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Research Article

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Solvent polarity-driven phytochemical profiling and antioxidant evaluation of Indian Persimmon (*Diospyros kaki* Thunb.) fruit extracts

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ABSTRACT

The present study investigates the phytochemical composition and antioxidant potential of Indian Persimmon (*Diospyros kaki* Thunb.) extracts obtained using ten different solvents with varying polarity. Soxhlet extraction was employed to obtain the extracts, with hydroethanolic solvent yielding the highest extractable content. Qualitative phytochemical screening revealed the presence of key bioactive compounds, including flavonoids, phenolics, alkaloids, terpenoids, saponins, tannins and significant levels of sugar across all extracts. Quantitative estimations indicated that Acetone extract of *D. kaki* (7.23±0.5 mg GAE/g) and Ethyl acetate extract of *D. kaki* (4.54±0.01 mg QE/g) were particularly rich in total phenolics and flavonoids, while Aqueous extract of *D. kaki* (1016±0.36 mg GE/g) and Hydroethanol extract of *D. kaki* (1015±0.79 mg GE/g) exhibited higher carbohydrate content. Uronic acid and reducing sugar levels were also prominent in the Acetone extract of *D. kaki* (395.14±0.32 & 1255±2 mg GluA/g), suggesting efficient extraction of pectic and simple sugar fractions. Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assays. Among the tested extracts, Ethyl acetate extract of *D. kaki* (28.96 & 33.19 µg/mL) and Acetone extract of *D. kaki* (127.6 & 18.43 µg/mL) demonstrated notable antioxidant capacities, with IC_{50} values closely approaching that of the standard BHT in both assays. These findings underscore the influence of solvent polarity on phytochemical recovery and bioactivity, with mid-polar solvents proving most effective in extracting antioxidant constituents. The results highlight *D. kaki* fruit as a promising natural source of antioxidants with potential therapeutic applications, particularly in managing oxidative stress-related disorders such as colon cancer.

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INTRODUCTION

Free radicals are extremely reactive, characterized by the presence of unpaired electrons. They perform crucial functions in numerous chemical and biological processes, encompassing aging, disease development and environmental interactions. Free radicals naturally generated by activities such as respiration, foods, alcoholic metabolic processes and lipid conversion, can initiate detrimental reactions within the body. If inadequately regulated, these can compromise and disrupt all cellular activities (Sharifi-Rad *et al.*, 2020). Oxidative stress denotes a discrepancy in the generation of Reactive Oxygen Species (ROS) and the cellular antioxidant defence systems. Under typical physiological settings, the generation of ROS is counterbalanced by antioxidants, which mitigate these reactive entities and preserve cellular equilibrium (Iqbal *et al.*, 2024).

Dietary antioxidants, especially those derived from fruits and vegetables are widely recognized for their health-protective effects. Plants-based foods are rich in polyphenols (flavonoids, phenolic acids, tannins, etc.), vitamins (e.g., vitamin C, E, carotenoids) and other bioactive compounds that can quench free radicals and inhibit oxidative damage (Singh *et al.*, 2022). Polyphenols, in particular, can donate electrons or hydrogen atoms to ROS, converting these reactive species into more stable molecules and thereby interrupting radical chain reactions (Andrés *et al.*, 2023). Diets abundant in fruits and vegetables are strongly associated with a lower incidence of oxidative stress-

related disease. For example, high polyphenol intake has been linked to reduced lipid oxidation and cardiovascular risk (Iqbal *et al.*, 2023). These findings have spurred interest in identifying potent natural antioxidants and optimizing their extraction from dietary sources. Compared to synthetic antioxidants like BHT (butylated hydroxytoluene), natural antioxidants tend to have fewer side effects and added nutritional benefits, making them attractive for functional foods and nutraceutical development.

Persimmon (*Diospyros kaki* Thunb.), a nutrient-dense fruit has gained attention as a promising source of natural antioxidants. Belonging to the Ebenaceae family, persimmon is widely cultivated in Asia (with China producing ~79% of the global supply) and valued for its sweet, palatable fruit, as reported by the United Nations Food and Agriculture Organization (UNFAO) (Dong *et al.*, 2024). This fruit is rich in essential nutrients like vitamins C & A, minerals, amino acids, sugar, lipids and pectin and also contains diverse phytochemicals such as carotenoids, flavonoids, tannins, terpenoids and phenolic acids (Polat *et al.*, 2024). Notably, persimmon has a high polyphenol content relative to many other fruits (Zhao *et al.*, 2024). The fruit occurs in both astringent and non-astringent varieties; astringent types are especially high in tannins, which are responsible for the puckering taste and are potent antioxidants (Giri *et al.*, 2024; Taguiam *et al.*, 2024). Studies have shown that persimmon extracts and isolated compounds exhibit strong antioxidant activity (González *et al.*, 2022) and anti-inflammatory properties (López-Bermudo *et al.*, 2024), along with disease-prevention capabilities. Consuming persimmons may have hypolipidemic effect (Hwang *et al.*, 2017), hypoglycemic activity (Han *et al.*, 2024), antiageing (Yokozawa *et al.*, 2014), immunomodulatory effect (Shin *et al.*, 2022), proper digestion (González *et al.*, 2021), antiobesity activity (Kim *et al.*, 2020) and anticancer activity (Al-hameed & Mohammed, 2022). For instance, persimmon tannins can significantly reduce oxidative damage to biomolecules and oligomeric proanthocyanidins from persimmon have been implicated in anti-aging mechanisms (Rauf *et al.*, 2019). Such pharmacological activities highlight persimmon as not only a nutritious fruit but also a potential source of therapeutic agents against oxidative stress-related disorders.

Effective extraction of persimmon's phytochemicals is essential to harness its full therapeutic potential. Solvent extraction is a crucial factor influencing extraction yield and phytochemical profile (Gil-Martín *et al.*, 2022). Different solvents (ranging from non-polar to polar) variably solubilize compounds depending on their polarity: for example, water and other polar solvents readily extract sugars and hydrophilic phenolics, whereas moderately polar organic solvents may better recover medium-polarity phenolics and flavonoids (Menegazzo & Fonseca, 2019). Prior research demonstrates that optimizing solvent polarity can maximize the recovery of antioxidants from plant materials. In persimmon and other botanical sources, solvents like methanol or ethanol often yield high total phenolics, while mixtures (e.g. hydroalcoholic solvents) can improve extraction efficiency by targeting a broader range of compounds (Lee *et al.*, 2024). For instance, one study on persimmon fruit reported that 70% ethanol extracts showed superior DPPH radical scavenging compared to less polar solvents (Choe *et al.*, 2014). Additionally, comparisons of persimmon seed or peel extracts indicate that solvent choice can markedly affect measured antioxidant activity, correlating with the types of phenolic compounds extracted (Jang *et al.*, 2011). Common antioxidant assays such as DPPH and ABTS are frequently employed to evaluate the radical-scavenging capacity of extracts, providing insight into how effectively the extracted phytochemicals can neutralize free radicals. The DPPH assay uses a stable free radical whereas the ABTS assay measures the quenching of a blue-green radical cation which can be scavenged by both hydrophilic and lipophilic antioxidants (Floegel *et al.*, 2011). The complementary assays help characterize the antioxidant potency of extracts in different chemical environments.

Considering the above, the present work systematically investigates how extraction solvent polarity influences the phytochemical yield and antioxidant activity of persimmon (*D. kaki*) fruit. Ten solvents ranging from non-polar (hexane, chloroform, petroleum ether) to polar (water, alcohols) were used to prepare fruit extracts, which were then profiled for their phytochemical composition (qualitatively and quantitatively) and evaluated for antioxidant activity using DPPH and ABTS assays. The aim is to determine which solvents maximize the recovery of key antioxidant constituents (phenolics and flavonoids, etc.) and how these constituents translate into radical-scavenging efficacy. By relating solvent polarity, phytochemical content and antioxidant performance, we provide insights into optimal extraction strategies for persimmon and shed light on the mechanisms by which different phytochemical classes contribute to antioxidant activity. This knowledge is valuable for developing persimmon-derived nutraceuticals or functional ingredients to combat oxidative stress and associated diseases.

