

# Methylkarranjic acid and Pongamol from *Derris indica* Seeds and their Antibacterial Activity

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**ABSTRACT:** Two phenolic compounds, methylkarranjic (1) acid and pongamol (2), were isolated from the petroleum ether soluble fraction of an ethanolic extract of *Derris indica* seeds. The structures of these compounds were confirmed by LC-MS and a series of 1D and 2D NMR data. The solvent solvent partitionates of the ethanolic extract and compounds (1 and 2) exhibited moderate antibacterial activity against several test organisms. The minimum inhibitory concentrations (MICs) of 1 and 2 were found to be in the range of 32-128 µg/ml against *Bacillus megaterium*, *Streptococcus β-haemolyticus*, *Shigella dysenteriae* and *E. coli*.

**Key words:** *Derris indica*; Leguminosae; Methylkarranjic acid; Pongamol; Antibacterial activity

## INTRODUCTION

*Derris indica* (Lam.) Bennet (Syn. *Pongamia pinnata* (L.) Pierre, *P. glabra* Vent.), locally known as Karanja, is a mangrove plant belonging to the family Leguminosae. It is a medium size glabrous tree with a short bole and attaining a height of around 18 m and is habitat in the littoral regions of Southeast Asia, Australia and Fiji.<sup>1-3</sup> Traditionally, its bark is used in pile; leaves are effective as medicated bath and rheumatic pains; seeds are used in hypertension, bronchitis, whooping cough, skin diseases and rheumatic arthritis; roots are effective in fistulous sores and gonorrhoea.<sup>2,3</sup> The plant is reported to produce a wide range of flavanoids.<sup>3-15</sup> Here, we report the isolation of two phenolic compounds, methylkarranjic (1) acid and pongamol (2), from the petroleum ether soluble fraction of an ethanolic extract of *Derris indica* seed and the antibacterial activity of the extractives.

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## MATERIALS AND METHODS

**General Experimental Procedures.** NMR spectra (both 1D and 2D) were obtained on a DPX (500 MHz for <sup>1</sup>H and 125 MHz for <sup>13</sup>C) spectrometer, using the residual solvent peaks as internal standard. *J*-modulated <sup>13</sup>C spectra were acquired with a relaxation time (d1) of 6s. HMBC spectra were optimized for a long range *J*<sub>H-C</sub> of 7Hz (d6=0.07s). Column chromatography (CC) was conducted over Si gel (Merck, mesh 80-230) while TLC and PTLC were carried out using Merck Si gel 60 PF 254 on glass plates at a thickness of 0.5 mm. Spots on TLC plates were visualized under UV light (254 and 366 nm) and spraying with 1% vanillin-H<sub>2</sub>SO<sub>4</sub> followed by heating at 110 °C for 5-10 min.

**Plant material.** The seeds of *Derris indica* Lam. were collected in August 2001 from the adjoining areas of Rajshahi University Campus, Bangladesh and were identified by Prof. Naderuzzaman, Department of Botany, Rajshahi University, Bangladesh where a voucher specimen of this collection has been deposited.

**Extraction and isolation.** The matured seeds were washed, chopped, sun dried and ground. The ground seeds (300 gm) were extracted with ethanol (3 L) using a Soxhlet apparatus followed by solvent-solvent partitioning of the concentrate extract with petroleum ether (60-80 °C), ethyl acetate, acetone and methanol. The petroleum ether extract (2 gm) was then fractionated by column chromatography over silica gel eluting with petroleum ether and chloroform of increasing polarity. Preparative TLC (mobile phase- n-hexane: EtOAc = 7:1) of CC fractions eluted with 5-25% CHCl<sub>3</sub> in petroleum ether yielded **2** (90 mg; R<sub>f</sub> = 0.64 in 12.5% EtOAc in n-hexane) while **1** (85 mg; R<sub>f</sub> = 0.18 in 50% EtOAc in n-hexane) was isolated from another CC fraction eluted with 50-75% CHCl<sub>3</sub> in petroleum ether followed by PTLC (mobile phase- n-hexane: EtOAc = 7:1).

