Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 7 (2): 125-129, 2013
ISSN 1819-3455 / DOI: 10.3923/rjmp.2013.125.129

Steroids and Triterpenoids from *Corypha taliera* Roxb: A Critically Endangered Palm Species of Bangladesh

[1]Akhtaruzzaman Chowdhury, [1]Md. Ashraful Alam, [2]Ridwan Bin Rashid, [3]Muhammad Abdullah Al-Mansur, [4]Mohammad S. Rahman and [4]Mohammad A. Rashid

[1]Department of Chemistry, Rajshahi University of Engineering and Technology, Rajshahi, Bangladesh
[2]Department of Microbiology, University of Dhaka, Dhaka-1000, Bangladesh
[3]Bangladesh Council of Scientific and Industrial Research, Dr. Qudrat-I-Khuda Road, Dhanmondi, Dhaka-1205, Bangladesh
[4]Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

Corresponding Author: Mohammad A. Rashid, Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh Tel: +880-2-9661900

ABSTRACT

Corypha taliera Roxb. is a critically endangered palm species. The tree grown in the campus of the University of Dhaka, Bangladesh used to be considered as the only living species in the world. This palm tree raised some interests due to several bioactivities observed earlier and later systematic scientific studies have been undertaken to explore for its secondary metabolites. In the present study, a thorough phytochemical investigation was conducted to explore its chemical features. Initially, the unripe fruits of this palm was extracted with methanol. Later on, it was fractioned by the modified Kupchan partitioning method in to n-hexane, carbontetrachloride, chloroform and aqueous soluble fractions. The n-hexane soluble fraction was subjected to Column Chromatography (CC) over Sephadex LH-20 and the column was eluted with n-hexane-dichloromethane-methanol (2:5:1) mixtures. From the collected fractions stigmasterol (1), β-sitosterol (2), β-amyrin (3), lupeol (4) and betulinic acid (5) were isolated by Preparative Thin Layer Chromatography (PTLC). The structures of these purified compounds were established by extensive spectroscopic analysis and by comparison of their spectral data with published values as well as co-TLC with authentic samples. This is the first report of isolation of steroids and terpenoids from this plant.

Key words: *Corypha taliera*, stigmasterol, β-sitosterol, β-amyrin, lupeol, betulinic acid

INTRODUCTION

Palm trees are renowned for their contribution to the human race. They can provide food and arrange landscaping for pleasing appearance. Medicinal scientists also put some light on their chemical and biological aspects for treating various pathological states (Asase *et al*., 2010; De Smet, 2002; Agbabiaka *et al*., 2009). Many palm species are now in a very challenging and endangered condition for their existence. In Bangladesh, *C. taliera* is considered as a very rare species and the only one found in the campus of University of Dhaka, Bangladesh. The tree was first discovered in 1919 in the then Bengal. The only record of *C. taliera* was reported in the University of Dhaka, Bangladesh. In the absence of any other record, the solitary tree was considered the last living species in the World (Johnson, 1998; Chowdhury *et al*., 2010).

C. taliera (Bengali name-tali palm; Family:Palmae) is a giant monocarpic palm that grows very slowly upto 10 m in height and 70 cm in diameter near the base. It is thought to be distributed in Bangladesh, India and throughout the tropical Asia and takes close to a century for flower to blossom, which will ultimately lead to gradual death. As a part of the continuous studies on medicinal plants of Bangladesh (Begum *et al*., 2010; Rahman *et al*., 2011), the preliminary antimicrobial, antioxidant and cytotoxic activities of the unripe fruits of this palm was reported earlier (Chowdhury *et al*., 2010). No other biological or chemical work has been conducted on this plant. In the present manuscript, the isolation stigmasterol (1), β-sitosterol (2), β-amyrin (3), lupeol (4) and betulinic acid (5) was reported for the first time from this palm species.

MATERIALS AND METHODS

General experimental procedures: Gel permeation chromatography was performed on Sephadex LH-20 while column chromatographic separation was achieved over silica gel (mesh 70-230). The ^{1}H NMR spectra were recorded using a Bruker AMX-400 (400 MHZ) instrument in $CDCl_3$ and the δ-values for ^{1}H and ^{13}C data were referenced to the residual non-deuterated solvent signals. All solvents were of analytical grade.

