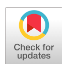
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Special Issue

Antioxidant and anti-browning activities of leaf extracts from paper mulberry (*Broussonetia kazinoki*)

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Abstract This study evaluated the antioxidant and anti-browning properties of paper mulberry (PM, *Broussonetia kazinoki*) leaf extracts prepared using different solvents, water, 30% ethanol, and 70% ethanol. The total polyphenol and flavonoid contents of the extracts varied between 37.00 to 46.91 mg tannic acid equivalents per gram and 26.32 to 46.73 mg quercetin equivalents per gram, respectively, with the PM 70% ethanol extract (PME70) showing the highest levels. PME70 exhibited remarkable radical scavenging activity, with RC_{50} values of 39.88 for the ABTS radical and 56.30 for the DPPH radical, and demonstrated greater antioxidant stability than L-ascorbic acid over two weeks. Additionally, PME70 effectively inhibited potato polyphenol oxidase (PPO) in a dose-dependent manner, reaching an inhibition rate of 86.51% and an IC_{50} of 1.16 mg/mL. In anti-browning tests on apple slices, PME70-treated samples maintained significant color stability (ΔL and ΔE values) compared to controls, including L-ascorbic acid, after 24 h of storage. These results highlight the potential of paper mulberry leaf extracts as a rich source of natural anti-browning agents for food applications.

Keywords anti-browning, antioxidant, *Broussonetia kazicoki*, free radical, paper mulberry leaf

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1. Introduction

The growing awareness of the health benefits associated with fruit and vegetable consumption has significantly increased consumer preference for fresh produce. Fresh-cut products, which are peeled, cored, sliced, washed, and hygienically packaged for convenience, are gaining popularity. However, fruits and vegetables are prone to tissue damage during processing, leading to softening and browning due to exposure of cut surfaces to air. This results in a shorter shelf life compared to whole produce (Park et al., 2021). To address this issue, synthetic chemicals such as sulfur dioxide and sulfites have been used as anti-browning agents. However, concerns over potential health risks and increasing consumer rejection of synthetic additives have driven demand for natural anti-browning agents (Chang et al., 2011). Although studies have explored the use of natural materials such as prickly pear (*Opuntia ficus-indica*), onion, apple, citrus peels, and plum seeds as anti-browning agents, their practical application to agricultural products remains limited (Chang et al., 2011; Park et al., 2021; Seo, 2012).

Paper mulberry (*Broussonetia kazinoki*), a plant native to Asia and commonly used as a raw material for traditional paper production, has various applications in traditional medicine and modern industries, including food, pharmaceuticals, and cosmetics. The fruit of paper mulberry is believed to strengthen joints, lower blood pressure, protect the liver, and promote diuresis, while its bark is used in the treatment of diabetes, hypertension, arthritis, and anemia. Extracts from

the fruit, roots, and leaves contain bioactive compounds such as arbutin, which exhibit high radical scavenging activity and are utilized in whitening cosmetic products (Jan et al., 2021). Additionally, studies have shown the medicinal potential of paper mulberry for diuretic, tonic, and edema treatments.

Notably, the leaves of paper mulberry contain flavonoids, glycosides, phenolics, organic acids, and tannins (Jung et al., 2013). These compounds exhibit pharmacological properties such as antioxidant, hypoglycemic, hypocholesterolemic, anticancer, and anti-inflammatory activities (Guangqun et al., 2022). Despite their potential, paper mulberry leaves remain underutilized, with most discarded as waste, and related biological activity studies remains insufficient.

This study aims to enhance the potential value of paper mulberry leaves, an underutilized resource, by preparing hot water and ethanol extracts and evaluating their antioxidant and anti-browning activities. Through this approach, the study seeks to explore the feasibility of using paper mulberry leaves as a natural anti-browning agent, paving the way for developing natural products and promoting the utilization of discarded resources.

