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Research Paper

Lupeol and 3β-hydroxy-urs-12-en-28-oic acid isolated from *Macphersonia gracilis* (Sapindaceae) and their anti-inflammatory activity in mice.

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Abstract
The present study focused on isolating active metabolites from Macphersonia gracilis and examining their anti-inflammatory activity. Ethanolic, hexanic, ethyl acetate, and butanolic extracts were screened in vivo for acute anti-inflammatory effects at 250 mg/kg, using the carrageenan-induced paw edema test in mice. The hexane extract displayed the highest activity, and active compounds were purified from it through chromatographic procedures. Structural elucidation using UV, 1D and 2D NMR, identified the compounds as β-hydroxy-urs-12-en-28-oic acid and lupeol. These isolates were tested at 12.5, 25, and 50 mg/kg against carrageenan-induced acute paw edema, cotton pellet-induced subchronic inflammation, and Freund's adjuvant-induced arthritis in mice. At 50 mg/kg, both compounds reduced carrageenan-induced paw edema by 76.00%, inhibited subchronic inflammation by 37.00%, and alleviated chronic arthritis inflammation by 67.50%.

Keywords: *Macphersonia gracilis, anti-inflammatory, Hexanic extract, β-Hydroxy-urs-12-en-28-oic acid and Lupeol*

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I. Introduction

Macphersonia gracilis (Sapindaceae) is a small endemic tree species found in the dry deciduous forests of Madagascar [1]. It is known by the local vernacular name "fano" and has a rich history in traditional medicine [2]. The plant's bark and leaves are traditionally prepared as a decoction or poultice to treat a wide range of ailments, including inflammatory conditions, fevers, and aches [3]. This long-standing ethnomedicinal use has inspired scientific investigation into its therapeutic potential, suggesting it may contain novel compounds with anti-inflammatory properties [4].

Inflammation is a fundamental physiological defense response that enables the body to protect itself. However, when this process becomes excessive or persists over time, it can contribute to the development of several chronic diseases [5]. Considerable research efforts have therefore focused on identifying anti-inflammatory agents derived from natural products [6]. In this study, biologically active compound(s) were isolated from the most active extracts of *Macphersonia gracilis* using different chromatographic techniques. The chemical structures of β-hydroxy-urs-12-en-28-oic acid and lupeol were determined through NMR spectroscopy. Their anti-inflammatory effects were evaluated in vivo in experimental models of acute, subchronic, and chronic inflammation induced by physiological stimuli. To our knowledge, this represents the first chemical and biological investigation conducted on this plant and its isolated constituents.

Fig1: *Macphersonia gracilis* O. Hoffm.

II. Materials And Methods

2.1 Plant material

Macphersonia gracilis was collected from a dry deciduous forest, in the Region of Menabe in the Western part of Madagascar. This species was identified at the Department of Botany of Botanical and Zoological Park of Tsimbazaza (Antananarivo). A voucher specimen was deposited at the Analytical Chemistry and Formulation Laboratory, Faculty of Sciences, University of Antananarivo and Geosciences, Physics, Environmental Chemistry and High Pathogenic System Doctoral School (GPCEHP), University of Toliara, Toliara 601 Madagascar for future references.

2.2 Extraction and isolation procedures

The dried stem leaves of *Macphersonia gracilis* were ground into a fine powder using a grinder. The plant powder (500 g) was then extracted by maceration with an Ethanol-water mixture (80:20) (3000 ml) for 96 h, and the solution was concentrated to dryness under vacuum at low pressure and at 60 °C. The crude extract was dissolved in water and was successively partitioned with hexane, ethyl acetate, and n-butanol, which gave hexanic, ethyl acetate, and butanol extracts.

One gram of the hexanic extract was placed on a column chromatography and was eluted with a gradient of cyclohexan–CH_2Cl_2 (100:0; 95:5; 90:10; 80:20). A total of 100 fractions of 10 ml each were collected and were analysed by thin layer chromatography (TLC), using precoated silica gel plates (60 F254, aluminium base, Merck) with 0.2 mm thickness. Five microliters of each fraction were spotted on TLC plates and were developed with a mobile phase cyclohexan–CH_2Cl_2 (70:30). After drying, the separated compounds were detected under a UV lamp (254/365 nm) or by spraying with a freshly prepared vanillin reagent (0.1 g vanillin dissolved in 28 ml methanol and 1 ml sulphuric acid) [7], followed by heating at 110 °C for 1 min. Spots that showed the same Rf on TLC were combined [8]. The fractions from 99 to 120, which were eluted with cyclohexan–CH_2Cl_2 (80:20), were grouped together and precipitated with MeOH. The structure of the isolated compound was confirmed by NMR data (UV, 1D- and 2D-NMR).

