ANTI-INFLAMMATORY ACTIVITY OF FLAVONOID COMPOUND ISOLATED FROM *GMELINA ARBOREA* FRUITS EXTRACT

**Bhabani Shankar Nayak**¹, **P. Ellaiah**¹, **Subas Chandra Dinda**², **Mansi Khadanga**, **Haragouri Mishra**, **Sarala Nayak**

¹Department of Pharmaceutical Technology, Jeypore College of Pharmacy, Rondapalli, Jeypore, Koraput, Odisha, India.
²College of Health Sciences, Mekelle University, Mekelle, Ethiopia

**ABSTRACT**

Background: The plant *Gmelina arborea* has been traditionally used in India for several medicinal purposes like anthelmintic, diuretic, antibacterial, hepatoprotective, anti-inflammatory, antioxidant and antidiabetic. It contains phytoconstituents like alkaloids, carbohydrates, anthraquinone glycosides, gums, mucilages, tannins, phenolic compounds and flavonoids. Aims: The objective of present study is to isolate a compound from ethanolic extract of *G. arborea* and to explore the anti-inflammatory activity of isolated compound.

Material and methods: The isolation of compound was done by column and thin layer chromatographic methods. The isolated compound was characterized to elucidate its structure by spectroscopic methods like ultraviolet, infrared, nuclear magnetic resonance and mass spectroscopy. The anti-inflammatory activity of phytoconstituent was evaluated by Carrageenan-induced rat paw edema test method using Wistar rats as animal model. Statistical analysis used: All data are verified for statistically significant by using one way ANOVA at 5 % level of significance (p < 0.05). Results: A flavonoidal compound was isolated as yellow colour crystal with melting point 177±1 °C with molecular formula C₁₆H₁₅O₅ and IUPAC name 5,7-dihydroxy-4-methoxy flavone. The compound was able to show good anti-inflammatory activity in comparison with standard drug Diclofenac sodium. Conclusion: It could be concluded that the isolated compound is a flavonoid and it possess anti-inflammatory activity.

**Key words** - *Gmelina arborea*, Flavonoid, Spectroscopy, Carrageenan, Inflammation

**INTRODUCTION**

*Gmelina arborea* Roxb (Family Verbenaceae) fruits are oval in shape, ¾ inches in length and are yellow in color. The fruits are sweet in taste and sometimes astringent [1,2]. The plant, *G. arborea* was reported to have several medicinal properties such as aphrodisiac, astringent, analgesic, antipyretic, antidiabetic, diuretic, anti-inflammatory and tonic characteristics [3]. The literature survey reveals that fruits of *G. arborea* contain cardiac glycosides, flavonoids and steroids. The ethanol extract contains alkaloids, carbohydrates, anthraquinone glycosides, gums, mucilages, tannins, phenolic compounds and flavonoids [4]. Inflammation is a local response of living mammalian tissues to the injury, which is a body defense reaction in order to eliminate or
limit the spread of injurious agents. However, inflammation that is unchecked leads to chronic inflammatory disorders. Oedema formation, leukocyte infiltration and granuloma formation represent components of inflammation [5,6]. The present study was aimed to isolate the chemical compound and to evaluate anti-inflammatory activity of isolated compound.

MATERIALS AND METHODS

Chemicals used
Diclofenac sodium was procured as a gift sample from JB Chemicals, Kolkata, West Bengal. The solvents, ethanol AR and ethyl acetate AR 60-80°C (Emsure® ACS) were procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. Other solvents, ethyl acetate, chloroform, methanol and petroleum ether AR 40-60°C were procured from Loba Chemie Pvt. Ltd., Mumbai, India. Silica gel G (Qualigen) and Carrageenan was procured from Fisher Scientific, Mumbai, India.

Instruments used
The ultraviolet spectrophotometer (UV-1700 Schimadzu, Japan), infrared (8400S Schimadzu IR spectrophotometer, Japan), nuclear magnetic resonance (Bruker AM-400 NMR) and mass spectrometer (Bruker APEXIII, APEX Technologies Inc.) were procured from Shimazu Pvt. Ltd., Japan.

Collection of plant materials, identification and size reduction
The fruits of *G. arborea* were collected from local area of Koraput district (India) and was authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M.S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Odisha [Letter no. MJ/DBT (15)/1067, dated 12.04.2015]. The fruits were shade dried and were pulverized to form coarse powder by using electrical grinder and stored in a closed air tight container for further use.

Preparation of solvent extracts
The coarse powder form of dried fruits was extracted by Soxhlation method by using ethanol as solvent. A total amount of 1500 g coarse powdered fruits was extracted with 1200 ml of each solvent. The crude extract was evaporated to dryness in a in a rotary flash evaporator, with the percentage yield being 2.2 %. Crude extract was kept in closed air tight containers under cool and dark place for further study.

