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Research Article

Quantitative Analysis of Phenolic Compounds in Citrus Peels Using UV-Visible Spectrophotometry

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ABSTRACT

Citrus fruit peels, often discarded as waste, are abundant sources of polyphenolic compounds with recognized antioxidant potential. This study was undertaken to comprehensively quantify polyphenols present in lemon (Citrus limon) and kaffir lime (Citrus hystrix) peels. A systematic approach was employed involving maceration extraction followed by ultraviolet-visible (UV-Vis) spectrophotometric analysis. Additionally, infrared (IR) spectroscopy was used to confirm the presence of key phenolic functional groups. Ethanol and ethyl acetate were selected as solvents due to their polar nature and effectiveness in extracting phenolics. Tannic acid served as a standard for the calibration curve, with the maximum absorbance observed at 208.2 nm. The quantification yielded polyphenol contents of 1.201 g/g and 1.057 g/g in lemon and kaffir lime peels, respectively. These results reinforce the potential application of citrus peels as sustainable and cost-effective sources of natural antioxidants in pharmaceutical, nutraceutical, and food industries.

INTRODUCTION

Polyphenols are naturally occurring secondary plant metabolites comprising multiple phenolic structures. Known for their antioxidant and therapeutic efficacy, polyphenols neutralize reactive oxygen species (ROS), chelate pro-oxidant metal ions, and modulate cellular signaling pathways involved in inflammation,

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cancer progression, and neurodegenerative diseases.[1–4] These bioactivities have sparked interest in identifying alternative sources of polyphenols, particularly from agricultural by-products. Citrus fruits are globally cultivated and extensively consumed. Post-consumption, large volumes of peel residues are generated and often discarded. These peels, however, are rich in bioactive phytoconstituents including flavonoids (e.g., hesperidin, naringin), phenolic acids (e.g., ferulic acid, caffeic acid), pectins and essential oils.[5–7] Valorizing citrus peels offers both environmental and economic advantages by converting waste into high-value antioxidant-rich extracts.

Among various extraction techniques, maceration remains a widely adopted method in phytochemistry due to its operational simplicity and solvent compatibility. Despite the development of more advanced methods like microwave-assisted extraction (MAE) and supercritical fluid extraction (SFE), maceration is suitable for pilot-scale studies and comparative analysis, especially in resource-limited settings.[8–10] The efficiency of extraction hinges on solvent polarity. Ethanol and ethyl acetate are frequently used for their effectiveness in dissolving phenolic compounds without compromising extract integrity. Their relative safety also permits use in food and cosmetic applications.[11,12] Quantitative analysis of extracted phenolics is conventionally carried out using UV-Vis spectrophotometry due to its accuracy, reproducibility, and cost-efficiency. The method involves measuring absorbance of polyphenol solutions at a defined wavelength and calculating concentration using the Beer-Lambert law.[13,14] Additionally, IR spectroscopy can identify functional groups within complex mixtures, thus serving as a structural confirmation tool. [15–17] This study aimed to develop an accessible and precise method to extract and quantify polyphenols from lemon and kaffir lime peels, combining traditional maceration with UV-Vis spectrophotometry and IR spectroscopy.

MATERIALS AND METHODS:

Chemicals and Reagents

Analytical-grade ethanol and ethyl acetate, tannic acid, double-distilled water, and Whatman No. 42 filter paper were used.

Instruments and Equipment

- UV-Visible Spectrophotometer (Shimadzu UV-1800)
- Fourier Transform Infrared Spectrophotometer (Shimadzu IR-8400)
- Digital Analytical Balance (Shimadzu BL-200 H)
- Ultrasonicator (PCI 3.5L100)
- Hot Air Oven (Universal Instruments)

Sample Collection and Preparation

Lemon and kaffir lime fruits were purchased from a local vendor. Peels were manually separated, thoroughly rinsed, chopped, and air-dried under shade for 7–10 days. To ensure moisture removal, peels were subjected to additional drying in a hot air oven at 40°C for 20 minutes. The dried material was pulverized using a mortar and pestle, then sieved to obtain a fine powder and stored in sterile containers at 4°C.

Extraction Procedure

For extraction, 3 g of powdered peel was added to 45 ml of ethanol (for lemon) or ethyl acetate (for kaffir lime) in iodine flasks. The flasks were sealed and left in a dark environment at room temperature for 7 days with intermittent shaking. After extraction, the mixture was filtered and the solvent evaporated at room temperature to yield crude extracts.