2.3 Animals

Male Swiss mice with an average weight of 30 g were used for the anti-inflammatory experiments. The animals were kept under laboratory conditions for one week to acclimatize before the study. They were fasted overnight prior to each test but were allowed free access to water. Each mouse was used only once, and all procedures were carried out following the ethical rules for the care of laboratory animals.

2.4 Antiinflammatory activity

Preliminary assay to determine the active fraction of *Macphersonia gracilis*: A preliminary assay was carried out to identify the most active extracts of *Macphersonia gracilis* (ethanolic, hexanic, ethyl acetate, and butanolic) using carrageenan-induced paw edema. All extracts were tested at the same dose of 250 mg/kg. The mice were divided into six groups of six animals each (four test groups, one standard group, and one control group).

The extracts of *Macphersonia gracilis* and the standard drug were freshly prepared in Tween 80–distilled water (10:90). Phenylbutazone (100 mg/kg) was used as the standard drug. All samples were given orally. The test

groups received *Macphersonia gracilis* extracts (250 mg/kg), the standard group received phenylbutazone, and the control group received the vehicle (Tween 80–distilled water (10:90)) at 10 ml/kg[9].

Acute inflammation activity of Lupeol and 3β-hydroxy-urs-12-en-28-oic acid: Carrageenan-induced paw edema was used as a model of acute inflammation, following the method of Winter et al. (1962) [10] and as previously described [11]. The initial volume of the right hind paw of each mouse was measured using a plethysmometer (Ugo Basile, Model 7140). All substances were administered orally to the animals.

Lupeol and 3β-hydroxy-urs-12-en-28-oic acid were tested at three doses (12.5 mg/kg, 25 mg/kg, 50 mg/kg) in the test groups. Phenylbutazone and Indomethacin were used as standard drugs, while the control group received the vehicle (Tween 80–distilled water, 10:90) at 10 ml/kg. Thirty minutes after administration, paw edema was induced by injecting 0.05 ml of 1% carrageenan into the subplantar tissue of the right hind paw of each mouse. The paw volume was measured at 1, 2, 3, 4, 5, and 6 hours after carrageenan injection.

The percentage inhibition of paw edema was determined using the following formula:

Percentage inhibition = [(1 – (a/b)) x 100]

where *a* represents the increase in paw volume in the test group, and *b* the increase in paw volume in the control group [12].

Activity of Lupeol and 3β-hydroxy-urs-12-en-28-oic acid on Subchronic inflammatory:

The subchronic inflammation model was carried out using cotton pellet granuloma in mice following the method of Meier et al., (1950) [13]. The animals were anesthetized with ether and the hair on their backs was removed. Cotton pellets (20 mg), previously soaked in 1% carrageenan prepared in physiological saline (0.9%), were implanted under the skin on both sides of the groin of each mouse [14, 15]. The treatments were given orally for 7 days in a row starting from the day of pellet implantation. Indomethacin (10 mg/kg) or phenylbutazone (100 mg/kg) was used for the standard groups, Lupeol and 3β-hydroxy-urs-12-en-28-oic acid (12.5 mg/kg, 25 mg/kg and 50 mg/kg) for the test groups, and solvent (Tween 80–distilled water, 10:90, 10 ml/kg) for the control group. On the 8th day, the mice were sacrificed and the cotton pellets with the surrounding tissue were collected. The granulomas were dried in an oven at 60 °C for 24 h, weighed, and compared with the control. The increase in dry pellet weight was taken as an indicator of granuloma formation [14]. The anti-inflammatory activity was calculated using the equation below.

Anti-inflammatory activity (%) = 1-(T/C) × 100.

Where, T represents the dry weight of the pellets in treated groups and C the dry weight in control groups.

