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Unveiling the Dual Anti-Inflammatory–Antioxidant Effect of Scopoletin and Myricetin: An In Vitro Evaluation

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ABSTRACT

AIM- The aim of the present investigation is to study the in-vitro effect of two bioactive flavonoids. MATERIAL & METHODS- Both flavonoids were analysed at concentrations of 100 to 500 µg/mL. Indomethacin and NS-398 were used as standards in COXs inhibition tests, whereas zileuton was used in 5-LOX assay. Cyclooxygenase inhibitory activity was assessed using a modified version of the method described by Jäger et al. (1996), based on measuring PGE_2 formation from arachidonic acid (AA) by COX-1 (from ram seminal vesicles) and human recombinant COX-2. COX-1 (1 unit/reaction) or COX-2 (0.5 unit/reaction) was added to 180 µL of an incubation mixture containing 100 mM Tris buffer (pH 8.0), 5 µM porcine hematin, 18 mM (−)-epinephrine, and 50 µM Na_2EDTA. The assay was carried out following the method described by Adams et al. (2004). Venous blood (45 mL) from healthy donors was allowed to sediment in 20 mL of a dextran solution (6% dextran T-500, 1% NaCl) at 4 °C. After 1 hour, the supernatant was collected, centrifuged at 1600 rpm for 10 minutes at 4 °C, and discarded. The resulting pellet was washed with a buffer solution (7.4% $CaCl_2 \cdot 2H_2O$, 0.1% D-glucose, 0.2% $MgCl_2 \cdot 6H_2O$, 0.04% KCl, and 1.75% Tris, pH 7.6) and centrifuged again. The DPPH assay was conducted following the in vitro procedure described by Sharma and Bhat (2009). Oxygen radical absorbance capacity (ORAC) assay was carried out following a slightly modified procedure of Ou et al. (2001). RESULTS- Among the both flavonoids Scopoletein was found to be the most active flavonoid towards COX-1 with an IC50 value of 6.65 µg/mL. Moderate efficacy was found for Myricetin with IC50 values ranging from 9.85 µg/mL. Indomethacin and NS-398 inhibited COX-1 with the IC50 values at 0.40 and 28.22 µg/mL, respectively. Likewise as in COXs assays, the Scopoletein and Myricetin were determined as the most effective inhibitor of 5-LOX enzyme and Scopoletein was found to be potent against LOX with IC50 value 4.80 µg/mL. CONCLUSION- These findings suggest that scopoletin and myricetin may serve as promising candidates for the development of new, safer plant-derived agents with anti-inflammatory and/or antioxidant activity. However, further investigations, including detailed chemical characterization and in vivo evaluations, are necessary to confirm their potential practical applications.

KEYWORDS: Unveiling effect, Dual Anti-Inflammatory–Antioxidant Effect, Scopoletin, Myricetin, In Vitro Evaluation, Bioactive Flavonoids

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INTRODUCTION

An acute inflammatory response typically resolves within two weeks, whereas inflammation that continues for months or years is considered chronic. Chronic inflammation is often associated with wounds containing necrotic tissue, persistent infections, or foreign materials that cannot be cleared during the acute phase. As a result, different regions within a wound may progress through healing at varying rates because the coordinated sequence of events required for effective repair is disrupted. Matrix metalloproteinases (MMPs) are crucial for tissue remodeling, yet imbalances between MMPs and their inhibitors can lead to excessive fibrosis or delayed healing. Irregular MMP activity may also reduce the capacity of cytokines to degrade bacteria and necrotic tissue, further impairing the progression of wound healing during inflammation (Li et al., 2007).

Traditional medicine serves as the primary healthcare resource for 70–95% of people in most developing countries and 70–90% in several industrialized nations (Demilew et al., 2018). Research indicates that the wound-healing potential of medicinal plant (MP) extracts and their phytochemicals stems from their antioxidant, anti-inflammatory, antibacterial, mitogenic, and tissue-regeneration properties (Alemu et al., 2020). Topical application of such extracts—particularly those with strong free radical–scavenging activity—has been shown to accelerate healing and protect tissues from oxidative damage (Kalaskar et al., 2024). MPs are abundant in polyphenols, which exhibit strong antioxidant activity (Paswan et al., 2023), and triterpenoid glycosides have also been recognized for their wound-healing and potential anti-aging benefits (Thong-on et al., 2024). Considering the toxicity concerns associated with some anti-inflammatory drugs, herbal remedies may provide safer alternatives for managing inflammation and facilitating wound repair (Paswan et al., 2023). Various phytochemicals, including phytosterols, flavonoids, triterpenoids, phenolics, and tannins, have demonstrated anti-inflammatory effects across multiple experimental systems (Gupta et al., 2022). Guided by traditional usage and prior findings, the present study investigates the in vitro anti-inflammatory activity of selected flavonoids.

MATERIAL & METHODS-

Procurement of Flavonoids-

The Scopoletein and Myricetin were procured as a gift samples from Himedia Chemical Pvt. Ltd., Mumbai (Maharashtra). The COA were also provided for the authenticity of the above said flavonoids.