Infrared Spectroscopic Analysis

IR analysis was conducted using the KBr pellet method. Spectra were scanned in the range of 4000–400 cm⁻1. Diagnostic peaks indicating phenolic compounds included O–H stretch (~2925 cm⁻1), C=C aromatic stretch (~1612 cm⁻1), and carbonyl (C=O) stretching vibrations (~1745 cm⁻1).

UV-Vis Spectrophotometric Quantification

Tannic acid standard solution (1 mg/ml) was prepared and serially diluted. Absorbance was recorded at 208.2 nm. Peel extracts were prepared similarly and compared against the standard curve to calculate polyphenol concentration. All samples were analyzed in triplicate, and data were expressed in g/g of extract.

RESULTS AND DISCUSSION:

IR Spectroscopy

Both lemon and kaffir lime peel extracts showed IR absorption bands characteristic of polyphenols. The broad O–H stretch peak at ~2925 cm⁻1 indicated hydroxyl groups, while peaks at ~1612 cm⁻1 and ~1745 cm⁻1 corresponded to aromatic ring and carbonyl functionalities respectively, confirming phenolic presence. The results are shown in table 1, 2 and figure 1,2.

Table 1: Interpretation of sample -1

INTERPRETATION	WAVELENGTH (1/CM)
C-OH stretching of phenol	2925.81
C-H stretching of hydrogen	2854.45
C=O stretching of ketone	1745.46
C=C stretching of phenol	1612.38
C-O stretching of ketone	1155.28

Table 2: Interpretation of sample - 2

INTERPRETATION	WAVE LENGTH (1/CM)
C-OH stretching of phenol	2925.81
C-H stretching of hydrogen	2854.45
C=O stretching of ketone	1743.53
C=C stretching of phenol	1679.88
C-O stretching of ketone	1157.21