The anti-inflammatory potential of lupeol and 3β-hydroxy-urs-12-en-28-oic acid was assessed using a chronic arthritis model in mice induced by Freund's adjuvant, according to the method described by Freund et al. (1937) [16]. The swelling of the right hind paw was determined with a plethysmometer (Ugo Basile, Italy).

All treatments, namely lupeol and 3β-hydroxy-urs-12-en-28-oic acid at doses of 12.5, 25, and 50 mg/kg, indomethacin (10 mg/kg), phenylbutazone (100 mg/kg), and the control vehicle (Tween 80–distilled water, 10:90, at 10 ml/kg), were given orally to mice 30 minutes before the administration of CFA (0.05 ml) into the metatarsal region of the right hind paw, and continued once daily for 28 days [17].

Paw volume was monitored on days 7, 14, 21, and 28 after Freund's adjuvant injection. The difference between the paw volume recorded on day 28 and the initial volume (V0) was used to evaluate the chronic inflammatory response, while the percentage inhibition of edema was determined according to the standard formula.

% inhibition = (Vc – Vt) / Vc × 100

Where, Vc is the mean changes in paw volume of control group and Vt is the mean changes in paw volume of treated group.

2.5 Statistical analysis

All experimental results were presented as mean ± SEM (standard error of the mean). The data were analyzed using the unpaired Student's *t*-test, and differences were considered statistically significant when $p < 0.05$.

III. Results and discussion

3.1 Results of extraction and structure of isolated compound

Fractionation of hexanic extract, 12 g (8.00%) of EtOH–H_2O extract (150 g) yielded 8 g (5.33%) of ethyl acetate extract and 10 g (6.67%) of butanolic extract.

The hexane extract was fractionated, and two compounds were isolated. **P1**, obtained from the aliquots eluted between 70 and 90 with cyclohexane–CH_2Cl_2 (80:20) and combined with MeOH, gave 15 mg of a compound (Rf = 0.49). Spectral analysis indicated that P1 is a triterpene, and the characteristic peaks corresponding to the carbonyl group of 3β-hydroxy-urs-12-en-28-oic acid were detected.

P2, obtained from the aliquots eluted at 95 under the same solvent system and combined with MeOH, yielded 10 mg of a compound (Rf = 0.47). Spectral studies revealed that P2 is also a triterpene, with peaks corresponding to the carbonyl group of lupeol. Thee data 1D and 2D NMR of this compound is given in table 1. [21]

The isolated compound was identified as 3β-hydroxy-urs-12-en-28-oic acid by comparison with previously reported spectral data in the literature [18-20] (Figure 2). Another compound was identified as lupeol based on comparison with published spectral data [21] (Figure 2). Lupeol has also been detected in this hexane extract and is among the most extensively studied triterpenes for its pharmacological properties [22, 23].

P1: 3β-hydroxy-urs-12-en-28-oic acid: White powder

^{1}H NMR spectral data (400.15 MHz, CDCl3): δ 0.96 (H-29), 0.96 (H-23), 1.04 (H-19), 1.13 (H-27), 1.27 (H-6), 1.27 (H-11), 1.30 (H-27), 1.31 (H-7), 1.36 (H-16), 1.39 (H-5), 1.43 (H-9), 1.47 (H-1), 1.52 (H-6), 1.52 (H-11), 1.56 (H-7), 1.60 (H-20), 1.61 (H-16), 1.63 (H-19), 1.69 (H-2a), 1.72 (H-1), 1.76 (H-18), 1.79 (H-15), 1.86 (H-2b), 2.01 (H-18), 2.04 (H-15), 2.42 (H-13), 3.15 (H-3), 3.58 (H-3), 5.29 (H-12), 11.0 (COOH). **^{13}C NMR spectral data (100.15 MHz, CDCl3)**: δ: 29,0 (C-15), 16.7 (C-26), 17.5 (C-27), 18.0 (C-6), 16.9 (C-28), 27.4 (C-23), 16.1 (C-24), 23.8 (C-11), 24.0 (C-16), 27.4 (C-2), 30.7 (C-21), 36.6 (C-22), 33.1 (C-7), 46,9 (C-17), 38.9 (C-4), 38.8 (C-20), 38.9 (C-19), 39.6 (C-8), 39.1 (C-1), 38.4 (C-10), 138,1 (C-13), 52,9 (C-18), 46.8 (C-9), 55.4 (C-5), 78.6 (C-3), 125.0 (C-12),41,6 (C-14), 17,0 (C-29).

