

synthesis of pongamol and lanceolatin *via* methyl ester of karanjic acid starting from diazacyclohexane-1,3-dione. Lee and Kang⁶ though adopted B-V approach, but harsh acid catalyzed cyclization was replaced with InCl_3 -catalyzed intramolecular dehydrative cyclization of furanochalcone to furanoflavone. Many such cyclization approaches reported in the literature is discussed by Sharma *et al.*⁷ Authors also reported a much simpler and efficient K_2CO_3 -mediated tandem O-arylation and C-O bond cleavage for the conversion of pongamol to lanceolatin⁷. Synthetic strategy adopted in the present manuscript is an amalgamation of some of these reported approaches. However, an alternate starting material, 4-hydroxy-2-methoxybenzaldehyde is proposed in the present work. Synthesis of pongamol and lanceolatin is achieved in 3 and 4 steps respectively in good yield. Structural confirmation of synthesized pongamol and lanceolatin B are based on NMR (^1H and ^{13}C) and mass and purity by HPLC. X-ray crystallographic study of synthesized lanceolatin B is also carried for the first time to confirm its structure. Synthesized compounds are evaluated for mosquito larvicidal activity against early 4th instar larvae of *Culex quinquefasciatus* strain.

Results and Discussion

Synthesis

Synthesis was initiated by reacting 4-hydroxy-2-methoxybenzaldehyde with bromoacetaldehyde

diethyl acetal in presence of anhydrous K_2CO_3 in dry DMF to give 4-(2,2-diethoxyethoxy)-2-methoxybenzaldehyde in 75% of isolated yield⁸. The cyclization of formed methoxy benzaldehyde was carried out by refluxing in toluene in presence of Amberlyst-15 giving a mixture of two benzofurans, 4-methoxybenzofuran-5-carbaldehyde (3a) as major and 5-methoxybenzofuran-6-carbaldehyde (3b) as minor. Column chromatographic purification of desired benzofuran (3a) afforded 77% of isolated yield⁹. Singh *et al.* reported a new N-heterocyclic carbene promoted intermolecular acylation of α -halo ketone with aromatic aldehyde for synthesis of 1,3-diketone¹⁰. The same umpoulong strategy was applied in the present synthesis of furanochalcone, pongamol. Intermolecular acylation of 2-bromoacetophenone with 4-methoxybenzofuran-5-carbaldehyde (3a) was conducted in presence of N,N-dibenzyl benzimidazolium chloride and DBU to get pongamol in 80% of isolated yield after column purification (Scheme 1). Conversion of pongamol to lanceolatin was achieved within 15 min at 27°C by a K_2CO_3 catalyzed intramolecular tandem O-arylation *via* C-O bond cleavage⁷ (Scheme 1). Synthesized pongamol and lanceolatin B were characterized by NMR (^1H and ^{13}C) and mass after ascertaining their purity by HPLC. HPLC analysis indicated that the synthesized pongamol is 99.6% pure whereas lanceolatin B is 99.8%.

Mosquito Larvicidal Assay

Karanjin, the principal furanoflavonoid compound present in different parts of karanja tree is found to possess excellent mosquito larvicidal activity against fourth instar larvae of *Culex quinquefasciatus* mosquito strain¹¹. In our earlier work, it was observed that a crude extract from deoiled seed cake containing 19-20% of karanjin along with karanja fatty acid methyl esters (48-52%), lanceolatin (6-7%) and other furanoflavonoids (22-26%) exhibited significantly higher larvicidal activity ($LC_{50} = 2.29 \pm 3.45$ ppm) compared to pure karanjin ($LC_{50} = 209.4 \pm 48.9$ ppm)^{11,12}. Though synergism of the crude extract may be the reason for showing exceptional larvicidal activity, but it is envisaged that both pongamol and lanceolatin B may show similar larvicidal activity like karanjin due to their structural similarity with karanjin, especially lanceolatin B. Standard bioassay procedure was adopted for ascertaining mosquito larvicidal activity of pongamol and lanceolatin B against early 4th instar larvae of *Culex quinquefasciatus* strain over a period of 24 h. Both these compounds exhibited very good mosquito larvicidal activity (Table 1). Though the activity of pongamol ($LC_{50} = 327.4 \pm 108$ ppm) was found to be at par with karanjin ($LC_{50} = 209.4 \pm 48.9$ ppm) but lanceolatin B exhibited poor activity compared to karanjin ($LC_{50} = 897.6 \pm 140$ ppm) despite its structural resemblance with karanjin.