Isolation of phytoconstituents
About 25 g of sample (Ethanol extract) was weighed and dissolved in 100 ml of chloroform for 3 h by continuous stirring. The suspension was filtered by using Whatmann filter paper no. 1 and the supernatant liquid thus collected in the beaker was concentrated by evaporating the solvent by heating at 60 °C for 10 min. The concentrated extract was again redissolved in chloroform, loaded at the top of the column and kept for 3 h. Mobile phases used were: Ethyl acetate - n-hexane (25:75), ethyl acetate - n-hexane (50:50), ethyl acetate - n-hexane (25:75), ethyl acetate (100 %), methanol - ethyl acetate (5:95), methanol - ethyl acetate (10:90), methanol - ethyl acetate (20:80), methanol - ethyl acetate (50:50) and methanol (100 %). About 25 ml fraction was collected at each time. The elutes were collected in test tubes and each fraction was subjected to TLC study [7-9].
Thin Layer Chromatography study
The slurry of adsorbent that is, Silica gel-G (Gypsum i.e. Calcium Sulphate) as stationary phase was transferred to various TLC plates by spreading method. The prepared TLC plates were air dried at room temperature for 30 min. The prepared TLC plates were activated by heating the plates at 105°C for 1 h in a hot air oven (ACM-22066-1, ACMAS Technocracy (Pvt.) Ltd., New Delhi). The solvent systems used for development of chromatogram in TLC plates were chloroform and ethyl acetate in the ratio of 5:95. The chromatograms were detected by spraying the above reagents. The resultant bands were also visualized by charring with a reagent containing methanol and concentrated sulphuric acid (85:15). The Rf values were recorded. The column with the solvent system methanol and ethyl acetate (5: 95) gave least number of fractions. The larger band was collected as eluent and subjected to for column chromatography once again, using chloroform and ethyl acetate in ratio of 5: 95. The column and thin layer chromatography studies were continued until the purification of compounds achieved, which was ensured by obtaining single spot on TLC plate.

Identification of isolated phytoconstituents

Physical and chemical evaluations
The properties like appearance, color, taste, odor, solubility and melting point of the isolated constituents was determined. The isolated constituent was dissolved in ethanol and evaluated chemically for detection of flavonoid by using Shinoda, zinc hydrochloride reduction and alkaline reagent test [8,9].

Structural elucidation of isolated phytoconstituent
The compound was dissolved in methanol and ultra violet absorption spectrum was determined by using UV spectrophotometer by scanning in the range of 200 to 800 nm using methanol as blank. The $\lambda_{\text{max}}$ was determined from UV spectral analysis by considering that the corresponding wavelength at which maximum absorbance took place [10,11]. The FT-IR was used for IR analysis in the frequency range between 4000 and 600 cm$^{-1}$ and at 1 cm$^{-1}$ resolution. The sample of pure isolated compound was prepared by palletization technique in KBr using IR press. The IR peaks of the sample were analyzed and interpreted to elucidate the structure of isolated compound [12]. Nuclear magnetic resonance spectra (H$^1$ and C$^{13}$) were recorded at 400 MHz for H$^1$, 100 MHz for C$^{13}$ – nuclei respectively [13,14]. Electron impact (EI) mass spectra were recorded coupled with PDP 11/34 computer system. High resolution spectrometry (HR-MS) and field desorption mass spectrometry (FD-MS) were also performed on ApexIII mass spectrometer [15,16].

Evaluation of anti-inflammatory activity of isolated compound

Drugs
The extracts of G. arborea were tested in single dose in each group of experimental animals (300 mg/Kg b.w.). Diclofenac sodium was used as the standard drug in Wister rat model at a dose of 5 mg/Kg b.w.

Animals
Healthy Wistar rats of either sex, weighing 180–250 g were used in the present study. They were housed in standard conditions of temperature (25±2 °C), relative humidity of 45-55 % in animal
house of Gayatri College of Pharmacy, Sambalpur, Odisha. They were fed with a standard pellet diet and water *ad libitum*. All the animals were carefully monitored and maintained in accordance with CPCSEA guidelines on control and supervision of experimental animals for 15 days. Animals were caged in polypropylene cages and all operations on animals were done under aseptic condition.

**Experimental protocol**

Animals were selected, weighed and divided into six groups (n = 3), namely control, standard drug and four groups belonging to four different extracts of *G. arborea* at a single dose. Approval for the research work and ethical clearance was obtained from the Gayatri College of Pharmacy, Gayatri Vihar, Jamadarpali, Sambalpur, Odisha (Ethical Committee No. 1339/ac/10/CPCSEA).