Collection of plant materials: The fruits of *C. taliera* were collected from the campus of the University of Dhaka, Bangladesh in the month of April, 2009. A voucher specimen (accession No. DACB-34180) for this collection has been deposited in Bangladesh National Herbarium, Dhaka.

Extraction and isolation of compounds: The unripe sun dried and powdered fruits (500 g) of *C. taliera* was soaked in 1.5 L methanol for 10 days with occasional shaking and stirring and filtered through a cotton plug followed by Whatman filter paper No. 1. The extract was then concentrated by using a rotary evaporator at reduced temperature and pressure. A portion (5 g) of the concentrated methanol extract was fractioned by the modified Kupchan partitioning method (VanWagenen *et al*., 1993; Anjum *et al*., 2013). Evaporation of solvents yielded n-hexane (1.25 g), carbon tetrachloride (0.55 g), dichloromethane (0.60 g) and aqueous soluble (2.00 g) materials. An aliquot (1.10 g) of the n-hexane soluble fraction was subjected to Column Chromatography (CC) over Sephadex LH-20 and the column was eluted with n-hexane-dichloromethane-methanol (2:5:1) mixtures to give a total of 40 fractions, each 5 mL.

Compound 1 was obtained as colorless crystalline mass from fractions 6 and 7. Compound 2 was isolated as white amorphous powder from fraction 9 by preparative thin layer chromatography (PTLC, stationary phase silica gel F_{254}, mobile phase-10% ethyl acetate in toluene, thickness of plates-0.5 mm). Similar PTLC of fractions 10-12 and 17 over silica gel F_{254} using 15% and 20% ethyl acetate in toluene afforded compound 3 (10 mg) and 4 (8 mg), respectively as white amorphous mass. On the other hand, compound 5 was isolated as white gum from fractions 21-22 by PTLC over silica gel (F_{254}) using 30% ethyl acetate in toluene as the developing solvent.

RESULTS AND DISCUSSION

The ^{1}H NMR spectra of compounds 1 and 2 (Table 1, Fig. 1a) readily demonstrated the steroidal nature of these compounds and were superimposable to the ^{1}H NMR spectra previously recorded for stigmasterol and β-sitosterol (Jahan *et al*., 2010) from the same laboratory. The identity of the compounds was further confirmed by co-TLC with authentic samples.

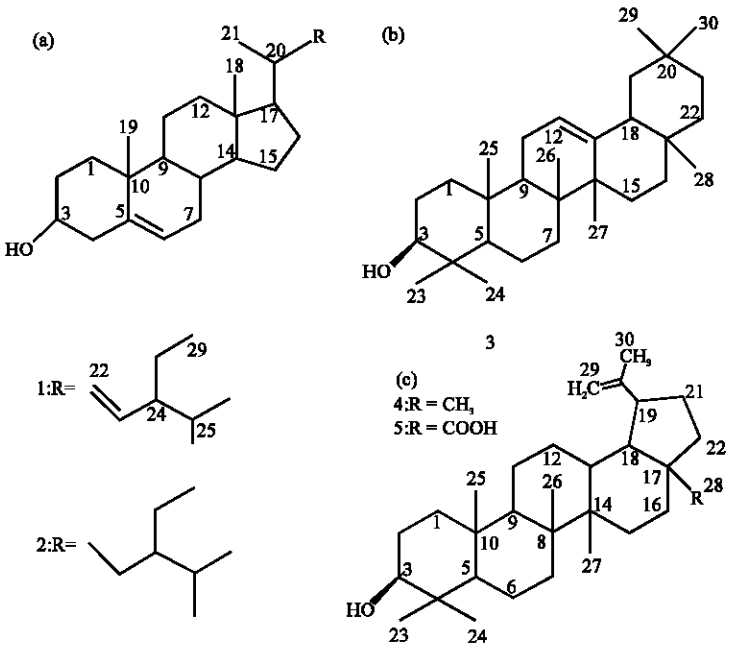

Fig. 1(a-c): Structure of the isolated compounds (a) 1 and 2 represent stigmasterol and β-sitosterol, respectively, (b) 3 represents α-amyrin and (c) 4 and 5 represent lupeol and betulinic acid, respectively

