Comparative Analysis of Methanol and Aqueous for Antioxidant Potential of Citrus Limon Leaf Extracts from Puliyankudi, India

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ABSTRACT

Plants constitute an important source of active natural products which differ widely in terms of structure and biological properties. Among these, the leaves of various plants, including citrus species, are particularly noteworthy due to their rich composition of bioactive compounds. When processed into powder, Citrus limon leaves offer a versatile form that can be easily incorporated into various applications, enhancing both their utility and efficacy. This study investigates the antioxidant activity of edible Citrus limon leaves powder collected from Puliyankudi, known as the "lemon city of India," situated in the Tenkasi district, Kadayanallur taluk, Tamil Nadu. The mineral composition of the botanical product was analyzed using methods including X-ray diffraction (XRD) and found out the qualitative and quantitative phytochemical analysis of citrus limon leaf powder by using to extract methanol and aqueous respectively. The antioxidant activity of Citrus limon leaves powder was assessed through a comprehensive range of assays, including DPPH, ABTS, H2O2, FRAP, and Total Antioxidant Activity, using both Aqueous and Methanol extracts. The results demonstrated significant antioxidant efficacy, showing high performance across all tested parameters. These findings highlight the potential of edible Citrus limon leaves powder as a valuable source of antioxidants, suitable for various applications in functional foods and nutraceuticals.

1. INTRODUCTION

Citrus limon, commonly known as lemon, is renowned for its rich composition of bioactive compounds, particularly flavonoids, phenolics, and essential oils, which are known to exhibit potent antioxidant properties (maqbool et al). Antioxidants play a crucial role in neutralizing free radicals, thereby preventing oxidative stress-related cellular damage, which is implicated in numerous chronic diseases including cancer, cardiovascular disorders, and neurodegenerative conditions (Jiao et al).

Among various parts of the lemon tree, the leaves have garnered interest for their potential health benefits, yet they remain underexplored compared to the more commonly studied fruit and peel. The present study focuses on evaluating the antioxidant potential of Citrus limon leaf extracts from Puliyankudi, India, an area known for its high-quality lemon production. Specifically, this study compares the efficacy of methanol and aqueous extracts in scavenging free radicals and inhibiting oxidative processes (González-Molina et al). Methanol, a polar solvent, is often effective in extracting a broad range of phenolic compounds, while aqueous extracts are more representative of traditional medicinal practices and consumer use (Sravanthi et al).

X-ray diffraction (XRD) analysis was conducted to determine the crystalline nature of the leaf powder, providing insight into the structural properties that may influence antioxidant activity. Preliminary XRD results indicate distinct peaks corresponding to crystalline phases, with the average crystallite size of the leaf nano powders being a key parameter (Toso et al). By elucidating the comparative antioxidant potential of methanol and aqueous extracts, this study aims to provide a scientific basis for the use of Citrus limon leaves in natural antioxidant formulations, potentially contributing to the development of novel therapeutic agents. The findings could have significant implications for both the nutraceutical industry and traditional medicine practices, promoting the utilization of this readily available botanical resource.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material:

Citrus limon leaves were collected without any infection from Puliyankudi, known as the "lemon city of India," situated in the Tenkasi district, Kadayanallur taluk, Tamil Nadu. The collected leaves were authenticated, washed thoroughly with running tap water to remove dust and contaminants, and air-dried in the shade for 7-10 days at room temperature.

2.2 Preparation of Edible Powder:

Plant material was divided into tiny bits, ground with a pestle and mortar, and then give one sieve and put into a container for storage until further analysis.

2.3 Preliminary phytochemical Analysis of Citrus Limon Leaf Powder:

Phytochemical examinationwas carried out for the aqueous and methanol extracts as per the HPSC method.

2.4 Antioxidant Activity of Citrus Limon Leaf Powder

2.4.1 DPPH:

Rumpf describe the procedure for assessing antioxidant capacity using the DPPH assay. Sample extracts were adjusted to 40 µL with DMSO, and 2.96 mL of 0.1 mM DPPH solution was added. The mixture was incubated in the dark for 20 minutes at room temperature, after which the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. A control using 3 mL of DPPH was also measured (rumpf et al).

$$\% \text{ RSA} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Surimi powder was mixed with 25 mL of distilled water and 25 mL of corn oil to achieve 0%, 0.5%, 1%, 1.5%, and 2% concentrations, blended for 1 minute, and centrifuged at 7500 g for 5 minutes. Emulsifying stability was determined similarly, but with an additional incubation step before centrifugation.

