Indian Journal of Chemistry
Vol. 55B, January 2016, pp.123-127

A new embelin from the mangrove *Aegiceras corniculatum*

Satya Prakasa Rao Thota[a], Nittala S Sarma*[a], Y L N Murthy[a],
V S S N Kantamreddi[b] & C W Wright[b]
[a]School of Chemistry, Andhra University, Visakhapatnam 530 003, India
[b]School of Pharmacy, University of Bradford, Bradford BD7 1DP, United Kingdom
E-mail: nssarma@rediffmail.com

Received 21 April 2015; accepted (revised) 11 October 2015

One new compound (**2**) and five known compounds (**1, 3**-**6**) have been isolated from the mangrove plant *Aegiceras corniculatum* collected from the Godavari mangrove forest, India. The structure of the new compound has been established by ^{1}H and ^{13}C NMR spectroscopy and mass spectral data as 2,5-didehydroxy-6-methylembelin (**2**). The known compounds have been characterized as 4-hydroxy-2-methoxybenzamide (**1**), embelin (**3**), 5-O-ethylembelin (**4**), 5-O-methylembelin (**5**) and 4-methoxyresorcinol (**6**). Compounds **2-5** exhibit moderate antimalarial activity. Embelin (**3**) shows activity against chloroquine-resistant strains better than against chloroquine-sensitive strains of *Plasmodium falciparum.*

Keywords: *Aegiceras corniculatum*, quinonoids, antimalarial activity

Mangroves grow on the fringe habitats of estuaries and lagoons with high salt content. Their root systems are regularly inundated with salt water. In many tropical and subtropical regions world-wide, they occur as forests and are important both ecologically and economically as they protect coastlines from erosion and provide various direct and indirect resources[1-3]. *Aegiceras corniculatum* (Linn) Blanco (Aegiceratataceae) is a shrub or a small tree that grows in mangrove swamps of Asia and Australia[3]. In mainland China, *A. corniculatum* is one of the two species of the *Aegiceras* genus and is widely distributed along the coastline in tropical and subtropical areas[4]. It is also widely distributed in the Godavari estuary, Andhra Pradesh, India.

The extracts of leaves and bark of *A. corniculatum* possess ichthyotoxicity; juvenile fish (*Tilapia nitolica*) were killed instantly when exposed to the extracts[4]. Four triterpenes were reported from this species collected from Sanya of Hainan province, Southern China[5]. Eight phenols and their derivatives were isolated from this species collected at the coastline close to Xiamen, Fujian Province, from China[6]. Three compounds, also phenols were reported from the plant of Philippines coast. Biological activity of these components has not been evaluated[7-11]. Four isomeric macrolides of combrestatin D-2 congeners, named isocorniculatolides, have been recently reported from this species collected from Nizampatnam coast of India[12]. With this background, we undertook a coupled investigation of isolation and pharmacological activity evaluation of *A. corniculatum* principles from the Godavari estuary.

Results and Discussion

Compound **2** (Figure 1) was obtained as a reddish brown coloured microcrystalline powder from methanol (with traces of chloroform), m.p. 140-42°C. The molecular formula was established as $C_{18}H_{28}O_2$ by elemental analysis and *m*/*z* 276 ($M^{+\cdot}$) in the EIMS spectrum. The UV-Vis spectrum shows significant absorption at 295 nm. The IR spectrum (KBr) showed intense bands at 1628 and 1624 cm^{-1} for quinone C=O groups. The ^{1}H NMR spectrum (Table I) showed a sharp signal in the aromatic region at δ 7.57 (2H, s, H-5, H-2). Two methyl groups, one at δ 1.91 (3H, s, 6-CH_3) and another at δ 0.89 (3H, t, J = 6.9 Hz, 11"-CH_3) were also present. A deshielded methylene appeared at δ 2.42 (2H, t, J = 7.5 Hz, 1"-CH_2) adjacent to quinonoid carbon and shielded methylene protons, which are part of long methylene chain *i.e.*, $(-CH_2)_8$, appeared at δ 1.29 (16H, br s). Another methylene group appeared at δ 1.45 (2H, m, 2"-CH_2). The ^{13}C NMR spectrum showed 9 signals, which include four sp^2 carbons (aromatic), and five sp^3 carbons. Two aromatic carbons (C-2 and C-5) and both carbonyls (C-1 and C-4) did not appear in the ^{13}C NMR spectrum due to loss of signals by

