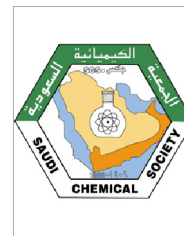




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ORIGINAL ARTICLE

# Phytochemical investigation of ethyl acetate extract from *Curcuma aromatica* Salisb. rhizomes

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## KEYWORDS

*Curcuma aromatica* Salisb.;  
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Rhizomes;  
Ethyl acetate extract;  
Phytochemical investigation

**Abstract** Three phytochemicals, curcumin **1**, demethoxycurcumin **2** and  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranoside **3** have been isolated from the ethyl acetate extract of rhizomes of *Curcuma aromatica*. Chemical structures of all the three isolates were determined using spectroscopic and chemical analyses.  $\beta$ -Sitosterol-3-*O*- $\beta$ -D-glucopyranoside has been isolated for the first time from this plant.  
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## 1. Introduction

*Curcuma aromatica* Salisb. (Family: Zingiberaceae) is a species occurring wild throughout India and cultivated chiefly in West Bengal and Travancore. The plant is commonly known as Jungalee Haldi (wild turmeric). The rhizomes are light yellow (internally orange) and possess a camphoraceous odour. They are sometimes used as a substitute for turmeric (*Curcuma*

*longa*). *C. aromatica* is never used as a spice but only as an aromatic for cosmetic purposes and in indigenous medicine for external applications on skin diseases, bruises and sprains (Anon., 1950; Maheshwari and Singh, 1965).

The colouring matter in the rhizomes of *C. aromatica* is predominantly curcumin (Rao and Shintre, 1928) and demethoxycurcumin, a trace of bis-demethoxycurcumin can be found when high levels of samples are taken for thin layer separation (Tonnesen et al., 1992). It has generally a higher level of volatile content (4–8%) and lower curcuminoids (~1.5%). The chemical and aroma characteristics of the volatile oil of two species are so different that *C. aromatica* cannot be substituted for *C. longa* in its food use. Thin-layer chromatography (TLC), gas chromatography (GC) could easily detect the accidental or deliberate admixture of *C. aromatica* in *C. longa* due to the presence of camphene and camphor and a high boiling alcohol in the volatile oil of *C. aromatica*, which are absent in *C. longa* (Govindarajan, 1980; Raghuveer and Govindarajan, 1979; Sen et al., 1974).

*C. aromatica* is used for preventing and treating coronary heart disease (Chemical Abstracts, 1990, 1985a,b), for epilepsy (Chemical Abstracts, 1989), as anti-allergic and in autoimmune diseases (Chemical Abstracts, 1996a,b; Dai et al., 1982).

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Rhizomes are used in combination with astringents and aromatics for bruises, sprains, hiccup, bronchitis, cough, leucoderma and skin eruptions (Warrier et al., 1993-1995). Rhizomes yield 6.1% essential oil (Chopra et al., 1980) and exhibited anti-tumor activity (Wu et al., 2000). Oil is also used for treatment of early stage of cervix cancer (Asolkar et al., 1992). The constituents identified in the oil were:  $\alpha$ -pinene,  $\beta$ -pinene, camphene, 1,8-cineol, isofurano-germacrene, borneol, isoborneol, camphor, germacrone and tetramethylpyrazine (Guo et al., 1980). The extracts of *C. aromatica* roots find uses for the treatment of cholecystitis, biliary calculi and other related diseases (Chemical Abstracts, 1987). Various other reported activities includes anti-microbial (Banerjee et al., 1978) and anti-tumor (Su et al., 1980) activities of essential oil, anti-inflammatory activity due to curcumin (Chan et al., 1998), anti-diabetic due to (4S,5S)-(+)-germacrone-4,5-epoxide (Chemical Abstracts, 1994) and anti-arrhythmic activity of aqueous extract due to the presence of dipotassium magnesium dioxalate dihydrate (Zhen et al., 1982; Chemical Abstracts, 1985a,b). Curdione, neocurdione, curcumol, tetramethyl pyrazine and (R)-(+)-1, 2-hexadecanediol were isolated from *C. aromatica* (Huang et al., 2000). Recently, the chemical composition of the hydro-distilled essential oil from leaves of *C. aromatica* was established, where 23 compounds representing 94.29% of the total oil were identified. The antioxidant activities of the oil and various extracts of *C. aromatica* were evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide radical-scavenging assays.