**Experimental method (Carrageenan induced rat paw edema)**

Anti-inflammatory activity of acacetin was evaluated by Carrageenan-induced rat paw edema method [17,18]. Twelve rats were divided into four groups of 3 rats each for various treatments. The first group (I) served as normal control (Vehicle) which received normal saline water (2 ml/Kg b.w.) only. The second group (II) served as standard control which received Diclofenac sodium (5 mg/Kg b.w.). Groups (III) and (IV) received acacetin at doses of 5 and 10 mg/Kg b.w respectively. The inflammation inducing agent carrageenan, standard and test drugs were administered in solution form using normal saline water as vehicle. In this study, initially animals are treated with drugs (control, standard and four extracts) as per the groups mentioned above. The standard and test drugs were administered intraperitoneally. Subsequently 30 min after above treatment, 0.05 ml of 1% solution of carrageenan was injected subcutaneously into the planter region of right hind paw to induce oedema. The edema was expressed as the increment in paw thickness due to carrageenan administration. The paw volume was measured initially and at 2, 3, 4 and 6 h after carrageenan injection using Plethysmometer (Ugo Basile, Italy) [19,20]. The percentage inhibition of paw thickness was calculated using the following formula,

\[
\text{Inhibition of paw thickness (\%)} = \left[ 1 - \left( \frac{V_t}{V_c} \right) \right] \times 100
\]  

Where, \(V_t\) is the mean relative change in paw volume in test groups and \(V_c\) is the mean relative change in paw volume in the control group. The reduction in the paw volume was compared to the vehicle-treated control animals and it was considered as anti-inflammatory response [21]. For determining the statistical significance, mean, standard deviation and standard error mean was used. All observations were done three times and data are represented as mean, standard deviation and standard error mean. All data were analyzed for statistical significance by using one way ANOVA at 5% level of significance (p < 0.05). The significance between two proportions was also tested by z-Test in comparison to standard drug, Diclofenac sodium.

**RESULTS AND DISCUSSIONS**

All phytochemical tests for flavonoid such as shinoda (Magnesium turning), ferric chloride, lead acetate, zinc hydrochloric acid reduction, sodium hydroxide and sulphuric acid were found to be positive. The appearance of the compound was crystalline powder and yield was 2.4%. The color
of the compound was yellowish white. It is soluble in methanol, chloroform and n-hexane. The melting point of compound was 176 to 178 °C and optical rotation was $^{30}\alpha_n = -32.5^\circ$ (C = 0.84 in methanol).

The infrared spectroscopy study (Fig 1) of isolated compound reveals that the major peaks were obtained at wave numbers ($\nu$) of 3405.57, 3211.76, 2954.96, 2834.58, 2722.24, 1594.95, 1497.82, 1348.62, 1302.16, 1252.69, 1168.52, 1090, 1020.49, 831.94, 721.76 and 559.15 cm$^{-1}$ respectively. It signifies that compound has hydroxy (OH), methoxy (OCH$_3$), keto groups etc. The compound presented the characteristic intensities of C=O absorption band at $\nu$ of 1594.95 cm$^{-1}$ and the OH stretching band at $\nu$ of 3405.57 cm$^{-1}$. The $\nu$ of 1497.82 and 1348.62 cm$^{-1}$ shows the absorption band of C=C and C-OH. The wave number of frequency 1252.69 cm$^{-1}$ shows the stretching band for C-O-C. The other wave number frequencies show characteristic bands for C-C and C=C.

![Infrared spectral data of isolated compound in the frequency range between 4000 and 600 cm$^{-1}$](image)

$^1$H NMR and $^{13}$C NMR (DMSO, 400 Hz) is shown in Fig 2 and 3. $^1$H NMR (DMSO, 400 Hz): $\delta$ 2.514 (1H,ddJ=12.4.2.4Hz, H-2), 2.676 (1H,ddJ=16.8.2.4Hz, H-3a), 3.247 (1H,ddJ =17.0.4.4Hz, H-3b), 5.895 (1H,dJ=8.2Hz, H-6), 5.895 (1H,dJ =2.8Hz, H-8), 7.445 (1H,dJ =8.8Hz, H-2'), 6.981 (1H,dJ =8.4Hz, H-3'), 6.981 (1H,dJ =8.4Hz, H-5'), 7.445 (1H,dJ =8.8Hz, H-6'), 12.141(1H,brs, OH-5), 10.801 (1H,brs,OH-7) and 3.776 (3H,s, OMe-4'). $^1$H NMR spectra showed the presence of thee protons attached to C-4' bearing methoxy group appeared at $\delta$ 55.16. The proton attached to C-5 and C-7 carbon bearing hydroxyl groups appeared at $\delta$ 12.141 and 10.801 as multiplet. The $^1$H NMR signals at 12.141 ppm should be attributed to hydroxyl protons of isolated constituent, which participated in a strong intramolecular hydrogen bond, between the hydrogen atom of the hydroxyl group OH (C-5) and the oxygen atom of the carbonyl group CO (C-4).
$^{13}$C NMR (DMSO, 400 Hz): $\delta$ 78.14 (CH-2), 41.99 (CH$_2$-3a), 196.10 (C-4), 163.47 (C-5), 95.85 (CH-6), 166.66 (C-7), 95.00 (CH-8), 130.62 (C-1’), 128.15 (CH-2’), 113.88 (CH-3’), 159.45 (C-4’), 113.88 (CH-5’), 128.15 (CH-6’), 101.78 (C-4a), 162.80 (C-8a), 55.16 (CH$_3$-OMe-4’). $^{13}$C
NMR showed the presence of one quartet, one triplet, seven doublets and seven singlets corresponds to one methyl, one methylene, seven methine groups and seven tertiary carbon atoms. The spectrum of isolated constituent showed product on aromatic groups ranging from 6 to 8 ppm and a strong intramolecular hydrogen bonding at 12.141 ppm.