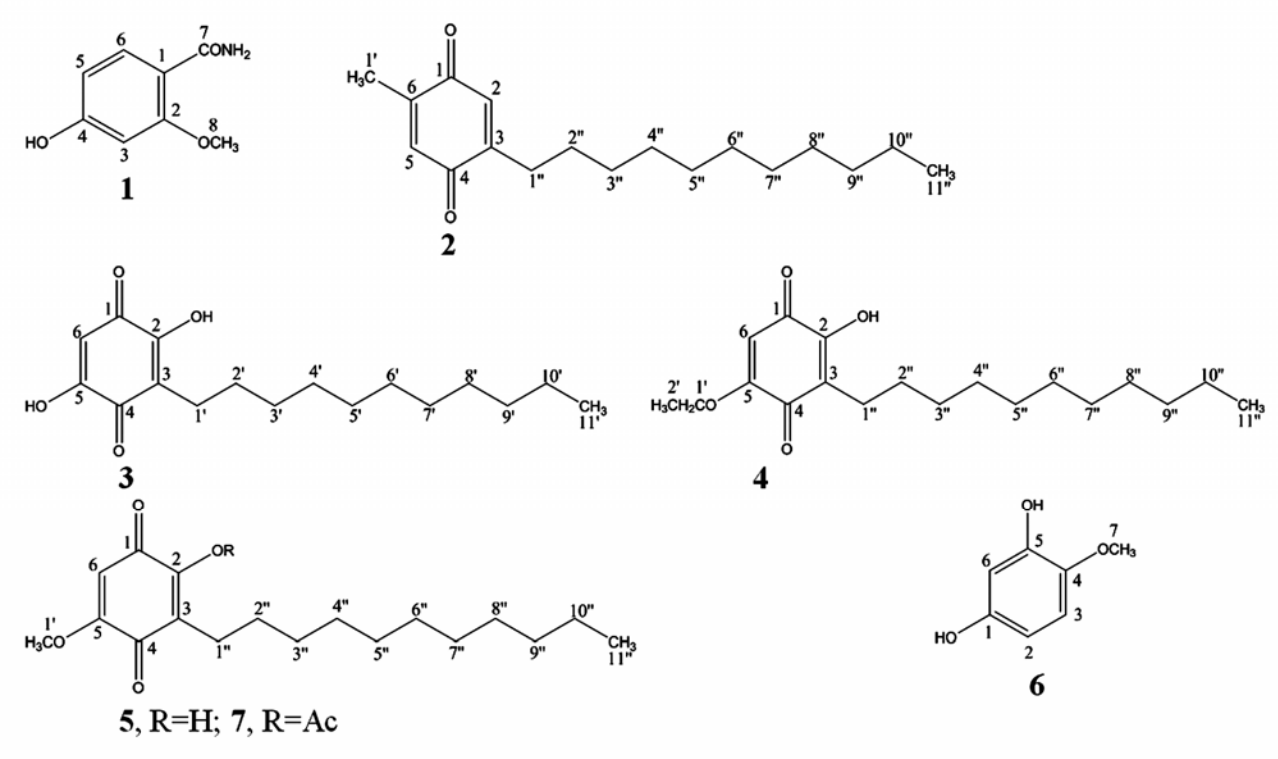

Figure 1 — Compounds isolated from *Aegiceras corniculatum*

saturation[13]. Among the sp^3 carbons, two are primary (methyl carbons) at δ 14.1 (11"-C) and 7.4 (1'-C). The latter is in an abnormally shielded position attributed to the shielding cone influence of the neighbouring quininonoid carbonyl[7], which is also behind the shielded position of the 6-methyl (1'-CH_3) protons (δ 1.91). The former methyl carbon is at the usual position (δ 14.1) for a primary methyl carbon (DEPT) at the end of an alkyl side chain[6,14]. The methylene carbon signal at δ 31.9 is consistent with an allylic carbon (1"-C), and the signal at δ 22.3 (10"-C) is consistent for methylene attached to the terminal methyl. The signal at δ 29.4 should contain seven methylene carbons, as usually observed in the case of 2,5-dihydroxy-3-undecyl-1,4-benzoquinone (embelin) which contains the same aliphatic long methylene chain[6] as compound **2**. The extra aromatic methyl group of **2** (1'-CH_3) and the absence of O-H or O-Alkyl substitution at C-2 and C-5 of the quinine cycle are novel structural features in this class of quinones. The 1'-CH_3 could alternatively be placed at C-5 since this substitution would produce NMR and mass spectra that are identical with those of **2**. To our knowledge, no quinone with *m*-dialkyl substitution has been isolated from natural sources; on the other hand, the *p*-dialkyl substitution has been reported *e.g.*, the quinones isolated from the fruits of the plant *Maesa lanceolata*[15]. The structure of compound **2** is thus established as 2,5-didehydroxy-6-methylembelin, a new embelin.

