



COMPARATIVE EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SUBSTITUTED FLAVONES

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ABSTRACT

Objective: The flavonoids are a heterogeneous group of ubiquitous plant polyphenols that abound in the human diet and are endowed with several biological activities including anti-oxidant, anti-inflammatory, immunomodulatory, antiviral, antimutagenic and anticarcinogenic properties. Flavones are believed to interfere with the different free radical-producing systems and enhance the functions of endogenous antioxidants. The aim of this study was to compare the antioxidant activity of synthesized substituted flavones by various free radical scavenging assays.

Method: The flavones used in the study, 6,3,4' – trihydroxy flavones (THF) and 3-hydroxy-6,3'-dimethoxy flavones (DMF) were synthesized using standard procedures and their anti-inflammatory activity was compared by inhibition of protein denaturation and proteinase inhibitory activity. The anti-inflammatory activity of THF was found to be higher than that of the DMF, as THF produced higher percentage inhibition of proteinase activity and protein denaturation.

Results: The anti-arthritic activity of THF was found higher than that of the DMF, as the THF produced higher percentage inhibition of protein denaturation in lower concentrations also. The anti-inflammatory activity of THF was found higher than that of the DMF, as the THF produced higher percentage inhibition of proteinase activity.

Conclusion: From the study it can be seen that the flavones showed significant anti-inflammatory activity that can be used for treatment of various diseases.

Keywords: Flavones, anti-inflammatory activity

1. Introduction

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruits, vegetables, grains, barks, roots, stems, flowers, tea and wine[1]. Flavonoids possess a common phenylbenzopyrone structure (C6-C3-C6). They are classified mainly into flavones, flavanols, isoflavones, flavonols, flavanones and flavanonols[2,3]. Flavonoids are associated with a broad spectrum of health promoting effects. They are capable of modulating the activity of enzymes and affect the behavior of many cell systems and exerting beneficial effects on body. They are an indispensable component in a variety of pharmaceutical, medicinal and cosmetic applications. This is attributed to their antioxidative, anti-inflammatory, antimutagenic and anticarcinogenic properties[4].

Nowadays, interest in their possible health benefits has increased owing to their potent antioxidant and free radical scavenging activities observed in vitro[5].

Flavonoids are a diverse class of plant-derived polyphenolic secondary metabolites recognized as potent natural anti-inflammatory agents capable of mitigating chronic inflammation, which is a key driver in diseases such as cancer, cardiovascular disease, neurodegenerative disorders, and arthritis. These compounds, including quercetin, luteolin, and apigenin, function primarily by targeting inflammatory signaling pathways, such as suppressing the nuclear factor kappa-light chain enhancer of activated B cells (NF- κ B) and activator protein-1 (AP-1) pathways, and downregulating the production of pro-inflammatory cytokines like TNF- α , IL-6, and IL-1 β . Additionally, flavonoids act as robust antioxidants that chelate iron and inhibit inflammatory enzymes, specifically cyclooxygenase-2 (COX-2) and lipoxygenase (LOX), thereby reducing the synthesis of inflammatory mediators like prostaglandins and leukotrienes[6]. Structure-activity relationship studies indicate that the presence of hydroxyl groups at specific positions, along with the C2–C3 double bond and a 4-oxo group on the heterocyclic C ring, are critical for maximizing this anti-inflammatory activity[7].

Since most of the scientific reports are on the natural flavonoids, the present work was undertaken to determine whether the synthetic flavonoids also possess significant anti-inflammatory activity.

2. Materials And Methods

The flavones used in the study, 6,3',4' – trihydroxy flavones (THF) (HW1) and 3-hydroxy-6,3'-dimethoxy flavones (DMF) (HW2) were synthesized using standard procedures at Research Organics, Chennai, TamilNadu. The melting point, UV, IR and NMR data of the synthesized compounds were compared with standard samples.