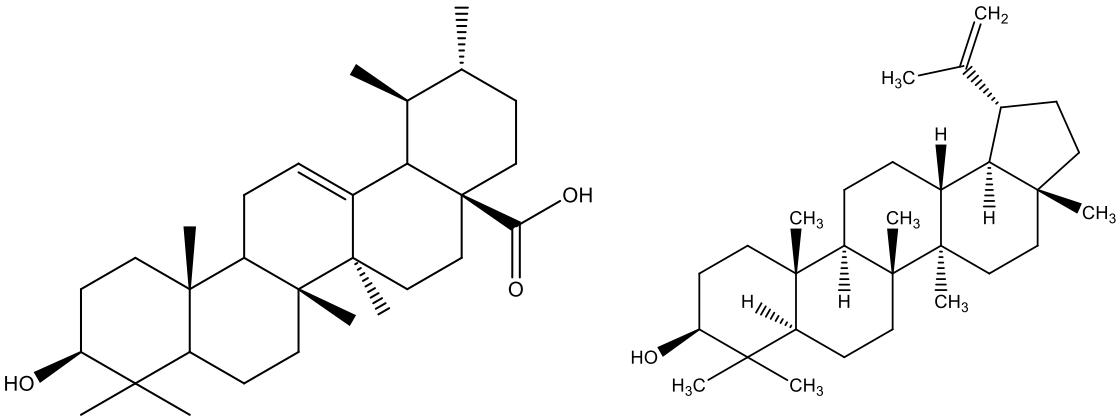
P2: lupeol: **δ (ppm) ^{1}H NMR (600 MHz, CD_3OD)**: δ 0.92, 1.35 (H-1); 1.49, 1.67 (H-2); 3.5 (H-3); 0.60 (H-5); 1.357, 1.50 (H-6); 1.40 (H-7); 1.27 (H-9); 1.21, 1.42 (H-11); 1.00, 1.65 (H-12); 1.59 (H-13); 1.00, 1.70 (H-15); 1.37, 1.47 (H-16); 1.40, 1.39 (H-18); 2.42, 1.42 (H-19); 1.31, 1.95 (H-21); 1.10, 1.35 (H-22); 1.00 (H-23); 0.90 (H-24); 0.93 (H-25); 1.05 (H-26); 0.94 (H-27); 0.79 (H-28); 4.56, 4.69 (H-29); 1.65 (H-30).

δ (ppm) ^{13}C NMR (125 MHz, CD_3OD): : δ 38.9 (C-1); 27.4 (C-2); 77.8 (C-3); 37.9 (C-4); 55.2 (C-5); 19.1 (C-6); 35.2 (C-7); 40.8 (C-8); 50.4 (C-9); 36.9 (C-10); 20.9 (C-11); 25.1 (C-12); 38.0 (C-13); 41.8 (C-14); 27.4 (C-15); 35.7 (C-16); 43.0 (C-17); 48.3 (C-18); 47.978 (C-19); 150.966 (C-20); 29.841 (C-21); 41.0 (C-22); 28.1 (C-23); 15.3 (C-24); 16.1 (C-25); 16.1 (C-26); 14.7 (C-27); 17.8 (C-28); 109.3 (C-29); 19.3 (C-30).

Table (1) :^{1}H and ^{13}C NMR Spectral Data of the compound and that of Lupeol from Literature (at 600 and 150 MHz resp. in $CDCl_3$; δ in ppm, J in Hz) [21]