Compound **1** was obtained from chloroform-methanol as orange-yellow crystals after repeated crystallization from methanol. The UV-Vis spectrum showed absorption maxima at 284 nm. Its molecular formula was derived as $C_8H_9O_3N$ from elemental analysis and *m/z* at 167 ($M^{+\cdot}$) in the EI-MS spectrum. The IR spectrum showed hydroxyl absorption band at 3485.1 cm^{-1}. The most prominent bands to be identified were the bands at 1628 cm^{-1} (C=O) and 1683.7 cm^{-1} (C=O stretch, amide I and N-H bending, amide II, overlapped). The aromatic C=C stretching is seen at 1598.9 cm^{-1}. The ^{1}H NMR spectrum of **1** shows signals at low field for aromatic protons at δ 7.74 (1H, d/d, 5-C*H*), δ 6.99 (1H, d, 6-C*H*), and δ 7.61 (1H, s, 3-C*H*). A characteristic three-proton singlet in the higher field region at δ 3.98 (3H, s, 8-OCH_3) indicated aromatic methoxy group[16]. A broad signal at δ 6.09 was attributed to amide protons (2H, br s, NH_2). The aromatic amide carbon appeared downfield at δ 170.2 (C-7) in the ^{13}C NMR spectrum. Apart from this, seven carbons are shown, one sp^3 (δ 56.1, methoxy carbon at C-8) and others

Table I — ^{1}H and ^{13}C NMR spectral data of compound **2***

	^{1}H NMR (400 MHz)	^{13}C NMR (100 MHz)
1		#
2	7.57 (1H, s)	#
3		116.1
4		#
5	7.57 (1H, s)	#
6		111.5
1'	1.91 (3H, s)	7.4
1"	2.42 (2H, t, *J* = 7.5Hz)	31.9
2"	1.45 (2H, m)	29.4
3"	1.29 (2H brs)	29.4
4"	1.29 (2H brs)	29.4
5"	1.29 (2H brs)	29.4
6"	1.29 (2H brs)	29.4
7"	1.29 (2H brs)	29.4
8"	1.29 (2H brs)	29.4
9"	1.29 (2H brs)	22.7
10"	1.29 (2H brs)	22.3
11"	0.89 (3H, t, *J* = 6.9 Hz)	14.1

*Solvent: $CDCl_3$; #No signal due to fluxional effect; Assignments are confirmed by DEPT experiments

sp^{2}, *e.g.*, δ 150.8 (C-4, phenolic), δ 146.2 (C-2, phenolic methyl ether), δ 112.1 (C-1), δ 121.1 (C-5), δ 125.2 (C-3) and δ 114.2 (C-6). Compound **1** is thus characterized as 4-hydroxy-2-methoxybenzamide. To our knowledge, 4-hydroxy-2-methoxybenzamide as a natural product is reported for the first time.