The comparative anti-inflammatory activity of the synthesized flavones were evaluated by the following methods

2.1 Proteinase inhibitory activity

Proteinase inhibitory activity was performed according to the modified method of Oyedepo et al and Sakat et al. The reaction mixture (2ml) contained 0.06mg trypsin, 1ml 20Mm TrisHCl buffer(pH 7.4) and 1ml test sample of different concentration. The mixture was incubated at 37°C for 5 minutes and then 1ml of 0.8%(w/v) casein was added. The mixture was incubated for an additional 20 minutes. Then 2ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was read at 200 nm against buffer as blank. The experiment was performed in triplicates.

The percentage of proteinase inhibitory activity was calculated using the following formula,
Percentage inhibition = $(100 - ((A \text{ of test} - A \text{ of product control}) / A \text{ of Control}) \times 100)$

2.2 Protein denaturation method

Anti arthritic drugs act not only by the inhibition of production of endogenous prostaglandins but also by protecting endogenous proteins against denaturation. So the anti arthritic activity of the test substances can be assessed by the evaluation of their ability to inhibit the protein denaturation. In this method, the protein denaturation was induced by the heat treatment.

Preparation of reagents

0.5 % Bovine Serum Albumin (BSA): Dissolved 500 mg of BSA in 100 ml of water.

Phosphate Buffer Saline PH 6.3: Dissolved 8 g of Sodium chloride (NaCl), 0.2 g of Potassium chloride (KCl), 1.44 g of Disodium hydrogen phosphate (Na₂HPO₄), 0.24 g of Potassium dihydrogen phosphate (KH₂PO₄) in 800 ml distilled water. The pH was adjusted to 6.3 using 1N HCl and made up the volume to 1000 ml with distilled water.

Procedure

- Test solution (0.5 ml) consists of 0.45 ml of BSA and 0.05 ml of test solution of various concentrations.
- Test Control solution (0.5 ml) consist of 0.45 ml of BSA and 0.05 ml of distilled water.
- Product Control (0.5 ml) consists of 0.45 ml of distilled water and 0.05 ml of test solution.

- Standard solution (0.5 ml) consists of 0.45 ml of BSA and 0.05 ml of Diclofenac sodium of various concentrations.

0.05 ml various concentrations (62.5, 125, 250 and 500 µg/ml) of test drugs and standard drug diclofenac sodium (62.5, 125, 250 and 500 µg/ml) were taken respectively and 0.45 ml BSA was mixed. The samples were incubated at 37°C for 20 minutes and the temperature was increased to 57°C for 3 minutes. After cooling, added 2.5 ml of phosphate buffer to the solutions. The absorbance was measured using uv-visible spectrophotometer at 255 nm. The control represents 100% protein denaturation. The results were compared with standard.[10]

The percentage inhibition of protein denaturation was calculated as:

Percentage inhibition = $100 - \left[\frac{(\text{optical density of test solution} - \text{optical density of product control})}{\text{optical density of test control}} \times 100 \right]$

3. Results And Discussion

3.1 Proteinase inhibitory activity

The anti-inflammatory activity of the derivatives were evaluated by Inhibition of Proteinase method. The effect of THF, DMF and the standard Diclofenac sodium on proteinase inhibition can be seen in the figure 1. The percentage inhibition of proteinase activity produced by different concentrations of derivatives are summarized in the table 1. The values are expressed as Mean±SD. Both THF and DMF showed significant activity at concentrations 125, 250, 500, 1000 and 2000 µg/ml. The standard drug Diclofenac sodium showed significant activity at all concentrations used. At 125 µg/ml, THF, DMF and Diclofenac sodium showed percentage inhibition of 58.32±1.036%, 40.82±0.9696% and 60.87±0.3845% respectively. At 500 µg/ml, THF, DMF and Diclofenac sodium showed percentage inhibition of 74.07±1.504%, 58.06±0.8860% and 78.23±0.4179% respectively. At 2000 µg/ml, THF, DMF and Diclofenac sodium showed percentage inhibition of 81.41±0.3845%, 76.40±0.3845% and 91.78±0.5350% respectively. In all the three cases, the effect was found to be concentration dependent. ie; the percentage inhibition increases with increase in concentration. At, higher concentrations, the effect produced by THF was almost same as that of Diclofenac sodium. This shows the anti-inflammatory activity of the derivatives. The anti-inflammatory activity of THF was found to be higher than that of the DMF, as the THF produced higher percentage inhibition of proteinase activity.

