**In vitro Antiplatelet Activity-Guided Fractionation of Aerial Parts of Melothria maderaspatana**

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**Melothria maderaspatana** (Linn) Cogn, a plant drug of Siddha medicine, is an annual monoeious tendril climber, belonging to the family Cucurbitaceae, mostly prevalent in South India. It is commonly called *Musumusukkai* in Tamil. Preliminary phytochemical screening of the plant revealed the presence of phytochemical constituents such as coumarins, flavonoids. Hence an attempt has been made to screen the effect of *Melothria maderaspatana* in platelet aggregation. Successive extracts of aerial parts of *Melothria maderaspatana* were used in increasing polarity of solvents (i.e., hexane, chloroform, ethyl acetate and methanol). The antiplatelet activity of different fractions of *Melothria maderaspatana* was studied using platelet-rich plasma in presence of ADP. Each extract was tested in concentrations of 100 µg/ml, 200 µg/ml, 400 µg/ml and 500 µg/ml for the study. The ethyl acetate extract showed a dose-dependent antiplatelet activity. Hexane and methanol extracts showed antiplatelet activity only at 200 µg/ml and 400 µg/ml concentrations. Chloroform extract showed negligible antiplatelet activity. However, the inhibition of platelet aggregation was only 50% when compared to the standard Aspirin.

Blood platelets are involved in haemostasis. The normal haemostatic system limits blood loss by precisely regulated interactions between components of vessel wall, circulating blood platelets and plasma proteins. Platelets can adhere to the walls of the blood vessels, release bioactive compounds and aggregate to each other. These properties increase to a well established level in conditions of arterial thrombosis and atherogenesis. Several agonists such as ADP, thrombin, collagen and serotonin induce the release of arachidonic acid, after phospholipase activation through calcium mobilization. Several drugs have been developed to block the different steps in platelet activation pathways. Inhibition of platelet function by Aspirin has been very well established.

One of the most potent mechanisms by which flavonoids appear to inhibit platelet aggregation is by mediating increase in platelets’ cyclic AMP levels by either stimulation of adenylate cyclase or inhibition of cAMP phosphodiesterase activity. Therefore the experiments in this study were designed to evaluate the antiplatelet activity of various fractions of *Melothria maderaspatana* and try to elucidate the inhibitory mechanism of coumarins or flavonoids on platelet aggregation.

The aerial parts of *Melothria maderaspatana* were collected from the herbal garden of Sri Ramachandra Medical College and Research Institute, Chennai, and were cleaned, air dried and powdered. The powdered aerial parts were subjected to exhaustive maceration with hexane, chloroform, ethyl acetate and methanol. The filtrates were collected, distilled and concentrated to get pure and dry extracts. The various fractions of the successive extracts were weighed and preliminary phytochemical screening was performed. The result is shown in Table 1.

![Table 1](image-url)

Various fractions of *Melothria maderaspatana* in different concentrations in DMSO were used. ADP, platelet-rich plasma and Tyrode buffer were used. Tyrode buffer was prepared using sodium chloride 149 mM, potassium chloride 2.6 mM, sodium bicarbonate 9.5 mM, glucose 5.5 mM, sodium dihydrogen phosphate 0.5 mM, magnesium chloride 0.6 mM and gelatin 0.25%.

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TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Hexane</th>
<th>Chloroform</th>
<th>Ethyl Acetate</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonones</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reducing Sugars</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolics</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
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</tr>
</tbody>
</table>

The platelet-rich plasma 0.13 × 10^7 for each assay was resuspended in Tyrode buffer (pH adjusted to 7.4 with 0.25 M HCl). Aggregation of the platelets was induced by ADP at a final concentration of 5 µM. Platelet aggregation was recorded by increasing transmittance value of spectrophotometric measurements. To determine the in vitro antiplatelet aggregation property, 100 µl of the various fractions of the plant, in different concentrations (100 µg/ml, 200 µg/ml, 400 µg/ml, 500 µg/ml), respectively was added to the platelet suspension at different concentrations for 1 min exposure at 37° before treatment with platelet aggregating agents. Aspirin at 100 µg/ml was used as standard. The results are depicted in figs. 1, 2, 3 and 4.

The hexane and methanol fractions showed maximum antiplatelet activity at 400 µg/ml concentration, whereas ethyl acetate fraction showed a dose-dependent antiplatelet activity. However, the prevention of platelet aggregation was lesser when compared to standard aspirin at 100 µg/ml. Chloroform fraction did not show protection against platelet aggregation. The coumarins and flavonoids present in ethyl acetate extract and flavonoids in hexane extract might have prevented the adhesion and aggregation of platelets, besides release of

Fig. 1: Antiplatelet activity of hexane extract
(-○-) shows antiplatelet activity of hexane extract at 100 µg/ml,
(-□-) shows antiplatelet activity of hexane extract at 200 µg/ml,
(-△-) shows antiplatelet activity of hexane extract at 400 µg/ml,
(-×-) shows antiplatelet activity of hexane extract at 500 µg/ml,
(-●-) shows antiplatelet activity of Aspirin at 100 µg/ml and
(-●●-) shows complete aggregation of platelets by ADP

Fig. 2: Antiplatelet activity of chloroform extract
(-○-) shows antiplatelet activity of chloroform extract at 100 µg/ml,
(-□-) shows antiplatelet activity of chloroform extract at 200 µg/ml,
(-△-) shows antiplatelet activity of chloroform extract at 400 µg/ml,
(-×-) shows antiplatelet activity of chloroform extract at 500 µg/ml,
(-●-) shows antiplatelet activity of Aspirin at 100 µg/ml and
(-●●-) shows complete aggregation of platelets by ADP

Fig. 3: Antiplatelet activity of ethyl acetate extract
(-○-) shows antiplatelet activity of ethyl acetate extract at 100 µg/ml,
(-□-) shows antiplatelet activity of ethyl acetate extract at 200 µg/ml,
(-△-) shows antiplatelet activity of ethyl acetate extract at 400 µg/ml,
(-×-) shows antiplatelet activity of ethyl acetate extract at 500 µg/ml,
(-●-) shows antiplatelet activity of Aspirin at 100 µg/ml and
(-●●-) shows complete aggregation of platelets by ADP
Simultaneous Estimation of Valdecoxib and Tizanidine Hydrochloride in Tablets by RP-HPLC

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A simple, fast, precise and accurate RP-HPLC method was developed for the simultaneous estimation of valdecoxib and tizanidine hydrochloride in tablet formulations. The separation was achieved by C18 Intersil column and acetonitrile: 0.02 M phosphate buffer (pH 3.5) (60:40 v/v) as mobile phase, at a flow rate of 1.5 ml/min. Detection was carried out at 240 nm. The retention time of valdecoxib and tizanidine hydrochloride was found to be 4.21 and 2.16 min respectively. The validation of the proposed method was also carried out for linearity, accuracy and precision. The linear dynamic range for valdecoxib and tizanidine hydrochloride was 0-100 µg/ml and 0-20 µg/ml respectively. The mean percentage recoveries obtained for valdecoxib and tizanidine hydrochloride were 99.10 and 100.19% respectively. The developed method was found to be accurate, precise, selective and rapid, and it can also be used for routine quality control analysis of these drugs in combination tablets.

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