Compound **6** was obtained from chloroform-methanol (1:1) as colourless needles, m.p. 178-80°C and the UV-Vis spectrum showed absorption maxima at 226 nm. Its molecular formula was $C_7H_8O_3$ from elemental analysis and *m*/*z* at 140 [$M^{+\cdot}$]. The IR spectrum showed hydroxyl absorption bands at 3446 and 3389 cm^{-1} and the olefinic (C=C) stretching bands at 1615 cm^{-1}. Intense bands were also seen at 1270-1121 cm^{-1} and 1145-1165 cm^{-1} ranges for the C-O-C stretching (phenyl methyl ether). The ^{1}H NMR spectrum of **6** showed signal at δ 3.7 (3H, s, 4-OC*H*$_3$) for aromatic methoxy group. Another three proton singlet was shown in the aromatic region at δ 7.1 (3H, s, H-2, H-3 and H-6). Two phenolic hydroxyl protons were noticed at δ 9.1 (2H, s, 1-O*H*, 5-O*H*). The ^{13}C NMR spectrum showed seven peaks. The two quaternary (DEPT) aromatic oxygenated carbons were downfield at δ 167.2 (C-1) and 147.4 (C-5), and the aromatic ether carbon at δ 140.3 (C-4). The signals at δ 106.9, 120.1 and 106.9 can be assigned to C-2, C-3 and C-6. The absorption at δ 56.8 is for methoxy carbon (C-7). These spectral features suggest the compound to be 6-methoxyresorcinol[6]. To our knowledge, 6-methoxyresorcinol is reported as a natural product for the first time.

Table II — Antimalarial testing results*

Compd	IC_{50} (μg/mL) = Mean±SD (n)	
	3D7	K1
1	Inactive	Inactive
2	25.95±0.66	21.82±1.12
3		
3	16.52±0.59	18.86±0.92
4	25.45±1.17	28.11±0.73
5	24.51±0.93	23.46±1.23
6	Inactive	Inactive
CQ	0.02±0.01	0.37±0.02

*n = 3, CQ = Chloroquine; Inactive: IC_{50}> 30 μg/mL.

The *in vitro* antimalarial activity of all the six isolated compounds was performed on two different strains of *Plasmodium falciparum*, chloroquine sensitive (3D7) and resistant (K1) strains using chloroquine as control. Compounds **1** and **6** were inactive. The remaining four compounds (**2**-**5**) exhibited moderate activity (Table II). Compound **3** (embelin) was found to be relatively more potent against both 3D7 and K1 strains with lower IC_{50} values (Table II). This compound shows a slightly better activity against chloroquine-resistant strain than against the chloroquine-sensitive strain of *P. falciparum*. Embelin has enjoyed application in traditional medicine[17] and has recently gained prominence as a selective inhibitor of cancer cells / inducer of apoptosis[18] and as a suppressor of osteoclastogenesis[19]. Embelins are so far isolated from plants, and their isolation in good yield from the mangrove plants together with the varied biological activities established for embelin promise to provide opportunities for testing of its various natural and synthetic analogues.

Experimental Section

Column chromatography (CC) was performed over 100-200 mesh silica gel (Merck, India). Melting points were determined on Kumar hot plate and are uncorrected. Optical rotations were measured on Rudolph Autopol III polarimeter. UV-Vis spectral data were determined on Milton Roy Spectronics-1201 spectophotometer. IR spectra were recorded on Shimadzu FT-IR 1640 spectrometer. NMR spectra were recorded on either JEOL 400, 270, 90 MHz or Bruker AV-300 MHz spectrometer with TMS as internal standard. EI-MS were recorded on Finnigan

MAT90 or MS-MS spectra were recorded on a Micromass Quattro Ultra HPLC-MS-MS electrospray spectrometer in positive or negative ionization modes.

Plant material

The stem and leaves of the mangrove plant were collected from the mangrove forest area of the Godavari estuary (8°28′ N and 78°08′ E) coming under the revenue village of Kandikuppa, East Godavari district, India in April 2004. The stem was washed with water and shade-dried, cut into pieces and powdered. The plant was identified as *Aegiceras corniculatum* by Prof. B. Kondala Rao of the Department of Marine Living Resources, Andhra University, Visakhapatnam. The voucher specimens (AU1-289) have been deposited at the museum of School of Chemistry, Andhra University, Visakhapatnam, and National Institute of Oceanography (NIO), Goa, India.