Table 1 :Effect of derivatives on inhibition of proteinase activity

Group	Drug	Concentration (µg/mL)	Absorbance of Test (200 nm)	Absorbance of Product control (200 nm)	Percentage inhibition(%)
Control	-		0.5982±0.0103		
Test	THF (HW1)	125	0.4413±0.0062***	0.1920±0.0030	58.32±1.036
		250	0.4009±0.0147***	0.1711±0.0012	61.58±2.457
		500	0.2782±0.0090***	0.1231±0.0027	74.07±1.504
		1000	0.2100±0.0042***	0.0936±0.0035	80.54±0.7021
		2000	0.1632±0.0023***	0.0520±0.0004	81.41±0.3845
	DMF (HW2)	125	0.5432±0.0058***	0.1892±0.0017	40.82±0.9696
		250	0.4333±0.0023***	0.1710±0.0011	56.15±0.3845
		500	0.3101±0.0053***	0.0592±0.0038	58.06±0.8860
		1000	0.2729±0.0038***	0.0396±0.0012	61.00±0.6353
		2000	0.1532±0.0023***	0.0120±0.0019	76.40±0.3845
Standard	Diclofenac sodium	125	0.4132±0.0023***	0.1791±0.0026	60.87±0.3845
		250	0.2882±0.0026***	0.0819±0.0002	65.51±0.4347
		500	0.2210±0.0025***	0.0908±0.0007	78.23±0.4179
		1000	0.1572±0.0010***	0.0705±0.0010	85.51±0.1671
		2000	0.1271±0.0032***	0.0779±0.0014	91.78±0.5350

N=3, Values are expressed as Mean ± SD; Data were analysed by One-way ANOVA

Comparative Evaluation Of Anti-Inflammatory Activity Of Substituted Flavones
 followed by Dunnett's Multiple Comparison Test; F value: 1721; R2: 0.9988
 ***: $p < 0.0001$ compared with control

