Triterpenoids and Steroids Isolated from Aerial Parts of *Glochidion multiloculare*

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Abstract. This study presents the chemical investigation of the leaves and stem barks of *Glochidion multiloculare* (Roxb. ex Willd.) Muell.-Arg., Phyllanthaceae. Classic phytochemical investigation of organic extracts of the aerial parts of *Glochidion multiloculare* together with spectroscopic methods led to the isolation and characterization of four triterpenes, namely; glochidonol (**1**), glochidiol (**2**), glochidone (**3**), lupeol (**4**) and two steroids, namely; daucosterol (**5**) and stigmasterol (**6**).

Keywords: triterpenes, glochidonol, glochidone, glochidiol, *multiloculare*, *Glochidion*

Introduction

Glochidion multiloculare (Roxb. ex Willd.) Muell.-Arg., Phyllanthaceae (synonym: *Phyllanthus multilocularis*) is an evergreen shrub or small tree; bark is brownish, peeling off in thin, papery transparent flakes, tuberculated or watery; leaves are stipulated, leaf blade is elliptic; flowers are small, greenish yellow, on short with stout pedicles. No ethnobotanical information is known for this plant. The plant is found in Bhutan, India, Myanmar, Nepal and Bangladesh. *Glochidion* was regarded as a genus of the family Euphorbiaceae, which consists of monoecious, rarely dioecious trees or shrubs. But molecular phylogenetic studies have shown that *Phyllanthus* is paraphyletic over *Glochidion*. A recent revision of the family Phyllanthaceae has subsumed *Glochidion* into *Phyllanthus* (Hoffmann *et al.,* 2006). Traditionally many *Phyllanthus* species are used in haemorrhoids, diarrhoea, dysentery, anaemia, jaundice, dyspepsia, insomnia etc. and some of them can induce diuresis (Ghani, 1998). In Chinese traditional medicine *Glochidion puberum* is used in dysentery, jaundice, leukorrhagia, common cold, sore throat, toothache, carbuncle, furuncle, rheumatic arthralgia (Fenglin *et al.,* 2004).

Biological investigations of *Phyllanthus* species revealed that many members of the genus possess anti-tumor promoting ability (Huang *et al.,* 2006; Tanaka *et al.,* 2004; Rajeshkumar *et al.,* 2004), apoptosis inducing ability (Puapairoj *et al.,* 2005; Huang *et al.,* 2004), antiviral activity against hepatitis B virus (Lam *et al.,* 2006; Venkateswaran *et al.,* 1987), anti-angiogenic effect (Huang *et al.,* 2006), analgesic effect (Santos *et al.,* 2000, 1994), diuretic effect (Srividya and Periwal, 1995), lipid lowering activity (Khanna *et al.,* 2002), hypocholesterolemic activity (Adeneye *et al.,* 2006), antioxidative effect (Sabir and Rocha, 2008; Harish and Shivanandappa, 2006; Raphael *et al.,* 2002;), antidiabetic effect (Adeneye *et al.,* 2006; Raphael *et al.,* 2002; Srividya and Periwal, 1995), antiherpetic activity (Álvarez *et al.,* 2009; Yang *et al.,* 2007), hepatoprotective effect (Sabir and Rocha, 2008; Harish and Shivanandappa, 2006), anti-inflammatory action (Kassuya *et al.,* 2006; Kiemer *et al.,* 2003), antiatherogenic effect (Duan *et al.,* 2005), anti-HIV activity (Notka *et al.,* 2004, 2003; Ogata *et al.,* 1992); antiplasmodial activity (Luyindula *et al.,* 2004), antibacterial activity (Meléndez and Capriles, 2006) and hypotensive activity (Leeya *et al.,* 2010; Srividya and Periwal, 1995) etc .

Several secondary metabolites have been isolated from different *Phyllanthus* species, including flavonoids, lignans, alkaloids, triterpenes, phenols and tannins (Chang *et al.,* 2003; Calixto *et al.,* 1998; Ishimaru *et al.,* 1992). Many secondary metabolites were isolated from *Glochidion* species, including tannins (Chen *et al.,* 1995), glycosides (Otsuka *et al.,* 2003), lignans (Otsuka *et al.,* 2000), terpenoids (Hui and Li, 1976). Previous

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investigation of *Glochidion multiloculare* revealed glochidiol, glochilocudiol, glochidone and dimedone (Talapatra *et al.,* 1973).

This study was taken to describe the chemical characterization of steroids and triterpenes obtained from *G. multiloculare* (synonym: *Phyllanthus multilocularis*) along with a small review regarding the importance of these compounds. This work supports the subsumption of *Glochidion* into *Phyllanthus*.

Materials and Methods

General experimental procedure. Silica-gel chromatography was performed using kieselgel 60 (mesh 70-230). Prep TLC: glass plates preparative with silica gel 60 PF_{254} (0.5 mm thickness, Merck); detection with vanillin spray. Gel permeation chromatography was performed using Sephadex LH-20. ^{1}H NMR spectra were acquired in $CDCl_3$ (δ values were reported in reference to $CHCl_3$ at 7.25 ppm) on a Bruker Avance 400 MHz Ultrashield NMR spectrometer equipped with broadband and selective (^{1}H and ^{13}C) inverse probes.

