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A new cyclopentane derivative from *Ficus pomifera* Wall. and *Curcuma leucorrhiza* Roxb.

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Two new compounds **1** and **2** have been isolated along with a known compound **3** from both *Ficus pomifera* Wall and *Curcuma leucorrhiza* Roxb. The compound **1** is a cyclopentane derivative, 5-(3,4,5,6-tetrahydroxyhexan-2-ylidene)cyclopentane-1,2,3,4-tetraol; compound **2** is a natural polymer compound and compound **3** is β-sitosterol glucoside. The similarity of compound **1** has been studied by Reversed Phase-High Performance Liquid Chromatography (RP-HPLC). The compounds **2** and **3** have further been identified by comparison with spectral data. The compound **1** has been tested for anti-microbial and antioxidant activities. The isolation of compound **1**, **2** and **3** have been reported from different plants of different families-Moraceae and Zingiberaceae for the first time.

Keywords: Ficus, Curcuma, RP-HPLC, antioxidant, antimicrobial activity

The plants of the genus *Ficus* (Moraceae) which consist of 800 species are used all over the world traditionally as herbal medicine[1,2]. Trace elements and minerals in *F. microcarpa*, *F. lacor*, *F. palmata*, *F. rligiosa L.*, *F. nerrifolia*, *F. auriculata* Wall., *F. benjamina*, *F. racemosa* Roxb., *F. bengalensis*, *F. johannis*, *F. sarmentosa*, *F. hispida* and *F. pomifera* were studied[3,4]. The barks of the plants *F. hispida* and *F. racemosa* had antidiabetic effect[5,6]. The ethanolic extracts of *F. vogelli* leaves were studied and found to have anti-hepatotoxic effect in liver damaged rats[7]. The GC-MS and GC-FTIR study of hexane extract of *F. bengalensis* showed hydrocarbons[8]. A glycoside of leucopelargonidin was isolated from *F. bengalensis* which had hypoglycemic effect[9]. The plants, *F. microcarpa*, *F. glomerata*, *F. bengalensis* Linn., *F. pumila*, *F. carica* and *F. racemosa* had good antioxidant properties[10]. The ethanol extract of stem bark of *F. religiosa* had antiulcer potential[11]. *F. platyphylla* contained triterpenes, steroids and tannins. *F. platyphylla* showed anticancer property without non-toxic character which is rarely the case[12]. *Curcuma* (Zingiberaceae) is a genus comprising of about 80 species distributed in most temperate parts of India, and in tropical and subtropical regions of Asia. The rhizomes of these plants are used in traditional medicines[13,14]. Essential oil from rhizomes is antiseptic, antacid, antifungal and carminative[15]. It has been found to have antioxidant properties[16-19]. Antifungal, antibacterial and anti-inflammatory activities have been reported for species, such as *C. longa*[20-22], *C. amada*[23], *C. malabarica*[24] and *C. zedoaria*[24,25]. The rhizomes of *C. leucorrhiza* Roxb. are used in Shingbhum, Bihar, India for enlarged liver and spleen, and ulcer in stomach[26]. The antioxidant and antimicrobial activities of different solvent extracts of the rhizome of this plant against four human pathogenic bacterial strains and four plant pathogenic fungal strains were reported[27]. The essential oils obtained by hydro distillation from the leaves and rhizomes of *C. leucorrhiza* were mainly germacrone (9.6–19.7%), curdione (19.1–19.5%), camphor (7.2–8.1%), 1,8-cineole (4.0–7.4%), curzerene (3.0–5.7%), linalool (5.2–5.4%), *neo*-curdione (2.8–4.6%) and isoborneol (2.0–3.8%) and showed antimicrobial activity[28]. Herein, we report three compounds **1**, **2** and **3** (Figure 1) isolated from both plants − *C. leucorrhiza* and *F. pomifera*. The compound **1** has been tested for anti-microbial and antioxidant activities.