No.	C/H	δ_H ($CDCl_3$)	δC ($CDCl_3$)	COSY correlation	J correlation (HMBC)
1	CH_2	0.92, 1.35 (o)	38.9	H-2a,2b	H-2a, 3, 5, 9, 25
2	CH_2	1.49, 1.67 (o)	27.4	H-1a,1b, 3	H-3, 23
3	CH	3.5 (dd)	77.8	H-2b	H-2a,2b, 5, 23,24
4	C		37.9		H-3,5,6a,6b,23,24
5	CH	0.60 (o)	55.2	H-6a,6b	H-1a,1b,6b,7,25
6	CH_2	1.357, 1.50 (o)	19.1	H-5, 7	H-5, 7
7	CH_2	1.40 (o)	35.2	H-6a,6b	H-5,6a,6b,9,26
8	C		40.8		H-6a,6b,7,9,13,26, 27
9	CH	1.27(o)	50.4	H-11a,11b	H-1a, 5, 11a, 11b, 25, 26
10	C		36.9		H-1a,1b,2a,2b,6a,6b,5,9,25
11	CH_2	1.21, 1.42 (o)	20.9	H-9, 12a, 12b	H-9, 12b,13
12	CH_2	1.00, 1.65 (o)	25.1	H-11a,11b, 13	H-11a, 11b,13
13	CH	1.59 (o)	38.0	H-12a, 12b, 18	H-11b,12a,19,27
14	C		41.8		H-12a, 13, 15a,16a,18,26,27
15	CH_2	1.00,1.70 (o)	27.4	H-16a, 16b	H-13, 16a, 16b
16	CH_2	1.37, 1.47 (o)	35.7	H-15a	H-18, 22a, 22b, 28
17	C		43.0		H-16a, 16b, 18, 21a, 21b, 22a, 22b, 28
18	CH	1.40, 1.39 (o)	48.3	H-13,19	H-19, 21b
19	CH	2.42 (m), 1.42(o)	47.978	H-21a, 21b	H-21b, 29a,29b
20	C		150.966		H-19,30

21	CH_2	1.31(o), 1.95(m)	29.841	H-19,22a, 22b	H-19, 22a,
22	CH_2	1.10, 1.35 (o)	41.0	H-21a,21b	H-18, 21b, 28
23	CH_3	1.00 (s)	28.1	H-3	H-3
24	CH_3	0.90 (s)	15.3	H-3, 5, 23	15.36
25	CH_3	0.93 (s)	16.1	H-1a, 1b, 9, 11b	16.11
26	CH_3	1.03 (s)	16.1	H-9, 13	15.96
27	CH_3	0.94 (s)	14.7	H-13, 15a	14.54
28	CH_3	0.79 (s)	17.8		H-16b, 22a, 22b
29	CH_3	4.56, 4.69 (d, J=1.91 Hz)	109.3	H-30	H-19,30
30	CH_3	1.65 (s)	19.3	H-29a,29b	H-19,29a,29b



P1 : 3β-hydroxy-urs-12-en-28-oic acid **P2** : lupeol

Figure 2: Isolated compounds chemical structures from *Macphersonia gracilis* O. Hoffm.

3-2 Anti-inflammatory activity

Active fraction of *Macphersonia gracilis* O.

Preliminary tests showed that all fractions of *Macphersonia gracilis* O. at 250 mg/kg reduced carrageenan-induced paw edema, with the hexane extract being the most active. Edema inhibition was 50 % for the ethanolic extract, 68% for the hexane extract, 21 % for ethyl acetate, and 27% for butanolic extract ($P < 0.05$). Phenylbutazone, used as reference, produced 85% inhibition. These results highlight the strong anti-inflammatory potential of the hexane extract in acute conditions (Figure 3).