In-Vitro Anti-inflammatory Activity

Both flavonoids were analysed at concentrations of 100 to 500 µg/mL. Indomethacin and NS-398 were used as standards in COXs inhibition tests, whereas zileuton was used in 5-LOX assay. The antiinflammatory assays were evaluated using the microplate reader Infinite 200 (Tecan, Männendorf, Switzerland).

COX-1 and COX-2 assays

Cyclooxygenase inhibitory activity was assessed using a modified version of the method described by Jäger et al. (1996), based on measuring PGE_2 formation from arachidonic acid (AA) by COX-1 (from ram seminal vesicles) and human recombinant COX-2. COX-1 (1 unit/reaction) or COX-2 (0.5 unit/reaction) was added to 180 µL of an incubation mixture containing 100 mM Tris buffer (pH 8.0), 5 µM porcine hematin, 18 mM (−)-epinephrine, and 50 µM Na_2EDTA. Test samples dissolved in DMSO, or pure DMSO for blank reactions, were added at a volume of 10 µL, and the mixture was pre-incubated for 5 minutes at room temperature. The reaction was initiated by adding 5 µL of 10 µM AA. After incubating for 20 minutes at 37 °C, the reaction was terminated with 10 µL of 10% formic acid. PGE_2 production was quantified using a Prostaglandin E_2 ELISA kit (Enzo Life Sciences International, Inc., Plymouth, USA). The reaction mixture was diluted 1:15 with assay buffer and immediately analyzed. Absorbance was recorded at 405 nm, with reference measurements taken at 570 and 590 nm.

5-LOX assay

The assay was carried out following the method described by Adams et al. (2004). Venous blood (45 mL) from healthy donors was allowed to sediment in 20 mL of a dextran solution (6% dextran T-500, 1% NaCl) at 4 °C. After 1 hour, the supernatant was collected, centrifuged at 1600 rpm for 10 minutes at 4 °C, and discarded. The resulting pellet was washed with a buffer solution (7.4% $CaCl_2 \cdot 2H_2O$, 0.1% D-glucose, 0.2% $MgCl_2 \cdot 6H_2O$, 0.04% KCl, and 1.75% Tris, pH 7.6) and centrifuged again. The pellet was then lysed in a solution containing 0.17% NH_4Cl and 0.2% Tris (pH 7.2) for 5 minutes at room temperature, followed by centrifugation at 1400 rpm for 5 minutes at 4 °C. After an additional washing and centrifugation step (1400 rpm, 15 minutes, 4 °C), the final pellet was resuspended in assay buffer (1.75% Tris, 0.9% NaCl, pH 7.4), and cell viability was confirmed (final concentration: 4500 cells/µL). The incubation mixture contained 225 µL of the cell suspension, 10 µL of 2 mM $CaCl_2$, 10 µL of 10 µM ETYA, 5 µL of the test compounds dissolved in DMSO, 10 µL of 21 µM calcium ionophore A23187, and 5 µL of 120 µM AA. After incubating for 10 minutes at 37 °C, the reaction was terminated by adding 20 µL of 10% formic acid. Samples were diluted 40-fold in ELISA buffer, and LTB_4 levels were quantified using a commercial LTB_4 ELISA kit (Enzo Life Sciences) following the manufacturer's protocol. Absorbance was recorded at 405 nm.

DPPH radical-scavenging assay

The DPPH assay was conducted following the in vitro procedure described by Sharma and Bhat (2009). Two-fold serial dilutions of each sample were first prepared in 175 µL of methanol within 96-well microtiter plates. Then, 25 µL of a freshly prepared 1 mM DPPH solution in methanol was added to each well, producing a series of final concentrations for initiating the radical–antioxidant reaction. The plate was kept at room temperature in the dark. Trolox served as the reference standard at concentrations of 0.5 to 512 µg/mL, while pure methanol (MeOH) acted as the negative control. Absorbance was measured at 520 nm, and antioxidant capacity was reported as Trolox equivalents (TE).

Oxygen radical absorbance capacity (ORAC) assay

The assay was carried out following a slightly modified procedure of Ou et al. (2001). To enhance thermal stability, the peripheral wells of black 96-well absorbance plates were filled with 200 µL of distilled water, as recommended by Held (2005). Stock solutions of AAPH (153 mM) and fluorescein (FL, 540 µM) were freshly prepared in 75 mM phosphate buffer (pH 7.0). For the assay, 25 µL of each sample was mixed with 150 µL of FL (54 nM) and incubated at 37 °C for 10 minutes. The oxidative reaction was then initiated by adding 25 µL of AAPH to each well. Fluorescence readings were recorded at 0.5-minute intervals over 1 hour, using excitation and emission wavelengths of 490 nm and 519 nm, respectively. Trolox calibration curves were generated at five concentration levels, with 75 mM phosphate buffer serving as the blank. Results were reported as Trolox equivalents (TE).

