

Antiinflammatory activity of Flavonoids isolated from *Rhus Mysorensis*

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Abstract: Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, and is a protective response involving immune cells, blood vessels, and molecular mediators. Medicinal plants have been long lastingly recognized as potential sources of natural drugs. The present study was framed to evaluate anti-inflammatory activity of the compounds isolated from *Rhus mysorensis*. The study was carried out using *in vitro* and *in vivo* methods. *In vitro* anti inflammatory activity of isolated compounds was screened using egg albumin. The inhibition of protein denaturation was found to be high **90.5, 84.1** with the compounds **2 and 4** at 100µg/mL respectively. Compounds **2 and 4** found active in reducing the paw volume 0.88 ± 0.26 , 1.35 ± 0.11 at fourth hour of treatment.

Keywords: egg albumin, Inflammation, paw volume, *Rhus mysorensis*

I. Introduction

Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, and is a protective response involving immune cells, blood vessels, and molecular mediators^[1]. In the modern concept Inflammation is associated with common symptoms generally are swelling, loss of function and heat^[2]. Acute transient phase, delayed sub acute phase and chronic proliferate phase are the three different phases which are usually categorized in the reactions of inflammation^[3]. In Ayurveda and Allopathic, antagonistic mediators are commonly used to prevent the inflammatory processes. The anti-inflammatory drugs which are most widely used as modern drugs and are proven as potential inhibitors especially of that produces prostaglandins is cyclooxygenase COX pathway (arachidonic acid metabolism). In Indian Ayurvedic system *Boswellia serrata* Roxb has been extensively and widely used medicine as the source of potential inflammatory drugs^[4-7]. Plant derivatives viz., phenolic compounds and flavonoids are observed with anti-inflammatory activity regulates the levels of various inflammatory cytokines or mediators (IL-1, IL-6, IL-10, TNF- α , NF- κ B, NO, iNOS and COX-2) have been reported by number of studies on plant derived compounds^[8]. Medicinal plants have been long lastingly recognized as potential sources of natural drugs^[9]. In traditional medicine for the treatment of inflammatory diseases several medicinal plants are widely used. In the treatment of inflammatory disorders many of these medicinal plants have been already established preliminary and clinical evidence. Isolation and characterization of a number of anti-inflammatory compounds structurally as well as pharmacologically have been perpetuating^[10]. Among such valuable and therapeutically immense important medicinal plants, it is known that *Rhus mysorensis* belongs to *Anacardeaceae* also exhibits pharmacological properties. Thus, in context to the hazardous effects of synthetic anti inflammatory drugs, the present study was framed to evaluate the anti-inflammatory activity of four flavonoids which were previously isolated from hydroalcoholic fractions of leaf and rhizomes of *Rhus mysorensis* (The data of isolation is yet to be published). The evaluation was carried in two different ways initially these hydroalcoholic extracts were investigated for *in vitro* anti-inflammatory activities by inhibition of egg albumin denaturation process and based on these results we have followed *in vivo* anti-inflammatory activities.

II. Material and Methods

2.1 Anti-inflammatory activity

2.1.1 Preliminary studies on anti-Inflammatory activities of compounds isolated from *Rhus mysorensis*

Preliminary studies on anti-inflammatory activities of the isolated from *Rhus mysorensis* leaves and roots were determined by the described method^[11]. About of 5 mL reaction mixture includes of 0.2 mL of egg albumin, 2.8 mL of phosphate buffered saline (PBS, pH 6.4) add 2 mL of isolated compounds at 10, 20, 40, 60,

80 and 100 µg/mL. Double-distilled water with same volume used as control. The mixtures were incubated at (37±2) °C in BOD incubator for about 15 min and followed by heating at 70 °C for 5 min. After attaining room temperature, absorbance was measured at 660 nm using vehicle as blank and viscosity was determined by using Ostwald viscometer. Ibuprofen at the final concentration of (10, 20, 40, 60, 80, 100 µg/mL) was used as reference drug. The inhibition percentage of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition} = 100 \times (V_t / V_c - 1)$$

Where, V_t = absorbance of test sample, V_c = absorbance of control.

2.1.2 *In vivo* anti-inflammatory activity

Animals

Albino rats of Wistar strain, of male sex, weighing 150 – 250 g were purchased from National Institute of Nutrition, Hyderabad, India and housed under standard environmental conditions (temperature: 24 ± 1o C, light / dark cycle: 10/14 h). The rats were fed with standard pellet diet (Amrut laboratory animal feed, Maharashtra, India) and water *ad libitum*. Animals were acclimatized to laboratory conditions at least 1 week before conducting the experiments according to the guide lines of CPCSEA – New Delhi, (**Registration No. - 915/ac/05/CPCSEA**).

Activity

The isolated compounds were assessed for their anti-inflammatory activity using carrageenan-induced hind paw edema method. The rat paw edema was induced by subcutaneous injection of 0.1 ml of 1% freshly prepared saline solution of carrageenan into the right hind paw of rats ^[12]. The standard drug Ibuprofen (100 µg/kg/ p.o) was given orally as a positive control. The control group was administered orally with 0.9% of 0.1 ml of saline solution only. The test groups were administered orally with the isolated compounds at the equimolar dosage of the standard drug, 1 h before the administration of carrageenan. The paw volumes were measured using plethysmometer at interval of 24 h.