2.4.2 ABTS:

Imeneo describe the preparation of the ABTS•+ radical working solution, which was made by combining 9.5 mL of ABTS (7 mM) with 245 µL of potassium persulfate (100 mM) and adjusting the volume to 10 mL with distilled water. After incubating in darkness at room temperature for 18 hours, the solution was diluted to an absorbance of 0.70 (±0.02) at 734 nm using 0.1 M potassium phosphate buffer (pH 7.4). Samples were diluted in methanol to various concentrations (µg/mL). A 10 µL aliquot of each sample was mixed with 2.99 mL of the ABTS radical working solution in a test tube, and the absorbance was measured at 734 nm (Imeneo et al). Percent antioxidant activity was calculated using the formula:

$$\%\text{Antioxidant activity} = [(Ac - As)/Ac] \times 100$$

where Ac and As are the absorbances of the control (methanol) and the sample, respectively.

2.4.3 H_2O_2:

Imeneo describe the hydroxyl radical scavenging activity of the extracts was determined following the method described by Klein et al. In each reaction mixture, 1.0 mL of extract at various concentrations (5-100 µg/mL), 1.0 mL of iron-EDTA solution (0.13% ferrous ammonium sulfate, 0.26% EDTA), 0.5 mL of 0.018% EDTA, 1.0 mL of DMSO (0.85% in 0.1 M phosphate buffer pH 7.4), and 0.5 mL of 0.22% ascorbic acid were combined. The mixture was capped tightly and heated in a water bath at 80-90°C for 15 minutes (Imeneo et al). Jeong describe the reaction was stopped by adding 1.0 mL of ice-cold trichloroacetic acid (TCA, 17.5%). After cooling, 3.0 mL of Nash reagent (containing 75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid, and 2.0 mL of acetyl acetone in distilled water to a total volume of 1 L) was added to the reaction mixture. The samples were then incubated at room temperature for 15 minutes to develop color. The intensity of the yellow color formed was measured at 412 nm against a reagent blank (Jeong et al). The percent inhibition of hydroxyl radicals was calculated using the formula:

$$\%\text{ inhibition} = [(\text{Control- Test}) / \text{control}] \times 100$$

2.4.4 FRAP:

Chatzimitakos describe the FRAP reagent was prepared by mixing 25 mL acetate buffer (30 mM, pH 3.6), 2.5 mL TPTZ solution (10 mM), and 2.5 mL ferric chloride solution (20 mM), incubated at 37°C for 15 minutes. Ascorbic acid (vitamin C) served as a standard, with a calibration curve ranging from 10 µg to 50 µg in water. In test tubes, 2.85 mL of FRAP reagent was mixed with various concentrations of sample extract (in methanol) or standard, incubated for 30 minutes in the dark, and absorbance measured at 593 nm. Results were expressed as µg of ascorbic acid equivalents (AAE) per gram (Chatzimitakos et al).

2.4.5 Total Antioxidant Activity:

Ehiobu JM describe the total antioxidant capacity of the sample extract was assessed using the phosphomolybdenum method (Ehiobu JM). Extract (0.3 mL) was mixed with reagent solution (3 mL; 0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate), and incubated at 95°C for 90 minutes. After cooling, absorbance was measured at 695 nm against a blank of solvent (0.3 mL). Total antioxidant activity was quantified as gram equivalents of ascorbic acid, with a calibration curve ranging from 31.25 to 1000 µg/mL prepared using ascorbic acid in solvent.

3. RESULTS

3.1 Preliminary of Phytochemical Analysis of Citrus Limon Leaf Powder

Qualitative phytochemical screening of citrus limon leaf powder reviewed the presence of tannin, alkaloids, phenolic compounds, flavonids, glycosides, terpenoids, saponins, limonoids, carotenoids, coumarins and essential oils where presence and Quantitative analysis of phytochemical of two solvent extractions like aqueous and methanol. The values are obtained from kaempferol, Quentin, Hesperia, naringin, rutin, limonin, nomilin, beta carotene, phenolic acids, caffeic acid, chlorogenic, coumarins, essential oils respectively.

