



Phytochemical Investigation and Anti – Inflammatory Activity of *Chrysanthemum Dendranthema*

KEYWORDS

Chrysanthemum dendranthema, Anti – Inflammation, Kampferol, Sheep blood, SRBC membrane.

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ABSTRACT

The fresh flowers of *chrysanthemum dendranthema* belongs to Asteraceae family is found to contain Kampferol – 7 – O – Glucoside. The isolated compound is characterized by chromatography and UV spectral techniques. The glycoside isolated from the flowers of *chrysanthemum dendranthema* showed highest anti – inflammatory activity.

1. Introduction

Chrysanthemum dendranthema is belonging to Asteraceae family. It is used in ayurveda and yunani. The fresh flowers contain Kampferol – 7 – O – Glucoside. The white flowers from *chrysanthemum dendranthema* were chosen for phytochemical investigation and anti inflammatory activity.

2. Experimental methods

2.1 Extraction and fractionation

The fresh flowers of *chrysanthemum dendranthema* were collected from Kumbakonam at thanjavur district during the month of november. They were extracted with 85% methanol [4 X 500 ml]. The alcoholic extract was concentrated in vacuo and the aqueous extract was fractionated with Benzene, (2 X 250ml), peroxide free dieithyl ether (3 X 250ml) and ethyl acetate (4 X 250ml). Only ethyl acetate fraction was taken up for further studies.

2.2 Characterization

Kampferol: Yellow solid M.P. 276-278°C .In UV spectroscopy it had λ max MeOH 264,293,336, NaOMe 270,325,387 + AlCl₃ 275,336, 385, AlCl₃/HCl 275, 340, 385 + NaOAc 265, 293, 331. It appeared deep purple under UV and turned yellowish green when fused with NH₃. It gave positive Wilson's boric acid Molish test and Hammer – Hansel test and also it gave a bluish green solution in Gibb's test. It was homogenous on PC and Rf values are depicted in Tab – I

Kampferol – 7 – O – Glucoside:

Yellow solid M.P. 224-226°C. It had λ max MeOH 266, 280, 342 + NaOMe 276, 282, 378, + NaOAc 273, 282,350, + NaOAc/H₃BO₃ 270, 300, 340 nm and had Rf values are recorded in Tab – II and was identified as kampferol and the identity confirmed by mixed PC.

3. Anti inflammatory activity of Kaempferol – 7 – O – glucoside

Experimental methods:

Blood was collected from sheep. The collected blood was mixed with equal volume of sterilized Alsever solution (containing 2% dextrose, 0.87% Sodium citrate 0.05% Citric acid and 0.427% Sodium Chloride) and stored at 4°C. Saline at different concentration was prepared (Isosaline and hyposaline).

Preparation of SRBC suspension:

The blood was centrifuged at 3000RPM and the packed cells obtained were washed with isosaline (0.85% pH 7.2) 3 times and a 10% suspension was made with isosaline.

Determination of SRBC membrane

Stabilization:

Solutions of different concentration of kaempferol 7 – O – glucoside are prepared. Assay mixture contained the drug (flavonoid in concentration are mentioned in Tab – 3) 1ml of phosphate buffer (0.5% pH 7.4) 2ml of hyposaline (0.25% and 0.5ml of 10% SRBC suspension. In another tube instead of drug 2ml distilled water was taken and this served as the control. All the tubes were incubated at 37°C for 30 minutes. Then they were centrifuged and the haemoglobin content in the supernatant was estimated using a photoelectric colorimeter at 560nm.

Results:

The isolated compound from *chrysanthemum dendranthema* has Kaempferol -7 – O –glucoside was screened for their anti inflammatory activity. In general the flavonoids were found to be effective in stability the SRBC membrane against hypotonicity induced haemolysis. The percentage of protection is parallel to the concentration axis (from 10 μ g to 50 μ g). The stabilization capacities decreases gradually with increasing concentration. Therefore the drug is active at 50 μ g beyond which it loses its anti inflammatory activity.

Discussion:

The flowers of *chrysanthemum dendranthema* were found to contain Kaempferol and its glycoside Kaempferol – 7 – O – glucoside. The structure of the compound have been ascertained by chemical reactions, PC and UV spectroscopic values. The results observed in the present study indicates the anti inflammatory activity of Kaempferol – 7-O – glucoside was a dose dependent one. This conclusion is supported by many of the earlier reports.

Table I Rf values of aglycone from the white flowers of *chrysanthemum dendranthema* (whatmann No 1 Ascending 30 \pm 2°C)

Compound	Developing Solvents							
	a	b	c	d	e	f	g	h
Glycoside	33	42	54	63	65	55	52	76

Compound	Developing Solvents							
	a	b	c	d	e	f	g	h
Kaempferol-7-O-glucoside	32	43	54	62	66	54	51	75
Aglycone	04	02	06	18	55	94	95	65
Kaempferol (Authentic)	03	02	04	17	53	92	95	65

Solvent Key

a = 5% aq. HOAC, b = 15% aq. HOAC, c = 30% aq. HOAC, d = 60% aq. HOAC, f = n. BuOH: HOAC: H₂O = 4:1:5 (upper phase), g = Phenol saturated with water, h = HOAC : Conc. HCl: H₂O = 30:3:10

Table II

R_f (X100) values of the sugar from the glycoside from *chrysanthemum dendranthema* (whatmann No 1 Ascending 30± 2°C)

Compound	Developing Solvents			
	e	f	g	h
Glucose	77	09	39	90
Sugar from Glycoside	70	10	40	91

Spray reagent : Aniline hydrogen phthalate

Solvent key

e = 60% aq. HOAC, f = n. BuOH: HOAC: H₂O = 4:1:5 (upper phase),

g = Phenol saturated with water, h = HOAC : Conc. HCl: H₂O = 30:3:10

Table III

SRBC membrane stabilization of *Chrysanthemum dendranthema*

S. No	Conc. of drug in µg	Transmittance
1.	10	88
2.	25	88
3.	30	88
4.	75	86
5.	100	86
6.	200	84

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