2. Materials and methods

2.1. Materials and reagents

The paper mulberry (*Broussonetia kazinoki*) leaves used in this study were obtained from Cheongmyeong Yakcho (Chungju, Korea). Green tea leaves (*Sejak*) were purchased online in dried form from Osulloc Co., Ltd. (Seoul, Korea). All samples were stored at -20℃ until use. Chemicals used in the study included arbutin (TRC, Toronto, Canada), L-ascorbic acid, aluminum nitrate nonahydrate, potassium acetate, dipotassium hydrogen phosphate dibasic, phenol reagent (Junsei Chemical Co., Ltd., Tokyo, Japan), potassium phosphate monobasic, prethanol A, methyl alcohol, sodium carbonate anhydrous (Duksan Co., Ltd., Ansan, Korea), dimethyl sulfoxide, and ethyl alcohol (Daejung Chemicals, Siheung, Korea). Other reagents not specifically mentioned were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of paper mulberry leaf and green tea extracts

The paper mulberry (PM) leaves were ground into small pieces for easy extraction. Ten times of sterile distilled water was added to the samples, and PM hot water extracts (PMEW) were prepared by reflux extraction at 80℃ for 3 h, repeated twice. For ethanol extracts, 10 times of 30% or 70% ethanol (v/w) was added, and the samples were incubated in a shaking incubator (IST3075, Jeio Tech. Co., Ltd., Daejeon, Korea) at 25℃, 120 rpm, for 18 h, repeated twice, to obtain PME30 and PME70, respectively. All extracts were filtered using Whatman No. 3 filter paper (Little Chalfont, England), concentrated using a rotary vacuum evaporator at 55℃, and freeze-dried at -110℃ for 72 h. The dried extracts were stored at -80℃ until use. Extraction yields were calculated as the percentage of the weight of freeze-dried extracts Srelative to the initial weight of the paper mulberry leaves (w/w). Green tea extracts were prepared using the same method as PMEW. For all subsequent analyses, the 70% ethanol extract was dissolved in dimethyl sulfoxide (DMSO), while the other extracts were dissolved in water. The solvents used to dissolve each extract were also employed as control solvents for their respective extracts.

2.3. Measurement of total polyphenol and flavonoid contents

The total polyphenol content was determined using a modified Folin-Denis method on a 96-well plate (Park et al., 2021). Each well was filled with 60 μL of 1 mg/mL sample solution, followed by 60 μL of diluted Folin-Ciocalteu reagent, and mixed on a shaker (SH2000, Finepcr, Gunpo, Korea) for 3 min. Then, 60 μL of 10% sodium carbonate (Na_2CO_3, Duksan Co., Ltd.) solution was added, and the mixture was incubated in the dark for 1 hour. Absorbance was measured at 700 nm using a microplate spectrophotometer (Bio-Tek Instruments Inc., Winooski, VT, USA). The total polyphenol content was determined using tannic acid, calculated from a standard curve prepared using the same method described above, and expressed as μg tannic acid equivalents (TAE)/mg.

Total flavonoid content was determined following the method of Park et al. (2021). The samples diluted to 1 mg/mL in 80% ethanol were mixed with 20 μL of 1 M potassium acetate, 20 μL of 10% aluminum nitrate, and 860 μL of 80% ethanol. Aliquots of 200 μL were dispensed into 96-well plates and incubated for 40 min on a shaker, and absorbance was measured at 415 nm. The total flavonoid content was determined using quercetin, calculated from a standard curve prepared using the same method described above, and

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expressed as μg quercetin equivalents (QE)/mg.

2.4. 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity assay

ABTS radical scavenging activity was measured following the method of Yoon et al. (2023). ABTS radicals were generated by reacting 2.45 mM potassium persulfate with 7 mM ABTS for 12 h in the dark at room temperature. The solution was diluted with phosphate-buffered saline (PBS, pH 7.4) to an absorbance of 0.70±0.02 at 734 nm. Each well of a 96-well plate was filled with 20 μL of appropriately diluted sample and 180 μL of the ABTS radical solution. The mixture was incubated for 1 min at room temperature, and absorbance was measured at 734 nm. Results were expressed as the concentration required to reduce the absorbance by 50% (RC_{50}) and μg trolox equivalents (TE)/mg.

2.5. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay

DPPH radical scavenging activity was evaluated by mixing 160 μL of appropriately diluted sample with 40 μL of 0.2 mM DPPH in methanol. The mixture was incubated on a shaker for 30 min, and absorbance was measured at 517 nm. Results were expressed as RC_{50} values and μg TE/mg.

