

CHEMICAL INVESTIGATION OF *CORYPHA TALIERA* ROXB.

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Abstract

β -sitosterol was isolated by silica gel column chromatography from the dichloromethane soluble extract of pericarp of *Corypha taliera* Roxb. (Talipalm), a rare species of Arecaceae family. The compound was characterized by IR, ¹H and ¹³C NMR spectroscopic studies. Fatty acid compositions of oil of the pericarp was determined as their methyl esters and identified by gas chromatography having flame ionization and the gas chromatograph was coupled to mass spectroscopic detectors. Myristic, palmitic, oleic, linoleic, stearic, arachidic and lignoceric acids were found to be present in the oil. The palmitic acid was predominant (50.75%) in the oil. The relative percentage of unsaturated acid i.e., linoleic and oleic acids was found to be 15.58 and 20.89%, respectively.

Introduction

Corypha taliera Roxb. belongs to the family Arecaceae. William Roxburgh discovered the plant in 1819 and reported it to be endemic to Bengal (Roxburgh 1820, Roxburgh 1832, Basu 1991). There are four species of the genus *Corypha*, namely *Corypha taliera*, *C. unbraculifera*, *C. elata* and *C. macropoda* available in the Indian subcontinent. *C. taliera* locally known as Talipalm, is a globally endangered species (Khondker *et al.* 2010). The existence of *C. taliera* in Bangladesh was identified by late Prof. Salar Khan in 1950 in the Dhaka University Campus, Dhaka (Khondker *et al.* 2010). This palm grows for ca. 80 years without producing flower. There is no information on the phytochemical work with this plant. However, literature survey showed that flavonoid, flavonoid glycosides, phenolics and carotenoids are present in plants of Arecaceae family (Kang *et al.* 2010). Some species of *Corypha* are also traditionally used for bowel complaints, diarrhoea and cough (Gunasekaran and Balasubramanian 2012). Palm trees are also well-known as one of the sources of vegetable oil. In the present study, we report for the first time the fatty acid composition of the oil and isolation of β -sitosterol from *C. taliera*.

Materials and Methods

Instruments: IR spectrum was recorded on Shimadzu IR-470 spectrometer. The ¹H and ¹³C NMR spectra were recorded on a Bruker 400 MHz spectrometer using tetramethylsilane (TMS) as the internal reference. Gas chromatographic (GC) analyses were performed on a Shimadzu 2025 GC connected with a flame ionization detector (FID). Nitrogen and hydrogen and air were used as carrier gas and flame, respectively. Separations were performed on HP-5 capillary WCOT quartz columns (30 m long and 0.25 inner diameter; film thickness was 0.25 μ m). Column flow rate was 2 ml/min and split ratio was 1 : 79. The column oven temperature was programmed for analysis: 120°C (1 min hold time) to 270°C (6 min) and 7°C rise per min. Injector and detector temperature were set 280 and 290°C, respectively. Gas chromatographic and mass spectrometric analyses were carried out on an Agilent Mass Spectrometer (Model No. 6890 N) coupled with a Gas Chromatograph (Model No. 5975 B). Separations were performed on a HP-5 MS 5% phenyl methyl siloxane column (30 m, i.d.: 0.25 mm, film thickness: 0.25 microns) at temperature 120°C

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(1 min) in a rate 7°C rise min to 270°C (6 min). Injector temperature was fixed at 280°C and compounds were detected in scan the mode.

Collection of plant material: The fruits of *C. taliera* were collected from the lone individual existed in the campus of the University of Dhaka (Khondker *et al.* 2010). The pericarp were separated from the seeds of dried fruits and made into powder.

Extraction: Pericarp powder (25 g) was successively extracted with *n*-hexane (100 ml × 3; 24 h), dichloromethane (DCM, 100 ml × 3; 24 hr) and methanol (MeOH, 100 ml × 3). The extracts were collected by suction filtration from the residual pericarp and were evaporated to dryness by rotary vacuum evaporator followed by a freeze-dryer. Thus, *n*-hexane (2.0 g), DCM (2.5 g) and MeOH (2.0 g) extracts were obtained. The present studies were done with *n*-hexane and dichloromethane extracts.

Isolation of compound: The DCM extract was fractionated by silica gel column chromatography. A glass column was made with the slurry of silica gel 60 (0.063 - 0.200 mm) and the column was equilibrated with *n*-hexane. The DCM extract was passed through the column and the column was eluted with 0 to 100% DCM in hexane followed by 2 to 10% MeOH in DCM and finally eluted with 50% MeOH in DCM. All fractions were collected in conical flasks and monitored by TLC. On the basis of the pattern of TLC, eight fractions were made. The fraction obtained from 100% DCM gave a single spot on TLC and was purified by washing with *n*-hexane several times to get compound **1** (10 mg) as white needles.

Properties of compound 1: White needles (10 mg), R_f value 0.80 (100% DCM), FT-IR (KBr pellets): ν_{max} 3432, 2928, 2858, 1639, 1055 cm^{-1} .

1H NMR ($CDCl_3$): δ 5.34 (s, 1H), 3.51 (m, 1H), 2.27 (m, 2H), 1.99 (m, 2H), 1.48, 1.24 (m), 1.00 (s, 3H), 1.82 (m, 2H), 0.67 (s, 3H), 0.85 (t, $J=7.2$ Hz, 3H), 0.84, 0.82, 0.92 (each d, $J=6.4$ Hz, $3H \times 3$).

