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PHENOLIC COMPOUNDS AND BIOACTIVITY OF LEAVES OF *MAYODENDRON IGNEUM* KURZ.

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Abstract

Four apigenin glycosides were isolated from the ethyl acetate extract of the leaves of *Mayodendron igneum* Fam.*Bignoniaceae*. They were identified as apigenin 7-O-glucoside; 6-methoxy apigenin-7-O-glucoside; 6-methoxy apigenin-7-O-rhamnoglucoside and 6-hydroxy apigenin-7-O-rhamnoglucoside. In addition an isoflavone glycoside was isolated, and identified as genistin 5,4\`-methyl ether. Ethanol (80%) extract of *Mayodendron igneum* leaves exhibited significant anti-inflammatory and analgesic activities. LD_{50} determination of the extract indicated the safety of the leaves of the plant.

Key words. *Mayodendron igneum*, Bignoniaceae, apigenin glycosides, anti- inflammatory activity.

Introduction

Mayodendron igneum Kurz (Bignoniaceae*)*, also known as *Radermarchera ignea* (Kurz), grows wildly in north Myanmar, north Thailand, Laos, Vietnam and south China. It is also planted as a vegetable, for medicinal uses and in home gardens of south Yunnan, as ornamental, Chun-Lin, (1990). Few reports have been published, Poser *et al*, (2000), about iridoids in Bignoniaceae being mainly C-4 carboxylated. Subramanian et al, (1972) examined the flavonoids of eight Bignoniaceous species comprising *Bignonia gracilis* and *B. megapotamica* Spreng which were found to contain quercetin-3-galactoside. Harborne, (1967), reported that sixteen representative species failed to reveal these rare plant pigments in the Bignoniaceae. Investigation of other flavonoids in leaves and petals of bignoniads showed that most species contained flavones rather than flavonols. The nectary structure and chemical nectar composition of 15 species of *Bignoniaceae* were analysed by Graletto (1995). The biological screening of fractions derived from leaves of some plants of *Bignoniaceae* as *Macfadyena unguis-cati* revealed antitumoral, antitrypanosomal and anti-inflammatory activities (Duarte et al, 2000). It is also used in folk medicine to treat snakebite (Houghton and Osibogun, 1993), dysentery, inflammation and rheumatism (PioCorrea, 1978). This work aims at investigating the constituents and biological activity of this plant.

Materials and methods

Plant material

The fresh leaves of *Mayodendron igneum* (Kurz), (Bignoniaceae) were collected from Giza Zoo of Egypt in June 2005. A voucher specimen of the plant (voucher number M.20) was identified by Mrs. Treeze Labib, Specialist in Plant Taxonomy,and kept in Orman Botanical Garden .

Isolation of flavonoids

500 g of air-dried powder of *Mayodendron igneum* was successively extracted in a Soxhlet apparatus using

petroleum ether, chloroform, ethyl acetate and ethanol 95%. Solvents were evaporated to dryness under vacuum at 40^{o}C yielding dark oily residues. The ethyl acetate extract was examined by paper chromatography (Whatmann No.1) using the solvent system n-butanol: acetic acid: water (3:1:1) (a) and 15% acetic acid (b).Chromatograms were visualized under UV before and after exposure to NH_3 and spraying with $AlCl_3$. R_f values and colours of the detected spots were recorded. Flavonoid compounds were isolated by preparative paper chromatography (PPC) on Whatmann 3MM, using solvent system (a), and purified by repeated PPC using solvent system (b). Final purification was performed on Sephadex LH-20 column and elution with methanol.

Apparatus

- ^{1}H-NMR Spectrophotometer: Jeol EX-270 NMR spectrometer.
- ^{13}C-NMR Spectrophotometer: Jeol EX-270, 75 MHz.
- Ultraviolet visible recording spectrophotometer, UV-VIS Double Beam, UVD-3500. Lambomed Inc.

Identification of compounds

The purified compounds were subjected to UV spectral analysis, ^{1}H-NMR and ^{13}C-NMR spectra were run for compounds I and III. The chromatographic and UV data of isolated compounds were compared with published data (Mabry et al., 1970 and Markham, 1982).

Compound I: obtained from the ethyl acetate extract, deep purple under UV, R_f = 0.75 and 0.39 (in solvent systems a & b, respectively) which changed to yellowish green on exposure to ammonia vapour.