Table 1: ^{1}H NMR (400 MHZ, $CDCl_3$) data of compounds 1-5

	δ (ppm), mult, J in Hz				
Proton position	Compound 1 (Stigmasterol)	Compound 2 (β-sitosterol)	Compound 3 (α-amyrin)	Compound 4 (Luepol)	Compound 5 (Betulinic acid)
H-3	3.51, m	3.50, m	3.23, dd (11.0, 5.0)	3.20, dd (11.5, 5.02)	3.17, dd (11.2, 5.2)
H-6	5.35, m	5.34, m (7.0)			
H-12	0.67, s		5.12, t (3.7)		
H-18	1.00, s	0.67, s			
H-19	2.28, m	1.00, s		2.28, m	2.85, m
H-21	0.92, d (6.0)	0.91, d (6.4)			
H-22	5.14, dd (15.0, 6.5)				
H-23 or H_3-23	5.04, dd (15.0, 6.5)		0.87, s	0.95, s	0.93, s
					0.81, s
H_3-24			0.79, s	0.78, s	0.74, s
H_3-25			0.85, s	0.84, s	
H_3-26	0.84, d (6.0)	0.83, d (6.0)	1.04, s	1.02, s	0.96, s
H_3-27	0.82, d (6.0)	0.81, d (6.0)	1.07, s	0.93, s	0.97, s
H_3-28			0.95, s	0.82, s	
H_3-29	0.82, t (6.5)	0.85, d (6.0)	1.00, s	4.67, br. s	4.73, s
				4.55, br. s	4.60, br. s
H_3-30			1.00, s	1.67, s	1.68, s

δ (ppm): Chemical shift expressed in parts per million, J in Hz: Coupling constant expressed in Hertz, mult: Multiplet, s: Singlet, br.s: Broad singlet, d: Doublet, dd: Double doublet, t: Triplet

The ^{1}H NMR spectrum (400 MHZ, $CDCl_3$) of compound 3 (Table 1, Fig. 1b) revealed eight singlets at δ 0.95 (H_3-28), 1.00 (H_3-29, H_3-30), 0.79 (H_3-24), 0.87 (H_3-23), 0.85 (H_3-25), 1.04 (H_3-26) and 1.07 (H_3-27). Integration of the singlets demonstrated the intensity for three protons. It suggested the presence of eight methyl groups in the molecule. It also revealed a triplet (J = 3.2 Hz) of one proton intensity centered at δ 5.12, which could be assigned to the olefinic proton (H-12). The double doublet (J = 11.0, 5.0 Hz) centered at δ 3.23 could be ascribed to the oxymethine proton at C-3. The splitting pattern of this proton suggested the beta orientation of the hydroxyl group. The above spectral features are in close agreement to those observed for α-amyrin (Parvin *et al*., 2009). Again the identity of this compound was substantiated by co-TLC with authentic sample.

The ^{1}H NMR spectrum (400 MHZ, $CDCl_3$) of compound 4 (Table 1, Fig. 1c) showed a double doublet (J = 11.5, 5.02 Hz) of one proton intensity at δ 3.20 typical for H-3 of a triterpene type carbon skeleton. The broad singlets at δ 4.67 and 4.55 (1H each) were assigned to the vinylic protons at C-29. A multiplet of one proton intensity at δ 2.28 was attributed to H-19. The spectrum also displayed six singlets at δ 0.78, 0.82, 0.84, 0.93, 0.95, 1.02 of three proton intensity. A broad singlet of three proton was also appeared at δ 1.67. All of these resonances are assignable to the methyl group protons at C-4 (H_3-24), C-17 (H_3-28), C-10 (H_3-25), C-14 (H_3-27), C-4 (H_3-23), C-8 (H_3-26) and C-20 (H_3-30), respectively. By comparing these with published ^{1}H NMR data for lupeol as well as by co-TLC with authentic sample established its identity as lupeol (4) (Sultana *et al*., 2010).

The ^{1}H NMR spectrum of compound 5 (Table 1, Fig. 1c) revealed the presence of a lupene type carbon skeleton. It displayed signals attributable to an exomethylene group at δ 4.60 and 4.73 (1H, each, br. s), which together with an allylic methyl at δ 1.68 indicated an isopropenyl functionality. The double doublet centered at δ 3.17 could be assigned to H-3. The large couplings (11.2 and 5.2 Hz) of this H-3 with the vicinal methylene protons suggested the β (beta) orientation of the hydroxyl group at C-3. In addition, the spectrum also showed a multiplet at δ 2.85 for the methine proton at C-19 and five methyl group resonances as singlets at 0.74 (H_3-24), 0.81, 0.93 (H_3-23), 0.96 (H_3-26) and 0.97 (H_3-27). On this basis, compound 5 (Fig. 1) was characterized as betulinic acid. The identity of 5 as betulinic acid was confirmed by comparison with published values (Parvin *et al*., 2009) as well as by co-TLC with an authentic sample.