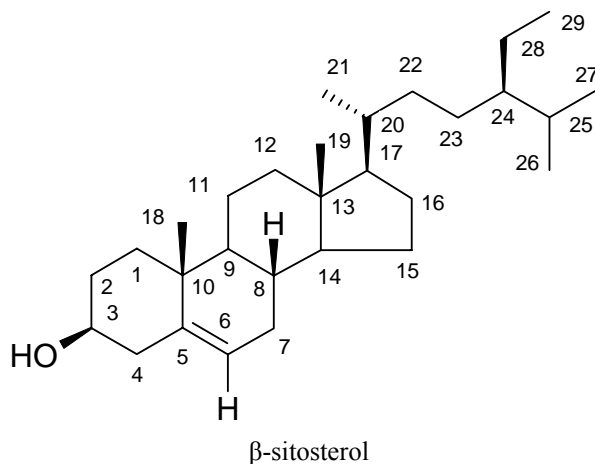
^{13}C NMR ($CDCl_3$): δ 140.8, 121.7, 71.9, 56.8, 56.1, 50.2, 45.9, 42.6, 43.3, 39.8, 37.3, 36.5, 36.2, 34.0, 31.9, 31.8, 31.7, 29.2, 28.3, 26.2, 24.3, 23.1, 21.1, 19.8, 19.4, 19.1, 18.8, 12.0, 11.9.

Analysis for fatty acid composition: Hexane extract (100 mg) was saponified with 1.0 ml of 0.5 M methanolic NaOH solution in a boiling water bath at about 96°C for 3 hours and evaporated to dryness. The dried material was dissolved in water (~ 1.0 ml) acidified with a few drops 2M HCl and partitioned with *n*-Hexane. The hexane extract (free fatty acids) was evaporated to dryness under blowing nitrogen. Borontrifluoride-methanol (BF_3 -MeOH, 1 ml) complex was added to the dried free fatty acids, ultrasonicated (vortexed 30 sec.), heated in a boiling water bath for 30 min and evaporated into dryness. *n*-hexane (~ 1 ml) was added to the dried methyl esters, filtered through Pasteur pipette containing cotton, transferred to a GC-MS vial and analyzed by GC-FID and GC-MS. Certified methyl ester of fatty acids purchased from Sigma-Aldrich were used as reference samples for GC-FID.

Results and Discussion

Silica gel column chromatography of the DCM extract of *C. taliera* afforded a pure compound (**1**). The 1H NMR spectrum of **1** gave signals at δ 5.34 (s, 1H), 3.51(m, 1H) which were characteristics of a steroidal nucleus (Greca *et al.* 1990). The signals at 1.00 (s, 3H), 0.92 (d, 6.4 Hz, 3H), 0.85 (t, 7.2 Hz, 3H), 0.84, 0.82 (each d, 6.4 Hz, $3H \times 2$) and 0.67 (s, 3H) ppm in the 1H NMR spectrum indicated the presence of 6-methyl groups. The signals at 2.24 - 1.48 ppm were for the presence of methylene groups. The ^{13}C NMR spectral data revealed the presence of 29 carbon atoms; two quaternary carbons (δ_c 42.6 and 36.5 ppm), two olefinic (δ_c 140.8 and 121.7 ppm) including a quaternary carbon, eight methine (δ_c 71.9, 56.8, 56.1, 50.2, 45.9, 36.2, 31.9 and 29.2 ppm), 11 methylene (δ_c 42.3, 39.8, 37.3, 34.0, 31.9, 31.7, 28.3, 26.2, 24.3, 21.1 and 23.1

ppm) and six methyl groups (δ_c 19.8, 19.4, 19.1, 18.8, 12.0 and 11.9 ppm) were present. The signal at δ_c 71.9 was due to the attachment of a hydroxyl ($-OH$) group. 1H and ^{13}C NMR spectral data of **1** were compared with reported NMR data of steroidal compounds (Greca *et al.* 1990) and were found to be consistent with β -sitosterol. Thus, **1** was confirmed as β -sitosterol. The isolation of β -sitosterol from *C. taliera* is reported here for the first time.



The fatty acids in hexane extract were made into their methyl ester by saponification with methanolic NaOH followed by esterification with BF_3 -MeOH complex, and analyzed by GC-FID. The relative percentage of methyl ester of fatty acids in oil was identified by comparing their retention time with that of methyl ester of standard fatty acids. It was found that myristic, palmitic, oleic, linoleic, stearic, arachidic and lignoceric acid were found in the hexane extract of pericarp of *C. taliera*. All fatty acids were confirmed by GC-MS. The methyl ester of palmitic acid gave m/z at 270 while linoleic acid had m/z at 295. The palmitic acid was predominant (50.75%) in the oil. The relative percentage of unsaturated acid i.e., linoleic acid and oleic acid were found to be 15.58 and 20.89%, respectively. Unsaturated fatty acids are good for health. If *C. taliera* is propagated in a large scale, there is a good possibility to get oil from the seeds of the plant which is rich in unsaturated fatty acids.

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