UV $\lambda_{\max}^{methanol}$: 268,332;NaOMe:266,300sh, 388; $AlCl_3$: 274, 298, 347, 379; $AlCl_3$/HCl: 275, 297, 340, 377; NaOAc: 266, 390; NaOAc/H_3BO_3: 267, 287 sh, 339. ^{1}H-NMR (270 MHz, DMSO), 3.27-3.59 (6H,M, 6 protons of glucosyl); 5.29 (1H, d.,J=6.7 H-1glucosyl); 6.41(1H, d.,J=2.7 H-6); 6.82 (2H, J=2.7, H-3, H-8); 6.90-6.93 (2H, d., J=8.1, H-3`,H-5`); 7.90-7.93 (2H, d.,J= 8.1, H-2`, H-6`).

^{13}C-NMR (75 MHz, DMSO): 166 C-2, 103.4 C-3,181.8 C-4, 160.3 C-5, 97.1 C-6, 162.3 C-7, 95.4 C-8, 156.0 C-9, 102.2 C-10, 121.0 C-1`, 128.6 C-2`/6`, 117.1 C-3`/5`, 160.6 C-4`, 99.8 C-1``,73.4 C-2``, 77.0 C-3``, 69.3 C-4``, 77.7 C-5``, 61.2 C-6``.

Compound II: isolated from the ethyl acetate extract, $R_{f\,=}$ 0.36 and 0.31(in solvent systems a & b respectively), purple colour under UV changed to yellowish green on exposure to ammonia vapour.

UV $\lambda_{\max}^{methanol}$: 272, 331; NaOMe: 269, 388; $AlCl_3$: 279, 298 sh, 357; $AlCl_3$/HCl: 259, 280, 300 sh, 347; NaOAc: 241, 334; NaOAc/ H_3BO_3: 271, 335, 400. ^{1}H-NMR (270 MHz, DMSO), 3.20-3.51(6H, m, 7-O-glucosyl); 3.75 (3H, s, OCH_3-6); 5.45 (1H, d.,J=6.7, O-glucosyl , H-1``); 6.83 (1H, s, H-3); 6.89 (1H, s, H-8); 6.92 -6.95 (1H,d.,J=8.1, H-3`, H-5`); 7.92-7.95 (2H, d.,J=8.1, H-2`,H-6`).

Compound III: isolated from the ethyl acetate extract, R_f = 0.82 and 0.42(in solvent systems a & b respectively), purple colour under UV, changed to yellowish green on exposure to ammonia vapour.

UV $\lambda_{\max}^{methanol}$: 230 sh, 274, 333; NaOMe: 272, 302 sh, 384; $AlCl_3$: 232 sh, 281, 300, 360; $AlCl_3$/HCl: 280, 298 sh, 349; NaOAc: 271, 389; NaOAc/H_3 BO_3 :273, 337. ^{1}H-NMR (270 MHz, DMSO), 1.21 (3H, m, rhamnosyl, CH_3); 3.35-3.48 (10H,m, rhamnoglucosyl, 10 protons); 3.87 (3H, s, OCH_3 at position 6); 5.13 (1H, br. rhamnosyl, H-1```); 5.44 (1H, m, glucosyl, H-1``); 6.82 (1H, s, H-3), 6.85 (1H, s, H-8); 6.89-6.92 (2H, d.,J=8.1, H-3`, H-5`).;7.91-7.94 (2H, d.,J = 8.1, H-2`, H-6`).

^{13}C-NMR (75 MHz, DMSO): 164.5 C-2, 105.0 C-3, 182.0 C-4, 161.2 C-5, 132.5 C-6, 163.1 C-7, 95.0 C-8, 156.2 C-9, 102.5 C-10, 121.6 C-1`, 128.6 C-2`/6`, 116.3 C-3`/5`, 160.2 C-4`, 99.3 C-1``, 73.2 C-2``, 77.1 C-3``, 69.1 C-4``, 77.7 C-5``, 60.5 C-6``, 100.4 C-1```, 70.2 C-2```, 70.6 C-3```, 75.1 C-4```, 68.2 C-5```, 17.7 C-6```, 60.0 – OCH_3.

Compound IV: isolated from ethyl acetate extract**,** R_f =0.71 and 0.75(in solvent systems a & b respectively) , purple colour under UV,changed to yellowish green when exposed to amm. vapour.