2.6. Determination of antioxidant stability of paper mulberry leaf extracts

To evaluate the antioxidant stability of the extracts, samples were stored in a 25℃ incubator, and DPPH radical scavenging activity was measured every two days over a 14-day period. L-ascorbic acid was used as a control to compare antioxidant stability. Antioxidant activity was expressed as a percentage relative to the initial activity measured on day 0.

2.7. Preparation of potato polyphenol oxidase (PPO) crude extract

Potatoes were purchased from a local supermarket (Daegu, Korea). After washing and peeling, the potatoes were blended with an equal weight (v/w) of 50 mM potassium phosphate buffer (pH 7.0). The homogenate was centrifuged at 13,000 ×*g* for 20 min at 4℃, and the supernatant was filtered through a 0.45 μm membrane filter (Adventec, Tokyo, Japan). The filtrate was aliquoted and stored at -80℃ until use.

2.8. Polyphenol oxidase (PPO) inhibition assay

The PPO inhibition assay was performed following the method of Park et al. (2021). Each reaction mixture contained 20 μL of PPO crude extract, 20 μL of appropriately diluted sample, and 100 μL of 50 mM potassium phosphate buffer (pH 7.0). The mixture was preincubated for 15 min at 25℃, and the initial absorbance was recorded at 334 nm. After adding 60 μL of 4 mM pyrogallol substrate solution, the reaction was incubated for 5 min, and the absorbance was measured at 334 nm. PPO inhibition was calculated as a percentage of the control absorbance.

2.9. Anti-browning activity assay on apple slices

Anti-browning activity was evaluated using the method of Park et al. (2021). Apples (*Malus pumila* Mill) were sliced into 0.5 cm thick pieces. Each slice was treated with 3 mL of 500 μg/mL extract or control solution (DMSO) for 1 min, followed by air-drying for 3 min. The slices were stored at 4℃ for 24 and 48 h, and their surface color was analyzed using a chromameter (CR-400, Minolta Co., Osaka, Japan). The Hunter L, a, and b values were measured at three points on each slice, and color differences (ΔE) were calculated.

2.10. Statistical analysis

All experiments were performed in triplicate, and results were expressed as mean±standard deviation (SD). Statistical analyses were conducted using SPSS Statistics 25 (SPSS Inc., Chicago, IL, USA). Differences among groups were evaluated using one-way analysis of variance (ANOVA), followed by Duncan’s multiple range test. Statistical significance was considered at $p<0.05$.

3. Results and discussion

3.1. Comparison of total polyphenol and flavonoid contents in paper mulberry leaf extracts by solvent

The bioactive compound content and physiological activity of natural extracts generally vary based on extraction conditions such as the plant part and solvent used. Extraction yield, expressed as the percentage of solid residue relative to the initial sample weight, was determined for paper mulberry leaf extracts. The yields of PMEW, PME30, and PME70 were 22.52%, 23.21%, and 21.06%, respectively, with no significant

differences among the extracts (Table 1).

Polyphenolic compounds, including phenolic acids, flavonoids, tannins, and coumarins, are plant-derived substances that exhibit diverse physiological activities (Kang et al., 2024). They contribute to pigmentation and cell wall structure and reduce reactive oxygen species and reactive nitrogen species, which are associated with aging and various diseases, thereby exhibiting antioxidant, anti-aging, and anti-browning effects (Kim et al., 2023). Flavonoids, characterized by a C6-C3-C6 structure, include catechins, flavones, flavanones, flavonols, isoflavonoids, and anthocyanidins, and are known for their potent antioxidant, anticancer, antiviral, and anti-inflammatory properties (Yun and Kang, 2021).

In this study, the total polyphenol and flavonoid contents of paper mulberry leaf extracts were quantified using tannic acid and quercetin as standards, respectively (Table 2). The total polyphenol content ranged from 37.00 to 46.91 mg TAE/g, with PME70 showing significantly higher levels than PMEW and PME30. Similarly, the total flavonoid content ranged from 26.32 to 46.73 mg QE/g, with PME70 exhibiting the highest levels. These differences are attributed to the polarity of the solvents, as 70% ethanol, being less polar, facilitated the extraction of flavonoids more effectively than water or 30% ethanol. Ethanol's dual hydrophilic and hydrophobic nature and its influence on solubility and osmotic pressure may explain the variation in the extraction of major compounds (Kim and Hong, 2021).