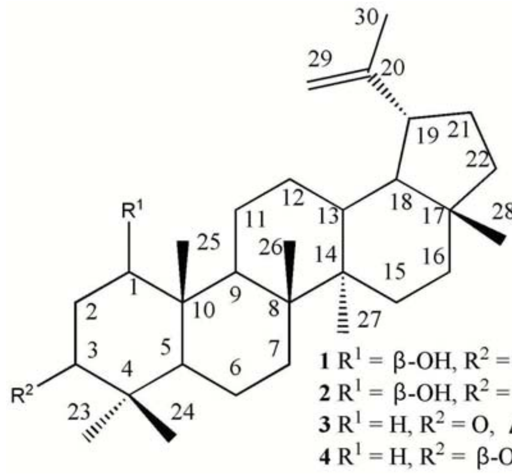
Plant material. *Glochidion multiloculare* (Roxb. ex Willd.) Muell.-Arg., Phyllanthaceae plant was collected from Rajendrapur, Gazipur, Bangladesh in the month of August, 2009, and was identified by the Bangladesh National Herbarium, Dhaka, Bangladesh. A voucher specimen (ACC. No. 34391) of the plant has been deposited in the Herbarium.

Extraction and isolation. The powdered leaves (1000 g) of *G. multiloculare* were soaked in methanol (3 L) for 15 days. Part of the residue (5 g), obtained from methanol extract on removal of the solvent at reduced pressure, was subjected to modified Kupchan partitioning (VanWagenen *et al.,* 1993) to obtain 1.2 g *n*-hexane soluble fraction; it was again subjected to gravity column chromatography (*n*-hexane, *n*-hexane–EtOAc, EtOAc and EtOAc-MeOH in increasing order of polarity). As a result, 167 fractions were obtained and combined on the basis of TLC. Here, fractions 67-76, 85-91 and 160-167 yielded compounds (**6**) (12.0 mg), (**1**) (3.0 mg) and (**5**) (5.0 mg) respectively.

The powdered barks (700 g) of *G. multiloculare* were soaked in methanol (3 L) for 15 days. Part of the residue (15 g) obtained from methanol extract on removal of the solvent at reduced pressure was subjected to gravity column chromatography (*n*-hexane, *n*-hexane-EtOAc, EtOAc and EtOAc-MeOH in increasing order of polarity). As a result, 111 fractions were obtained and combined on the basis of TLC. Fractions 45-47 and 58-59 afforded compounds (**3**) (6.0 mg) and (**4**) (5.8 mg), respectively. Fractions 108-111 were combined and subjected (380 mg) to Sephadex LH-20 chromatography using 2:5:1 *n*-Hexane/ CH_2Cl_2/ MeOH, 1:9, 1:1 MeOH/CH_2Cl_2 and finally 100% MeOH as mobile phases. As a result, 47 fractions were obtained and combined on the basis of TLC. Here, fractions 20-21 yielded 10.0 mg of compound (**2**).

Results and Discussion

Chemical study of the leaves and stem barks of *G. multiloculare* (Roxb. ex Willd.) Muell.-Arg., Phyllanthaceae led to the identification of four triterpenes, namely; glochidonol (**1**) glochidiol (**2**), glochidone (**3**) and lupeol (**4**) and two steroids, namely; daucosterol (**5**) and stigmasterol (**6**).



1 R^1 = β-OH, R^2 = O
2 R^1 = β-OH, R^2 = α-OH
3 R^1 = H, R^2 = O, Δ^{1-2}
4 R^1 = H, R^2 = β-OH

The ^{1}H NMR (400 MHz, $CHCl_3$-d_1) spectrum of compound (**1**) displayed methyl group resonances at δ 0.79, 0.83, 0.97, 1.03, 1.05, 1.05 and 1.69 for H_3-28, H_3-25, H_3-27, H_3-24, H_3-23, H_3-26 and H_3-30, respectively. The spectrum displayed two singlets at δ 4.68 and 4.56 (1H each) assignable to protons at C-29. A doublet of triplets at δ 2.38 (1H, *J* = 11.2, 6.0 Hz) was indicative of H-19. A multiplet at δ 3.89, integrating one proton, was indicative of H-1. Two doublets of doublets at δ 2.98 (1H, *J* = 14.4, 8.0 Hz) and 2.21 (1H, *J* = 14.4, 3.6 Hz) were assignable to H_{ax}-2 and H_{eq}-2, respectively. The spectral features of compound (**1**) were in close agreement to those observed for glochidonol (Hui and Li, 1976). This is the first report of this compound from *G. multiloculare*.