X-ray Crystallography

X-ray crystallographic study of natural pongamol is already reported in the literature confirming its enol form rather than β -diketo form¹³. Thorough literature survey indicated no such study was carried out on lanceolatin B. In order to reconfirm its structure, X-ray study of lanceolatin B was carried out as per the standard protocol (Fig. 1).

Experimental Details

All the chemicals used in this study were obtained from different commercial sources and were used without any further purification. Reactions were monitored on micro TLC with UV detection. Final

purifications were carried out using silica gel (Rankem) 60-120 mesh. All ¹H and ¹³C NMR spectra were recorded on ADVANCE-300 and 400 (300 and 400 MHz for ¹H NMR and 75 MHz for ¹³C NMR). Chemical shifts are reported in δ (ppm) with reference to TMS as internal standard. Molecular weights of unknown compounds were identified by ES-MS and HR-MS (Electron Spray Ionization Technique). HPLC analysis was performed on HPLC equipment (Agilent 1260) equipped with Diode Array Detector. Analytical separation was carried out on reversed phase column, RP-C18 (250 \times 4.6 mm i.d. \times 3 μ m) (Merck) using 90:10 (v/v) of Methanol:water in isocratic elution mode. Data and chromatograms were collected using Open lab Chemstation chromatopac software system.

Synthesis of 4-(2,2-diethoxyethoxy)-2-methoxy benzaldehyde, 2

To a stirred solution of 4-hydroxy-2-methoxybenzaldehyde (5.0 g, 32.86 mmol) in DMF (50 mL) was added K₂CO₃ (9.0 g, 65.72 mmol) followed by bromoacetaldehyde diethyl acetal (5.93 mL, 39.43 mmol). Then reaction mixture was warmed to 50°C and stirred for 3 h. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to RT, diluted with water and extracted with EtOAc. The organic layer was dried over anhyd. Na₂SO₄, filtered and concentrated. The residue was purified by column

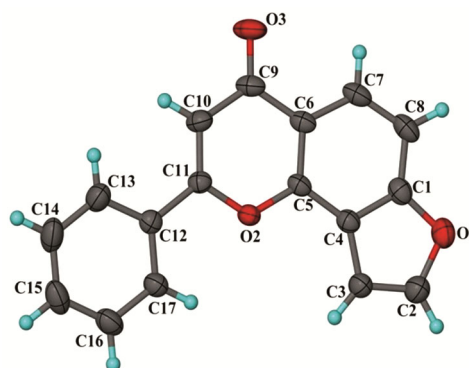


Fig. 1 — ORTEP diagram of synthesized lanceolatin B with the atom-numbering. Displacement ellipsoids are drawn at the 35% probability level and H atoms are shown as small spheres of arbitrary radius

Table 1 — Larvicidal activity (in ppm) for 24 h of pongamol and lanceolatin B against 4th instar larvae of *Culex quinquefasciatus* mosquitoes

Compd	Mosquito Larvicidal Activity (Mean \pm SE)		Chi square	Regression coefficient
	LC ₅₀ (ppm)	LC ₉₀ (ppm)		
Pongamol	327.4 \pm 108	953.2 \pm 822.4	0.48	2.76
Lanceolatin	897.6 \pm 1404	3113.5 \pm 9186	0.54	2.37

Negative control (acetone) – no activity; *level of significance: 0.05

chromatography on silica gel by eluting with ethyl acetate: hexane (5:95 v/v) to yield 4-(2,2-diethoxyethoxy)-2-methoxybenzaldehyde as light yellow colour liquid (6.6 g, 75%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.2 (s, 1H, Ar-CHO), 7.9 (s, 1H, Ar-H), 7.1 (d, 2H, Ar-H), 4.9 (m, 1H, O-CH-O), 4.2 (m, 6H, 3 × O-CH₂), 3.9 (m, 3H, O-CH₃), 1.2 (m, 6H, 2 × -CH₃); ¹³C NMR (75 MHz, CDCl₃): δ 188.2, 165.0, 163.4, 130.5, 119.2, 106.3, 101.3, 100.1, 98.4, 68.5, 62.7, 62.3, 55.3, 31.7, 15.2; HR-MS (ESI): *m/z* [M+H⁺] Calcd for C₁₄H₂₁O₅: 269.13835. Found: 269.13835.