MATERIALS AND METHODS

Sample Collection and Authentication

The popular Indian persimmon fruit (*Diospyros kaki* Thunb.) cultivar 'Delotus' was obtained at the Government of Tamil Nadu's Pomological station, Coonoor, Tamil Nadu, India in July (Figure 1a). The authenticity of the fruit was confirmed (Ref. No: S.M.S.001) by the Raphinat Herbarium at St. Joseph College (Autonomous) in Tiruchirappalli, Tamil Nadu- 620002, India. The entire fruit underwent a thorough washing process using reverse osmosis water, followed by wiping and subsequent storage at a temperature of -80 °C. The fruit sample was cut into small pieces and subjected to freeze drying at a temperature of -50 °C using a Lyophilizer (Esquire Biotech, EBT 12N) (Figure 1b). The desiccated fruit material was pulverized into granular form and set aside for subsequent analysis (Figure 1c-e).

Preparation of Solvent Extracts

Soxhlet extraction (Organic solvents)

The freeze-dried material of Persimmon fruit was subjected to extraction utilizing several solvents including ethanol, methanol,

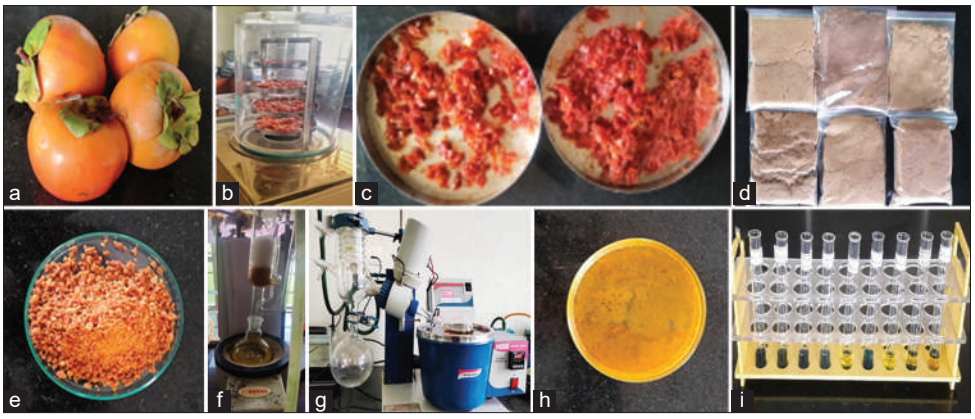

Figure 1: a) Fresh persimmon fruit, b) Lyophilization of the sample, c) Freeze-dried persimmon pieces, d, e) Lyophilized persimmon powder, f) Soxhlet extraction of fruit sample, g) Solvent condensation using rotary vacuum evaporator, h) Crude extract of the sample and i) Qualitative phytochemical analysis of extracts

acetone, hexane, ethyl acetate, chloroform and petroleum employing the Soxhlet equipment (Figure 1f). 20 g of freeze-dried powdered sample was placed in an extraction thimble and extraction was performed at a controlled temperature of 50-60 °C for 6-8 cycles for each extraction for 2-3 days. Post extraction, solvents were evaporated using a rotary evaporator maintained at 50-60 °C (Figure 1g). The resulting extracts were stored at -20 °C until analyses (Figure 1h).

Maceration method (Aqueous, Hydroethanolic & Hydromethanolic)

Aqueous extraction involved soaking 5 g of powdered persimmon fruit in 150 mL distilled water. For hydroethanolic (50%) and hydromethanolic (50%) extractions, 20 g of powdered samples were extracted with respective solvents (200 mL). Extractions were carried out at room temperature (25 °C) under continuous shaking for 48 hours. Extracts were then filtered through Whatman No.1 filter paper using Buchner funnel. The aqueous extract was dried in a hot air oven at 40 °C for 48 hours. All final extracts were adjusted to a standard concentration (1mg/mL) (w/v) using respective solvents and stored at -20 °C until analyses. The percentage yield for each solvent extraction was calculated as follows:

$$\text{Yield (\%)} = (\text{Weight of the dry extract/Initial weight of the powdered sample}) \times 100$$

Phytochemical Screening

Qualitative analysis

Preliminary phytochemical analyses were conducted (Widjajakusuma *et al.*, 2019). Tests were performed for alkaloids (Mayer's reagent) (Figure 1i), phenolics (5% ferric chloride), flavonoids (magnesium turnings with conc. HCl), terpenoids (acetic acid with conc. Sulfuric acid), saponins (foam test), reducing sugars (Fehling's solution), tannins (ferric chloride), phlobatannins (ammonia solution), quinones (conc. Sulfuric acid), general sugars (Benedict's reagent) and cardiac glycosides (glacial acetic acid and conc. Sulfuric acid with $FeCl_2$).

Quantitative analysis

Total phenolic content (TPC)

TPC of 10 different extracts acquired from the fruit of *D. kaki* was assessed using a Folin-Ciocalteu's reagent assay (Ayele *et al.*, 2022) and a UV spectrophotometer (Shimadzu/UV-2600, Japan). Approximately 100 µL of extract (1 mg/mL) was mixed with 250 µL of FC reagent, followed by the addition of 7.5% sodium carbonate solution. The volume was adjusted to 2.5 mL with distilled water and the mixture was vigorously vortexed for 15 seconds. After incubating in darkness for 1 hour, the absorbance was measured at 760 nm. Gallic acid served as the reference standard drug for the analysis. The aforementioned GA concentrations helped to determine/express the TPCs as mg of GAE/g of the extract and to obtain the regression equation curve from the absorbance. The outputs of this triple TPC estimation of the standard and sample were completed and presented as mean±standard error mean (SEM).

Total flavonoid content (TFC)

TFC in 10 different extracts attained from fruits of *D. kaki* was determined using the $AlCl_3$ method (Ali *et al.*, 2018). A stock solution of quercetin was prepared in methanol, and the sample extracts were also dissolved in their respective solvents to achieve a concentration of mg/mL. Subsequently, 20-100 µL of the quercetin stock solution/100 µL of the sample solution were mixed with 600 µL of methanol, 40 µL of a 10% $AlCl_3$ solution prepared in ethanol, 40 µL of 1M potassium acetate and the volume was made up to 2 mL with distilled water. The mixture was then incubated at room temperature for 30 minutes, with methanol serving as the blank solvent. The TFC of both the standard and sample solutions was measured spectrophotometrically at 420 nm. The various concentrations of quercetin used in this process were used to construct a standard curve based on absorbance and the TFC of the samples was expressed in milligrams of quercetin equivalent per gram of the extract (mg QE/g). The quantification was performed in triplicate and the results were presented as the mean value with standard error of the mean (SEM).

Total carbohydrate content (TCC)

TCC of 10 extracts earned from the fruits of *D. kaki* was determined using the Anthrone method (Kaur *et al.*, 2018). To reach a concentration of 1 mg/mL, the sample extracts were also dissolved in the corresponding solvents after a stock solution of glucose was produced in distilled water. 100-1000 µL of stock solution/1000 µL of sample solution was added to 4 millilitres of anthrone reagent and the mixture was boiled for 15 minutes. The TCC of both the standard and sample was measured spectrophotometrically at 620 nm after cooling the tubes, ideally in ice. Calculate the amount of carbohydrates in the standard curve by utilizing the standard glucose solution. The TCC of the samples was reported in milligrams of glucose equivalent per gram of extract (mg GE/g), and a standard curve on absorbance was constructed using the different amounts of glucose utilized in this method. The results of this triplicate quantification were displayed as the mean value together with the standard error of the mean (SEM).