Table 1. The yield of paper mulberry leaf extracts

Samples	Yield (%)[1]
PMEW	22.52±11.24[NS2)]
PME30	23.21±0.97
PME70	21.06±3.47

[1]Yields (%) = total paper mulberry extract powder weight/total paper mulberry weight × 100.
[2]NS, not significant.
PMEW, the water extract; PME30, the 30% ethanol extract; PME70, the 70% ethanol extract.

Table 2. Total polyphenol and flavonoid contents of paper mulberry leaf extracts

Samples	Total polyphenols (mg TAE/g)[1]	Total flavonoids (μg QE/mg)[2]
PMEW	41.92±1.16[b3)]	26.32±0.59[c]
PME30	37.00±0.20[c]	31.84±2.40[b]
PME70	46.91±1.79[a]	46.73±0.65[a]

[1]Tannic acid equivalent.
[2]Quercetin equivalent.
[3]All values are mean±SD (n≥3), and different superscripts ([a-c]) in the same column are significantly different at p<0.05 by Duncan's multiple range test.
PMEW, the water extract; PME30, the 30% ethanol extract; PME70, the 70% ethanol extract.

3.2. ABTS radical scavenging activity

The ABTS radical scavenging activity of the extracts was evaluated based on the discoloration of ABTS radicals by antioxidants (Yoon et al., 2023). Antioxidant activity was expressed as TEAC values, and RC_{50} values (the concentration required to reduce ABTS absorbance by 50%) were calculated (Table 3). L-ascorbic acid was used as a control. The RC_{50} values for PMEW, PME30, PME70, and L-ascorbic acid were 72.27 μg/mL, 78.01 μg/mL, 39.88 μg/mL, and 3.93 μg/mL, respectively. PME70 exhibited significantly higher ABTS scavenging activity compared to PMEW and PME30. The superior performance of PME70 is consistent with its higher flavonoid content.

3.3. DPPH radical scavenging activity

The DPPH radical scavenging activity was assessed by measuring the reduction in absorbance at 517 nm upon reaction with antioxidants (Kim et al., 2019). TEAC values and RC_{50} values were determined for the extracts (Table 4). PME70 showed the lowest RC_{50} value (56.30 μg/mL) and the highest TEAC value (27.01 μg TAE/mg), followed by PMEW (RC_{50}: 64.35 μg/mL; TEAC: 24.65 μg TAE/mg) and PME30

Table 3. ABTS radical scavenging activity of paper mulberry leaf extracts

Samples	RC_{50} (μg/mL)[1]	TEAC (μg TE/mg)[2]
PMEW	72.27±0.53[a3)]	74.37±0.50[c]
PME30	78.01±6.76[a]	64.97±1.09[c]
PME70	39.88±2.63[b]	109.18±1.30[b]
L-ascorbic acid	3.93±0.04[c]	1,484.36±30.46[a]

[1]Concentration required for 50% reduction of $ABTS^+$ at 1 min after starting there action.
[2]Trolox equivalent antioxidant capacity.
[3]All values are mean±SD (n≥3), and different superscripts ([a-c]) in the same column are significantly different at p<0.05 by Duncan's multiple range test.
PMEW, the water extract; PME30, the 30% ethanol extract; PME70, the 70% ethanol extract.

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Table 4. DPPH radical scavenging activity of paper mulberry leaf extracts

Samples	RC_{50} (μg/mL)[1)]	TEAC (μg TE/mg)[2)]
PMEW	64.35±2.40[b3)]	24.65±0.31[b]
PME30	93.08±1.20[a]	17.30±0.58[b]
PME70	56.30±4.51[c]	27.01±1.22[b]
L-ascorbic acid	1.98±0.16[d]	1,416.18±708.42[a]

[1)]Concentration required for 50% reduction of $DPPH^+$ at 30 min after starting the reaction.
[2)]Trolox equivalent antioxidant capacity.
[3)]All values are mean±SD (n≥3), and different superscripts ([a-d]) in the same column are significantly different at $p<0.05$ by Duncan's multiple range test.
PMEW, the water extract; PME30, the 30% ethanol extract; PME70, the 70% ethanol extract.