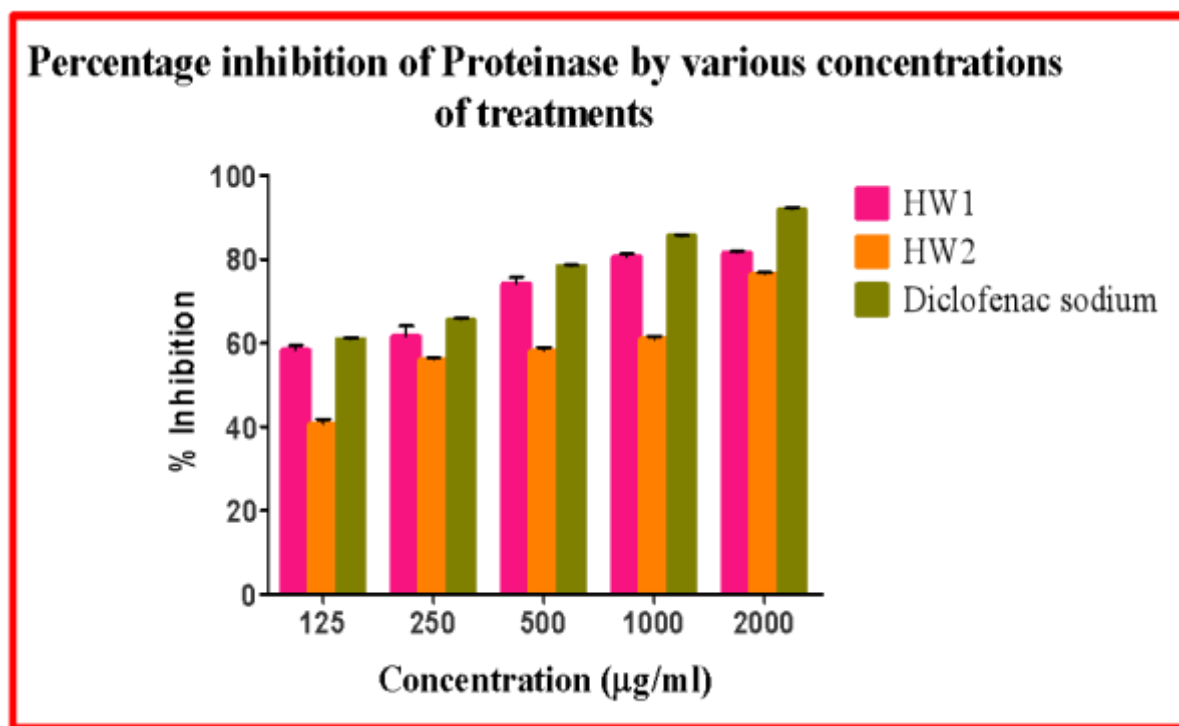


Fig 1: Effect of derivatives on inhibition of proteinase activity

3.2 Inhibition of Protein denaturation method

The anti arthritic activity of the derivatives were evaluated by Inhibition of Protein Denaturation method. The effect of THF, DMF and the standard Diclofenac sodium on protein denaturation can be seen in the figure 2. The percentage inhibition of protein denaturation produced by different concentrations of derivatives are summarized in the table 2. The values are expressed as Mean $\pm$ SD. THF showed significant activity at concentrations 125, 250 and 500 μ g/ml. At the same time, DMF showed significant activity at 62.5, 250 and 500 μ g/ml. The standard drug Diclofenac sodium showed significant activity at all concentrations used. At 500 μ g/ml, THF, DMF and Diclofenac sodium showed percentage inhibition of 77.58 ± 0.8800 % , $73.50 \pm 2.290\%$ and 78.30 ± 2.265 % respectively. In all the three cases, the effect was found to be concentration dependent. ie; the percentage inhibition increases with increase in concentration. At, higher concentrations, the effect produced by the derivatives were almost same as that of Diclofenac sodium. This shows the anti-arthritic activity of the derivatives. The anti arthritic activity of THF was found higher than that of the DMF, as the THF produced higher percentage inhibition of protein denaturation in lower concentrations also.

Table 2: Effect of derivatives on inhibition of protein denaturation

Group	Drug	Concentration (µg/mL)	Optical density of Test (255 nm)	Optical density of Product control (255 nm)	Percentage inhibition(%)
Control	-		0.4541 $\pm$ 0.015	-	
Test	THF	62.5	0.4420 $\pm$ 0.0078	0.1031 $\pm$ 0.018	25.36 $\pm$ 2.245

Comparative Evaluation Of Anti-Inflammatory Activity Of Substituted Flavones

	(HW1)	125	0.4051±0.0072***	0.1734±0.0075	48.97±3.240
		250	0.0442±0.0045***	0.1457±0.0034	77.64±0.2450
		500	0.0434±0.0013***	0.1452±0.0053	77.58±0.8800
	DMF (HW2)	62.5	0.4799±0.012*	0.0522±0.0076	5.797±4.290
		125	0.4520±0.0092	0.1553±0.046	34.66±8.060
		250	0.1669±0.011***	0.0447±0.0026	73.08±3.085
		500	0.1548±0.0050***	0.0345±0.0054	73.50±2.290
	Diclofenac sodium	62.5	0.3368±0.012***	0.0922±0.0077	46.13±0.9250
		125	0.2337±0.0095***	0.0469±0.0046	58.86±1.080
		250	0.1330±0.00010***	0.0102±0.0014	72.95±0.3300
		500	0.1003±0.0096***	0.0018±0.0007	78.30±2.265