Uronic acid content

The amount of uronic acid present in 10 different extracts of the fruits of *D. kaki* was determined (Oshima *et al.*, 2021). After making a stock solution of glucose in distilled water, the sample extracts were also diluted to achieve a concentration of 1 mg/mL. To 20-100 µL of stock solution/mL of sample solution, add 40 µL of 4M sulfamic acid and 2.4mL of concentrated sulfuric acid and mix thoroughly. After adding 100 µL of 0.1% carbazole, place it in a water bath for 20 minutes and allow it to cool down. Then the uronic acid in the extracts and standard was determined using a spectrophotometer at 525 nm. Calculate the amount of uronic acid present in the standard curve using the standard glucose solution. A standard curve based on absorbance was created using the various amounts of glucose used in this procedure and the uronic acid content of the samples was expressed in milligrams of glucuronic acid equivalent per gram of extract (mg GluA/g). The mean value and standard error of the mean (SEM) were presented as the outcomes of this triplicate quantification.

Reducing sugar content

The amount of reducing sugars present in the 10 different extracts of *D. kaki* fruits was determined (Ayala *et al.*, 2021). To obtain a concentration of 1 mg/mL, the sample extracts were additionally diluted with suitable solvents after preparing a stock solution of glucose in distilled water. Add 1 mL of DNS reagent to 100-1000 µL of the stock solution or sample solution and then place the mixture in a water bath for 15 minutes before allowing it to cool in an ice bath for 2 minutes. Following this, introduce 9 mL of distilled water and the reducing sugars in the extracts and standard were measured in the absorbance at 540 nm. Determine the concentration of reducing sugars in the standard curve by employing the standard glucose solution. A standard curve was constructed by measuring absorbance with different concentrations of glucose during this procedure. The reducing sugar content of the samples was expressed in milligrams of glucose equivalent per gram of extract (mg GE/g). The results in this triplicate quantification were present as the mean value and standard error of the mean (SEM).

Antioxidant Assays

It is widely acknowledged that oxidative stress plays a pivotal role in the pathogenesis of several types of cancer, including colon cancer. Oxidative stress arises from a disparity between the generation of reactive oxygen species (ROS) and the organism's capacity to mitigate or restore the resulting damages inflicted by these deleterious chemicals (Di Carlo & Sorrentino, 2024). The occurrence of this imbalance has the potential to result in cellular harm, alterations in DNA, and an elevated susceptibility to the development of cancer (Alhmoud *et al.*, 2020).

DPPH free radical scavenging activity

The DPPH assay evaluates antioxidant activity by measuring the reduction of the stable, purple-coloured 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical to its yellow-coloured non-radical from upon receiving an electron or hydrogen atom from an antioxidant (Akar *et al.*, 2017). This study discovered that *D. kaki* fruit extracts inhibited DPPH free radicals by the modified method (Baliyan *et al.*, 2022). 1,1-diphenyl 2-picry hydrazyl (DPPH) was used to determine the antioxidant activity of all ten extracts of *D. kaki* fruit through a spectrophotometer. The standard commercial antioxidant, which was ascorbic acid, was prepared in methanol (w/v), while the sample crude extracts were diluted to achieve the concentration of 1 mg/mL. Add 50-250 µL of standard or sample solution to a 0.1 mM DPPH solution, then adjust the total volume to 3 mL by introducing methanol. Thoroughly mix the entire combination and store it in a dark place at room temperature for approximately 30 minutes. A lower optical density (OD) value indicates a higher scavenging activity. The control group used the same quantity of DPPH solution, with the volume adjusted to 3 mL through the addition of methanol. Methanol was used as the baseline correction solvent. The decrease in the absorbance of each extract was observed at 517 nm using a UV-spectrophotometer (Shimadzu/UV-2600, Japan) and the percentage of inhibition was calculated using the formula,

$$\text{Inhibition (\%)} = [(\text{Absorbance of control} - \text{Absorbance of Sample})/\text{Absorbance of control}] \times 100$$

The IC_{50} value, representing 50% inhibition, was determined for the standard and sample (µg/mL) using linear regression analysis, with a lower value indicating stronger antioxidative properties. This experiment was conducted in triplicate, and the results were presented as the mean value±SEM.

ABTS radical cation scavenging activity

The reduction of the ABTS radical cation ($ABTS^{*+}$) in *D. kaki* fruit extracts was consistently observed (Hussen & Endalew, 2023). The preparation of the $ABTS^{*+}$ stock solution involved combining 10 mL of 7 mM ABTS (dissolved in 100 mL distilled water) with 10 mL of 2.45 mM potassium persulfate

(PPS, dissolved in 100 mL distilled water) in a light-protected container. The mixture was left undisturbed for 12 to 16 hours at room temperature, allowing PPS to generate a stable bluish-green $ABTS^{*+}$ solution. The absorbance of the solution was adjusted to 0.700±0.10 at 734 nm by diluting with methanol if the absorbance was below this threshold. The prepared stock solution remained stable for up to fifteen days. For the assay, 1.0 mL of the working $ABTS^{*+}$ stock (with an absorbance of 0.700±0.10 at 734 nm) was mixed with 50-250 µL of either the standard (BHT) or sample dilutions. The total volume was adjusted to 2.0 mL using methanol. A control was prepared by combining 1.0 mL of the $ABTS^{*+}$ stock solution with 1.0 mL of methanol, serving as a baseline correction agent. After thorough mixing, the reaction solution was incubated at room temperature for five minutes and the scavenging activity of the standard or sample was measured spectrophotometrically at 734 nm. The optical density (OD) values of the standard and sample concentrations were correlated with their antioxidant capacities, following a pattern consistent with the DPPH assay. The calculation of the inhibition percentage, IC_{50} values, the number of experimental replicates and their presentation were performed using the same methodology as in the DPPH assay.

Statistical Analysis and Data Visualization

All experiments were conducted in triplicate. Data were analysed using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) to determine statistically significant differences. Statistical significance was considered at $p \leq 0.05$. All statistical analyses were performed using SPSS version 20 (IBM SPSS Inc., Armonk, New York, USA).

Data visualization and plotting were carried out using Python 3.11 in Jupyter Notebook, employing Seaborn and Matplotlib libraries for violin plots, bubble charts, heatmaps and bar plots. Customizations such as bubble scaling, annotation and violin overlays were implemented to highlight key trends and statistical features. Final figures were rendered on a Windows 10 operating system.

RESULTS AND DISCUSSIONS

Extraction Yield by Solvent Type

The choice of solvent had a profound impact on the yield of extractable solids from persimmon fruit. Yields ranged from very low with non-polar solvents to exceptionally high with polar mixtures. Notably, the 50% hydroethanolic solvent produced the highest yield (approximately 65% of dry weight), significantly outperforming both pure water (30%) and pure methanol (58%). This suggests a synergistic effect of the water-ethanol mixture, which can solubilize a broad spectrum of compounds. Hydromethanol (50% methanol) also gave a moderate yield (~35.7%). In contrast, non-polar solvents (hexane, petroleum ether) yielded <1% (0.2-0.3%), reflecting the low content of purely lipophilic constituents in the fruit (Figure 2). These trends align with general expectations that polar or mid-polar solvents recover the bulk of fruit phytochemicals (which include water-soluble sugars, organic acids and polyphenols), whereas non-polar solvents extract only hydrophobic compounds (e.g., lipid carotenoids) present in smaller quantities (Gil-Martín *et al.*, 2022). The superior performance of 50% ethanol in particular corroborates earlier reports that mixture of polar and less-polar solvents can improve extraction efficacy by targeting a wider range of constituents (Tourabi *et al.*, 2023). A study observed that intermediate polarity solvents optimally solubilize diverse antioxidants, which is consistent with our findings (Okur *et al.*, 2023).