(RC_{50}: 93.08 μg/mL; TEAC: 17.30 μg TAE/mg). These results appears to be correlated with flavonoid content and align with previous findings that higher flavonoid levels correspond to greater DPPH scavenging ability.

According to previous study (Zhang et al., 2001), paper mulberry leaves contain various prenylated flavonoids, flavans, and alkaloids. Notably, prenylated flavonoids such as broussonols A-E and 1,3-diphenylpropanes like kazinol F and kazinol J have been identified. These compounds are highly non-polar, likely contributing significantly to the strong antioxidant activity observed in the 70% ethanol extract. Although research on the specific types and amounts of phenolic acids in paper mulberry leaves is limited, studies on the stem bark (Xu et al., 2010) suggest that phenolic acids with higher polarity, such as caffeic acid and coumaric acid, may contribute to the antioxidant activity of the water extract. In contrast, the 30% ethanol extract exhibited relatively weaker antioxidant activity compared to the other two extracts. This suggests that the extraction conditions of 30% ethanol may be less favorable for isolating these antioxidant compounds, and the compounds extracted under these conditions may have lower antioxidant potential than the aforementioned compounds.

3.4. Antioxidant stability of paper mulberry leaf extracts

Antioxidant stability was evaluated by monitoring changes in DPPH radical scavenging activity over two weeks at two-day intervals. The initial activity was set at 100%, and the reduction in activity was recorded over 14 days (Fig. 1).

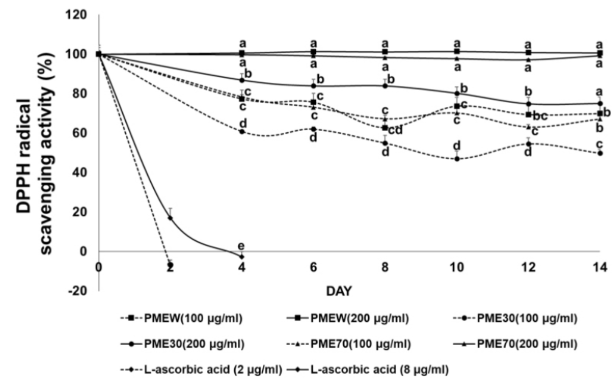

Fig. 1. Changes in DPPH radical scavenging activity of paper mulberry leaf extracts compared with L-ascorbic acid during the storage at 25°C for 2 weeks. All values are mean±SD (n≥3) and different letters ([a-e]) on the same day are significantly different at $p<0.05$ by Duncan's multiple range test. PMEW, the water extract; PME30, the 30% ethanol extract; PME70, the 70% ethanol extract.

The relative stability of the samples was observed as follows: PMEW at 200 μg/mL and PME70 at 200 μg/mL showed the highest stability, followed by PME30 at 200 μg/mL. Next in stability were PMEW and PME70 at 100 μg/mL, which demonstrated similar stability levels, and PME30 at 100 μg/mL. L-ascorbic acid at both 8 μg/mL and 2 μg/mL exhibited the lowest stability among the samples. L-ascorbic acid lost its antioxidant activity after four days, showing significantly lower stability compared to the paper mulberry extracts. Among the extracts, PMEW and PME70 demonstrated high stability. Combining the strong antioxidant capacity of L-ascorbic acid, which has very low stability, with the high stability of paper mulberry leaf extracts could create a complementary effect, potentially resulting in synergistic benefits, and further research on this combination is warranted.