From the mass spectroscopy study (Fig 4), the result showed a peak at 286 (molecular peak ion - m/z value) which corresponds to molecular weight of isolated compound was found to be 286 (M+) with molecular formula of C_{16}H_{15}O_{5}.

The results of anti-inflammatory activity of acacetin are shown in Table 1. Both the doses (5 and 10 mg/Kg b.w.) of acacetin showed anti-inflammatory activities (Fig 6). The anti-inflammatory effects of acacetin are well comparable with the standard drug, Diclofenac sodium. When the dose of the acacetin is increased, a gradual increase in anti-inflammatory activity was observed. Acacetin at a dose of 10 mg/Kg b.w. exhibited greater anti-inflammatory activities than the
standard drug, Diclofenac sodium. By employing one-way ANOVA, all data were found to be statistically significant ($F$ value $< F$ crit) at 5 % level of significant ($p < 0.05$ that is $p = 0.04887284$) followed by $z$-Test.

![Fig 6: Comparative study on anti-inflammatory activities of acacetin](image)

Group II - Standard (Diclofenac sodium - 5 mg/Kg b.w.), groups III and IV - Acacetin (At 5 and 10 mg/Kg b.w.) respectively.

**Table 1: Anti-inflammatory activity of acacetin in Wister rats by carrageenan induced rat paw edema method**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg</th>
<th>Carrageenin induced rat paw edema volume in mL (% inhibition) (X±S.D.)</th>
<th>0 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>6 h</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>2 ml/kg</td>
<td>1.75±0.67</td>
<td>1.86±0.92</td>
<td>1.89±0.48</td>
<td>1.79±0.88</td>
<td>1.75±0.56</td>
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<tr>
<td>II</td>
<td>5</td>
<td>1.84±0.97</td>
<td>1.44±1.06 (22.6)</td>
<td>1.25±1.11 (33.9)</td>
<td>1.10±0.76 (38.5)</td>
<td>1.05±1.03 (40.2)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>1.79±1.03</td>
<td>1.48±0.97 (20.43)*</td>
<td>1.29±0.81 (31.75)*</td>
<td>1.18±0.99 (34.08)*</td>
<td>1.11±1.14 (36.57)*</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>10</td>
<td>1.82±0.93</td>
<td>1.36±0.77 (26.88)*</td>
<td>1.14±0.82 (39.68)*</td>
<td>1.06±0.69 (40.78)*</td>
<td>1.01±0.97 (42.28)*</td>
<td></td>
</tr>
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ANOVA

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<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.83787</td>
<td>4</td>
<td>0.20947</td>
<td>2.593597</td>
<td>0.04887284</td>
<td>3.05557</td>
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<tr>
<td>Within Groups</td>
<td>1.21145</td>
<td>15</td>
<td>0.08076</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.04932</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Each value represents as mean ± standard deviation (n = 3). Standard error of mean < 0.658. Group I – Control (Normal saline water), group II - Standard (Diclofenac sodium - 5 mg/Kg b.w.), groups III and IV – Acacetin (At 5 and 10 mg/Kg b.w.) respectively. *$P<0.05$ (Test of significance between two proportions by $z$-Test) in comparison to standard. Data are found to be significant ($F$ value $< F$ crit) by testing through one way ANOVA at 5 % level of significance ($p < 0.05$ that is $p = 0.04887284$).

**CONCLUSION**

It could be concluded that the isolated compound is a derivative of flavonoid with a chemical structure “5, 7-dihydroxy-4 methoxy flavone” and it possess anti-inflammatory activity. The isolated flavonoid, acacetin is having reasonably good anti-inflammatory activity.
Acknowledgement
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REFERENCES