RESULTS

Anti-inflammatory activity

Among the both flavonoids Scopoletein was found to be the most active flavonoid towards COX-1 with an IC50 value of 6.65 µg/mL. Moderate efficacy was found for Myricetin with IC50 values ranging from 9.85 µg/mL. Indomethacin and NS-398 inhibited COX-1 with the IC50 values at 0.40 and 28.22 µg/mL, respectively. Likewise as in COXs assays, the Scopoletein and Myricetin were determined as the most effective inhibitor of 5-LOX enzyme and Scopoletein was found to be potent against LOX with IC50 value 4.80 µg/mL. Results on the anti-inflammatory activities are presented in Table 1 & 2.

Table No.1: Effect of Scopoletein & Myricetin on COX-1 and COX-2 enzymes

S No.	Flavonoids	Concentration (µg/ml)	COX-1 Inhibition	COX-2 Inhibition
1	Scopoletein	100 µg/ml	30.83±2.45	81.11±2.44
2		200 µg/ml	34.39±2.67	87.44±3.46
3		300 µg/ml	40.22±3.89	91.65±3.53
4		400 µg/ml	44.49±2.22	98.41±2.53

5		500 µg/ml	48.91±3.65	121.33±3.76
6	Myricetin	100 µg/ml	33.11±3.49	77.34±2.66
7		200 µg/ml	36.55±2.22	79.12±4.78
8		300 µg/ml	38.22±2.58	82.35±3.79
9		400 µg/ml	41.33±3.22	90.56±3.11
10		500 µg/ml	49.11±2.44	94.18±3.31
11	Indomethacin	---	41.11±3.16	126.30±3.11
12	NS-398	---	40.36±4.49	128.98±2.87

Table No.2: Effect of Scopoletein & Myricetin on LOX-5 enzymes

S No.	Flavonoids	Concentration (µg/ml)	LOX-5 Inhibition
1	Scopoletein	100 µg/ml	48.54±0.18
2		200 µg/ml	60.22±4.65
3		300 µg/ml	76.47±3.98
4		400 µg/ml	84.11±6.34
5		500 µg/ml	97.87±4.19
6	Myricetin	100 µg/ml	40.23±3.45
7		200 µg/ml	45.28±2.67
8		300 µg/ml	51.16±3.98
9		400 µg/ml	59.76±3.69
10		500 µg/ml	66.89±4.28
11	Zileuton	---	86.34±3.77

Antioxidant activity

In DPPH-radical scavenging assay, the highest antioxidant activity was detected for Scopoletein and moderate effect was observed in the case of Myricetin. The respective IC50 values for Trolox were detected at concentrations of 6.5 and 4.4 µg/mL in DPPH and ORAC assay, respectively. The results were expressed in the Table no 3.

Table No.3: Effect of Scopoletein & Myricetin on In vitro antioxidant activities

S No.	Flavonoids	Concentration	DPPH TEa	ORAC TEa
1	Scopoletein	100 µg/ml	0.101±0.010	0.236±0.061
2		200 µg/ml	0.123±0.011	0.267±0.078
3		300 µg/ml	0.140±0.015	0.340±0.033
4		400 µg/ml	0.180±0.016	0.412±0.056
5		500 µg/ml	0.240±0.012	0.518±0.067
6	Myricetin	100 µg/ml	0.98±0.012	0.190±0.045
7		200 µg/ml	0.121±0.016	0.210±0.089
8		300 µg/ml	0.138±0.034	0.230±0.011
9		400 µg/ml	0.149±0.057	0.256±0.045
10		500 µg/ml	0.195±0.061	0.310±0.025

DISCUSSION

Inflammation is essential for wound healing, as it helps remove necrotic tissue, cellular debris, and bacteria, while also facilitating fibroblast recruitment to support tissue repair. Although this response should subside naturally, prolonged or excessive inflammation can impair proper healing (Menke et al., 2007). In the present study, we explored the potential therapeutic value of compounds relevant to conditions in which inflammation and oxidative stress play a primary or secondary role. To achieve this, we employed a relatively novel strategy that evaluates combined anti-inflammatory and antioxidant activities. Notably, both flavonoids exhibited strong and selective inhibition of the COX-2 and 5-LOX enzymes, along with marked activity in the DPPH and ORAC assays. The remaining extracts showed some degree of selective anti-inflammatory potential; however, their IC_{50} values for COX-2 and 5-LOX were near or above 100 µg/mL, which is considered pharmacologically insignificant according to Hostettmann et al. (1991).

CONCLUSION

The present study demonstrated the in vitro anti-inflammatory properties of scopoletin and myricetin. Both compounds showed the most potent inhibitory effects on COX enzymes and 5-LOX. These findings suggest that scopoletin and myricetin may serve as promising candidates for the development of new, safer plant-derived agents with anti-inflammatory and/or antioxidant activity. However, further investigations, including detailed chemical characterization and in vivo evaluations, are necessary to confirm their potential practical applications.

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