UV $\lambda_{\max}^{methanol}$: 275, 332; NaOMe: 230 sh, 272, 300 sh, 387; $AlCl_3$: 230 sh, 281, 299, 361; $AlCl_3$/HCl, 279, 289 sh, 349; NaOAc: 272 , 336, 400; NaOAc/ H_3BO_3: 273, 338. ^{1}H-NMR (270 MHz, DMSO), 1.08-1.20 (3H, m, rhamnosyl, CH_3); 3.20-3.52 (10H, m, rhamnoglucosyl, 10 protons); 5.22 (1H, rhamnosyl, H-1```); 5.32 (1H, m, glucosyl, H-1``); 6.79 (1H, s, H-3); 6.86 (1H, s ,H-8); 6.98-7.10 (2H,d.,J= 8.1, H-3`,H-5`); 7.87-7.90 (2H, d.,J=8.1, H,2`, H-6`).

Compound V:isolated from the ethyl acetate extract at R_f = 0.71 and 0.67 (in solvent systems a & b respectively), yellow colour under UV and still yellow when exposed to ammonia vapour.

UV $\lambda_{\max}^{methanol}$: 274, 318; NaOMe: 271, 305 sh, 350 sh, 393; $AlCl_3$:230sh, 270, 300sh, 350; $AlCl_3$/HCl: 376, 339; NaOAc: 270, 285, 318, 335; NaOAc/H_3 BO_3: 266, 280, 320 sh, 332.

^{1}H-NMR (270 MHz, DMSO), 3.21-3.48 (6H, m, glucosyl); 3.62 (3H, s, OCH_3-4`); 3.76 (3H, s, $OCH_3$-5); 5.32 (1H,m, O-glucosyl, H-1); 6.21(1H, d.,J=2.9 H-6); 6.55 (1H, d.,J=2.9 H-8); 6.91 (2H, d.,J=7.9, H-3`, H-5`); 7.50 (2H, d.,J=7.9, H-2`, H-6`); 7.89 (1H, s, H-2).

Investigation of biological activity

Experimental animals: Adult male albino rats weighing 130-150 g. and albino mice of 25-30 g body weight, female albino rats of average body weight 100 g, were obtained from the animal house of National Research Centre, Egypt. They were kept under the same hygienic conditions and well balanced diet and water.
Normal diet: vitamin mixture 1%, mineral mixture 4%, corn oil 10%, sucrose 20%, cellulose 0.2%, casein (95% pure)10.5% and starch 54.3%. Ethical issue was followed as reported by Ney et al. (1988).

Drugs

Indomethacin; (Eipico, Egyptian Int. Pharmaceutical Industries Co., A.R.E.,), Carrageenan (Sigma Co)., Dipyron metamizol (Aventis , Cairo, under lisence of Aventis Frankfurt, Germany) and Paracetamol (Misr Co, Mataria, Cairo).
Doses of the drugs used were calculated according to Paget and Barnes, (1964) and were administered orally by gastric tube.

Determination of LD_{50}

Total ethanol extract of *Mayodendron igneum* 500 g of dried powder was percolated with 80% ethanol till exhaustion, filtered, evaporated under vacuum to yield a gummy residue. Applying the procedure of Miller and Tainter, (1944), albino mice (25-30g) were divided into groups each of 6 animals. Preliminary experiments were carried out to determine the minimal dose that kills all animals, (LD_{100}) and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in between these two doses; each dose was given orally to a group of six animals. The mice were then observed for 24 h. after administration and symptoms of toxicity and mortality rates in each group were recorded and LD_{50} was calculated.

Acute anti-inflammatory activity of 80% ethanol extract of *Mayodendron igneum*

The method described by Winter et al., (1962) was applied. Twenty-four male albino rats, (130-150 g) were divided into four groups, each of six animals: **Group 1:** received 1 mL of saline serving as control, **Group 2:** received orally a dose of 100 mg /kg of the extract, **Group 3:** received orally a dose of 150 mg /kg of the extract, **Group 4:** positive control received 20 mg /kg of the reference drug, indomethacin. One h after oral administration of the different doses, saline, plant extract and indomethacin, all the animals were given a sub-plantar injection of 0.1 mL of 1% carrageenan solution in saline in the right hind paw and 0.1 mL saline in the left hind paw. Four h. after administration, the rats were sacrificed; both paws were excised and weighed separately. The percentage oedema was calculated and compared with that of the control group.