The present study explored the presence of steroids and triterpenes from *C. taliera*. Among the isolated compounds, betulinic acid and lupeol have previously been reported for anticancer activity (Patocka, 2003; Saleem, 2009). It might have connection with the cytotoxicity exhibited in brine shrimp lethality bioassay by *C. taliera* fruits (Chowdhury *et al*., 2010). Preliminary TLC screenings revealed that the extractives of *C. taliera* fruits might have more bioactive triterpenes. Further bioactivity guided isolation is required to identify the bioactive principles.

CONCLUSION

The unripe fruits of *C. taliera* were subjected to phytochemical investigation, which yielded steroids and triterpenes stigmasterol (1), β-sitosterol (2), β-amyrin (3), lupeol (4) and betulinic acid (5) for the first time from this palm tree. Among these lupeol and betulinic acid are renowned for having anticancer activities. More extensive phytochemical and biological investigations are required to isolate the potential bioactive components from this plant species.

REFERENCES

Agbabiaka, T.B., M.H. Pittler, B. Wider and E. Ernst, 2009. *Serenoa repens* (saw palmetto): A systematic review of adverse events. Drug Saf., 32: 637-647.

Anjum, A., M.A. Sikder, M.R. Haque, C.M. Hasan and M.A. Rashid, 2013. *In vitro* antioxidant and thrombolytic activities of *Bridelia* species growing in Bangladesh. J. Sci. Res., 5: 343-351.

Asase, A., G.A. Akwetey and D.G. Achel, 2010. Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West District of Ghana. J. Ethnopharmacol., 129: 367-376.

Begum, R., M.S. Rahman, S. Chowdhury, M.M. Rahman, S. Gibbons and M.A. Rashid, 2010. A new 7-oxygenated coumarin from *Clausena suffruticosa*. Fitoterapia, 81: 656-658.

Chowdhury, A., M.A. Alam, M.S. Rahman, M.A. Hassan and M.A. Rashid, 2010. Antioxidant, antimicrobial and cytotoxic activities of *Corypha taliera* Roxb. Latin Am. J. Pharm., 29: 1231-1234.

De Smet, P.A.G.M., 2002. Herbal remedies. N. Engl. J. Med., 347: 2046-2056.

Jahan, I., M.S. Rahman, M.Z. Rahman, M.A. Kaisar, M.S. Islam, A. Wahab and M.A. Rashid, 2010. Chemical and biological investigations of *Delonix regia* (Bojer ex Hook.) Raf. Acta Pharm., 60: 207-215.

Johnson, D., 1998. *Corypha taliera*. IUCN Red List of Threatened Species. http://www.iucnredlist.org/details/38493/0

Parvin, M.N., M.S. Rahman, M.S. Islam and M.A. Rashid, 2009. Chemical and biological investigations of *Dillenia indica* Linn. Bangledesh J. Pharmacol., 4: 122-125.

Patocka, J., 2003. Biologically active pentacyclic triterpenes and their current medicine signification. J. Applied Biomed., 1: 7-12.

Rahman, M.S., M.Z. Rahman, B. Begum, R. Chowdhury, S.N. Islam and M.A. Rashid, 2011. Antidiabetic principle from *Eclipta prostrata*. Latin Am. J. Pharm., 30: 1656-1660.

Saleem, M., 2009. Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. Cancer Lett., 285: 109-115.

Sultana, S., M.S. Rahman, M.A. Hossain, M.K. Hossain and M.A. Rashid, 2010. Phytochemical and biological investigations of *Ixora lutea* Hutch. Dhaka Univ. J. Pharm. Sci., 8: 17-21.

VanWagenen, B.C., R. Larsen, J.H. Cardellina II, D. Randazzo, Z.C. Lidert and C. Swithenbank 1993. Ulosantoin, a potent insecticide from the sponge Ulosaruetzleri. J. Org. Chem., 58: 335-337.