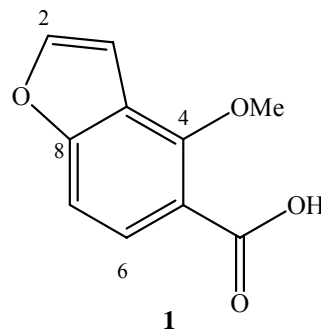
**Antibacterial activity.** Antibacterial assay was performed by disc diffusion technique.<sup>16,17</sup> The samples solution of the materials (fractions and pure compounds) were prepared by dissolving definite amounts of materials in appropriate solvent to attain the desired concentration and then applied on to sterile disc (6mm diameter, filter paper) followed by drying off the solvent in an aseptic hood. To compare the activity with standard antibiotics, kanamycin (30 µg/disc) was used. As negative control, a blank disc impregnated with 10µl solvent followed by drying off the discs, was used.

The minimum inhibitory concentrations (MICs) of the compounds **1** and **2** against *Bacillus megaterium*, *Streptococcus β-haemolyticus*, *Shigella dysenteriae* and *E. coli* were also determined by serial dilution technique.<sup>18</sup>

## RESULTS AND DISCUSSION

Column chromatography followed by preparative TLC of the petroleum ether soluble fraction of an ethanol extract of *D. indica* seeds yielded two phenolic compounds (**1** and **2**). The LCMS of **1** established its molecular formula as C<sub>10</sub>H<sub>8</sub>O<sub>4</sub>. The <sup>1</sup>H NMR spectrum (500 MHz, CDCl<sub>3</sub>,

Table 1) showed a three-proton singlet at δ 4.33 for a methoxyl group, an AB quartet (*J* = 2.2 Hz) at 7.69 and 7.02 of a distributed furan ring and two doublets (*J* = 8.7 Hz) resonating at δ 8.14 and 7.35 suggesting the presence of a tetra-substituted benzene ring. A *J*-modulated <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, Table 1) exhibited the presence of a carboxylic acid (δ<sub>C</sub> 166.2), a methoxyl group (δ<sub>C</sub> 61.8), four methines (δ<sub>C</sub> 105.4, 108.0, 129.6 and 145.7) and four quaternary carbons (δ<sub>C</sub> 114.2, 118.0, 153.6 and 160.0). The complete structure elucidation of this compound was achieved by 2D experiments. In the HSQC experiment, the furan ring protons (H-2 and H-3) showed direct correlation with methine carbons at δ<sub>C</sub> 145.7 (C-2) and 105.4 (C-3), respectively. The remaining two aromatic protons at δ 8.14 and 7.35 showed direct correlation to the methine carbons at δ 129.6 and 108.0, respectively. A common <sup>3</sup>*J* correlation by H-2 and H-3 and proton at δ 8.14 to an oxygenated quaternary at 160.0 confined its assignment as C-8. So the protons at 8.14 and 7.35 must be assigned to H-6 and H-7, respectively. The higher chemical shift of the former proton suggested its β-position to the carbonyl group. Further, a common <sup>3</sup>*J* correlation by H-6 and methoxyl protons (δ<sub>H</sub> 4.33; δ<sub>C</sub> 61.8 from HSQC) to at δ 153.6 confined its identity as C-4. The quaternary carbon at δ 114.2 was assigned as C-9 from its common correlation (<sup>3</sup>*J*) from H-2 and H-7. H-6 also showed a <sup>3</sup>*J* correlation to the carboxylic acid (166.2). On the basis of above spectral data, the compound was identified as methylcaranjic acid (**1**).<sup>19</sup> This is the first time report of its isolation from *D. indica* although it has previously been reported from *Tephrosia hamiltonii* (Family- Papilionaceae).<sup>19</sup>