Analgesic activity of 80% ethanol extract of *Mayodendron igneum*

It was evaluated according to the method of Charlier et al., (1961), by using an electric current as a noxious stimulus where electrical stimulation was applied to the rat's tail by means of 515 Master Shocker (Lafayette Inst. Co.) using alternative current of 50 cycles /sec. for 0.2 sec. Twenty-four male albino rats, weighing 130 -150 g. were divided into four groups, each of six animals:

1- First group: Negative control that received 1 mL saline.
2- Second group: Received 100 mg /kg b.wt. of plant extract.
3- Third group: Received 150 mg /kg b.wt. of plant extract
4- Fourth group: Positive control that received 50 mg / kg b.wt. of the reference drug, dipyron-metamizol.

The minimum voltage required for the animal to emit a cry was recorded for the four groups after one and two h. intervals.

Antipyretic activity of 80% ethanol extract of *Mayodendron igneum*

This effect was carried out following the method of Buch and Alexander (1960). Twenty-four female albino rats, of average body weight 100 g were divided into four groups of six animals each:

1- First group: negative control that received 1 mL saline.
2- Second group: Rats that received 100 mg /kg b.wt. of the plant extract.
3- Third group: Rats that received 150 mg /kg b.wt. of the plant extract.
4- Fourth group: positive control that received 20 mg/kg of the reference drug, paracetamol.

Normal vaginal temperature was recorded before the start of the experiment. Pyrexia was induced by intramuscular injection of 1 mg/100 g b.wt. of 44% yeast suspension. The site of injection was then massaged to spread the suspension beneath the skin. After 18 h., the vaginal temperature was recorded for all groups to serve as the baseline of elevated body temperature, with which the antipyretic effect will be compared. One and two h. later other records of vaginal temperature were determined.

Statistical analysis

The data obtained were statistically analysed using Student's "t" test (Sendecor and Cochran 1971).

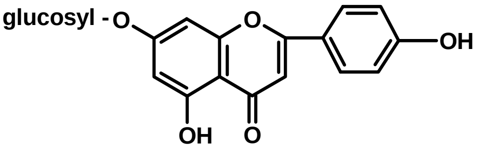
Results and Discussion

Characterization and Identification of Isolated Flavonoids .

From the ethyl acetate extract successive to petroleum ether and chloroform extraction of the dried leaves of *Mayodendron igneum*, five flavonoids were isolated. **Compounds I, II, III and IV** appeared purple when viewed on paper chromatography in UV light indicating a free 5- hydroxyl and the four compounds changed on exposure to ammonia vapour , indicating that the 4`- hydroxyls are unsubstituted. UV spectral analysis following standard procedures (Mabry et al., 1970) indicated that I, II, III and IV were based on an apigenin-like skeleton while V being an isoflavone. Sodium methoxide shift reagent indicated that the four compounds are substituted in position 7. In compounds I, II , III and IV, bathochromic shifts of band I about 45 to 65 nm were observed with increase in intensity after addition of NaOMe due to free 4` - OH .

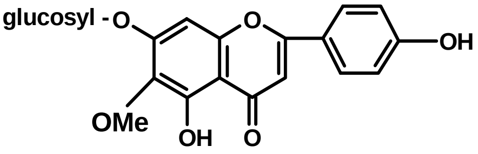
Compound I:

Bathochromic shift of band I from 332 to 377 (about 45 nm) after addition of $AlCl_3/HCl$ indicates 5-OH with no 6-oxygenation. Compound I afforded 7-O-glucosyl with H-1 of glucosyl at 5.29 as doublet with J=6.7, while the other six protons appeared as multiplet between 3.27-3.59. The doublet signal at 6.41 with J= 2.7 is ascribable to H-6 (due to meta coupling with H-8) and the signal at 6.82 is attributed to H-8 and H-3. The two equivalent protons at δ 6.90 -6.93 ppm (H-3`/5`) and 7.90-7.93 ppm (H-2`/6`) with J = 8.1 indicating 1, 4-disubstituted β-ring. ^{13}C-NMR of this compound confirmed the presence of 7-O, glucosyl, and the signals at 77.0 for C-3`` and 77.7 for C-5`` proved a glucose moiety. Compound I was identified as apigenin 7-O-glucoside.



I. Apigenin 7-O-glucoside.

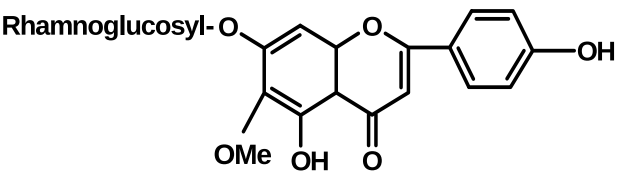
Compound II: exhibited a low bathochromic shift of band I after addition of $AlCl_3/HCl$ (16 nm, relative to methanol), indicating 5-OH with 6-oxygenation.The singlet signal at δ 3.75 ppm characteristic for $–OCH_3$, and absence of proton at position 6, indicated that compound II is 6-methoxy apigenin 7-O-glucoside.



