ria*. AO-1 was dissolved in a mixture of water and dimethyl formamide (1:4). Streptomycin Sulphate was employed as the reference drug. All the investigations were carried out in duplicate. Simultaneous control investigations were done to observe the solvent effect.

Results are expressed as mean±SD. The differences between experimental groups were compared by one-way ANOVA (control vs treatment) followed by Student-Neuman-Keuls test and were considered statistically significant at P<0.05.

The number of acetic acid induced writhings were significantly reduced by treatment with AO-1 in both the doses. The effect was found to be more than that of the standard, diclofenac (20 mg/kg) at a dose of 100 mg/kg (Table 1). AO-1 pretreatment significantly reduced the paw edema in rats. The effect was dose-dependent and at a dose of 100 mg/kg was comparable with that of ibuprofen 50 mg/kg (Table 1). AO-1 has not shown any absorbance per se (without DPPH). The absorbance value of DPPH blank was found to be 0.175. AO-1 exhibited a dose dependent antioxidant activity in the DPPH assay. At 3 mg/0.1 ml concentration the absorbance value was 0.06. An antibacterial activity against both gram positive and gram negative organisms was also evident. MIC values of E. coli and B. subtilis was found to be 100 µg/ml (0.172 µM) and for P. vulgaris and S. albus it was 90 µg/ml (0.154 µM). AO-1 exhibited antinociceptive activity against acetic acid – induced writhings in mice. At the highest tested dose it was found to be more effective than diclofenac. AO-1 also possesses significant antiinflammatory, antioxidant and antibacterial activities.

Acknowledgements

The first author wishes to thank the University Grants Commission, New Delhi, for the financial support. Thanks are also due to Dr. B. Ravi Kumar for the supply of Prunin-6-O-p-coumarate.

D. Harikrishna, A. V. N. Appa Rao, M. C. Prabhakar*

Departments of Pharmacology and Chemistry*, University College of Pharmaceutical Sciences, Kakatiya University, Warangal - 506 009, India.
E-mail: mc_prabhakar@yahoo.com

References


Antiplasmodial activity of seven plants used in African folk medicine

Sir,
 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 aseptic or alcoholic decoctions of barks, roots and leaves of different plants for relief form the clinical manifestation of malaria. China, on its part, added the artiminisin derivative of quinghoasu, (a Chinese medicinal herbal plant product) to the antimalaria armamentarium. However, depressing reports show that more than 5% of 65 isolates from South East Asia are resistant to artiminisin 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 diffimis, Cleome rutidosperma, Cymbopogon citratus, Piper umbellatum, Mellotus appositofolius, Mangifera indicus 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. diffimis 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.
The results are summarized in Table 1. While *Piper umbellatum* and *Mellotus appositolius* extracts had moderate activity against the parasite with 40 µg/ml of each giving 70% and 57% inhibition, respectively, extracts from *Cymbopogon citratus*, *Mangifera indicus* and *Annona muricata* were found to possess greater effects on the growth with 20 µg/ml of each giving 57.9%, 50.4% and 67% inhibition, respectively. *Achromanes difformis* and *Cleome rutidosperma* extracts showed the least antiplasmodial activity even with 40 µg/ml of each resulting in 32.4% and 31.6% inhibition, respectively (Table 1).

It is not known whether the in vitro effect of these extracts against *F32 P. falciparum* is due to the concerted activity of their components. This issue could be addressed after chemical fractionation and isolation of the principles. Toxicology studies in animals may provide additional information on the feasibility of their use in humans.

**Acknowledgement**

We acknowledge the advice of the late Prof. Johnson Ayafor on the plant extraction procedure, and of Mr. Nahou Ndam in identifying the plant extracts. This investigation received financial support from the University of Buea, Cameroon and the International Program in Chemical Sciences (IPiCS) Uppsala University, Sweden.

---

**Table 1**

<table>
<thead>
<tr>
<th>Drug/Extract</th>
<th>0.08</th>
<th>0.16</th>
<th>0.32</th>
<th>0.64</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage inhibition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Achromanes difformis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.0</td>
<td>11.4</td>
<td>25.6</td>
<td>32.4</td>
</tr>
<tr>
<td>2. Cleome rutidosperma</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.2</td>
<td>11.9</td>
<td>12.3</td>
<td>26.9</td>
<td>31.6</td>
</tr>
<tr>
<td>3. Cymbopogon citratus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>34.0</td>
<td>42.0</td>
<td>57.9*</td>
<td>75.2</td>
<td></td>
</tr>
<tr>
<td>4. Piper umbellatum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>36.2</td>
<td>43.0</td>
<td>70.0</td>
<td></td>
</tr>
<tr>
<td>5. Mellotus appositolius</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>40.0</td>
<td>43.0</td>
<td>50.4*</td>
<td>71.5</td>
</tr>
<tr>
<td>6. Mangifera indicus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>34.2</td>
<td>46.1</td>
<td>67.0*</td>
<td>73.6</td>
<td></td>
</tr>
<tr>
<td>7. Annona muricata</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>45.8</td>
<td>46.4</td>
<td>46.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Chloroquine</td>
<td>5</td>
<td>47</td>
<td>78</td>
<td>89</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Concentration of chloroquine (0.08-0.64) is in µM and those of plant extracts (2.5-40) are in µg/ml. Values are mean percentage inhibition at the concentration of extract given in the top row. *More than 50% inhibition of parasite growth at 20 µg/ml of extract. ‘ – ’ = Not determined.

---

**Piracetam attenuates minoxidil-induced antinociception in mice**

Sir,

Piracetam is a nootropic agent and has been used to treat various dementias for several years as it enhances or facilitates various learning and other cognitive functions. It has been shown to attenuate the opioid antinociception. Besides, piracetam increases the intracellular ATP concentration in the nerve cell which may have an inhibitory effect over the ATP-gated potassium channels (K\_ATP channels). Therefore, the present study has been designed to investigate the effect of piracetam on the K\_ATP channel opener-induced antinociception. Minoxidil is a selective K\_ATP channel opener and produces antinociception in mice when administered centrally.

Six to eight-weeks-old healthy inbred BALB/c mice (25±3 g) of either sex were used in the study. They were housed in an animal house provided with a 12 h light/dark cycle and had free access to food and water. Minoxidil (Dr. Reddy’s Laboratories Ltd., Hyderabad, India) and piracetam (Micro Labs Ltd., Pondicherry, India) were dissolved in normal saline immediately before use. The institutional ethical committee approved all experimental procedures.

Minoxidil was injected i.c.v. in conscious mice in a volume of 10 µl with Hamilton syringe as described by Haley and McCormick. Time course studies were used to ascertain peak antinociception as tested by the tail flick test. Peak time for minoxidil was 10 min after injection.

nociceptive threshold was measured by the tail flick test in mice. The tail flick latency was considered as the time between tail exposure to radiant heat and tail withdrawal. An electrically heated nichrome wire was used as a source of radiant heat in the analgesiometer. The intensity of radiant heat was regulated in order to obtain pretreatment latency between 2 to 3 s. A cut-off latency time was fixed at 10 s. Tail flick latency was expressed as a percentage of the maximum pos-