Synthesis of 4-methoxybenzofuran-5-carb-aldehyde, **3a**

To a stirred solution of 4-(2,2-diethoxyethoxy)-2-methoxybenzaldehyde (6.0 g, 22.39 mmol) in toluene (50 mL) was added amberlyst-15 (1.0 g). Then reaction mixture was stirred at 110°C for 16 h. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to RT, diluted with water and extracted with EtOAc. The organic layer was dried over anhyd. Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel by eluting with ethyl acetate: hexane (10:90 v/v) to yield **3a** as light white colour solid, m.p.93-95°C (3.0 g, 76%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.5 (s, 1H, Ar-CHO), 8.2 (d, 1H, Ar-H), 7.7 (d, 1H, Ar-H), 7.1 (d, 1H, Ar-H), 6.8 (d, 1H, Ar-H), 4.0 (s, 3H, O-CH₃); ¹³C NMR (75 MHz, CDCl₃): δ 188.6, 159.4, 159.58, 144.81, 120.9, 120.8, 119.8, 106.01, 93.7, 54.6, 28.5; HR-MS (ESI): *m/z* [M+H⁺] Calcd for C₁₀H₉O₃: 177.05462. Found: 177.05462.

Synthesis of (Z)-3-hydroxy-3-(4-methoxybenzo-furan-5-yl)-1-phenylprop-2-en-1-one, **4**

In a solution of benzimidazolium salt (1.36 g, 4.54 mmol) in THF was added **3a** (2.0 g, 11.36 mmol) under nitrogen atmosphere at 27°C and stirred for 10 min at the same temperature. After that DBU (0.690 g, 4.5 mmol) was added to the stirred reaction mixture with syringe followed by the addition of 2-bromo acetophenone (2.26 g, 11.36 mmol) in THF and stirred for 4 h at 27°C. After completion of the reaction, as judged by TLC the solvent was evaporated using rotary evaporator, water was added and the product was extracted with ethyl acetate and dried over anhyd. Na₂SO₄. The crude product was purified by using column chromatography by eluting with ethyl acetate: hexane (30:70 v/v) which afforded the title compound as brownish colour solid, m.p.135-137°C (2.35 g, 70%). ¹H NMR (400 MHz, CDCl₃): δ 8.0 (d, 2H, , Ar-H), 7.9 (d, 1H, Ar-H), 7.7(d, 1H, Ar-H), 7.65-7.60 (m, 1H,

Ar-H), 7.5-7.45 (m, 1H, Ar-H), 7.35 (s, 1H, COCH=CH), 7.2 (d, 1H, Ar-H), 7.0 (d, 1H, Ar-H), 4.2 (s, 3H, O-Me); ¹³C NMR (75 MHz, CDCl₃): δ 186.13, 184.30, 158.73, 153.70, 144.80, 135.61, 132.22, 128.70, 128.60, 128.30, 127.10, 126.50, 122.20, 119.50, 107.0, 105.30, 97.90, 61.16; HR-MS (ESI): *m/z* [M+H⁺] Calcd for C₁₈H₁₅O₄: 295.09640. Found: 295.09649.

Synthesis of 2-phenyl-4H-furo(2,3-h)-chromen-4-one, **5**

To a stirred solution of pongamol (0.5 g, 1.7006 mmol) in DMF was added K₂CO₃ (0.470 g, 3.401 mmol) and reaction mixture was stirred at 60°C for 12 h. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to RT, diluted with water and extracted with DCM. The organic layer was dried over anhyd. Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel by eluting with ethyl acetate: hexane (30:70 v/v) to yield Lanceolatin as white solid, m.p.125-127°C (0.360 g, 80%). ¹H NMR (400 MHz, CDCl₃): δ 8.2 (d, 1H, Ar-H), 7.9 (d, 2H, Ar-H), 7.7(d, 1H, Ar-H), 7.63-7.60 (m, 4H, Ar-H), 7.35-7.45 (d, 1H, Ar-H), 6.8 (s, 1H, Ar-H); ¹³C NMR (75 MHz, CDCl₃): δ 178.24, 162.63, 158.34, 150.80, 145.82, 131.72, 131.56, 129.17, 126.16, 121.74, 119.34, 117.14, 110.77, 107.98, 104.18; HR-MS (ESI): *m/z* [M+H⁺] Calcd for C₁₇H₁₁O₃: 263.07012. Found: 263.07027.