The molecular formula of **2** was established as C<sub>16</sub>H<sub>13</sub>O<sub>4</sub> by LC-MS. The presence of a methoxyl ( $\delta$  4.14, *s*), an AB quartet ( $J = 2.1$  Hz) at  $\delta$  7.64 and 7.01 of a disubstituted furan ring and another set of AB quartet at  $\delta$  7.88 and 7.32 ( $J = 8.7$  Hz) in the <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>, 500 MHz, Table 1) suggested that compound **2** was an analog of **1**. The <sup>13</sup>C NMR data (CDCl<sub>3</sub>, 500 MHz, Table 1) of this part of the molecule was almost identical to **1** except the presence of a ketone ( $\delta$  186.3) instead of a carboxylic acid (166.0). The <sup>1</sup>H NMR spectrum also showed a hydrogen-bonded hydroxyl at  $\delta$  16.9, an olefinic proton (7.17, *s*) and resonances for a monosubstituted benzene ring ( $\delta$  7.99, 2H, *d*,  $J = 7.4$  Hz; 7.49, 2H, *t*,  $J = 7.4$ ; 7.54, 1H, *t*,  $J = 7.4$  Hz). In

the HMBC experiment, the hydrogen bonded hydroxyl group showed <sup>3</sup>*J* correlations with the olefinic methine carbon at 91.8 (C-11) and a quaternary carbon at 135.9 (C-13) and <sup>2</sup>*J* correlations with another oxygen bearing quaternary carbon at 184.5 (C-12). So the higher carbon chemical shift of C-12 was due to the tautomerism between carbonyl and hydroxyl group. In the HMBC experiment, C-13 was also connected to H-15/17 by <sup>3</sup>*J* while C-16 (132.4) was connected to H-14/18 by <sup>3</sup>*J*. On this basis the compound was identified as pongamol (**2**).<sup>20</sup> This compound has previously been reported from the fruits of *D. indica* species before, however this is the first report of its isolation from the seeds.

**Table 1.** <sup>1</sup>H (500 MHz) and <sup>13</sup>C 125 MHz NMR data of **1** and **2** in CDCl<sub>3</sub>

Position	$\delta_{\text{H}}$		$\delta_{\text{C}}$	
	<b>1</b>	<b>2</b>	<b>1</b>	<b>2</b>
2	7.69, <i>d</i> , $J = 2.2$ Hz	7.64, <i>d</i> , $J = 2.1$ Hz	145.7	145.1
3	7.02, <i>d</i> , $J = 2.2$ Hz	7.01, <i>d</i> , $J = 2.1$ Hz	105.4	105.5
4	-	-	153.6	154.0
5	-	-	114.2	122.5
6	8.14, <i>d</i> , $J = 8.7$ Hz	7.88, <i>d</i> , $J = 8.7$ Hz	129.6	126.7
7	7.35, <i>d</i> , $J = 8.7$ Hz	7.32, <i>d</i> , $J = 8.7$ Hz	108.0	107.3
8	-	-	160.0	158.9
9	-	-	118.0	119.8
10	-	-	166.2	186.3
11	-	7.17, <i>s</i>	-	91.8
12	-	-	-	184.5
13	-	-	-	135.9
14, 18	-	7.99, <i>d</i> , $J = 7.8$ Hz	-	127.3
15, 17	-	7.49, <i>t</i> , $J = 7.4$ Hz	-	128.4
16	-	7.54, <i>t</i> , $J = 7.4$ Hz	-	132.4
MeO-4	4.33, <i>s</i>	4.15, <i>s</i>	61.8	61.4
HO-12	-	16.9, <i>br, s</i>	-	-