III. Result and discussion

In accordance to the data of the present investigation the anti-inflammatory activity of compounds isolated from *Rhus mysorensis* was found to be in concentrated dependent manner. *In vitro* anti inflammatory activity of isolated compounds were screened using egg albumin. The protein denaturation was found to be compounds concentration dependent. Among the compounds **2 and 4** are found highly active at all concentrations tested and noticed highest inhibition percentages **90.5, 84.1** at 100µg/mL respectively. The activity of these compounds is very much comparable with the known standard Ibuprofen. Results are shown in **table 1**.

All the isolated compounds showed *in vivo* anti-inflammatory activity by significant inhibition of edema (Table 2). The *in vivo* anti-inflammatory activity of isolated compounds is quite different compared to that from *in vitro* activity. Accordance to present investigation results, we observed that compounds which are active *in vitro* exhibited moderate *in vivo* activity. The reduction in the paw volume and subsequent anti-inflammatory activity by the compounds was noticed in fourth and fifth hour and slightly increased in sixth hour. Compounds **2 and 4** found active by reducing paw volume 0.88±0.26, 1.35±0.11 at fourth hour of treatment and comparable with the values of the positive control Ibuprofen.

Generally inflammatory diseases are treated using traditional remedies. Now-a-days, scientists are paying large amounts of hard work to evaluate the potential herbal remedies as novel bioactive compounds that leads for the development of anti inflammatory drugs ^[13]. The common mechanism involved in the reduction of edema in the rat paw is due to the release of histamine and serotonin, prostaglandins, protease and lysosome ^[14]. The anti-inflammatory of various flavonoid derivatives were previously reported using various models such as human neutrophil functions, eicosanoid release, TNF-α production, air-pouch ^[15-17]. In the current study we have evaluated the anti-inflammatory activity of four flavonoids which we were isolated from the *Rhus mysorensis* root and stem (Data was not shown). Compounds screened in the current study have been shown to possess anti-inflammatory activity by the inhibition of histamine induced vascular permeability and reverse passive Arthus reaction ^[18-19].

Table 1 *In vitro* Anti-inflammatory activity of compounds isolated from *Rhus mysorensis* plant extract

compounds	10 µg/mL	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
Control (-)	--	--	--	--	--	--
Ibuprofen	68.3±0.1	75.8±1.2	82.1±1.7	88.7±1.2	93.6±1.6	98.3±1.1
Compound 1	48.1±1.0	52.3±1.5	56.0±1.4	62.1±1.2	66.1±1.1 ^b	70.3±1.2 ^b
Compound 2	57.1±1.6	62.1±1.1	69.3±2.0 ^b	78.2±1.2 ^a	83.1±1.1 ^a	90.5±1.4 ^a
Compound 3	50.5±1.1	54.4±1.5	59.0±1.0 ^b	66.1±1.1 ^a	70.6±1.8 ^a	76.2±1.2 ^a
Compound 4	54.4±1.1	59.0±1.3	64.5±1.0 ^b	71.1±1.1 ^a	77.3±1.2 ^a	84.1±1.0 ^a

Compound 1; 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one, **Compound 2;** 2-(3,4-dihydroxyphenyl)-hydroxy-4*H*-chromen-4-one, **Compound 3;** 2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-4*H*-chromen-4-one, **Compound 4;** 5,6,7-trihydroxy-2-phenyl-4*H*-chromen-4-one

Values are expressed as Percentage ±SD from six observations and Data is analyzed by one way ANOVA followed by Dunnett's 't' test. ^aindicates P < 0.001 & ^b indicates P < 0.01. Concentration of the compounds in µg/mL.

Table 2 *In vivo* Anti-inflammatory activity of compounds isolated from *Rhus mysorensis* plant extract

Compounds µg/mL	Swelling (AI %)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Control (-)	1.25±0.039	1.40±0.011	1.65±0.020	1.90±0.011	2.78±0.015	3.91±0.011
Ibuprofen	0.07±0.019 (94.4)	0.08±0.021 (94.2)	0.06±0.017 (93.6)	0.10±0.021 (94.7) ^b	0.11±0.026 (96.0) ^a	0.11±0.025 (97.1)
Compound 1	3.59±0.10 (28.1)	3.32±0.08 (33.7)	3.09±0.012 (39.0)	2.81±0.20 (48.9) ^b	2.75±0.11 (55.0) ^a	2.89±0.29 (50.1)
Compound 2	1.78±0.10 (42.1)	1.50±0.03 (53.6)	1.33±0.18 (63.3)	0.88±0.26(76.8) ^a	0.69±0.07 (81.5) ^a	0.81±0.011 (72.2)
Compound 3	2.68±0.12 (32.4)	2.45±0.06 (39.7)	2.23±0.19 (45.4)	2.09±0.03 (52.1) ^b	1.85±0.14 (66.1) ^a	1.95±0.004 (60.7)
Compound 4	2.10±0.11 (37.2)	1.77±0.09 (41.9)	1.50±0.23 (49.6)	1.35±0.11 (58.4) ^b	1.11±0.10 (65.9) ^a	1.42±0.19 (53.2)

Compound 1; 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one, **Compound 2;** 2-(3,4-dihydroxyphenyl)-hydroxy-4*H*-chromen-4-one **Compound 3;** 2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-4*H*-chromen-4-one, **Compound 4;** 5,6,7-trihydroxy-2-phenyl-4*H*-chromen-4-one

Values are expressed as Percentage ±SD from six observations and Data is analyzed by one way ANOVA followed by Dunnett's 't' test. ^aindicates P < 0.001 & ^b indicates P < 0.01. Concentration of the compounds in µg/mL.

IV. Conclusion

Basing on our studies, we conclude that the compounds isolated from the *Rhus mysorensis* exhibit the anti-inflammatory activity. However, the further studies are required to use these compounds as anti-inflammatory agents.

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