II. 6-methoxyapigenen 7-O-glucoside.

Compound III:

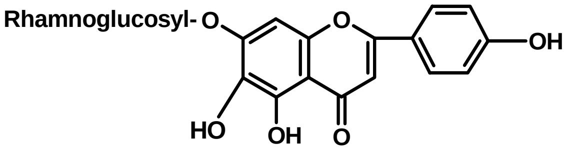
The purple colour which is changed on exposure to ammonia vapour to yellowish green indicated free hydroxyls at 5 and 4`. The chromatographic properties of compound III indicated 6-methoxy apigenin, 7-O-glycoside. UV spectrum in methanol gave the two characteristic bands of apigenin like-structure (band I and II at 333 and 274, respectively). Absence of bathochromic shift in band II on addition of NaOAc referred to substitution at 7-OH. Bathochromic shift of band I after addition of $AlCl_3$ /HCl from 333 to 349 (16 nm) was less than 44 nm, indicating 5-OH with 6-oxygenation. The signal at δ 3.87 ppm was attributed to 6-methoxy. Three protons of rhamnosyl (CH_3) appeared at 1.21 as multiplet and H-1``` as br. signal at 5.13 which is specific for rhamnosyl moiety. The appearance of H-1`` at 5.44 as multiplet confirmed the glycosidation at OH-7. ^{13}C-NMR of this compound indicates the presence of glucosyl and rhamnosyl moieties at position 7, the methoxy group being confirmed by the signal at 60.0 (not terminal). The downfield of C-6 from δ 97.0 to 132.5 ppm indicates the substitution of proton 6 by OMe. Compound III was accordingly identified as 6-methoxy apigenin 7-O-rhamnoglucosyl.



III. 6-Methoxyapigenin-7-O-rhamnoglucoside.

Compound IV:

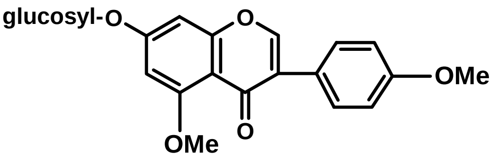
The bathochromic shift of band I, on addition of $AlCl_3$/HCl from 332 to 349 (relative to methanol), less than 44 nm indicates 5-OH with 6-oxygenation. ^{1}H-NMR spectrum exhibited AA`XX` spin coupling system of two equivalent protons at δ 6.98-7.10 ppm (H-3`/5`) and δ 7.87-7.90 ppm (H-2`/6`) indicated 1,4-disubstituted β-ring. The terminal rhamnosyl moiety on OH-2`` was confirmed by the characteristic position of H-1``` as single signal at 5.22, while the multiplet of glucosyl H-1``being at 5.32 ppm. It was identified as 6-hydroxy-apigenin 7-O- rhamnoglucosyl,



IV. 6-Hydroxyapigenin-7-O-rhamnoglucoside
IV. 6-hydroxy, apigenin-7-O-neohesperidoside

Compound V:

The R_f values of this compound, with yellow colour under UV, unchanged on exposure to ammonia vapour indicated that hydroxyls at positions 5 and 4` are substituted. UV spectral analysis indicated an isoflavone. Sodium methoxide and sodium acetate shift reagents showed substituents on the 7-and 4`-hydroxyls, by addition of NaOMe no shift in band II (274 and 271) due to the absence of –OH in A-ring. Bathochromic shift of band I from 318 to 393 with decrease in intensity indicats absence of free 4`-OH. The presence of two singlet signals at δ 3.62 ppm (terminal methoxy) and 3.76 are attributable to two methoxy groups at 4` and 5. The presence of H-6 and H-8 as doublets at 6.21 and 6.55 with J= 2.9 and singlet signal at δ 7.89 ppm is ascribable to H-2, suggesting that compound V is genistin 5,4`-methyl ether.



V-Genistin 5,4`- methyl ether.

LD_{50} of 80% ethanol extract of *Mayodendron igneum*

Study of LD_{50} of 80% ethanol extract of *Mayodendron igneum* proved that oral administration of 6.7 g/kg b.wt. kills 50% of the tested animals, indicating that this plant is relatively non toxic and explains its use as vegetable in homegardens of south Yunnan.