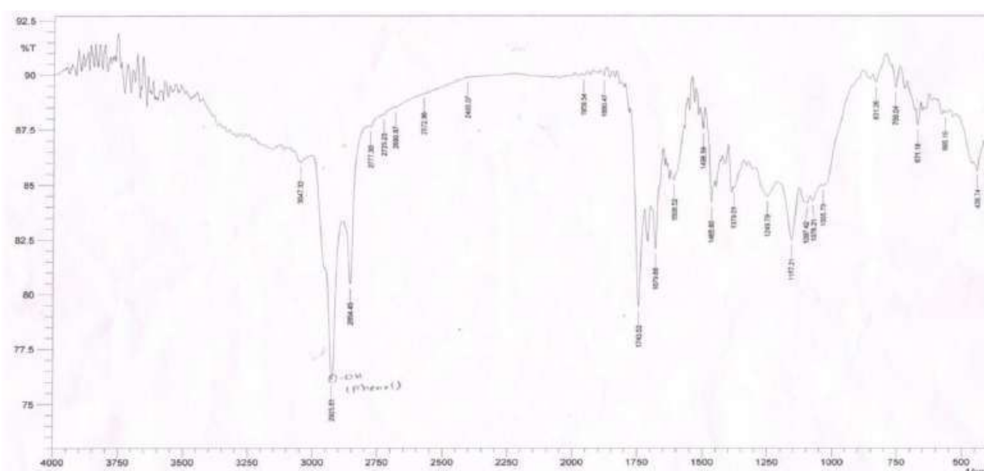

Figure 1: Interpretation of sample -1

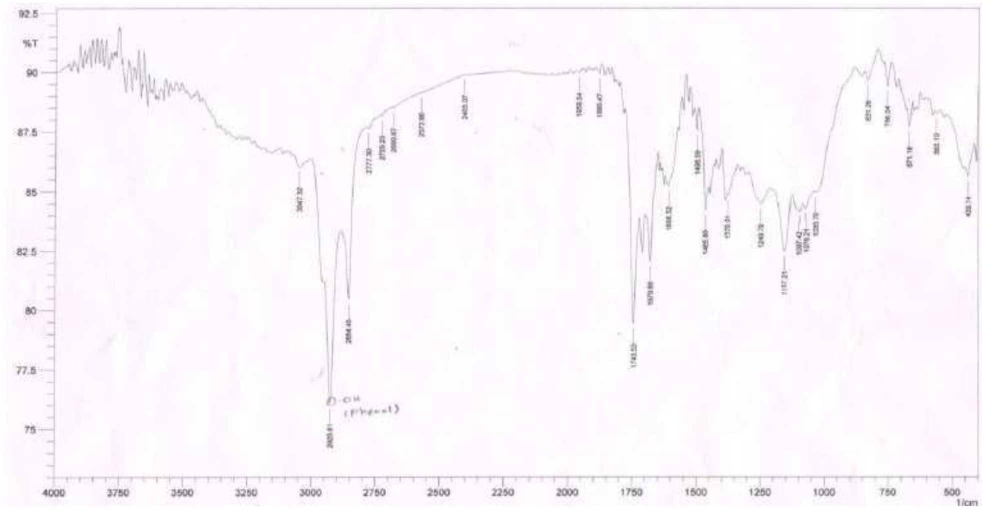

Figure 2: Interpretation of sample – 2

UV-Visible Analysis

Tannic acid displayed a maximum absorbance at 208.2 nm. The calibration curve exhibited linearity ($R^2 > 0.99$) across the tested concentrations. Quantitative estimation revealed 1.201 g/g of polyphenols in lemon peel and 1.057 g/g in kaffir lime peel. These findings were consistent with those reported by Nishad *et al*., [18] and Saini *et al*., [19] who documented high polyphenolic content in citrus peel extracts obtained via ethanol-based extraction.

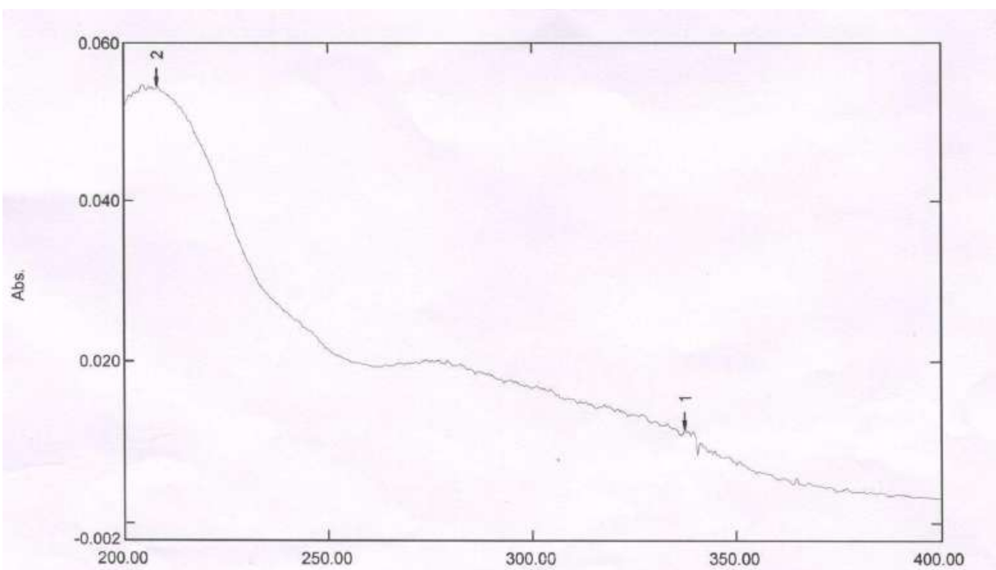

Figure 3: Determination of wavelength

Table 3: Absorptivity value for polyphenols in citrus peels powder

SAMPLE	λ max	ABSORBANCE	CONCENTRATION OF CITRUS PEELS (g)
Sample 1	208.2	0.025	**1.201**
Sample 2	208.2	0.022	**1.057**

Comparative Analysis

The yield of polyphenols from both citrus species demonstrates the efficacy of the extraction process. Although modern techniques like ultrasound-assisted extraction (UAE) have shown higher yields, maceration remains valuable for preliminary screening and comparative phytochemical analysis. [20–22] Additionally, the observed antioxidant potential aligns with prior studies suggesting that citrus peels are suitable candidates for developing natural antioxidant formulations. [23–25]

CONCLUSION

This investigation successfully established a cost-effective and reproducible method for the extraction and quantification of polyphenols from citrus peels. The integration of maceration, UV-Vis spectrophotometry, and IR spectroscopy provides a robust analytical platform for polyphenolic assessment. The significant polyphenol yield highlights the value of lemon and kaffir lime peels as bioresource materials in functional food and pharmaceutical formulations. Further studies may include bioactivity profiling, antioxidant assays (DPPH, FRAP), and scale-up of extraction methods for industrial use.

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