3.5. PPO inhibition activity

Polyphenol oxidase (PPO), a copper-containing enzyme commonly found in fruits and vegetables, catalyzes polyphenol oxidation, resulting in browning (Chen et al., 1991). In this study, the browning inhibition activity of paper mulberry leaf extracts was evaluated by comparing their ability to inhibit potato PPO enzyme activity using pyrogallol as a substrate. The results showed significant inhibition of enzyme activity in the order of PME70, PMEW, and PME30 (Table 5). PMEW showed inhibitory activities of 15.75% at 1 mg/mL and

Table 5. Inhibitory effect of paper mulberry leaf extracts on PPO activity

Samples	Concentration (mg/mL)	Inhibition rate (%)	IC_{50} (mg/mL)[2)]
PMEW	0.5	10.26±0.64[d1)]	2.45±0.04[a]
	1	15.75±1.80[cd]	2.45±0.04[a]
	2	29.81±1.07[c]	2.45±0.04[a]
PME30	0.5	6.49±1.63[d]	2.67±0.02[a]
	1	6.27±0.19[d]	2.67±0.02[a]
	2	20.68±0.48[cd]	2.67±0.02[a]
PME70	0.5	24.26±2.01[cd]	1.16±0.26[b]
	1	47.34±20.71[b]	1.16±0.26[b]
	2	86.51±19.72[a]	1.16±0.26[b]

[1)]All values are mean±SD (n≥3), and different superscripts ([a-d]) in the same column are significantly different at $p<0.05$ by Duncan's multiple range test.
[2)]Concentration required for 50% inhibition of PPO activity.
PMEW, the water extract; PME30, the 30% ethanol extract; PME70, the 70% ethanol extract.

29.81% at 2 mg/mL, while PME30 exhibited 6.27% inhibition at 1 mg/mL and 20.68% at 2 mg/mL. PME70 demonstrated significantly higher inhibition, with 47.34% at 1 mg/mL and 86.51% at 2 mg/mL. While PMEW and PME30 did not exhibit significant differences in activity between 0.5 and 1 mg/mL, PME70 showed a dose-dependent increase in inhibitory activity. The IC_{50} values for PPO inhibition were 2.45 mg/mL for PMEW, 2.67 mg/mL for PME30, and 1.16 mg/mL for PME70, confirming that PME70 had significantly superior PPO inhibitory activity compared to PMEW and PME30.

Fennel (*Foeniculum vulgare*) seed extract (2.5 mg/mL) has been reported to exhibit 40.82% PPO inhibitory activity (Lee and Kim, 2020), while 1% dandelion hot water extract and 1% apple ethanol extract showed inhibition rates of 17.41% and 18.53%, respectively (Chang et al., 2011). Additionally, a study on the PPO inhibitory activity of plum seed extracts by cultivar (Park et al., 2021) reported significant differences among cultivars, with the Soldam cultivar (IC_{50}: 675.85 μg/mL) demonstrating superior activity compared to Formosa, Dodam, and other cultivars (IC_{50}: >1 mg/mL). When compared to these findings, PME70 exhibited relatively high PPO inhibitory activity, highlighting its potential as an anti-browning agent.

However, it was noted that the concentrations required for PPO inhibition were much higher than those required for radical scavenging activity, a trend consistent with previous studies (Lee and Kim, 2020; Park et al., 2021). Natural extracts generally require higher concentrations to achieve anti-browning effects. In this case, the dark color of the extracts themselves may pose a sensory challenge when applied to fresh-cut foods, emphasizing the need to explore strategies to address this issue.

3.6. Anti-browning activity on apple slices

The anti-browning activity of paper mulberry leaf extracts was evaluated using apples, a widely consumed fresh produce. Apples are commonly eaten either with or without their peel, but tissue damage caused by peeling, cutting, or other processing exposes the flesh to air, where polyphenols react with oxygen, resulting in browning (Park et al., 2021). Considering these characteristics, this study assessed the time-dependent anti-browning activity of paper mulberry extracts on apple slices. The slices were treated with the extracts, including the control groups (green tea extract and L-ascorbic acid) and PME70, which showed the highest PPO inhibitory activity. The treated apple slices were stored at 4℃ for 24 and 48 h, and changes in Hunter L values, expressed as ΔL and ΔE values, were observed.

According to Weller et al. (1997) and Ahn and Lee (2005), PPO becomes highly soluble and activated due to tissue aging or storage-induced stress, and increases in PPO activity are directly associated with changes in the brightness, as represented by the L value. In this study, the difference in L values (ΔL) was calculated by comparing the initial L value measured immediately after sample treatment with the values measured after 24 and 48 h.