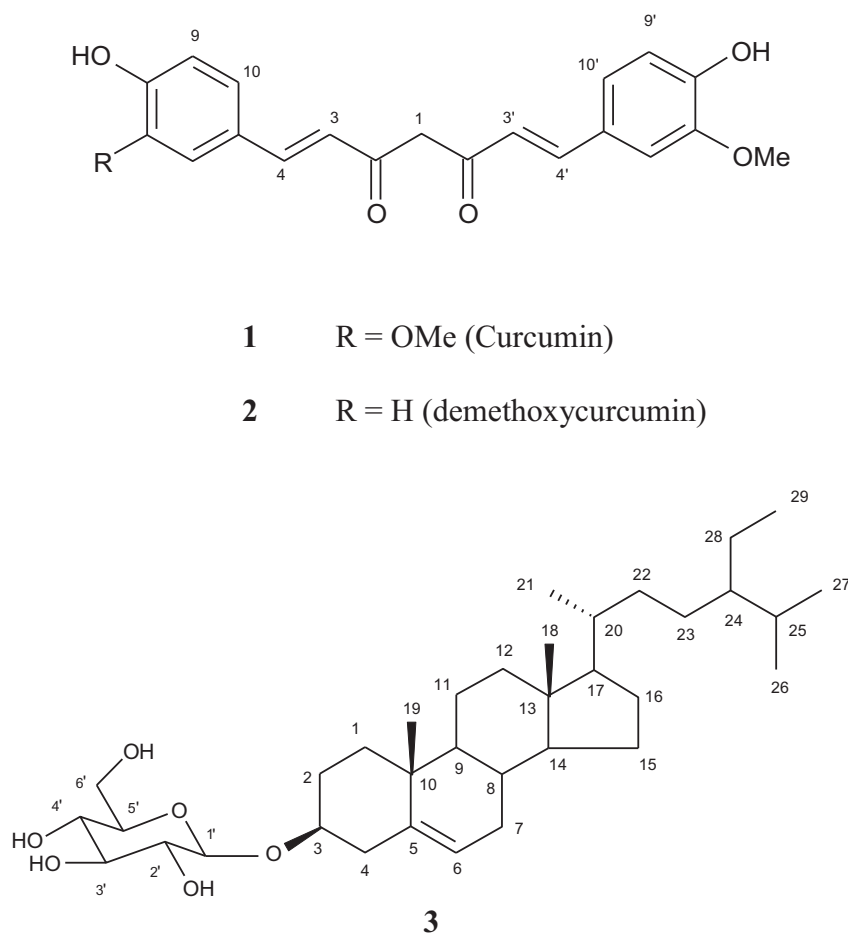
The oil and methanol extract showed potent DPPH radical-scavenging activities ( $IC_{50}$  = 14.45 and 16.58  $\mu\text{g/mL}$ , respectively), which were higher than butylated hydroxyanisole ( $IC_{50}$  = 18.27  $\mu\text{g/mL}$ ). The extracts also exhibited remarkable superoxide radical-scavenging activities ( $IC_{50}$  = 22.6–45.27  $\mu\text{g/mL}$  and the activity in the methanol extract was superior to all other extracts ( $IC_{50}$  = 22.6  $\mu\text{g/mL}$  (Al-Reza et al., 2010).

In course of search for biologically active substances, the present work was undertaken to isolate and characterize phytochemicals from the rhizomes of *C. aromatica*. Ethyl acetate extract, fractionated from hydromethanolic extract of plant material after defatting with hexane, yielded three secondary metabolites namely, curcumin **1**, demethoxycurcumin **2** and  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranoside **3** (Fig. 1). Of these compounds, **3** is isolated for the first time from *C. aromatica*.

## 2. Materials and methods

### 2.1. General procedures

Melting point was determined on electro thermal (UK-made) apparatus on centigrade scale and was uncorrected. Ultra-violet absorption spectrum was recorded on Perkin–Elmer Lambda Bio 20 UV spectrophotometer. Infrared spectrum was recorded on Perkin–Elmer 1710 infrared Fourier Transform spectrometer.  $^1\text{H}$  and  $^{13}\text{C}$  NMR was recorded on a Bruker AVANCE DRX-300 (300 and 75 MHz) equipped with 5 mm



**Figure 1** Chemical structures of Curcumin (**1**), Demethoxycurcumin (**2**) and  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranoside (**3**).

inverse multinuclear probe head. Tetramethyl silane (TMS) was used as an internal standard and chemical shift values are expressed in  $\delta$  (ppm) values, coupling constant ( $J$ ) in Hertz. ESMS, electro spray mass spectra, was recorded on a Micro-mass Quattro II triple quadrupole mass spectrometer. EIMS, electron impact mass spectra, was recorded at 70 eV on JEOL-JMS D-100 spectrometer. Column chromatography was performed over silica gel (60–120 mesh, Qualigen) and TLC was carried out on a silica gel G (10–40  $\mu$ m, Merck). Paper chromatography (PC) was carried out on Whatman paper number 1 in descending mode in *n*-BuOH:AcOH:H<sub>2</sub>O (4:1:5, v/v/v), developed by spraying with silver nitrate solution in acetone followed by NaOH solution in methanol.