Materials and Methods

The leaves and young buds of *F. pomifera* Wall. were collected from the northeastern part of Manipur, India. The rhizomes of *C. leucorrhiza* Roxb. were collected from Imphal West and Thoubal districts of Manipur, India. A voucher specimen (000206) of *F. pomifera* Wall. and a voucher specimen (000815) of *C. leucorrhiza* Roxb. were deposited in Herbarium Museum, Department of Life Sciences, Manipur University, Manipur, India. The leaves of *F. pomifera* and rhizomes of *C. leucorrhiza* were cleaned and air dried.

Preparation of plant extracts

The dried leaves of *F. pomifera* (1.5 kg) were ground to powder form and extracted in a Soxhlet apparatus using different solvents: petroleum ether, chloroform and methanol. The extracts were concentrated under vacuum using Buchi Rotavapor. Compound **1** was isolated from the methanol extract by treating with *n*-butanol and then, *n*-butanol soluble part was removed. The residue contained three components − a sandy compound which decomposed into black paste, a white crystal and a syrupy compound, **1**. The compound was also isolated from water extract.

The rhizomes of *C. leucorrhiza* were collected and washed with water, chopped into slices, dried in the shade and powdered. The powdered rhizomes (2.5 kg) were subjected to extraction in the same way as cited above with various organic solvents and water. The water extract was further separated using a separating funnel. The compound **1** was isolated from the chloroform insoluble part. Thus, the same compound was isolated from two different plants, from the leaves of *Ficus pomifera* and from the rhizomes of *Curcuma leucorrhiza*.

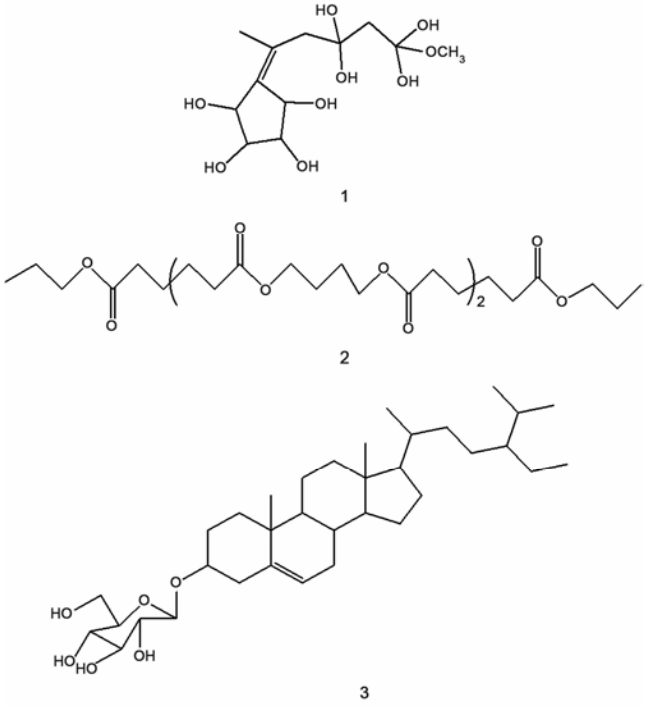


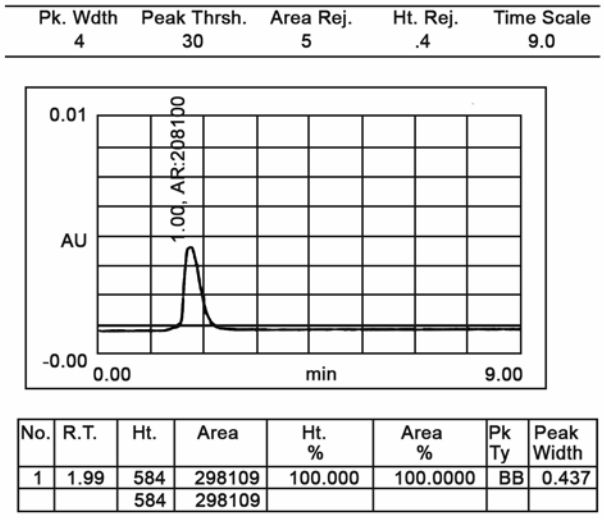
Figure 1 — Chemical structure of compounds **1-3**