Table 1: Mean and Standard Deviation of Citrus Limon Leaves Nano Powder using Aqueous and Methanol Extracts [9]

Parameters	*Citrus limon* nano leaf powder	*Citrus limon* nano leaf powder
	Flavnoids	
Kaempferol	27.1 ± 0.1	45.7 ± 0.1
Quentin	13.05 ± 0.05	22.05 ± 0.05
Hesperia	4.25 ± 0.05	14.45 ± 0.05
Naringin	10.15 ± 0.05	18.01 ± 0.1
	Alkaloids	
Rutin	118 ± 1	243.5 ± 1.5
	Limonoids	
Limonin	19.25 ± 0.05	35.7 ± 0.1
Nomilin	6.1 ± 0.1	10.45 ± 0.05
	Carotenoids	
Beta carotene	90.5 ± 0.5	102.5 ± 0.05
	Phenolic compounds	
Phenolic acid	141 ± 0.1	116 ± 1
Caffeic acid	115.5 ± 0.5	80.5 ± 0.1
Chlorogenic acid	95.4 ± 0.1	63.65 ± 0.05
Coumarins	102.5 ± 0.5	134 ± 1
Essential oils	1.1 ± 0.1	30.65 ± 0.15

3.2 Antioxidant Analysis of Citrus Limon Leaf Powder

The table 2, below provides a comparative analysis of the antioxidant activity of Citrus limon leaves powder was evaluated using both aqueous and methanol extracts across five different assays: DPPH, ABTS, H2O2, FRAP, and Total Antioxidant Activity, as illustrated in Fig 1, Fig 2, Fig 3 and Fig 4.

Table 2: Antioxidants Assay Activity of Plant Leaves

S.N0	Different A	Methanol Extract	Aqueous Extract
1	DPPH Scavenging Activity	63.45 ± 0.14	78.35 ± 0.42
2	ABTS Scavenging activity	66 ± 0.28	84.55 ± 0.42
3	H_2O_2 Scavenging activity	68.9 ± 0.28	90 ± 0.56
4	FRAP Assay	185.5 ± 1.41	120.5 ± 1.41
5	Total Antioxidant capacity	241 ± 2.82	193.5 ± 4.24