Phytochemical Screening

Qualitative analysis

The preliminary phytochemical tests revealed that all ten extracts contained a broad array of bioactive compound

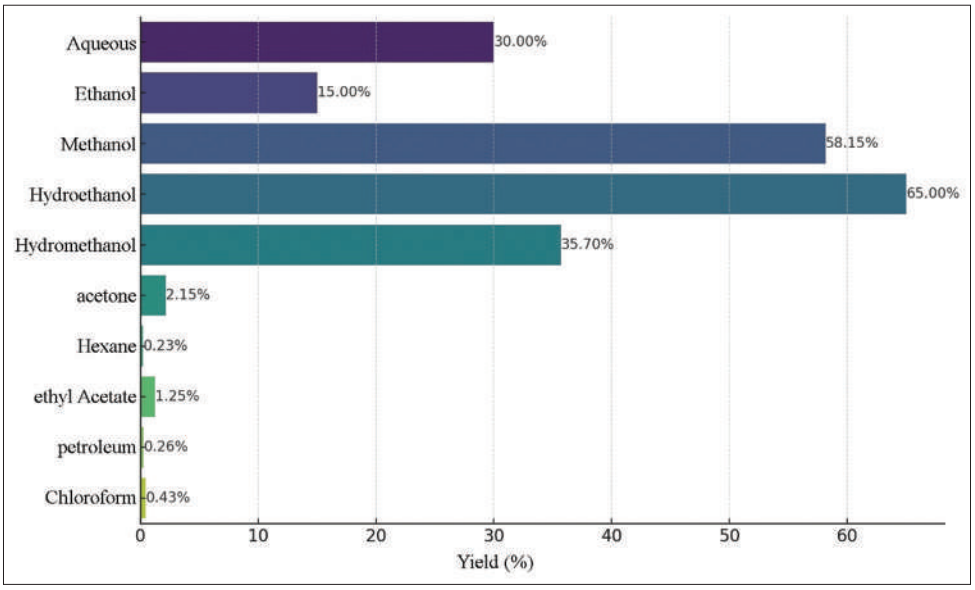

Figure 2: Extraction Yield Percentage by Solvent in *D. Kaki* fruit

classes with some variation in intensity (Figure 3). Phenolics, flavonoids, tannins, saponins, terpenoids, alkaloids, reducing sugars and other sugars were detected in every extract (at least in trace amounts). The ubiquity indicates that the persimmon fruit's metabolic profile is rich and diverse, even solvents with limited polarity can recover some of each class. For, example, alkaloids and phenolics were present (qualitative '+' test) across the board. All extracts tested very strongly positive for simple sugars, with the tests for total sugars and reducing sugars yielding intense reactions ('+++') in most extracts, the results showed the high natural sugar content of persimmon. The abundance of sugars corroborates earlier findings that persimmon fruits are rich in simple carbohydrates and polysaccharides (Dave *et al.*, 2021). Tannins were also universally present, though appearing more abundant (darker precipitates) in intermediate-polarity extracts (e.g., methanol, hydromethanol) than in non-polar ones (Fraga-Corral *et al.*, 2020). Overall, the qualitative profile confirms that no single solvent isolates a unique class exclusively, rather each solvent extracts a complex mixture, with polarity biases favouring certain compounds.

Quantitative analysis

Total phenolic content (TPC)

The total phenolic content of the 10 extracts was determined in this investigation using the regression equation derived from the gallic acid reference curve (Figure 4). Among the fruit extracts, TPC of Acetone extract of *D. kaki* fruit was comparatively higher (7.23±0.5 mg GAE/g), followed by HMEDK (Hydromethanol extract of *D. kaki* fruit) > HEEDK (Hydroethanol extract of *D. kaki* fruit) > PEDK (Petroleum extract of *D. kaki* fruit) >MEDK (Methanol extract of *D. kaki*) > EEDK (Ethanol extract of *D. kaki* fruit) > AEDK (Aqueous extract of *D. kaki* fruit) >AcEDK (Acetone extract of *D. kaki* fruit) > HEDK (Hexane extract of *D. kaki* fruit) > CEDK (Chloroform extract of *D. kaki* fruit). This value was markedly greater than that of most other extracts, suggesting that acetone (an intermediate-polarity solvent) is particularly effective at liberating polyphenolic compounds from persimmon. Acetone's ability to swell plant tissues and disrupt cell walls may facilitate the release of bound phenolics, including both polar phenolic acids and moderately polar flavonoids (Lee *et al.*, 2024). Following acetone, the next highest TPC values were observed in the HMEDK and HEEDK, which is logical given their mixed polarity. Pure methanol and even the petroleum ether extract also showed moderate TPC, whereas the chloroform extract had the lowest phenolic content. The aqueous extract was relatively low in TPC as well. These results support the view that solvents of intermediate polarity optimize phenolic extraction. This finding supports the view that acetone-based extraction may be particularly effective for phenolic compounds due to its intermediate polarity (Edo *et al.*, 2025). The high phenolic yield in acetone extract is significant because phenolics are known for their strong antioxidant properties and potential health benefits like anticancer activity (Maheshwari & Sharma, 2023).

Total flavonoid content (TFC)

The total flavonoid content (TFC) of 10 extracts was determined using the equation derived from the quercetin calibration curve (Figure 4). Ethyl acetate extract of *D. kaki* has higher flavonoid contents (4.54±0.01 mg QE/g of the dry weight of the extracts, respectively), followed by CEDK > AEDK > MEDK > AcEDK > HMEDK > HEEDK > HEDK > EEDK and PEDK. Ethyl acetate is a moderately polar aprotic solvent, which appears to selectively

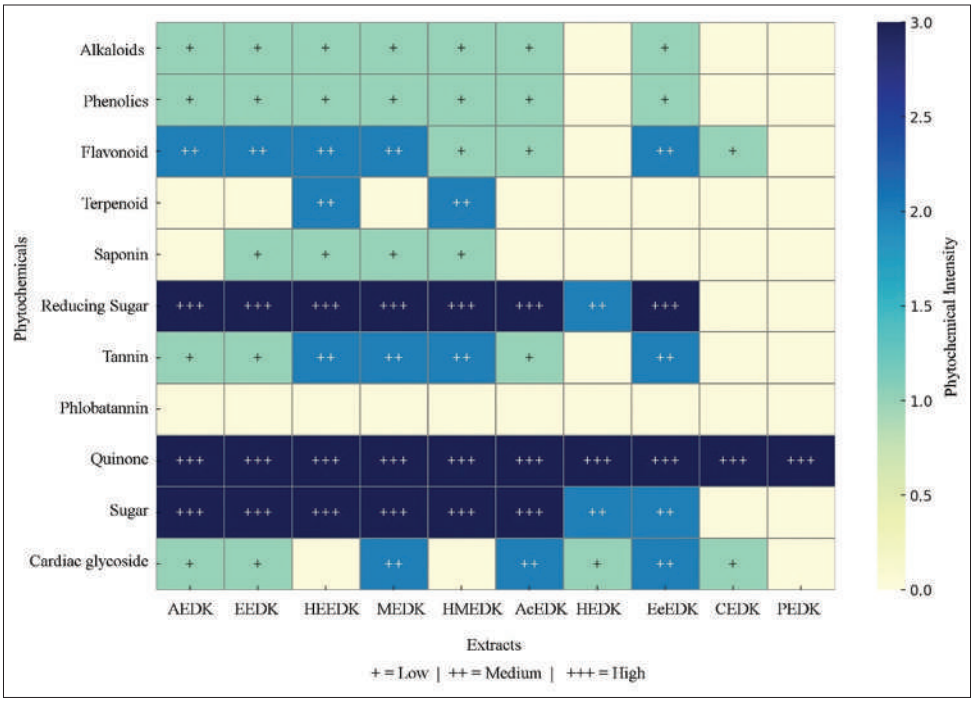

Figure 3: Heatmap of Phytochemical Screening in differential extracts of *D. kaki* Fruit