Acute anti-inflammatory effect of 80% ethanol extract of *Mayodendron igneum*

The results of the acute anti-inflammatory test revealed that a significant effect was exhibited by oral administration of 100 and 150 mg/kg b.wt. of the aqueous ethanol extract of *Mayodendron igneum*. They significantly decreased weight of the rat paw oedema induced by carrageenan to a level nearly similar to that of the positive control used (indomethacin), being 91.29% and 98.08% of that of indomethacin (Table 1).

Table(1): Acute anti-inflammatory effect of 80% ethanol extract of *Mayodendron igneum*.

Group	**Dose in mg/kg b.wt.**	**% Oedema**		
		Mean ± S.E.	**% of change**	**Potency**
1- Control	1 mL saline	61.8 ± 1.4		
2- 80% Alcohol extract of *M.igneum*	100	25.2 ± 0.8	*59.22	91.29
3- 80% Alcohol extract of *M.igneum*	150	22.6 ± 0.7	* 63.43	98.08
4- Indomethacin	20	21.7 ± 0.9	*64.67	100

* P< 0.01 vs control group.
% of change calculated as regard the control group.

Analgesic activity of 80% ethanol extract of *Mayodendron igneum*

In Table 2, it can be concluded that the highest analgesic activity produced from the aqueous alcohol extract of *Mayodendron igneum* was after two hrs. with concentration of 150 mg/kgm b.wt and its potency reached *ca*. 100.24% of that of the drug used in the test (dipyron – metamizol 100%).

Table (2): Analgesic activity of 80% ethanol extract of *Mayodendron igneum.*

Group	**Dose in mg/kg b.wt.**	**Volts needed Before treatment**	**Volts needed after single oral dose**					
			One hour			**Two hours**		
			***Mean±S.E.**	**% of change**	**Potency**	***Mean±S.E.**	**% of change**	**Potency**
1- Control	1 mL Saline	70.5 ± 1.9	72.1±2.3	2.27	--	71.9±1.4	1.99	
2- 80% ethanol extract of the plant	100	73.6 ± 2.8	129.5 ± 4.5	*75.95	68.29	145.1±5.3	*97.15	87.64
3- 80% ethanol extract of the plant	150	76.8 ± 1.4	140.3 ± 2.9	* 82.68	74.34	171.9 ± 7.5	*123.83	100.24
4- Dipyron metamizol	50	74.8 ± 2.4	158.7± 6.1	*111.22	100	167.2±5.8	*123.53	100.00

* Significantly different from zero time at P<0.01% of change calculated with reference to zero time.

Antipyretic activity of 80% ethanol extract of *Mayodendron igneum*

Results in Table 3, revealed that 80% ethanol extract of leaves of *Mayodendron igneum* showed a moderate antipyretic activity, being 57.54 and 75.32% with concentration 100 and 150 mg/kg b.wt. relative to paracetamol used as the positive control.

Table(3): Antipyretic activity of 80% ethanol extract of leaves of *Mayodendron igneum.*

Group	**Dose in mg/kg b.wt.**	**Induced rise in temperature**	**Body temperature change**					
			One hour			**Two hours**		
			Mean±S.E.	**% of change**	**Potency**	***Mean±S.E.**	**% of change**	**Potency**
1- Control	1 mL saline	38.9 ± 0.1	39.1±0.1	-	-	39.4±0.2	-	-
2- 80% ethanol extract of the plant	100	39.2 ± 0.3	38.4 ± 0.2	2.04	36.62	37.6±0.1	*4.08	57.54
3- 80% ethanol extract of the plant	150	39.3 ± 0.4	38.1 ± 0.3	3.05	57.76	37.2 ± 0.1	5.34	75.32
4- Paracetamol	20	39.5 ± 0.2	37.3± 0.1	5.57	100	36.7±0.2	7.09	100

*P<0.01 corresponding induced rise in temperature of the tested groups.

From Tables 1, 2 and 3 , it could be concluded that 80% ethanol extract of *Mayodendron igneum* leaves, showed very low toxicity, high potencies as anti-inflammatory and analgesic and moderate potency as antipyretic agent. It significantly inhibited the rat paw oedema weight induced by carrageenan ($p < 0.01$). The anti-inflammatory activity may possibly be attributed to the flavonoid, the triterpenoid content, β-sitosterol and/or β-sitosterol– glucoside. *Macfadyena unguis-cati* has been reported as anti-inflammatory by Duarte et al., (2000), being used in folk medicine to treat inflammation and rheumatism (PioCorrea, 1978).

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