Pharmacological investigation of prunin-6"-O-p-coumarate: A flavonoid glycoside

Sir,

Flavonoids possess a wide variety of biological activities and in recent times this group of natural products has gained much interest as bioactive compounds. Prunin-6"-O-p-coumarate a flavonoid glycoside was isolated first from the nut shells of *Anacardium occidentale* (Anacardiaceae) in 1978 by Wasiur Rahman and co-workers and later in 1999 in our natural products laboratory. This glycoside is peculiar in that it is esterified by p-coumaric acid in glucose part.

Literature survey reveals that this compound has not been screened for its pharmacological activity. We herewith report for the first time its antioxidant, antiinflammatory, anti-infective and antibacterial activities.

Prunin-6"-O-p-coumarate was isolated from the nut shells of *Anacardium occidentale* and its identity was established by spectroscopy (Mass, $^1$H-NMR and $^{13}$C NMR). Melting point was found to be 154-156°C and it coincides with the earlier report. Its structure is shown in Figure 1.

Male Wistar rats (150-200 g) and Swiss albino mice of either sex (22-25 g) were used in the study. The animals were maintained under standard environmental conditions during quarantine period.

Unless mentioned otherwise the test compound was administered as a suspension in distilled water containing 0.1% w/v of sodium carboxymethyl cellulose as suspending agent. Blank consisted of distilled water and 0.1% w/v of the suspending agent.

For antinociceptive activity writhings were induced by acetic acid (1.0% v/v, 0.1 ml/10 g, i.p.) in mice. Diclofenac (20 mg/kg, i.p.) was used as a reference. Two doses of *anacardium occidentale*-1 (AO-1) (30 and 100 mg/kg, i.p.) were used.

Antiinflammatory activity was tested by carrageenin induced rat paw edema. Ibuprofen (50 mg/kg, i.p.) was used as a reference, and the doses of AO-1 tested were same as above.

For the in vitro study of antioxidant activity of AO-1, a stable free radical α, α′-diphenyl-β-picryl hydrazyl (DPPH) was used at a concentration of 0.2 mM in methanol. In a set of clean, dry test tubes 1 ml of different concentration of AO-1 solution were taken (10, 30, 100, 300 µg and 1 and 3 mg). To each of these tubes 2.4 ml of methanol and 0.5 ml of DPPH were added and mixed thoroughly. Immediately the absorbance due to DPPH reagent solution was read at 517 nm. It was read against a blank prepared identically without the drug (i.e., only DPPH in methanol). Blank readings were recorded for each concentration.

The antibacterial activity of the test compound was investigated by an agar-well diffusion method against two gram positive (*Bacillus subtilis* and *Staphylococcus albus*) and two gram negative (*Escherichia coli* and *Proteus vulgaris*) bacte-

![Figure 1: Chemical structure of test compound](image)

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg, i.p. (mM)</th>
<th>Acetic acid-induced writhings in mice</th>
<th>Carrageenin-induced paw edema in rats</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>41.0 ± 2.8</td>
<td>1.00 ± 0.2</td>
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<tr>
<td>AO-1</td>
<td>30 (0.051)</td>
<td>25.0 ± 5.4**</td>
<td>0.74 ± 0.1*</td>
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<tr>
<td>AO-1</td>
<td>100 (0.172)</td>
<td>14.2 ± 4.4**</td>
<td>0.48 ± 0.2**</td>
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<tr>
<td>Diclofenac</td>
<td>20 (0.062)</td>
<td>20.3 ± 2.8**</td>
<td>0.44 ± 0.1**</td>
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<tr>
<td>Ibuprofen</td>
<td>50 (0.242)</td>
<td>-</td>
<td>-</td>
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One-way ANOVA

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>df</th>
<th>P</th>
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<tbody>
<tr>
<td>Acetic acid</td>
<td>48.4</td>
<td>3.20</td>
<td>&lt;0.0001</td>
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<tr>
<td>Carrageenin</td>
<td>13.7</td>
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</tr>
</tbody>
</table>

Values are expressed as mean±SD. *P<0.05, **P<0.001, Vs control, Student-Neuman-Keuls test.
Antiplasmodial activity of seven plants used in African folk medicine

Sirs,

Currently, there is a considerable increase in mortality caused by malaria due to the rapid spread of drug-resistant strains of P. falciparum. It is important, therefore, that new antimalarial drugs are developed to cope with the spread of resistance. In areas that are endemic for malaria in Africa a feverish patient may consume aqueous or alcoholic decoctions of barks, roots and leaves of different plants for relief from the clinical manifestation of malaria. China, on its part, added the artimisinin derivative of quinghoasu, (a chinese medicinal herbal product) to the antimalaria armamentarium. However, depressing reports show that more than 5% of 65 isolates from South East Asia are resistant to artemisinin and artesunate. This further underscores the need to search for new antimalaria. Herein, we tested the in vitro inhibition assay on the chloroquine-sensitive (F32) laboratory strain of P. falciparum with extracts from seven plants used in Cameroon by some traditional healers.

The plants, Achromanes difformis, Cleome rutidosperma, Cymbopogon citratus, Piper umbellatum, Mellotus appositofolius, Mangifera indica and Annona muricata were collected from various parts of Cameroon and identified in the Limbe Botanic Garden. The voucher specimens were deposited at the University of Buea Herbarium. The leaves of each plant were dried, ground and soaked in 1:1 chloroform/methanol mixture. The soluble extracts were sieved and concentrated in a speed vacuum concentrator. The plant extracts were then weighed and dissolved in a malaria culture medium (10.49 g RPMI, 2 g NaHCO3, 6 g HEPES, 10% AB sera and 0.025 mg gentamycin per liter). M. indicus, A. difformis and M. appositofolius could not dissolve directly and were first dissolved in dimethyl sulphoxide (DMSO) and were finally diluted in the malaria culture medium to a final DMSO concentration of 0.16% w/v. Serial dilutions of each extract were tested on an asynchronous F32 strain of P. falciparum in 96 flat-bottom well plates in duplicate using chloroquine as a positive control. Serial dilutions of DMSO were used to check the effect of DMSO. A control containing everything but the extract was included and used in calculating the percentage inhibition of parasite growth. In addition to the extract, each test well contained 1% parasitized blood Group O of 2% hematocrit in malaria culture medium. Growth inhibition was assessed after 24 h of culture under conditions as previously described. Assessment of inhibition was done by fluorescent microscopic examination of acridine orange-stained thin smears from each well on an eight-well multi-test slide. The number of parasitized erythrocytes was estimated by counting at least 6000 erythrocytes (25 microscopic fields) per well. Percentage inhibition was calculated as the number of parasites in the control well minus number of parasites in the test well divided by the number of parasites in the control well. Final percentage inhibition for each concentration of extract was obtained as mean of percentage inhibition for that concentration.

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References