Mosquito Larvicidal Assay

The larvicidal activity of the synthesized pongamol and lanceolatin B was determined by standard bioassay procedure. A laboratory colony of early 4th instar larvae of *Culex quinquefasciatus* was used for the larvicidal activity, which was maintained at 26±2°C, 70%±10% RH, under 14 L: 10 D photoperiod cycles. The larvae were fed with dog biscuits and yeast at 3:1 ratio. Ten of early 4th stage larvae of *Culex quinquefasciatus* were kept in a 100 mL of distilled water. Each treatment was replicated 6 times. Stock solutions of known concentration of synthesized pongamol and lanceolatin B were prepared in acetone. Different concentrations of 100, 200 and 300 ppm were prepared and efficacy was studied against larvae of *Culex quinquefasciatus* mosquito larvae. A negative (Acetone) control was set up along with the bio-efficacy studies. Larval mortality (in percentage) was recorded after 24 h treatment and probit analysis was performed for calculating the LC₅₀ and LC₉₀ values representing the concentration in ppm that killed 50% and 90% of larvae, respectively¹⁴.

Crystal Data of Lanceolatin B

$C_{17}H_{10}O_3$, $M = 262.25$, Monoclinic, space group P21/n (No.14), $a = 5.743(2)\text{\AA}$, $b = 17.265(6)\text{\AA}$, $c = 12.777(5)\text{\AA}$, $\alpha = 90^\circ$, $\beta = 95.043(7)^\circ$, $\gamma = 90^\circ$, $V = 1262.0(8)\text{\AA}^3$, $Z = 4$, $D_c = 1.380\text{ g/cm}^3$, $F_{000} = 544$, Bruker D8 QUEST PHOTON-100, Mo-K α radiation, $\lambda = 0.71073\text{ \AA}$, $T = 293(2)\text{K}$, $2\theta_{\text{max}} = 55^\circ$, $\mu = 0.095\text{ mm}^{-1}$, 15685 reflections collected, 2890 unique ($R_{\text{int}} = 0.0356$), 181 parameters, $R_1 = 0.0478$, $wR_2 = 0.1050$, R indices based on 1905 reflections with $I > 2\sigma(I)$ (refinement on F^2), Final GooF = 1.046, largest difference hole and peak = -0.114 and 0.119 e.\AA^{-3} .

Data collection and structure solution details

Single crystal X-ray data were collected at RT on a Bruker D8 QUEST equipped with a four-circle kappa diffractometer and Photon 100 detector. An μs microfocus Mo source ($\lambda = 0.71073\text{\AA}$) supplied the multi-mirror monochromated incident beam. A combination of Phi and Omega scans were used to collect the necessary data. Integration and scaling of intensity data were accomplished using SAINT program¹⁵. The structures were solved by Direct Methods using SHELXS97^(Ref. 16) and refinement was carried out by full-matrix least-squares technique using SHELXL-2014/7^(Ref. 16,17). Anisotropic displacement parameters were included for all non-hydrogen atoms. All H atoms were positioned geometrically and treated as riding on their parent C atoms, with C-H distances of $0.93\text{--}0.97\text{ \AA}$, and with $U_{\text{iso}}(\text{H}) = 1.2U_{\text{eq}}(\text{C})$ or $1.5U_{\text{eq}}$ for methyl atoms. The CCDC No 2202114 deposition number contains the supplementary crystallographic data for this paper which can be obtained free of charge at <https://www.ccdc.cam.ac.uk/structures/>.