Extraction and isolation

The air dried and powdered plant material (2.5 Kg) was exhaustively extracted in a Soxhlet apparatus first with *n*-hexane for defatting and then with chloroform: methanol (1:1) mixture. Removal of solvent from the latter extract under reduced pressure gave a residue (30 g), which was repeatedly extracted with ethyl acetate (6 × 500 mL). Removal of solvent from the ethyl acetate extract under reduced pressure gave a residue (20 g) which was subjected to column chromatography over silica gel (Acme, 100-200 mesh, 200 g) by gradient elution with *n*-hexane - ethyl acetate mixtures and collecting 250 mL fractions. Monitoring was by silica gel TLC and the spots visualized under UV lamp (254 nm), exposure of TLC plates to iodine vapour or by spraying with H_2SO_4 in methanol (5%) reagent followed by heating at 110°C for five minutes. Eluates with identical spots on TLC were pooled together, to get five fractions. They were from the eluates using 2.5% ethyl acetate (Fractions 18-32, residue 1, 3.1 g), 5% ethyl acetate (33-53, 2, 1.45 g and 54-68, 3, 1.25 g), 10% ethyl acetate (69-88, 4, 1.9 g) and 20% ethyl acetate (89-110, 5, 1.5 g). Residue 1 on column chromatography over silica gel using *n*-hexane and ethyl acetate mixtures as eluent gave a mixture of **1** and **2**, which on re-chromatography over silica gel (Acme, 100-200 mesh) column furnished the pure compounds **1** (orange-yellow semisolid, 21 mg) and **2** (reddish brown crystals m.p. 140-42°C, 28 mg). The Residue 2, on re-chromatography over a small column of silica gel gave compound **3** as pure red coloured needles when crystallized from chloroform (m.p.142-44°C, 52 mg). The Residue 3 showed a single spot on TLC and on repeated crystallization using hexane gave **4** as orange coloured plates (m.p. 59-61°C, 15 mg). The Residue 4 also showed a single spot on TLC with moderate tailing. It was re-chromatographed over a small column of silica gel using *n*-hexane and acetone mixture to give orange coloured residue which on repeated crystallization using methanol gave orange coloured needles of **5** in high purity (m.p. 95-96°C, 80 mg). The Residue 5 also showed a single spot on TLC plate but with a little tailing. Re-chromatography over a small column of silica gel of this residue and using *n*-hexane and acetone as eluant afforded pure compound **6**, which was repeatedly crystallized using chloroform-methanol (1:1) to give **6** as colourless crystals (m.p. 178-80°C, 34 mg).

4-Hydroxy-2-methoxybenzamide, 1: Orange yellow micro needles, 21 mg, m.p.153-55°C. UV-Vis ($CHCl_3$): 284 nm; IR ($CHCl_3$): 3485.1, 1683.7, 1628, 1598.9, 1473.5, 1298, 1113 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.61 (1H, s, H-3), 6.99 (1H, d, J = 8.4Hz, H-6), 7.74 (1H, dd, J = 8.4Hz, H-5), 6.09 (2H, br s, 7-CONH_2), 3.98 (3H, s, 8-OCH_3); ^{13}C NMR ($CDCl_3$): δ 150.8 (C-4), 125.2 (C-3), 146.2 (C-2) 112.1 (C-1), 114.2 (C-6), 121.1 (C-5), 179.2 (C-7), 56.1 (C-8); EI-MS [$M^{+\cdot}$]: *m*/*z* 167 ($C_8H_9O_3N$).

2,5-Didehydroxy-6-methylembelin, 2: Reddish brown coloured microcrystalline powder, 28 mg, m.p.140-42°C. UV-Vis ($CHCl_3$): 295 nm (log ε, 3.4); IR (KBr): 3327, 1628, 1624, 1126 cm^{-1}; For ^1H and ^{13}C NMR data, see Table I; EI-MS [$M^{+\cdot}$]: *m*/*z* 276 ($C_{18}H_{28}O_2$).

Embelin, 3: Orange flakes, 150 mg, m.p.142-44°C. UV-Vis ($CHCl_3$): 230, 292 nm; IR ($CHCl_3$): 3307.7, 1614.3, 1541 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.70 (1H, br s, OH-2), 7.70 (1H, br s, OH-5), 6.02 (1H, s, H-6), 2.46 (2H, t, J = 7.65 Hz), 1.28 (16H, br s, CH_2-), 0.88 (3H, t, -CH_3); ^{13}C NMR ($CDCl_3$): δ 116.9 (C-3), 111.5 (C-6), 31.9 (C-1'), 29.6 (C-2'), 29.5 (C-3'), 29.5 (C-4'), 29.5 (C-5'), 29.5 (C-6'), 29.5 (C-7'), 27.9 (C-8'), 22.6 (C-9'), 22.4 (C-10'), 14.1 (C-11'); EI-MS [$M^{+\cdot}$]: *m*/*z* 294 ($C_{17}H_{26}O_4$).