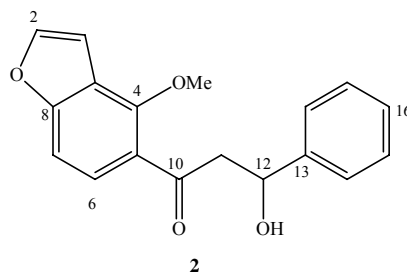
**Table 2.** HMBC data of **1** and **2** in CDCl<sub>3</sub>

Protons	<b>1</b>		<b>2</b>	
	<sup>2</sup> <i>J</i>	<sup>3</sup> <i>J</i>	<sup>2</sup> <i>J</i>	<sup>3</sup> <i>J</i>
H-2	105.4 (CH)	160.0 (C-O), 118.0 (C)	105.5 (CH)	119.8 (C), 158.9 (C-O)
H-3	145.7 (CH)	160.0 (C-O)	145.1 (CH), 119.8(C)	158.9 (C-O)
H-6	-	166.2 (CO), 153.6 (C-O)	-	186.3 (CO), 154.0 (C-O), 158.9 (C-O) 119.8 (C), 122.5 (C)
H-7	160.0 (C-O)	114.2(C), 118.0 (C)	-	-
H-11	-	-	186.3 (CO)	184.5 (C-O), 132.4 (CH)
H-14, 18	-	-	128.4 (CH)	135.9 (C)
H-15, 17	-	-	-	128.4 (CH)
H-16	-	-	-	154.0 (C-O)
MeO-4	-	153.6 (C-O)	-	135.9 (C), 91.8 (CH)
HO-12	-	-	184.5 (C-O)	-

The results of antibacterial activity are presented in Table 3. The fractions and pure compounds showed moderate antibacterial activity against the

test organisms. The minimum inhibitory concentrations (MICs), determined by serial dilution technique,<sup>18</sup> of compound **1** were found to be 128

$\mu\text{g/ml}$  against *Bacillus megatorium* and *Streptococcus  $\beta$ -haemolyticus* and 32  $\mu\text{g/ml}$  against *Shigella dysenteriae* and *E. coli* while the MIC of 2 was recorded as 128  $\mu\text{g/ml}$  against above mentioned bacteria.



**Table 3. Antibacterial activity of fractions (petroleum ether, ethyl acetate, acetone and methanol) of ethanol extract and compounds (1 and 2) of *D. indica* seed**

Bacteria	Diameter of the zone of inhibition (mm)						K 30 $\mu\text{g/disc}$
	PE	EA	AC	ME	1	2	
	200 $\mu\text{g/disc}$	200 $\mu\text{g/disc}$	200 $\mu\text{g/disc}$	200 $\mu\text{g/disc}$	200 $\mu\text{g/disc}$	200 $\mu\text{g/disc}$	
Gram positive							
<i>Bacillus subtilis</i>	15	9	12	13	12	11	24
<i>B. megaterium</i>	16	9	10	11	12	12	27
<i>Sarcina lutea</i>	15	9	12	12	13	12	29
<i>Staphylococcus aureus</i>	14	10	12	12	12	13	33
<i>Staphylococcus <math>\beta</math>-haemolyticus</i>	16	9	11	12	13	13	27
Gram negative							
<i>Esherichia coli</i>	14	10	11	11	15	13	27
<i>Psedomonas aeruginosa</i>	15	9	12	17	11	10	31
<i>Salmonella typhi</i>	14	8	19	13	13	11	28
<i>Shigella boydii</i>	16	9	11	13	15	12	31
<i>Sh. dysenteriae</i>	15	9	10	17	13	13	32
<i>Sh. flexneri</i>	14	8	12	11	15	13	27
<i>Sh. sonnei</i>	15	8	12	13	13	11	31
<i>Sh. shiga</i>	15	8	10	12	11	10	31
<i>Klebsiella</i> species	14	9	12	11	12	10	28

PE = Petroleum ether; EA= Ethyl acetate; AC= Acetone; ME= Methanol; K= Kanamycin

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