Similarity study of the compound 1 by RP-HPLC

The liquid compound was isolated and purified by repeated filtration. The purity of the compound was checked with RP-HPLC. The compound was dissolved in the mobile phase acetonitrile : water (75:25, v/v) and was injected in RP-HPLC. The wavelength of the UV-Vis detector was adjusted at 254 nm. The experiment was carried out at RT and the HPLC column used was RP-C-18 Lichocart (Licrospher Merck). The chromatogram showed only one peak (Figure 2). The compound from the two different samples was injected separately in RP-HPLC. The retention time was found to be same. When the compound from the two plant samples was mixed, the peak was spiked and peak area was found to be increased.

Seperation of enantiomers of compound 1

The separation of the optically active compound **1** was carried out with Chiral-HPLC (Figure 2). The compound was dissolved in the mobile phase methanol : water (75:25, v/v) and was injected in Chiral-HPLC. The wavelength of the UV-Vis detector



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Peak Width
1	1.99	584	298109	100.000	100.0000	BB	0.437
		584	298109				

Figure 2 — HPLC chromatogram

was adjusted at 390 nm. The experiment was carried out at RT and the HPLC column used was Chiradex-Lichocart. The chromatogram showed two peaks of nearly equivalent concentration (Figure 3). The positive dominated which was found out by collecting the enantiomer from the HPLC outlet and measuring the optical activity with a polarimeter.

Cyclopentane derivative : 5-(4,4,6,6-tetrahydroxy-6-methoxyhexane-2-ylidene)cyclopentane-1,2,3,4 tetraol, 1: Brownish syrupy liquid. $[\alpha]_D^{25}$ = +0.01° (c 0.08 percent, MeOH); IR (KBr): 3346-3294, 1620, 1420, 1078, 887 cm^{-1}; ^1H NMR (400 MHz, D_2O): δ_H 1.66 (s,3H), 2.15(s,2H), 2.93(s,2H), 3.10(s,3H), 3.25-3.35(m,2H), 3.36-3.45(m,2H); ^{13}C NMR (D_2O): δ_C 20.9, 46.7, 60.3, 60.4, 69.3, 69.7, 72.7, 73.5, 100.0, 133.1; EI-MS ($C_{12}H_{22}O_9$): *m/z* 278 [M-OCH_3]. Anal. Found: C, 47.45; H, 7.15; O, 45.41. Calcd for $C_{12}H_{22}O_9$: C, 46.45; H, 7.15; O, 46.41%.

Polymeric compound, 2: White solid, m.p. 62-64°C. $[\alpha]^{20}_D$ −18.94° (c 10 percent, chloroform), optical density −0.01; IR (KBr): 2918, 2849, 1734, 1462, 1177, 957, 920, 723 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ_H 4.05 (t, *J* = 7.5 Hz, 2H, OCH_2), 2.29 (t, *J* = 7.5 Hz, 2H, CH_2), 1.61 (m, 2H, CH_2), 0.88 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 14.1, 22.7, 25.0, 29.5, 31.9, 34.4, 64.4, 174.1; EI-MS ($C_{32}H_{54}O_{12}$): *m/z* 631 [M+1].

