant pharmaceutical product for jaundice, fever and liver pain, treatment of throat infections, rheumatic joint pain, ulcers, and immune boosters.<sup>[58,59]</sup>

### ACKNOWLEDGEMENT

The researchers received a great deal of support at Periyar University in Salem, Tamil Nadu, India, where the studies were carried out in the Department of Food Science and Nutrition. The Department of Environmental Science at Periyar University in Salem, Tamil Nadu, India, is also appreciated by the authors for granting permission to operate on the solar drying panel.

#### **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

GCMS: Gas Chromatography Mass Spectrometry; SEM-EDX: Scanning electron microscopy-energy dispersive X-ray analysis; FTIR: Fourier Transform Infrared Spectroscopy; AOAC: Association of Official Agricultural Chemists; ICP-MS: Inductively coupled plasma mass spectrometry; IS: Intermediate Solution; SEM: Scanning Electron Microscopy; ATR: Attenuated Total Reflection; DNA: Deoxyribonucleic acid

#### AUTHOR'S CONTRIBUTION

It was required by PV to conceptualize. Data gathering, formal analysis, inquiry, methodology, visualization, first draft, second draft, and editing support were all handled by RA. PV has reviewed the manuscript along evaluated its methodological structure.

#### SUMMARY

The solar dried Limonia acidissima powder showed an excellent nutritional quality. It had low moisture (12.27%), high protein (17.60%), fat (8.53%), and carbohydrate (54.62%) content comparable with fresh powder. High mineral compositions were noted in solar dried powder and through structural characterization, the identified bioactive compounds were acetic acid, propanoic acid, octadecanoic acid, and N-hexadecanoic acid and exhibited strong antioxidant, antibacterial, and anti-inflammatory properties. The seven elements, including C, O, Mg, P, Nb, Cl, and K, demonstrating the existence of trace elemental compounds in the solar-dried powder. The solar dried had good biological properties due to the presence of carbonyl compounds, aldehydes, ketones, carboxylic acids, and esters, as well as aromatic compounds, methylene groups, methyl groups, esters, ethers, carboxylic acids, and alcohols. According to these data, solar drying is the most often used drying technique in the current context, and it improves food therapeutic benefits. This study illustrates how underutilized fruit can be used to make high-quality commodities, particularly during off-peak seasons. To summarize, spectral and phytochemical studies confirm the existence of secondary metabolites that can help treat a variety of ailments. The fruit's antimicrobial properties imply that it could be an effective treatment for antibiotic-resistant illnesses. Because of its high concentration of bioactive components, this study supports the use of wood apple powder as a nutraceutical. Wood apple fruit powder could be exploited for developing therapeutic supplements.

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**Cite this article:** Amardeepa R, Vijayakumar P. Unveiling the Quantitative and Qualitative Traits of Solar-Dried Indian *Limonia acidissima* Powder and its Therapeutic Findings. Pharmacog Res. 2025;17(1):232-43.