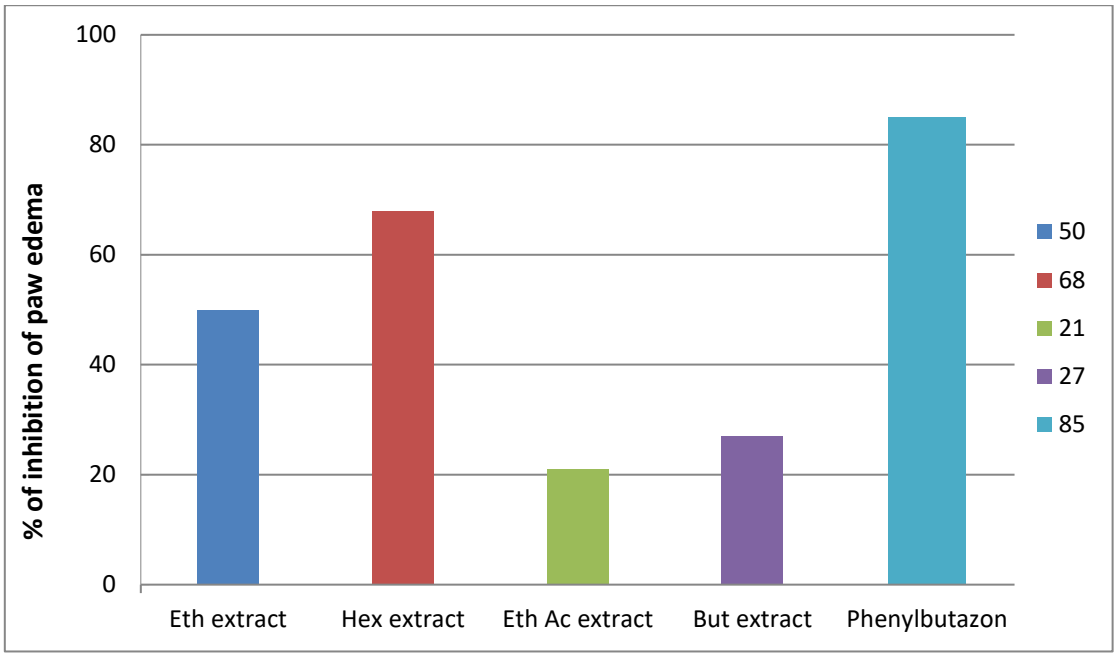


Fig. 3: Anti-inflammatory effect of *Macphersonia gracilis* extracts and phenylbutazone given orally in an acute inflammation model (carrageenan-induced paw edema in mice), assessed two hours after carrageenan injection (mean ± SEM; n = 6). Differences from control were statistically significant at $P < 0.05$.

Effect of Lupeol and 3β-hydroxy-urs-12-en-28-oic acid on acute inflammation

Lupeol and 3β-hydroxy-urs-12-en-28-oic acid, isolated from the hexane fraction, significantly reduced carrageenan-induced paw edema at different doses. After three hours, inhibition reached 36.80 % and 42.10 % at 12.5 mg/kg and 50 mg/kg, respectively. At 12.5 mg/kg, their effect was weaker than indomethacin (36.80 % vs. 48.50 %), and at higher doses, still lower than phenylbutazone, which produced 85.00 % inhibition compared to 76.00% at 50 mg/kg ($P < 0.05$) (Figure 4).

Lupeol and 3β-hydroxy-urs-12-en-28-oic acid effectively reduced carrageenan-induced edema, which is a two-phase inflammatory process. Their anti-inflammatory effect was most notable in the second phase (2–6 hours), which is sensitive to drugs like indomethacin. Although less potent than indomethacin and phenylbutazone, these compounds showed maximum activity at a dose of 50 mg/kg, three hours after injection.

Lupeol and 3β-hydroxy-urs-12-en-28-oic acid on Granuloma Formation

The anti-inflammatory effects of **Lupeol** and **3β-hydroxy-urs-12-en-28-oic acid** were tested on mice with granulomas induced by a cotton pellet. This type of inflammation is characterized by an influx of monocytes, macrophages, and exudation.

After five days, the compounds at doses of 12.00 mg/kg and 50 mg/kg reduced granuloma formation by 12.00 % and 15.78% respectively, when compared to the control group. In contrast, the standard drugs, indomethacin (10 mg/kg) and phenylbutazone (100 mg/kg), showed a stronger inhibition of 27.50% and 41.00 %.

These results suggest that Lupeol and 3β-hydroxy-urs-12-en-28-oic acid inhibit granuloma formation by decreasing the migration of mononuclear cells and preventing the development of giant cells.