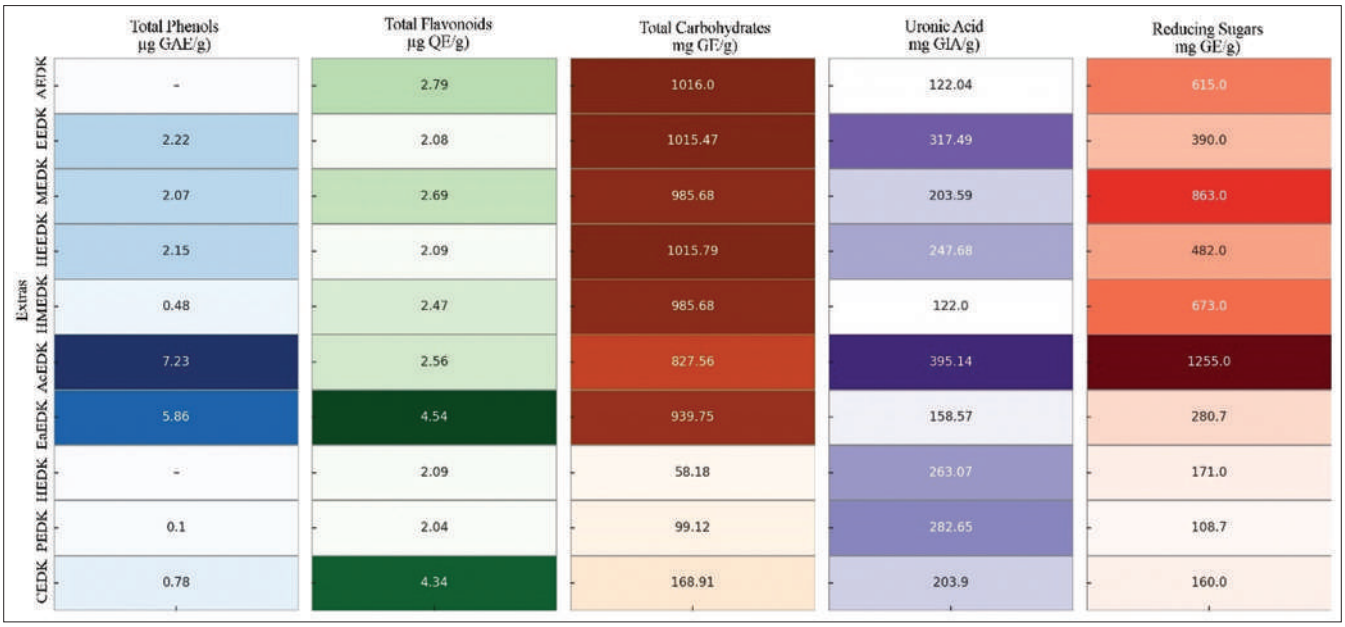

Figure 4: Heatmap of Phytochemical estimation in differential extracts of *D. kaki* Fruit

solubilize flavonoid aglycones and certain glycosides very well. This finding is consistent with literature indicating that ethyl acetate is often an excellent solvent for flavonoid-rich fractions (Ali Redha, 2021). It is interesting that the chloroform extract had a comparatively second high flavonoid content, which might indicate that certain flavonoid glycosides or non-polar flavonoid derivatives partition into moderately low-polarity solvents. The petroleum ether extract had the lowest TFC, as expected for a largely non-polar medium. These variations underscore that solvent polarity significantly influences not just the quantity of total flavonoids extracted, but also the specific types, ethyl acetate likely extracted a subset of flavonoids that are less polar than those extracted by water or methanol. The fact that water (high polar) and chloroform (very low polar) both extracted notable flavonoid levels suggests persimmon contains both flavonoid glycosides (soluble in water) and more lipophilic flavonoids or polyphenolic fragments (soluble in chloroform). Solvent-dependent selectivity is thus crucial for targeting desired antioxidant flavonoids. The variation in flavonoid content among extracts demonstrates that solvent choice significantly affects the type and quantity of extracted compounds.

Total carbohydrate content (TCC)

Persimmon fruit is known for its high sugar content and the determination of the total carbohydrate content (TCC) of 10 extracts was conducted by employing the regression equation that was generated from the glucose calibration curve (Figure 4). Among all the extracts, AEDK (1016±0.36 mg GE/g) HEEDK (1015.79±0.27 mg GE/g) and EEDK (1015.47±0.20) have higher sugar content than MEDK > HMEDK > EaEDK (Ethyl acetate extract of *D. kaki* fruit) > AcEDK > CEDK > PEDK > HEDK. Over 100% of the extract mass was equivalent to glucose, indicating that these polar extracts are largely composed of sugars and polysaccharides (including pectin). Such high values suggest that simple sugars and soluble polysaccharides constitute a major fraction of the persimmon fruit dry weight, reinforcing persimmon's role as an energy-rich fruit. Indeed, persimmon pulp is rich in fructose, glucose and sucrose as well as pectic substances (Dong *et al.*, 2024). The slightly lower TCC in methanol and mixed solvents suggests that while those solvents do extract sugars, their capacity is a bit less than water or pure ethanol for very polar saccharides. On the other hand, the non-polar extracts showed negligible carbohydrate content, which is expected since sugars are insoluble in non-polar media. The highest carbohydrate concentrations in aqueous and ethanolic extracts showed the carbohydrates are highly polar and water-soluble. The values observed suggest that the major fraction of fruit weight is constituted by sugars, reinforcing persimmon's traditional use as an energy-rich dietary fruit (Alara *et al.*, 2021).

Uronic acid content

The determination of uronic acid in 10 extracts was performed using the regression equation derived from the glucose calibration curve (Figure 4). Closely related to total sugars is the uronic acid content, which reflects pectic polysaccharides (rich in glucuronic acid units). AcEDK (395.14±0.32 mg/GE of the dry weight of the extract) exhibited a relatively higher value after EEDK > PEDK > HEDK > HEEDK > MEDK > CEDK > EaEDK > AEDK > HMEDK. Acetone's efficacy suggests, it was able to solubilize a significant amount of pectin or pectin fragments from fruit cell wall. Acetone is known to dehydrate and disrupt cell wall structure, which may facilitate pectin extraction (Bedzo *et al.*, 2024). The presence of uronic acids in some non-polar extracts is somewhat surprising, it could be due to co-extraction of pectin-associated lipophilic compounds or experimental error, but it might also indicate that during Soxhlet extraction some polysaccharide material carried over. Nonetheless, acetone's top performance for pectins underscores its ability to break the plant matrix and extract

large polar molecules. This has implications for antioxidant activity, as pectins and dietary fibers can indirectly contribute to health (e.g., via gut health and modulating absorption of sugars or lipids) and may also bind polyphenols (García-Pérez *et al.*, 2024). Prior work noted that stronger solvents can release matrix-bound compounds, enhancing yields of constituents like pectin that have gut-modulatory and antioxidant co-benefits (Akomeng & Adusei, 2021).