After 24 h, the untreated control group showed a ΔL value of 4.50, whereas the PME70 and green tea extract-treated groups had significantly lower ΔL values of 2.36 and 2.99, respectively, indicating less brightness change compared to the control (Table 6). After 48 h, PME70 and green tea extract-treated groups continued to exhibit significantly lower ΔL values than the control, demonstrating that PME70 effectively inhibited browning in apple slices, comparable to the effect of green tea extract. In contrast, the L-ascorbic acid-treated group showed no difference in ΔL values compared to the untreated control, suggesting no browning inhibition, likely due to its low stability (Fig. 1).

The ΔE value represents the degree of color change, calculated based on the initial L, a, and b values, with higher ΔE values indicating greater color changes (Park et al.,

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Table 6. Changes in color of apple slices treated with paper mulberry leaf extracts

Samples	0 h	After 24 h		After 48 h	
	L[1)]	ΔL[2)]	ΔE[3)]	ΔL	ΔE
Control	79.37	4.50±0.22[ab4)]	10.57±1.49[a]	6.29±0.10	7.61±0.39[a]
PME70	77.76	2.36±1.29[b]	5.96±2.06[b]	3.82±1.89	4.69±1.90[b]
Green tea extract	76.91	2.99±1.46[ab]	6.51±0.41[b]	3.65±0.84	4.61±2.78[b]
L-ascorbic acid	77.45	4.81±1.08[a]	10.83±1.53[a]	6.33±3.06	7.99±0.78[a]

[1)]L, lightness.
[2)]ΔL, degree of lightness of change.
[3)]ΔE, degree of color of change.
[4)]All values are mean±SD (n≥3), and different superscripts ([a,b]) in the same column are significantly different at $p<0.05$ by Duncan's multiple range test.
PME70, the 70% ethanol extract.

2021). After 24 h, the untreated control group exhibited a ΔE value of 10.57, while the PME70 and green tea extract-treated groups showed significantly lower ΔE values of 5.96 and 6.51, respectively, indicating less color change. Similarly, after 48 h, PME70 and green tea extract-treated groups maintained significantly lower ΔE values compared to the control, demonstrating their anti-browning activity.

These results highlight the potential of paper mulberry leaf extracts as a novel natural anti-browning agent. The extracts could effectively prevent surface browning, a critical factor affecting the marketability of fresh-cut products. Moreover, considering previous studies (Park et al., 2013) reporting enhanced anti-browning effects when combining natural extracts with L-ascorbic acid, further research on such combinations is recommended.

The limitations of this study include the lack of precise identification of active compounds and the absence of standardization of raw materials based on the geographical origin of paper mulberry. Future research should focus on isolating and identifying the active compounds in paper mulberry leaf extracts. Additionally, studies on the standardization of raw materials across different origins are necessary to ensure consistency and reliability in their application.

4. Conclusions

This study aimed to provide foundational data on the potential use of underutilized paper mulberry (*Broussonetia kazinoki*) leaves as a food additive for anti-browning applications. Solvent-based extracts of paper mulberry leaves (PMEW, PME30, and PME70) were prepared, and their antioxidant and anti-browning activities were evaluated. Among the extracts, PME70 exhibited the highest total polyphenol and flavonoid contents, as well as superior radical scavenging activity, while PME30 showed the lowest activity, confirming a positive correlation between antioxidant activity and total flavonoid content. In the PPO inhibition assay, PME70 demonstrated the most effective browning inhibition, and in the apple slice browning test, PME70 also showed excellent anti-browning effects. These findings suggest that PME70 possesses relatively higher antioxidant activity compared to PMEW and PME30, highlighting the potential of paper mulberry leaf extracts as natural anti-browning agents. Particularly, PME70 is expected to serve as a safe food additive for preventing quality deterioration and browning in raw agricultural products and fresh-cut foods, offering significant practical applications.

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Conflict of interests

The authors declare no potential conflicts of interest.

Author contributions

Conceptualization: Lee SO. Methodology: Kim CE, Cheon EY, Kim YJ. Formal analysis: Kim CE, Cheon EY, Kim YJ.

Validation: Kim CE, Cheon EY, Kim YJ, Lee SO. Writing - original draft: Kim CE, Cheon EY, Kim YJ. Writing - review & editing: Lee SO.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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