4. N=3, Values are expressed as Mean ± SD; Data were analysed by One-way ANOVA
 5. followed by Dunnett's Multiple Comparison Test; F value: 1046; R2: 0.9979
 6. ***: p<0.0001 compared with control
 7. *: p<0.01 compared with control

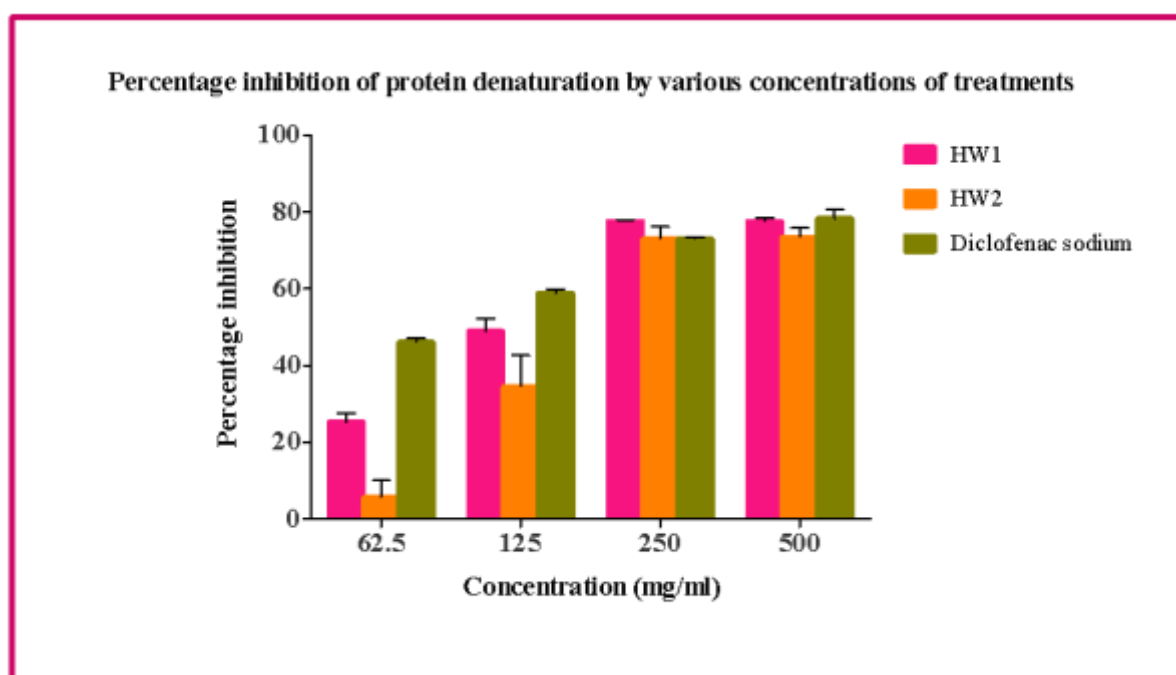


Fig 2: Effect of derivatives on inhibition of protein denaturation

4. Conclusion

The synthesized substituted flavones, 6,3',4'- trihydroxy flavone (THF) or (HW 1) and 3-hydroxy-6,3'-dimethoxy flavones (DMF) or (HW 2) exhibited varying degrees of anti-inflammatory activity towards different anti-inflammatory activity assays. Relatively better anti-inflammatory activity was shown by 6,3',4'- trihydroxy flavones than 3-hydroxy-6,3'-dimethoxy flavones, which may be due to the presence of more number of hydroxyl groups on B ring. Hence this study suggest that the compounds possess significant anti-inflammatory activity and could be further evaluated against free radical mediated diseases like anti-inflammatory, anticancer and neurodegenerative disorders.

5. References

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