The ^{1}H NMR (400 MHz, $CHCl_3$-d_1) spectrum of compound (**2**) showed methyl group resonances at δ 0.74, 0.78, 0.90, 0.93, 0.94, 1.04 and 1.66 to indicate H_3-24, H_3-28, H_3-25, H_3-27, H_3-23, H_3-26 and H_3-30, respectively. The spectrum displayed two singlets at δ 4.67 and 4.54 (1H each) assignable to protons at

C-29. A doublet of triplets at δ 2.36 (1H, J = 11.2, 6.0 Hz) was indicative of proton at C-19. A triplet of doublets at δ 3.41 (1H, J = 11.2, 5.6 Hz) was assignable to proton at C-1. A broad doublet at δ 3.23 (1H, J = 11.6 Hz) was indicative of H-3. The spectral features of compound (**2**) were in close agreement to those observed for glochidiol (Srivastava and Kulshreshtha, 1988; Hui and Li, 1976). It is the second report of this compound from *G. multiloculare*.

The ^{1}H NMR (400 MHz, $CHCl_3$-d_1) spectrum of compound (**3**) displayed methyl group resonances at δ 0.80, 0.95, 1.06, 1.07, 1.10, 1.12 and 1.70 for H_3-28, H_3-27, H_3-23, H_3-24, H_3-26, H_3-25 and H_3-30, respectively. The spectrum displayed two broad singlets at δ 4.69 and 4.59 (1H each) assignable to protons at C-29. A doublet of triplets at δ 2.39 (J = 11.2, 5.6 Hz), integrating one proton, was indicative of H-19. Two doublets at δ 5.78 (1H, J = 10.0 Hz) and 7.09 (1H, J = 10.0 Hz) were assignable to H-2 and H-1, respectively (Neto *et al.,* 1995). The spectral features of compound (**3**) were in close agreement to those observed for glochidone (Hui and Li, 1976). This is the second report of glochidone from *G. multiloculare*.

The ^{1}H NMR spectrum (400 MHz, $CHCl_3$-d_1) of compound (**4**) showed seven singlets at δ 0.96, 0.78, 0.75, 1.02, 0.94, 0.82 and 1.67 (3H each) assignable to protons of methyl groups at C-4 (H_3-23, H_3-24), C-10 (H_3-25), C-8 (H_3-26), C-14 (H_3-27), C-17 (H_3-28) and C-20 (H_3-30), respectively. The spectrum displayed two singlets at δ 4.67 and δ 4.55 (1H each) assignable to protons at C-29. A one proton doublet of triplets at δ 2.36 (J = 11.2, 5.6 Hz) is indicative of H-19. The spectrum displayed one multiplet of one proton intensity at δ 3.19 typical for H-3. By comparing the ^{1}H NMR data of compound (**4**) with previously published data (Arataneche-muge *et al.,* 2004), it was confirmed as lupeol. It is the first report of lupeol from the plant.

Two steroids were afforded from the leaves of *G. multiloculare*, namely; daucosterol (**5**) (Voutquenne *et al.,* 1999) and stigmasterol (**6**) (Forgo and Kövér, 2004). This is the first report of these compounds from *G. multiloculare*.

This work supports the subsumption of *Glochidion* into *Phyllanthus* as the compounds reported here, especially glochidonol and glochidone, were also reported in many *Phyllanthus* species e.g., *P. caroliniensis*, *P. stipulates*, *P. urinaria*, *P. fraternus* (Catapan *et al.,* 2000) and *P. pulcher* (Bagalkotkar *et al.,* 2011).

Functions of sterols *in planta* are related to cell membrane structure and hormonal action. But these compounds exert various biological effects on other organisms. For example, daucosterol possesses immunoregulatory activity (Lee *et al.,* 2007). Stigmasterol inhibits several pro-inflammatory and matrix degradation mediators typically involved in osteoarthritis-induced cartilage degradation (Gabay *et al.,* 2010). It also shows thyroid inhibitory, antiperoxidative and hypoglycemic effects in mice (Panda *et al.,* 2009); reduces plasma cholesterol levels and inhibits hepatic synthesis and intestinal absorption of cholesterol in the rat (Batta *et al.,* 2006). Moreover, stigmasterol and β-sitosterol neutralizes viper and cobra venom (Gomes *et al.,* 2007).

Glochidonol and glochidiol show strong antiproliferative activity against three human tumor cell lines, MCF-7, NCI-H-460 and SF-268, through the involvement of apoptosis (Puapairoj *et al.,* 2005). Glochidone shows pronounced antinociceptive properties in mice (Krogh *et al.,* 1999). Lupeol is reported to exhibit a spectrum of pharmacological activities against various disease conditions such as inflammation, arthritis, diabetes, cardiovascular ailments, renal disorder, hepatic toxicity, microbial infections and cancer (Siddique and Saleem, 2011; Saleem, 2009).

Compounds isolated from this plant have already gained the attention of medical professionals, researchers and pharmaceutical industries for their wide ranging crucial pharmacological activities. New sources of the isolated compounds and of their precursors have been sought to account for the increased demand of these compounds and of their analogs of medicinal value.

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