β-Sitosterol-α-D-glucoside, 3: White solid; m.p. 255-60°C (d); IR (KBr): 3360, 2940, 1460, 1369, 1070, 1024 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ_H 0.63, 0.76, 0.81, 0.88, 0.95, 1.11 (s, 3H each, 6×CH_3), 2.89 (m, 1H, H-3), 3.62 (m, 1H, CH of glucose), 3.10 (m, 1H, CH of glucose), 3.04 (m, 1H, CH of glucose), 4.20 (d, *J* = 7.5 Hz, 1H, CH of glucose), 4.48 (m, 1H, CH of glucose), 4.92 (m, 2H, CH_2 of glucose), 5.03 (m, 1H, H-1□'), 5.30 (m, 1H, H-6); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 36.7 (C-1), 29.2 (C-2), 76.9 (C-3), 40.0 (C-4), 140.4 (C-5), 121.2 (C-6), 31.3 (C-7), 29.2 (C-8), 49.5 (C-9), 36.2 (C-10), 20.9 (C-11), 39.2 (C-12), 41.8 (C-13), 56.1 (C-14), 25.3 (C-15), 28.6 (C-16), 55.3 (C-17), 12.1 (C-18), 18.9 (C-19), 35.4 (C-20), 19.1 (C-21), 36.1 (C-22), 33.2 (C-23), 45.1 (C-24), 29.2 (C-25), 19.6 (C-26), 20.5 (C-27), 28.6 (C-28), 11.7 (C-29), 100.7 (C-1′), 70.0 (C-2′), 76.9 (C-3′), 73.3 (C-4′), 76.7 (C-5′), 61.0 (C-6′); EIMS: *m/z* 576.8.

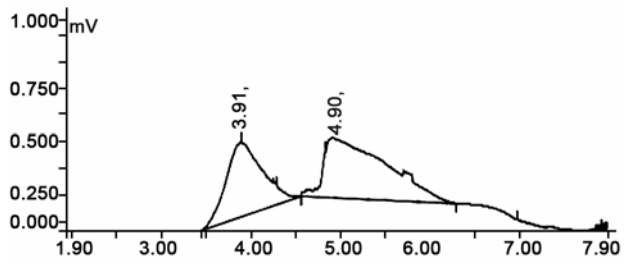


Figure 3 — Chiral-HPLC chromatogram of **1**

General procedures

Optical rotations were measured with a Rudolf Research Analytical Autopol-II automatic polarimeter. The purity of the compound was checked by reversed phase HPLC (Merck-Hitachi model with UV detector, C18 reversed-phase Merck column). The HPLC eluents were acetonitrile : water (3:1, v/v). IR spectra were recorded on a Shimazdu IR-408 spectrometer using a KBr disc and ν_{max} is given in cm^{-1}. ^1H NMR spectrum and ^{13}C NMR spectrum were recorded on a Bruker AC-400 spectrometer using residual non-deuterated solvent as an internal reference and all chemical shifts (δ_H and δ_C) are quoted in parts per million (ppm) downfield from tetramethylsilane (TMS). Mass spectra were recorded by Water ZQ-4000 mass spectrometer.

Microorganisms

Five species each of bacteria and fungi (*Proteus mirabilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella paratyphi* and *Pseudomonas aeruginosa*) and (*Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Fusarium oxysporum* and *Candida albicans*), respectively, were employed. Antimicrobial activity was carried out using agar-diffusion method. Petri plates (100 mm) were prepared with 20 mL of sterile nutrient agar (NA) (Hi-Media) and Potato-Dextrose Agar (PDA) (Hi-Media) and SDA (Hi-Media) for testing the bacterial and filamentous fungal and yeast activity. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. Stock solutions of each chemical were diluted. The dilutions were deposited (20 µL per well) which were subsequently placed on the inoculated petri plates and left for 10 min at RT for compound diffusion. Negative control was prepared using DMSO. Amphotericin-B (Hi-Media) for fungi and yeast, and Ciprofloxacin (Hi-Media) for bacteria were served as positive control. The plates with bacteria were incubated at 37ºC for 24 hr and for fungal cultures at 30ºC for 48-72 hr. The experiment was repeated thrice and the average results were recorded. The antimicrobial activity was determined by measuring the diameter of the inhibition zone around the well. The results are tabulated in Table I. The MICs were

Table I—Antimicrobial activity

Positive control at 2×Amphotericin B; Ciprofloxacin (20 µL)
Chemical conc. at 10000 µg/1000 µL; Agar Well 20 µL