5-O-Ethylembelin, 4: Orange plates, 15 mg, m.p.59-61°C. UV-Vis ($CHCl_3$): 290 nm; IR ($CHCl_3$): 3353, 1659 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.24 (1H, s, OH-2), 3.41(2H, q, 5-CH_3), 5.99 (1H, s, H-6), 2.22 (2H, t, J = 7.0 Hz, 7-CH_2), 1.58 (16H, s, CH_2-), 0.86 (3H, t, J = 7.0 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 183.0

(C-1), 151.4 (C-2), 119.2 (C-3), 181.8 (C-4), 160.3 (C-5), 102.4 (C-6), 65.8 (C-1'), 28.6 (C-2'), 28.0 (C-1"), 28.0 (C-2"), 28.0 (C-3"), 28.0 (C-4"), 28.0 (C-5"), 28.0 (C-6"), 28.0 (C-7"), 28.0 (C-8"), 22.7 (C-9"), 22.6 (C-10"), 14.1 (C-11"); EI-MS [$M^{+\cdot}$]: *m*/*z* 322 ($C_{19}H_{30}O_4$).

5-O-Methylembelin, 5: Orange crystals. 120 mg. m.p.95-96°C. UV-Vis ($CHCl_3$): 300 nm; IR ($CHCl_3$): 3352.1, 1598.9, 1201.6 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.28 (1H, s, OH-2), 3.87 (3H, s, 5-OCH_3), 5.68 (1H, s, H-6), 2.45 (2H, t, J = 9.0 Hz,), 1.27 (16H, br s, CH_2-), 0.89 (3H, t, J = 6.6 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 182.9 (C-1), 151.5 (C-2), 119.3 (C-3), 181.7 (C-4), 161.2 (C-5), 102.2 (C-6), 56.1 (C-1'), 31.9 (C-1"), 29.4 (C-2"), 29.4 (C-3"), 29.4 (C-4"), 29.4 (C-5"), 29.4 (C-6"), 29.4 (C-7"), 29.4 (C-8"), 22.7 (C-9"), 22.3 (C-10"), 14.1 (C-11"); EI-MS [$M^{+\cdot}$]: *m*/*z* 308 ($C_{18}H_{28}O_4$).

4-Methoxyresorcinol, 6: IR (KBr): 3446, 3389, 1615, 1253 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 9.1 (2H, s, OH-1, OH-5), 3.7 (3H, s, 4-OCH_3), 7.1 (3H, s, H-2, H-3, H-6); ^{13}C NMR (DMSO-d_6): δ 147.4 (C-5), 140.3 (C-4), 120.1 (C-3), 106.9 (C-2, C-6), 167.2 (C-1), 56.8 (C-7); EI-MS [$M^{+\cdot}$]: *m*/*z* 140 ($C_7H_8O_3$).

Acetylation of 5-O-methylembelin, 5: Compound **5** (10 mg) was acetylated with pyridine (one drop) and acetic anhydride (two drops). The reaction mixture was kept overnight at RT and usual work up followed by recrystallization yielded the acetate (Compound **7**, 5 mg, yellow coloured semi-solid). UV-Vis ($CHCl_3$): 233 nm (log ε 3.89), 285 (3.55); IR ($CHCl_3$): 1588 cm^{-1}; ^{1}H NMR: δ 2.39 (3H, s, 2-COCH_3), 2.45 (2H, t 4-CH_2), 3.89 (3H, s, 5-OCH_3), 5.95 (1H, s, H-6), 1.23-1.50 (20H, br s, 1'-10' (CH_2)$_{10}$), 0.90 (3H, t J = 6.6 Hz, 11'-CH_3); EI-MS [$M^{+\cdot}$]: *m*/*z* 350 ($C_{20}H_{30}O_5$).