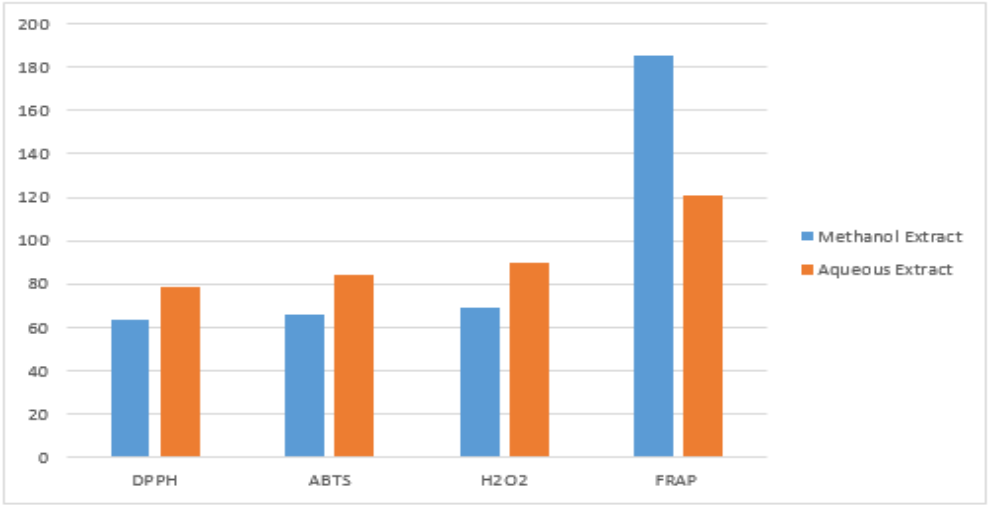

Fig 1: Different Antioxidant Activity of Citrus Limon Leaves Powder

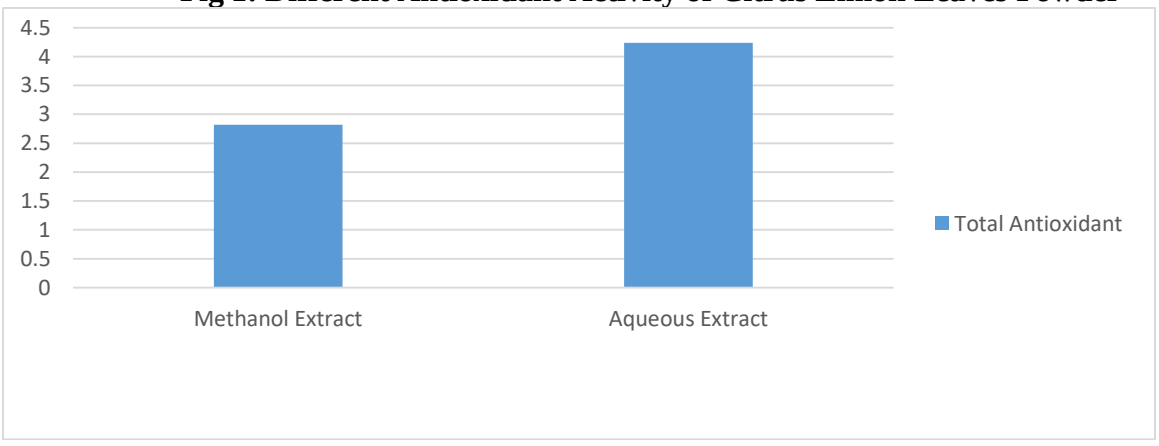

Fig2: Difference between Total Antioxidant in Standard Deviation Value

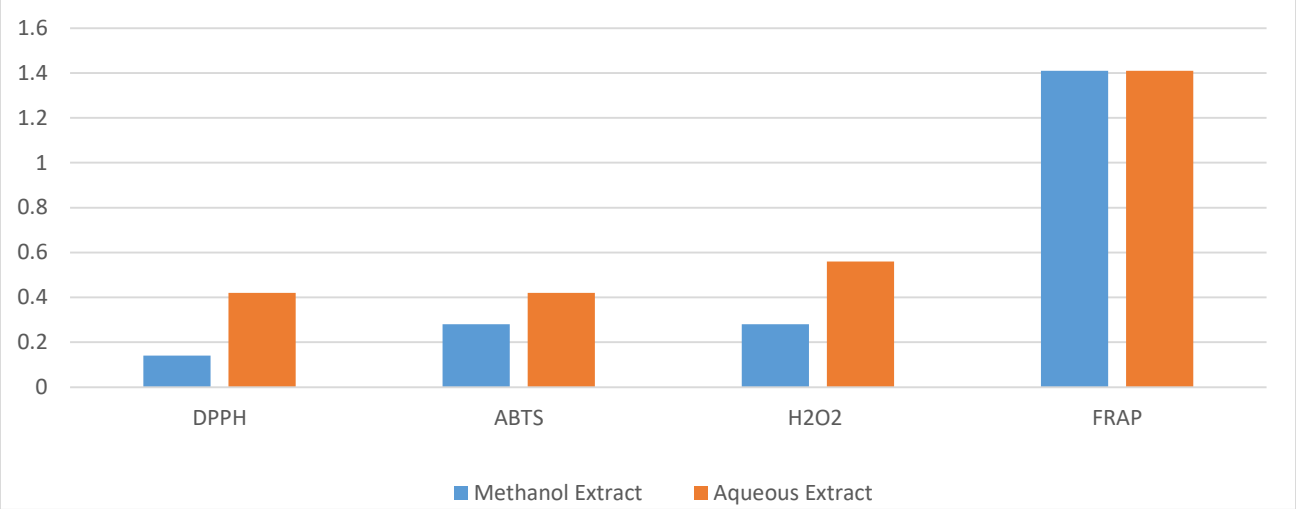

Fig 3: Different Antioxidant Activity of Citrus Limon Leaf Powder

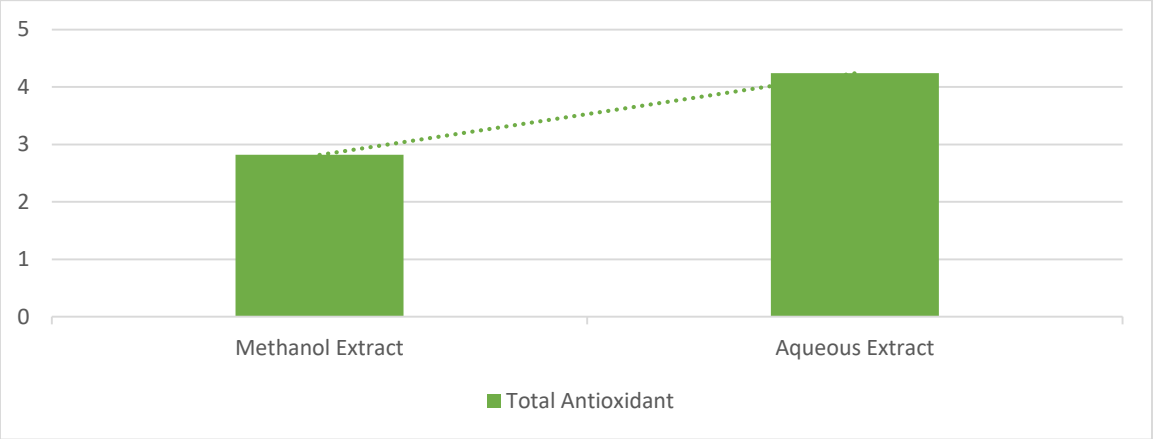

Fig 4: Difference between Total Antioxidant in Standard Deviation Value

The charts above depict the comparison of methanol and aqueous extracts for the antioxidant assay, including DPPH, ABTS, H2O2, FRAP and Total Antioxidant Capacity. Figure 1 represents the comparison of mean values, while Figure 3 represents the comparison of standard deviation values. Finally Figure 2 and Figure 4 represent the Total Antioxidant Capacity of Mean value and standard deviation values.

4. DISCUSSION

The results of this study demonstrate the significant antioxidant activity of Citrus limon leaves powder, with both aqueous and methanol extracts showing substantial efficacy across all tested assays. The X-ray diffraction (XRD) analysis revealed distinct peaks values, indicating the presence of crystalline phases in the Citrus limon leaf nano powders The results, consistent with findings by Irfin Fathima, show that the values of the average crystallite size for Citrus limon leaf nanopowders are, which further corroborates the high degree of crystallinity observed (Irfin Fathima et al). These

findings suggest that the nanoscale properties of the leaf powder may contribute to its potent antioxidant activity, as the high surface area of the nanomaterials could enhance interaction with free radicals. The findings are discussed in detail below:

The findings of the antioxidant assays reveal notable differences between the aqueous and methanol extracts of Citrus limon leaves. In the DPPH assay, the aqueous extract showed a higher free radical scavenging activity (78.35 ± 0.42) compared to the methanol extract (63.45 ± 0.14), suggesting that water-soluble compounds contribute significantly to the antioxidant potential. Similarly, the ABTS assay confirmed this trend, with the aqueous extract displaying greater radical scavenging activity (84.55 ± 0.42) than the methanol extract (66.00 ± 0.28), further highlighting the effectiveness of water-soluble antioxidants. In terms of hydrogen peroxide (H_2O_2) scavenging, the aqueous extract again outperformed the