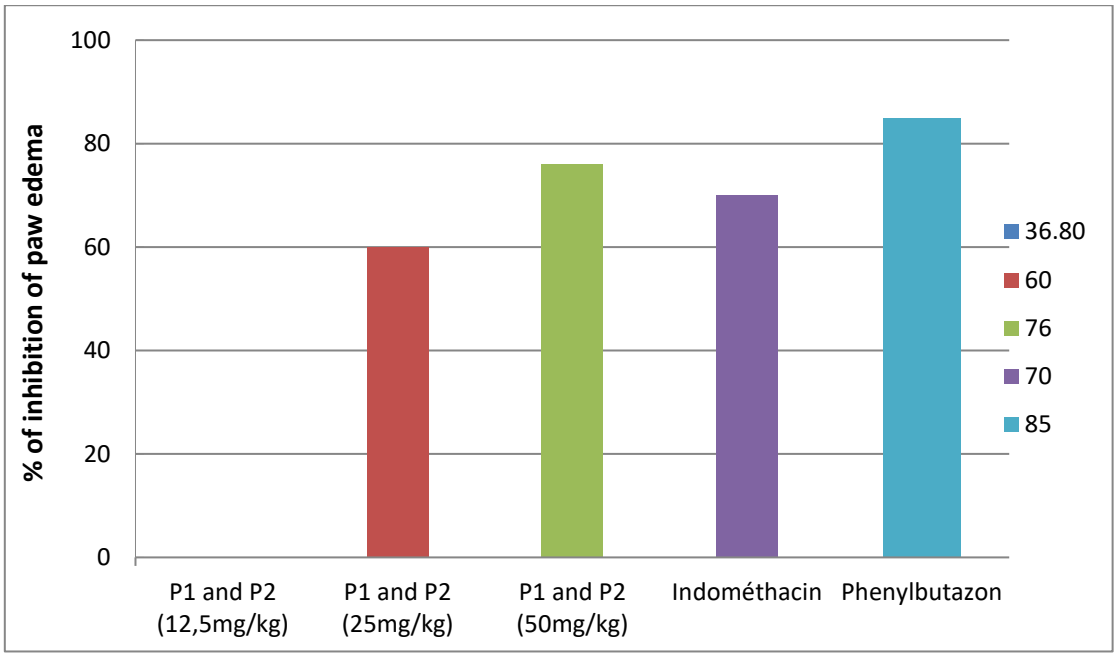


Fig4: This study evaluates the acute anti-inflammatory activity of **Lupeol** and **3β-hydroxy-urs-12-en-28-oic acid** in mice. Administered orally, the compounds reduced paw edema caused by a carrageenan injection. The results, measured three hours after the injection, were compared to the effects of indomethacin and phenylbutazone. [24-25].

(Graph shows % inhibition:

Lupeol and 3β-hydroxy-urs-12-en-28-oic acid (12.5 mg/kg) → 36.80%
Lupeol and 3β-hydroxy-urs-12-en-28-oic acid (50 mg/kg) → 42.10%
Indomethacin (10 mg/kg) → 48.50 %
Lupeol and 3β-hydroxy-urs-12-en-28-oic acid (100 mg/kg) → 76.00%
Phenylbutazone (100 mg/kg) → 85.00%)

Table 2 shows the statistically significant effects of orally administered Lupeol and 3β-hydroxy-urs-12-en-28-oic acid , indomethacin, and phenylbutazone on chronic inflammation (granuloma) in mice.

Groups	**Dose (mg/kg)**	**Weight of dry cotton pellet granuloma (mg ± SEM)**	**Percentage of inhibition**
Control		57.261 ± 1.927	
Lupeol and 3β-hydroxy-urs-12-en-28-oic acid	12.5	50.361 ± 2.171	12.00%
Lupeol and 3β-hydroxy-urs-12-en-28-oic acid	50	48.228 ± 1.350	16.00%
Indomethacin	10	41.803 ± 1.068	27.50%
Phenylbutazone	100	34.283 ± 1.062	41.00%

The cotton pellet granuloma method was used to assess the different stages of inflammation. Granuloma development involves macrophage and fibroblast proliferation, with fibroblasts producing collagen that forms the fibrous mass. When macrophages cannot eliminate pathogens, fibroblasts generate connective tissue, leading to persistent damage. Pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) stimulate cell recruitment, fibroblast aggregation, and fibrosis. In this study, the reduced granuloma weight indicates that Lupeol and 3β-hydroxy-urs-12-en-28-oic acid from *Macphersonia gracilis* inhibit the proliferative phase, likely by lowering cytokine activity. [26-28].