Reducing sugar content

The Dinitrosalicylic acid assay was conducted on all 10 extracts of *Diospyros kaki* fruit, utilizing the regression equation obtained from the glucose calibration curve (Figure 4). AcEDK (1255±2 mg GE/g) has a higher concentration of reducing sugar followed by MEDK > HMEDK > AEDK > HEEDK > EEDK > EaEDK > HEDK > CEDK > PEDK. This indicates an abundance of free monosaccharides and oligosaccharides in the acetone extract. It might seem counterintuitive that acetone (less polar than water) extracted more reducing sugars than water did. One plausible explanation is that acetone's disruption of cell walls and dehydration effect can break starch or larger sugars into smaller reducing sugars or release them more effectively, whereas an aqueous extraction might leave more of these sugars bound in complexes (Speir & Ross, 1981). Following acetone, methanol and hydromethanol extracts had the next highest reducing sugar levels and even the aqueous extract was fairly high. Polar solvents are generally expected to dissolve sugars well, so their strong performance is logical. The fact that acetone > methanol in this assay suggests that acetone may have caused partial hydrolysis of polysaccharides during extraction (acetone can sometimes promote acid hydrolysis if any organic acids are present). Alternatively, acetone might preferentially extract certain reducing polysaccharides and intermediates. Regardless, the high sugar content in many extracts (especially polar ones) is a double-edged sword: while sugars contribute to yield, they are essentially inactive in antioxidant assays and can dilute the concentration of phenolics per gram extract. This likely explains why some high-sugar extracts (like aqueous or ethanol) do not exhibit proportionally high antioxidant activity despite their yield, much of their mass is inert sugars rather than reactive antioxidants. Reducing sugars themselves generally do not scavenge free radicals (though they can participate in browning reactions), thus, their presence must be considered when correlating phytochemical content with bioactivity (Peng *et al.*, 2024).

Antioxidant Potential of Extracts

The antioxidant capacity of the persimmon extracts was evaluated by two complementary in vitro assays, DPPH and ABTS radical scavenging. These assays measure the ability of extracts to quench stable free radical species by donating an electron or hydrogen atom, thereby neutralizing the radical (Schaich *et al.*, 2015). The results are typically expressed as IC_{50} values (the concentration of extract required to scavenge 50% of the radicals) – lower IC_{50} indicates higher antioxidant potency. We compared the extracts against a known synthetic antioxidant standard, BHT (butylated hydroxytoluene), to gauge their efficacy.

DPPH Scavenging Activity

This study conducted an in vitro antioxidant test (DPPH) using extracts from all *D. kaki* fruit extracts to identify the active extracts possessing the highest capacity for reducing free reactive oxygen species (ROS). In the DPPH assay, the purple DPPH radical reduced to a yellow product upon accepting electrons or hydrogen from antioxidants (Njoya, 2021). As expected, BHT showed very strong activity with an IC_{50} of 25.08 μg/mL, serving as a benchmark for potent radical scavenging (Figures 5 & 6). Among the persimmon extracts, the ethyl acetate extract (EaEDK) exhibited the most powerful DPPH scavenging, with an IC_{50} of 28.96 μg/mL. This value is remarkably close to BHT, indicating that EaEDK contains a high concentration of radical scavenging compounds. The efficacy of the ethyl acetate extract in this assay aligns with its high flavonoid content, many flavonoids are excellent hydrogen donors due to their phenolic hydroxy groups and conjugated ring systems that stabilize the resulting radical. Indeed, flavonoids like quercetin and catechins (present in persimmon) can readily transfer a hydrogen atom to DPPH, quenching the radical and forming a relatively stable flavonoid radical in the process. The strong performance of EaEDK underscores ethyl acetate's ability to extract such active flavonoid antioxidants. In fact, previous studies on persimmon have noted that high catechin and phenolic acid content correlates with strong DPPH scavenging activity, particularly highlighting gallic acid and epicatechin gallate as key contributors (Lee *et al.*, 2012). Our findings are in line with those observations, suggesting that EaEDK likely contains a rich pool of similar phenolic antioxidants.

Several other extracts demonstrated moderate DPPH scavenging capacity. The hydromethanolic extract (HMEDK) had an IC_{50} of ~74 μg/mL, hexane extract (HEDK) ~93.8 μg/mL, ethanol extract (EEDK) ~102.6 μg/mL, methanol (MEDK) ~112.6 μg/mL and hydroethanol (HEEDK) ~113.7 μg/mL. These IC_{50} values, though higher (weaker activity) than BHT, still indicate substantial antioxidant activity given that they are in the low hundreds of μg/mL range. Notably, all of these extracts with moderate activity came from polar or mid-polar solvents. Their antioxidant performance can be attributed to the presence of phenolic and flavonoid compounds, though in slightly lower concentrations or different compositions than the ethyl acetate extract. For example, the hydromethanol and hydroethanol extracts, which combined water and alcohol, likely extracted a mix of phenolics and vitamin C along with sugars. Their IC_{50} ~74-114 μg/mL suggests they do scavenge DPPH radicals but less efficiently, possibly because the co-extracted sugars and other inert constituents dilute the effective concentration of antioxidants in the extract. Nonetheless, the fact that even the aqueous-alcohol mixtures have decent activity implies they did recover some of the polar antioxidant compounds (e.g., vitamin C, some polyphenols).

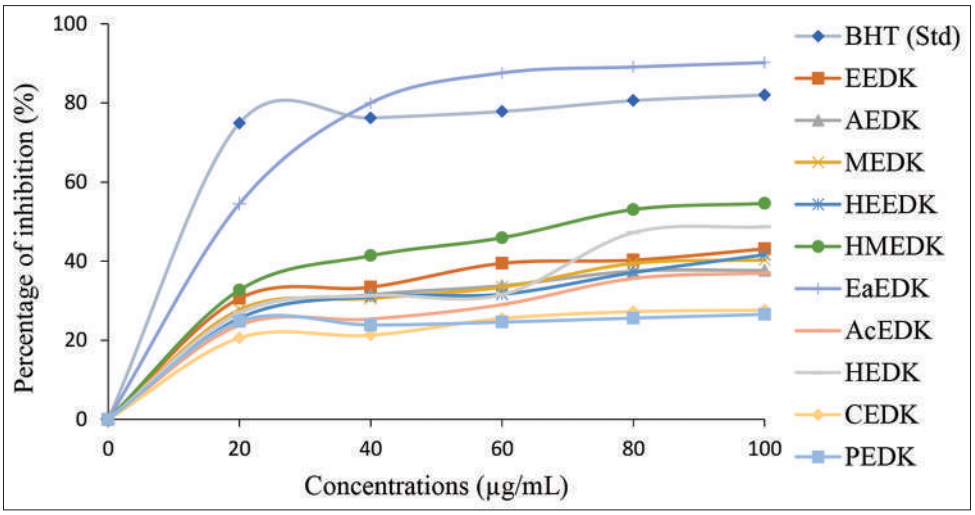

Figure 5: DPPH Radical scavenging activity of ten extracts of *D. kaki* fruit with different concentrations

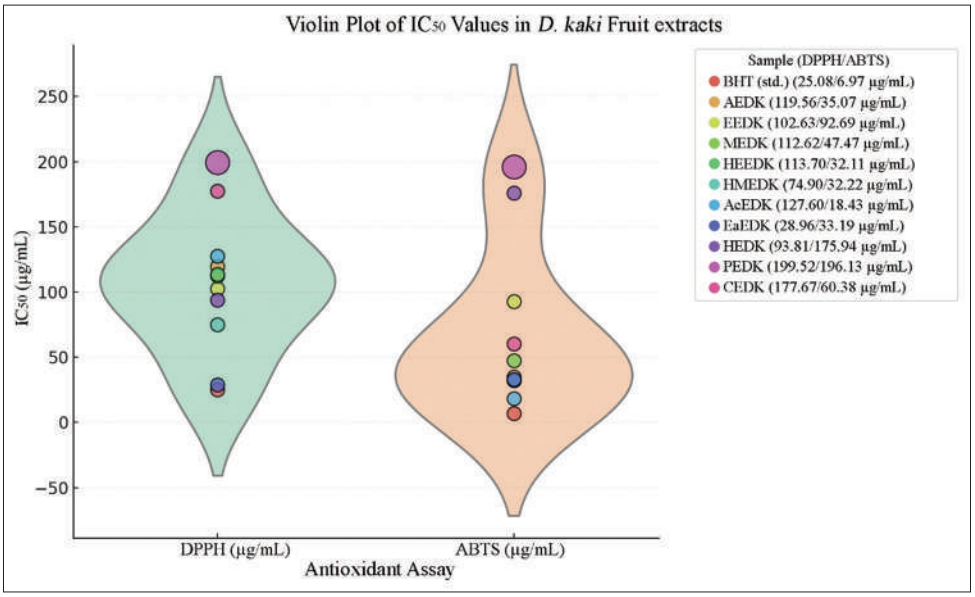

Figure 6: Violin Plot of IC_{50} estimation in differential extracts of *D. kaki* Fruit