Antimalarial activities

The antimalarial activity testing was carried out at the School of Pharmacy, University of Bradford, UK. Cultures containing predominantly early ring stages of *Plasmodium falciparum* were used for testing. Chloroquine diphospate was used as positive control and uninfected and infected erythrocytes without sample solutions were incubated in each test plate placed into a modular incubator gassed with 93% N_2, 3% O_2, and 4% CO_2 and incubated at 37°C for 48 h. Parasite growth was assessed as PLHD (parasite lactase dehydrogenase) activity as described[20]. The reagent used contained the following in each milliliter: acetylpyridine adenine dinucleotide (APAD), 0.74 mg; lithium lactate, 19.2 mg; diaphorase, 0.1 mg; Triton X-100, 2 µL; nitrobluetetrazolium, 1 mg and phenazine ethosulfate, 0.5 mg. Fifty microliters of this reagent was added to each well and mixed, and the plates were read at 550 nm and the percent inhibition of growth was calculated by comparison with control values. The IC_{50} values were determined using linear regression analysis. A minimum of three separate determinations were carried out for each sample.

Acknowledgements

Authors thank Prof. B. Kondala Rao, Department of Marine Living Resources, Andhra University, Visakhapatnam, India for identifying the plant. Financial assistance from the Ministry of Earth Sciences, New Delhi in the form of research project (YLN) and further financial assistance by CSIR, New Delhi (NSS), are gratefully acknowledged.

References

1 Miles D H, Kokpol U, Chittawong V, Tip-Payang S T P, Kwanjai T K & Nguyen C, *Pure Appl Chem*, 70 (1998) 2113.
2 Dodd R S, Fromard F, Rafii Z A & Blasco F, *Biochem Systemat Ecol*, 23 (1995) 859.
3 Li M S & Lee S Y, *Forest Ecol Management*, 96 (1997) 241.
4 Lin P & Fu Q, *Environmental Ecology and Economic Utilization of Mmangroves in China* (Higher Education Press, Beijing) (1995) 1-95.
5 Zhang D, Jun W, Si Z & Jianshe H, *Fitoterapia*, 76 (2005) 131.
6 Xu M, Deng Z, Li M, Li J, Fu H, Proksch P & Lin W, *J Nat Prod*, 67 (2004) 762.
7 Gomez E & De La Cruz-Giron O D L, *J Nat Prod*, 52 (1989) 649.
8 Hensens O D & Lewis K G, *Australian J Chem*, 19 (1966) 169.
9 Rao K V, *Tetrahedron*, 20 (1964) 973.
10 Rao K V & Bose P K, *Tetrahedron*, 18 (1962) 461.
11 Rao K V & Bose P K, *J Indian Chem Soc*, 36 (1959) 358.
12 Ponnapalli M G, Annam, S Ch V A R, Ravirala S, Sukki S, Ankireddy M & Tuniki V R, *J Nat Prod*, 75 (2012) 275.
13 Mahendran S, Badami S, Ravi S, Thippeswamy B S & Veerapur V P, *Chem Pharm Bull*, 59 (2011) 913.
14 Qin X D & Liu J K, *Helv Chim Acta*, 87 (2004) 2022.
15 Mossa J S, Muhammad I, Ramadan A F, Mirza H H, El-Feraly F S & Hufford C D, *Phytochemistry*, 50 (1999) 1063.
16 Abbas F A, Al-Massarany S M, Khan S, Al-Howiriny T A, Mossa J S & Abourashed E A, *Nat Prod Res*, 21 (2007) 383.
17 *Indian Herbal Pharmacopoeia* (Indian Drug Manufacturers Association, Mumbai) (2002) 206.
18 Nikolovska-Coleska C Z, Xu L, Hu Z, Tomita Y, Li P, Roller P P, Wang R, Fang X, Guo R, Zhang M, Lippman M E, Yang D & Wang S, *J Med Chem*, 47 (2004) 2430.
19 Reuter S, Prasad S, Phromnoi K, Kannappan R, Yadav V R & Aggarwal B B, *Mol Cancer Res*, 8 (2010) 1425.
20 Makler M T, Ries J M, Williams J A, Bancroft J E, Piper R C, Gibbins B L & Hinrichs D J, *Am J Trop Med Hyg*, 48 (1993) 739.