Anti-arthritic effect of Lupeol and 3β-hydroxy-urs-12-en-28-oic acid

Complete Freund's adjuvant (CFA) injection in the right hind paw of mice caused persistent joint inflammation up to 28 days. Repeated treatment with Lupeol and 3β-hydroxy-urs-12-en-28-oic acid from *Macphersonia gracilis* significantly reduced paw swelling in a dose-dependent manner. At 12.5 mg/kg, inhibition reached 20.00%, while at 50 mg/kg it increased to 41.50%. The effect at 12.5 mg/kg was weaker than indomethacin (61.50 % at 10 mg/kg), but at 50 mg/kg it surpassed phenylbutazone (57.50 % at 100 mg/kg) (Figure 5). [28].

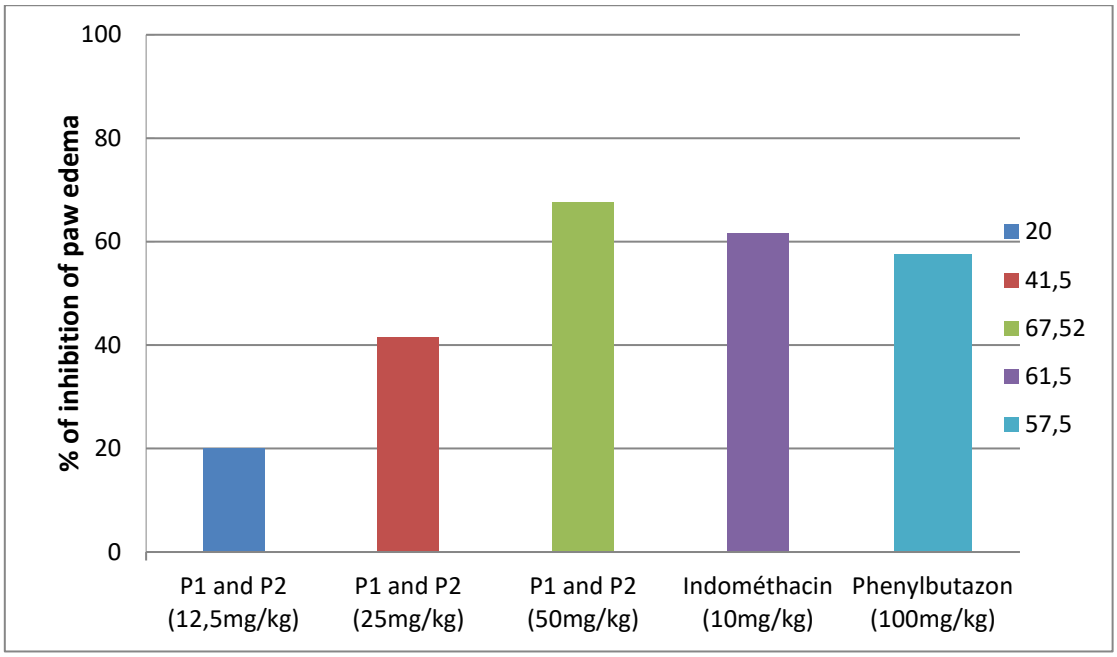


Figure 5. Anti-arthritic effect of Lupeol and 3β-hydroxy-urs-12-en-28-oic acid**, indomethacin, and phenylbutazone in mice with CFA-induced arthritis in the right hind paw, assessed 28 days after injection (mean ± SEM, n=6, *P<0.05).**

The compounds from *Macphersonia gracilis* inhibited paw swelling in a dose-dependent manner: 20.00% at 12.5 mg/kg, 41.50% at 50 mg/kg, and 61.50% at 100 mg/kg. In comparison, indomethacin (10 mg/kg) produced 61.8% inhibition, while phenylbutazone (100 mg/kg) reached 57.50%.[30-32].

IV. Conclusion

This study demonstrated that the hexane extract of *Macphersonia gracilis* exhibits strong anti-inflammatory effects. Bioassay-guided fractionation revealed that **Lupeol and 3β-hydroxy-urs-12-en-28-oic acid**, triterpenoid compounds, show significant activity in acute, granuloma, and chronic models, including cotton pellet- and Freund's adjuvant–induced arthritis. Further investigations are required to clarify the precise mechanisms underlying their anti-inflammatory action.

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