In contrast, the least active DPPH scavengers were the extracts from extreme-polarity solvents: AEDK, AcEDK, CEDK and PEDK showed higher IC_{50} values in the range between ~119.5 and 199.5 µg/mL. These higher IC_{50} values indicate significantly weaker radical scavenging. The PEDK and CEDK, which are low polarity, likely contain mainly non-polar compounds (e.g., carotenoids, fatty acids) and very few phenolics, explaining their poor performance. Interestingly, the AEDK also fell in this weak group despite water being polar, this can be explained by its composition as noted, the AEDK is dominated by sugars and lacks a high load of phenolics or flavonoids (it had one of the lowest TPC/TFC). Sugars do not have significant antioxidant activity and water might not extract less polar phenolics that contribute more strongly to DPPH reduction. The AcEDK's weak DPPH activity (~127.6 µg/mL) is somewhat surprising given that it had the highest TPC. We suspect that although acetone pulled out many phenolics, it also extracted a lot of other compounds (including those sugars and pectins) that do not participate in DPPH scavenging and may sterically or chemically interfere with the assay. It could also be that some of the phenolics in the acetone extract are larger tannins or complexes that react more slowly with DPPH (the DPPH assay is time-sensitive). Additionally, acetone's phenolics might require more polar conditions to dissociate and react, whereas the DPPH assay is conducted in methanol, any poor solubility of certain compounds in the assay medium can lead to underestimation of activity. Despite these nuances, a clear pattern emerges: mid-polar solvent extracts outperformed non-polar extracts in DPPH scavenging, confirming that the most potent antioxidants in persimmon are polar/semi-polar molecules (flavonoids, phenolic acids, etc.).

Mechanistically, the superior DPPH activity of flavonoid rich extracts can be explained by their chemical structure. Flavonoids

(such as quercetin, myricetin, catechins) have multiple hydroxy groups that can donate hydrogen atoms to free radicals. The resulting flavonoid radical is stabilized by resonance across the aromatic ring system, which dampens reactivity (Zahra *et al.*, 2024). Phenolic acids (gallic acid, caffeic acid) operate similarly, with galloyl or catechol groups providing hydrogen donors and forming stable semiquinone radicals after donation (Sun *et al.*, 2022). Tannins, which are essentially polymers of phenolics, can quench radicals at multiple sites, both hydrolysable tannins (gallic acid esters) and condensed tannins (proanthocyanidins) present in persimmon have been noted to exhibit strong radical-scavenging abilities (Lekha & Lonsane, 1997). These compounds may work by sequentially donating several hydrogens/electrons or chelating transition metals that catalyse radical generation (Tomasi *et al.*, 2022). In persimmon, condensed tannins (proanthocyanidins) are abundant and likely contribute significantly to the DPPH scavenging capacity, especially in extracts like acetone or methanol that would solubilize such polyphenols (Ozogul *et al.*, 2025). The presence of vitamin C (ascorbic acid) in persimmon could also play a role in polar extracts, ascorbic acid is known antioxidant that readily reduces DPPH by electron transfer, although our extraction condition (warm soxhlet) might degrade vitamin C (Gęgotek & Skrzydlewska, 2022). Neverthless, any residual ascorbate in the aqueous or hydroethanolic extracts could contribute to their activity. On the other hand, compounds like sugars or organic acids (malic, citric acids in fruit) have negligible direct radical-scavenging effect, which is why high-sugar extracts showed weak DPPH activity.

ABTS Radical Inhibition Activity

The ABTS assay involves the generation of a blue-green ABTS radical cation, which is then quenched by antioxidants leading to a decrease in absorbance (Bessada *et al.*, 2015). The assay is known to be more broadly applicable, as ABTS can be scavenged by both water soluble and fat-soluble antioxidants (Hao *et al.*, 2025). In our study, BHT again served as a positive control with an extremely low IC_{50} of 6.976 µg/mL, reflecting its potent activity in this assay (BHT is a small lipophilic molecule effective in both systems). Results show the ABTS antioxidant activity of various extracts of *D. kaki* fruit (Figures 6 & 7). Among various extracts of *D. kaki* fruit, AcEDK demonstrated higher activity at 18.43 µg/mL. This is a notable strong activity, even better than the ethyl acetate extract's performance in DPPH. The fact that AcEDK, which was middling in DPPH, excelled in ABTS suggests that the ABTS assay is capturing a broader range of antioxidants present in the acetone extract. Acetone likely extracted certain compounds that respond better to the ABTS radical than to DPPH. For example, larger polyphenols or more polar phenolics (including polymeric tannins, water-soluble phenolics) may be more effective against ABTS (an aqueous phase radical) than against DPPH (a lipophilic radical). ABTS also tends to be more sensitive to compounds like vitamin C and tocopherols, if any ascorbate or similar remained in acetone extract (7.23 mg GAE/g) directly translates to ABTS scavenging once the assay conditions allow those phenolics to fully interact. Our data indicate that AcEDK contained 'potent antioxidant compounds' capable of neutralizing both hydrophilic and lipophilic radical species. The broad-spectrum activity is a desirable trait for functional extracts, as it means the extract can work in multiple phases (which is relevant biologically since oxidative stress can occur in aqueous cytosol, lipid membranes, etc.).

EaEDK, HMEDK and HEEDK also showed strong ABTS scavenging with IC_{50} values at 33.19 µg/mL, 32.22 µg/mL and 32.22 µg/mL respectively. EaEDK in ABTS is slightly weaker relative to its DPPH result, but still quite good. This may indicate that some constituents in EaEDK (e.g., certain flavonoids) are less reactive toward ABTS than DPPH, possibly due to differences in reaction mechanism (ABTS reduction can involve single-electron transfer mechanisms that might favour different antioxidant structures). The hydroalcoholic mixtures (HMEDK and HEEDK) performing well here suggests that their diverse composition (mix of polar and semi-polar compounds) is effective against ABTS. Mixed solvent extracts likely contain both hydrophilic antioxidants

