Supporting Information to:

*Myristica malabarica* Heals Stomach Ulceration by Increasing Prostaglandin Synthesis and Angiogenesis

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**Preparation of the methanol extract of** *M. malabarica*

The dry fruit rinds (20 g) of *M. malabarica* were powdered with a grinder and extracted successively with ether, methanol and water (60 mL x 4 days with each solvent) at room temperature. The supernatants in each case were decanted. The entire process was repeated three times, each of the combined supernatants was filtered through a nylon mesh and evaporated at <40 °C in vacuo to obtain the respective extracts. These were designated as rampatri ether (7.30 g, 36.5%), methanol (5.77 g, 28.9%), and aqueous extracts (0.42 g, 0.02%), respectively, and stored in a vacuum desiccator. RM was used for the anti-ulcer experiments.

**Isolation of malabaricone B and malabaricone C**

Part of the methanol extract (3.7 g) was subjected to partial fractionation using a silica gel (25 g) column and eluting with hexane to collect five 300 mL fractions. These upon evaporation in vacuo gave subfractions F1-F5. Rigorous column chromatography (silica gel, 0-20% EtOAc/hexane) of F2 gave five compounds, including malabaricone B and malabaricone C, which were fully characterized using IR and 1H NMR spectroscopy. The IR spectra were scanned as KBr pellets with a JASCO model A-202 spectrophotometer. The 1H (200 MHz) spectra were recorded in CD₃OD with a Bruker AC-200 instrument and the values are provided in δ (ppm) scale along with the coupling constant (*J*) values in Hz. Standard abbreviations such as s: singlet, d: doublet, t: triplet, dd: doublet of doublet, have been used.

**1-(2',6'-Dihydroxyphenyl)-9-(4''-hydroxyphenyl)nonan-1-one (malabaricone B):** IR: 3619, 3579, 3163, 1586, 1250 cm⁻¹; 1H NMR (CD₃OD): δ 1.31 (s, 8H), 1.43-1.58 (m, 4H), 2.47 (t, *J* = 7.6 Hz, 2H), 3.08 (t, *J* = 7.54 Hz, 2H), 4.78 (broad s, 1H), 6.32 (d, *J* = 8.22 Hz, 2H), 6.66 (dd, *J* = 8.48, 2.2 Hz, 2H), 6.95 (dd, *J* = 8.48, 2.2 Hz, 2H), 7.17 (t, *J* = 8.22 Hz, 1H).

**1-(2',6'-Dihydroxyphenyl)-9-(3'',4''-dihydroxyphenyl)nonan-1-one (malabaricone C):** IR: 3547, 3145, 3116, 1580, 1184 cm⁻¹; 1H NMR (CD₃OD): δ 1.20 (s, 8H), 1.33-1.47 (m, 2H), 1.49-1.65 (m, 2H), 2.32 (t, *J* = 7.24 Hz, 2H), 2.99 (t, *J* = 7.54 Hz, 2H), 6.17 (d, *J* = 8.22 Hz, 2H), 6.35 (dd, *J* = 7.98, 2.0 Hz, 1H), 6.48 (d, *J* = 2.0 Hz, 1H), 6.53 (d, *J* = 7.98 Hz, 1H), 7.07 (t, *J* = 8.22 Hz, 1H).
Identification of phytoconstituents in the methanol extract of *M. malabarica*

The chemical constituents of the methanol extract were analyzed by high performance liquid chromatography (HPLC) with a Jasco model PU-2080 plus chromatogram using a Hypersil GOLD (250 × 4.6 mm, particle size 5 µm; Thermo Electron Corporation) column; the eluent was acetonitrile/water (60:40, flow rate 1.0 mL/min) and peaks were detected at 345 nm. The HPLC chromatograph of the extract showed a number of peaks and the data are shown in Table 1S. Some of these were identified as malabaricones A-D, and the glycosides of malabaricones B and C by comparing to the HPLC profiles of the pure compounds, isolated earlier by us from the extract [3]. The HPLC chromatograph of the extract is already available in reference [5] of the paper.

Table 1S

<table>
<thead>
<tr>
<th>peak no.</th>
<th>retention time (min)</th>
<th>compound</th>
<th>conc. (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.0</td>
<td>malabaricone C</td>
<td>21.2</td>
</tr>
<tr>
<td>2</td>
<td>11.9</td>
<td>malabaricone B</td>
<td>6.7</td>
</tr>
<tr>
<td>3</td>
<td>27.3</td>
<td>malabaricone D</td>
<td>1.2</td>
</tr>
<tr>
<td>4</td>
<td>36.0</td>
<td>malabaricone A</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>4.4-4.6</td>
<td>glycosides&lt;sup&gt;b&lt;/sup&gt;</td>
<td>--</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on the weight of RM.

<sup>b</sup> The minor peaks were not quantified due to their overlapping nature.

Ulceration experiments

All experiments were carried out with male Swiss albino mice, were approved by Post Graduate Institute of Basic Medical Sciences, Kolkata Animal Ethics Committee 507/CPCSEA (Sanction No. IAEC/SB-2/2004/UCM-16, dated 06.15.04) and BARC Animal Ethics Committee (BAEC), laboratory animal facility (sanction no. BAEC/03/05, dated 11.07.05) and were performed in accordance with the International Animal Ethics Committee Guidelines. The mice (25–30 g), grown on a balanced laboratory diet as per NIN, Hyderabad, India and given tap water ad libitum, were housed in plastic cages inside a well-ventilated room, maintained under standard condition (20 ± 2 °C, 65–70% humidity, and day/night cycle (12 h/12 h)). For carrying out experiments in a
blinded fashion, all animals were identified by typical notches in the ear and limbs and then randomized.

**Quantification of epidermal growth factor (EGF) expression**

After deparaffinization of the paraffin blocks in xylene, the sections were treated with a graded series of alcohol and subsequently rehydrated in PBS at pH 7.5. The sections were sequentially treated with 3% hydrogen peroxide in PBS and protein blockers (5% normal horse serum, 1% normal goat serum in PBS), and incubated overnight at 4 °C with primary antibody at the appropriate dilution. In control sections, antibodies were omitted, and only PBS was added. After incubation for 1 h at room temperature with peroxidase conjugated goat anti-rabbit IgG, a positive reaction was detected by exposure to DAB for 2 to 5 min. The slides were counterstained with Meyer’s hematoxylin, and the intensities of immunolocalized areas were quantified using Biovis MV500 software. Five areas from each section were scanned and the integrated optical density (IOD) in each area was calculated. The IOD of the negative control was subtracted from the IOD of each experimental section for each animal in all the groups.

**Quantification of von Willebrand factor VIII (vWF VIII)**

Following digestion of the tissue section with 0.1% trypsin, endogenous peroxidase activity as well as nonspecific protein binding sites were blocked. The sections were incubated with the polyclonal rabbit antihuman factor VIII-related antigen for 2 h at room temperature and the vWF was assayed using the peroxidase method. Any positive-staining endothelial cell or endothelial cell cluster that was clearly separated from adjacent microvessels was considered an angiogenic microvessel. The vascular areas immediately adjacent to the normal tissue of the stomach served as internal quality controls. The microvessels (under X 400 magnification) on coded slides in five randomly selected microscopic fields of mucosal erosions were counted, and the data were averaged.