Conclusion

The present work describes synthesis of pongamol and lanceolatin in an alternate and efficient pathway starting from 4-hydroxy-2-methoxybenzaldehyde. Both these compounds were characterized using NMR (^1H and ^{13}C) and mass and purity by HPLC. The structure of synthesized lanceolatin B is also confirmed by X-ray crystallography, which has not been reported earlier. Mosquito larvicidal activity of both the synthesized compounds were evaluated against early 4th instar larvae of *Culex quinquefasciatus* strain for 24 h. Pongamol and lanceolatin exhibited very good mosquito larvicidal activity against the studied strain. However the activity of pongamol is found to be better than lanceolatin B but at par with karanjin.

Supplementary Information

Supplementary information is available in the website <http://nopr.niscpr.res.in/handle/123456789/58776>.

Acknowledgement

The authors thank Director, CSIR-IICT for support in carrying out the research work (IICT/Pubs./2022/277) and Dr Jagadeesh Babu Nanubolu for carrying out the X-ray crystallographic study.

References

- (a) Jahana S, Md. Mahmuda H, Khana Z, Alama A, Khalilb A A, Raufc A, Tareqa A M, Nainud F, Tareqa S M, Emrane T B, Khanf M, Khang I N, Wilairatanah P & Mubarak M S, *Biomed Pharmacotherapy*, 142 (2021) 112109; (b) Sharma R, Williams I S, Gatchie L, Sonawane V R, Chaudhuri B & Bharate S B, *Bioorg Med Chem*, 26 (2018) 6076; (c) Phrutivorapongkul A, Lipipun V, Ruangrunsi N, Kirtikara K, Nishikawa K, Maruyama S, Watanabe T & Ishikawa, *Chem Pharm Bull*, 51 (2003) 187.
- Rangaswami B Y S & Seshadri T R, *Indian Acad Sci Section A*, 15A (1942) 417.
- (a) Mukerjee S K & Seshadri T R, *J Chem Soc*, 2048 (1955); (b) Srimannarayana G & Rao N V S, *Curr Sci*, 34 (1965) 581; (c) Sayed Alam, *Acta Chim Slov*, 51 (2004) 447; (d) Kumar P A, Mohapatra S, Das S & Basu S P, *J Innov Pharm Biol Sci*, 3 (2016) 116; (e) Lee Y R & Morehead A T Jr, *Tetrahedron*, 51 (1995) 4909; (f) Pirrung M C & Lee Y R, *Tetrahedron Lett*, 35 (1994) 6231.
- Chadha T C & Mahal H S, *Curr Sci*, 4 (1933) 214.
- Goel A & Dixit M, *Indian Patent* IN 2004DE01730 A 2006-09-22 (2006).
- Lee Y R & Kang K Y, *Lett Org Syn*, 4 (2007) 440.
- Sharma R, Vishwakarma R A & Bharate S B, *Org Biomol Chem*, 13 (2015) 10461.
- Yadav A P P, Srivastava A K & Maurya R, *Med Chem Res*, 20 (2011) 1465.
- (a) Dixit M, Tripathi B K, Tamrakar A K, Srivastava A K & Goel A, *Bioorg Med Chem*, 15 (2007) 727; (b) Goel A & Dixit M, *SYNLETT*, 11 (2004) 1990.
- Singh S, Singh P, Rai V K, Kapoor R & Yadav L D S, *Tetrahedron Lett*, 52 (2011) 125.
- Satyavani S R, Kanjilal S, Rao M S, Prasad R B N & Murthy U S N, *Med Chem Res*, 24 (2015) 842.
- Satyavani S R, Prasad R B N, Murthy U S N, Rao M S, Murthy M M & Kanjilal S, *J Lipid Sci Technol*, 44 (2012) 54.
- Parmar V S, Rathore J S, Jain R, Henderson D A & Malone J F, *Phytochem*, 28 (1989) 591.
- Finney D J, *Probit Analysis*, 3rd Edn. (Cambridge University Press, London) (1971).
- SMART & SAINT. Software Reference manuals. Versions 6.28a & 5.625, Bruker Analytical X-ray Systems Inc., Madison, Wisconsin, USA (2001).
- Sheldrick G M, SHELXS97 and SHELXL Version 2014/7, <http://shelx.uni-ac.gwdg.de/SHELX/index.php>
- Crystal Structure Refinement: A Crystallographer's Guide to SHELXL*, edited by Muller P, Herbst-Imer R, Spek A, Schneider S & Sawaya M (Oxford University Press, Oxford, New York), pp.57-91 (2006).