Chemical	Bacteria	Inhibition zone (mm), (µg/well)				MIC (µg/mL)	CIP
		5×10^3	2.5×10^3	1.25×10^3	0.625×10^3		
WE_3	PM	10	7	–	–	0.312×10^3	1
	KP	11	9	–	–	0.156×10^3	2
	EC	9	7	–	–	0.625×10^3	<0.5
	SP	7	-	–	–	1.25×10^3	2
	PA	11	8	–	–	0.156×10^3	0.5

PM -*Proteus mirabilis*; *KP* - *Klebsiella pneumonia;* EC - *Escherichia coli;* SP - *Salmonella paratyphi;* PA - *Pseudomonas aeruginosa;* CIP - Ciprofloxacin

determined by serial dilution against the fungal and bacterial organisms. The minimum concentrations at which no visible growth were observed were defined as the MICs, which were expressed in µg/mL[29-33]. The results of the antimicrobial study are shown in Table I.

Determination of free radical scavenging activity

The antioxidant property of **1** was screened for radical scavenging activity using DPPH method (Warjeet and Brajeshwari, 2010). The different extracts were measured in terms of hydrogen donating or radical scavenging ability using a stable radical DPPH. Each sample (3 mL. at 0.025 g/mL) was mixed with a DPPH solution (45 µg/mL, Sigma) in methanol, vortexed well at RT and left standing exactly for 5 min. The UV-Vis absorbance was then measured at 517 nm, serving the methanol without DPPH solution as blank solution. A reference solution (125 µg/mL) of butylated hydroxyl toluene (BHT, Sigma) in methanol was used as reference taking 100% radical scavenging activity. The scavenging percentage was calculated using the equation,

$$\% \text{ Scavenging} = (A'_{\circ} - A'_5)\times100/(A_{\circ} - A_5),$$

where $A'_{\circ}$, A'_5 are the absorbance values of DPPH + Sample solution at 0.0 min and after 5.0 min, respectively; $A_{\circ}$, A_5 are the absorbance values of DPPH + BHT at 0.0 min and 5.0 min. respectively[34,35].

Results and Discussion

Compound **1** was isolated as brownish syrupy liquid, the molecular formula was determined as $C_{12}H_{22}O_9$ by the presence of a peak at *m/z* 278 $[M\text{-}OCH_3]^+$. The IR absorption bands revealed the presence of hydroxyl (3346-3294cm^{-1}) and unsaturated double bond (1620 cm^{-1}). The ^1H NMR (D_2O) spectrum showed signals at δ 1.66, 2.15, 2.93, which are due to methyl and methylene protons and at δ 3.1 for the methoxy group. The ^1H NMR signals at δ 3.25-3.35(m,2H), 3.36-3.45(m,2H) are due to protons of the cyclopentane ring (Figure 1). The purity of the compound was checked by RP-HPLC. The similarity of the two syrup samples which are isolated from the two plants with same physical properties were studied by RP-HPLC and found to have the same retention time of 1.9 min under the same conditions. When the compound from the two plant samples was mixed and injected, the peak was spiked and the peak area was found to be increased. This is a preliminary confirmation to the similarity of the compound isolated from two different plants[36].

Antioxidant activity of **1** which was determined by using DPPH method was found to be 151%. The antioxidant activity is more than the standard BHT, which proves the medicinal properties of the leaves of *F. pomifera* and the rhizomes of *C. leucorrhiza*. The antimicrobial activities of **1** were determined by minimum inhibitory concentration (MIC) with five species each of fungi and bacteria. It was found to be effective against *Proteus mirabilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella paratyphi* and *Pseudomonas aeruginosa* and no antifungal activity of the extracts had been shown, except that *Aspergilus flavus* showed less zone of inhibition activity (8 mm). The effectiveness of the compound against *Klebsiella pneumonia* and *Salmonella paratyphi* correlates to the traditional uses of both the plants for cure of cold and cough.

A bioactive novel compound, **1** of diverse activity with higher antioxidant activity from BHT was separated and identified along with compound **2** and **3** from the leaves of *Ficus pomifera* Wall. and from the rhizomes of *Curcuma leucorrhiza* Roxb. Thus, for the first time, the compounds **1**, **2** and **3** have been isolated from two different plants of different families.

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