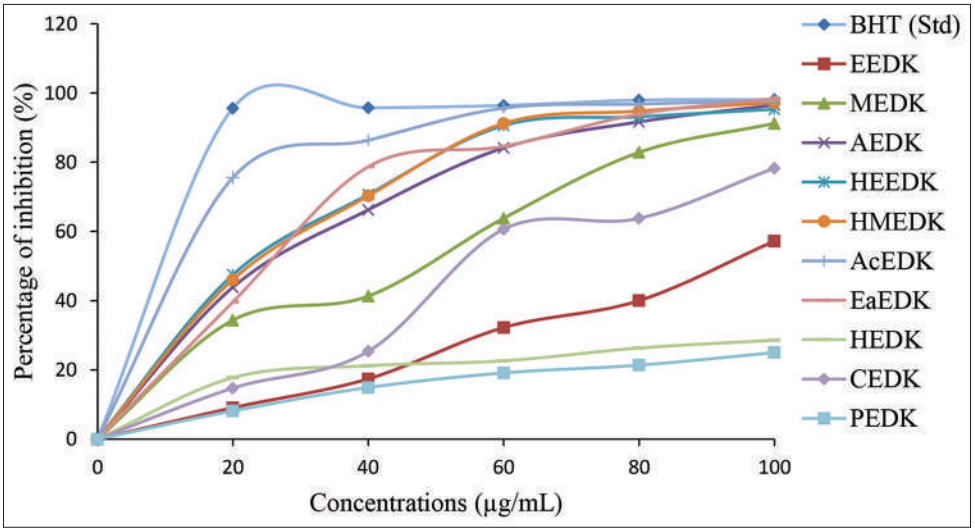

Figure 7: ABTS Radical scavenging activity of ten extracts of *D. kaki* fruit with different concentrations

(phenolics, ascorbate) and moderately lipophilic ones, giving them a broad action spectrum. This is supported by the idea that intermediate polarity solvents extract a broader range of antioxidant molecules. In fact, our ABTS results echo the notion that solvent polarity balance is key: too polar or too non-polar miss certain compounds, whereas mid-polar gets many. The AEDK also had fairly good ABTS activity (IC_{50}=35.07 µg/mL), better than its DPPH result. This could be attributed to any water-soluble antioxidants (like vitamin C or certain phenolic glycosides) exerting an effect in the aqueous ABTS system. It might also be that the ABTS, being generated in a water-rich environment, is more accessible to compounds in the aqueous extract compared to the DPPH radical in methanol.

On the other hand, MEDK and CEDK showed moderate ABTS activity (IC_{50} ~47.5 and 60.4 µg/mL, respectively). Methanol's activity is somewhat lower than one might predict from its phenolic content, possibly due to the presence of interfering compounds or simply a lower concentration of the most effective phenolics compared to acetone or ethyl acetate extracts. Chloroform's moderate result is interesting, while low in total phenolics, the chloroform extract might contain some carotenoids or other compounds that respond in the ABTS assay (carotenoids can quench ABTS through electron transfer). Still, the activity is not high, indicating limited antioxidants in that extract. Finally, the EEDK, HEDK and PEDK were the weakest in the ABTS assay, with IC_{50} values of ~92.7, 175.9 and 196.1 µg/mL, respectively. Hexane and petroleum extracts, being almost devoid of polar antioxidants, expectedly showed very poor scavenging of the polar ABTS radical. Ethanol's relatively high IC_{50} (despite being polar) again reflects that the ethanol extract was dominated by sugars and lacked sufficient phenolic concentration, as evidenced in lower TPC and TFC.

SUMMARY

The antioxidant evaluation clearly demonstrated that solvent polarity profoundly influences not only what phytochemicals are extracted, but also the resulting bioactivity. Mid-polarity solvents yielded extracts with the best overall antioxidant performance in vitro. Specifically, ethyl acetate and acetone extracts had IC_{50} values approaching that of the synthetic standard BHT in the DPPH and ABTS assays, respectively. These findings underscore that the most effective antioxidant constituents in persimmon (likely polyphenols such as flavonoids, tannins and phenolic acids) are optimally recovered by solvents of intermediate polarity. Polar solvents do extract antioxidants as well, but the high co-extraction of sugars dilutes their potency. Non-polar solvents fail to extract most phenolics, resulting in feeble antioxidant activity. Importantly, our results align with the broader understanding that dietary polyphenols are major contributors to antioxidant activity. Persimmon fruit, being rich in these compounds, can be considered a potent natural source of antioxidants when extracted appropriately. This has practical implications, for minimal antioxidant yield (e.g., to create a persimmon extract supplement or functional food ingredient), using a solvent like 50% ethanol or acetone or ethyl acetate or sequential extraction combining polarity steps, would be advisable to ensure a high phenolic content and strong radical-scavenging ability.

From a mechanistic perspective, the extracts likely neutralize DPPH and ABTS radicals through electron transfer (ET) and hydrogen atom transfer (HAT) mechanisms (Siddeeg *et al.*, 2021). Polyphenols can undergo one-electron oxidation to form phenoxyl radicals, effectively 'donating' an electron to reduce ABTS or DPPH (Andrés *et al.*, 2023). They can also donate a hydrogen atom (a proton and electron together) to DPPH in HAT mechanism (Xiang *et al.*, 2025). Flavonoids with ortho-dihydroxyl (catechol) structure in the B-ring (like quercetin) are particularly effective, as the semiquinone formed after donation is stabilized by intramolecular hydrogen bonding and resonance (De Martino *et al.*, 2012). Tannins, with multiple gallol or catechol units, can neutralize several radical molecules per tannin molecule, which contributes to their high antioxidant equivalence (Mal & Pal, 2021). In addition, some antioxidants can work by secondary mechanisms such as chelating metal ions (thus preventing Fenton-type ROS generation), persimmon tannins might play a role here by binding iron or copper, though this isn't measured by DPPH/ABTS assays (Rodríguez-Arce & Saldías, 2021). The assays used are direct radical quenching tests, so the performance directly reflects the concentration and reactivity of radical-scavenging in each extract.

Correlating IC_{50} values with phytochemical content reveals logical relationships. The ethyl acetate extract, with the highest flavonoid content, had the lowest (best) IC_{50} in DPPH, highlighting flavonoids contribution to that assay. The acetone extract, highest in total phenolics, achieved the lowest IC_{50} in ABTS, underscoring phenolics effectiveness against the ABTS radical. In fact, statistically, one would expect a negative correlation between TPC/TFC and IC_{50} across these samples. The extracts richer in phenolic compounds tend to exhibit stronger antioxidant effects. This is in agreement with the consensus in phytochemical research, for many plant extracts, TPC is often a good predictor of antioxidant activity. Our work reinforces that notion in the context of *D. kaki*. That said, the nuances observed (like differential assay response) also emphasize that no single measure of 'antioxidant content' can perfectly predict activity in all systems, thus comprehensive profiling as we have done is valuable.

CONCLUSION

The persimmon fruit extracts demonstrated significant antioxidant potential, strongly dependent on the extraction solvent used. Mid-polar solvents such as acetone and ethyl acetate were the most successful in extracting antioxidant phytochemicals, yielding extracts with low IC_{50} values in DPPH and ABTS assays, approaching the efficacy of pure antioxidant compounds. The findings highlight persimmon fruit as a promising natural source of antioxidants with potential applications in managing oxidative stress-related conditions. For example, persimmon extracts (especially those from acetone or ethyl acetate) could be explored as functional food ingredients or nutraceutical supplements to bolster antioxidant defences

in humans. The relevance is particularly notable for chronic diseases like colon cancer, where oxidative stress is implicated, the ability of persimmon extracts to quench free radicals might confer protective effects. Of course, further *in vivo* studies and characterization of individual active compounds would be worthwhile to translate these results into health recommendations or therapies. Nonetheless, our study provides a detailed understanding of how solvent-driven extraction modulates the phytochemical yield and antioxidant capacity of persimmon, guiding future efforts in natural antioxidant extraction and utilization. The strong antioxidant activity observed (especially in acetone and ethyl acetate extracts) underscores that *D. kaki* fruit, often consumed fresh or dried, indeed contains compounds of high bioactive value. By selecting appropriate solvents, one can maximize the recovery of these bioactives for potential therapeutic or nutraceutical applications targeting oxidative stress and associated diseases.

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AUTHORSHIP CONTRIBUTIONS

MSS performed the sample collection, writing the original draft. AC performed data curation and diagrammatic representation. ST revised, finalized the manuscript and also supervised the project. DB, VN and VV helps in formal analysis. All authors approved the final version of the manuscript.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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