methanol extract, exhibiting a higher activity level (90.00 ± 0.57 vs. 68.90 ± 0.28), which suggests its superior capacity to neutralize reactive oxygen species. However, the methanol extract demonstrated greater efficacy in the FRAP assay, with a ferric reducing antioxidant power of 185.50 ± 1.41, compared to 120.50 ± 1.41 for the aqueous extract, indicating that methanol may extract more potent electron-donating antioxidants. Additionally, the total antioxidant capacity was higher in the methanol extract (241.00 ± 2.83) than in the aqueous extract (193.50 ± 4.24), suggesting a broader range of antioxidants with diverse mechanisms of action are present in the methanol extract. These findings collectively highlight the distinct antioxidant profiles of the aqueous and methanol extracts, each offering unique contributions to the overall antioxidant activity of Citrus limon leaves.

The findings from this study highlight the potential of Citrus limon leaves powder as a valuable source of natural antioxidants. The high levels of bioactive compounds, particularly in the methanol extract, make it an excellent candidate for use in functional foods and nutraceuticals. The rich mineral composition further enhances its nutritional profile, supporting its use as a dietary supplement.

5. CONCLUSION

This study demonstrates the significant antioxidant properties of Citrus limon leaves powder, particularly from Puliyankudi, Tamil Nadu. The comprehensive range of antioxidant assays confirmed high performance across all tested parameters, with both aqueous and methanol extracts showing substantial efficacy. The methanol extract generally exhibited higher antioxidant activity, except for the DPPH and H2O2 assays where the aqueous extract showed superior results. The rich mineral composition and potent antioxidant properties suggest that Citrus limon leaves powder could be effectively utilized in the development of functional foods and nutraceuticals, offering both health benefits and nutritional value. Future research should focus on the bioavailability and long-term health effects of these compounds in vivo to fully realize their potential in human health applications. Additionally, exploring the synergistic effects of combining Citrus limon leaves powder with other natural antioxidants could provide insights into developing more effective antioxidant formulations.

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