## 2.2. Plant material

The rhizome of *C. aromatica* was collected from Assam (Goal Para, Lakhimpur) and was identified by the Botany and Pharmacognosy Division of Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow (India), where a voucher specimen has been retained.

## 2.3. Extraction and isolation

The air-dried and powdered rhizomes (4.50 kg) of *C. aromatica* were extracted with *n*-hexane (2.5 L  $\times$  6) and defatted marc was then extracted with aqueous methanol (90%) at room temperature (3 L  $\times$  10). The aqueous methanolic extract thus obtained was concentrated to dryness (150 g) and subjected to fractionation with different solvents of increasing polarity viz., chloroform, ethyl acetate, methanol and *n*-butanol in a soxhlet apparatus. Evaporation of solvent under reduced pressure yielded different extracts 117.96 g chloroform extract, 5.71 g ethyl acetate extract, 7.11 g methanol extract and 17.04 g *n*-butanol extract, respectively. We have carried out investigation of ethyl acetate extract as it showed six spots on TLC plate developed in chloroform:methanol (95:5). Spots were visualized by spraying TLC plate with 10% H<sub>2</sub>SO<sub>4</sub> followed by heating at 110 °C for 5–10 min. Ethyl acetate extract (5.71 g) was subjected to column chromatography. Elution of the column made by using chloroform:methanol [100:0 (fractions 1–7), 99:1 (fractions 8–12 and 13–19), 97:3 (fractions 20–24 and 25–30), 95:5 (fractions 31–38), 93:7 (fractions 39–42) and 90:10 (eluted further)].

### 2.3.1. Compound 1 (curcumin)

Preparative TLC of fraction no. 13–19 yielded compound 1, which was crystallized in chloroform. Yield of 1 (5 mg), m.p. 184–186 °C; IR<sub>vmax</sub>: 3448 (OH), 2967, 2865, 1631 (C=O), 1605, 1524, 1460, 1431, 1381, 1280, 1233, 1205, 1182, 1155 cm<sup>-1</sup>. UV  $\lambda_{\text{max}}$ : 240, 420 nm. <sup>1</sup>H NMR (CDCl<sub>3</sub>,  $\delta$ ): 1.30 (2H, br s, H-1), 6.52 (2H, brs, H-3/H-3'), 6.90 (2H, br s, H-4/H-4'), 7.14 (2H, d,  $J$  = 10.5 Hz, H-9/H-9'), 7.65 (2H, d,  $J$  = 10.5 Hz, H-10/H-10'), 3.30 (6H, s, OCH<sub>3</sub>  $\times$  2). EIMS:  $m/z$  368 [M]<sup>+</sup> C<sub>21</sub>H<sub>20</sub>O<sub>6</sub>.

### 2.3.2. Compound 2 (demethoxycurcumin)

Compound 2 was obtained along with compound 1 in very low yield (3.0 mg) by preparative TLC of fraction no. 13–19 and re-crystallized in chloroform. m.p. 168–170 °C, IR<sub>vmax</sub>: 3448 (OH), 2967, 2865, 1631 (C=O), 1605, 1524, 1460, 1431,

1381, 1280, 1233, 1205, 1182, 1155 cm<sup>-1</sup>; UV  $\lambda_{\text{max}}$ : 251, 423 nm. <sup>1</sup>H NMR (CDCl<sub>3</sub>,  $\delta$ ): 1.30 (2H, br s, H-1), 6.53 (2H, br s, H-3/H-3'), 6.93 (2H, br s, H-4/H-4'), 7.46 (2H, br s, H-9/H-9'), 7.66 (2H, br s, H-10/H-10'), 3.93 (3H, s, OCH<sub>3</sub>); EIMS:  $m/z$  338 [M]<sup>+</sup> C<sub>20</sub>H<sub>18</sub>O<sub>5</sub>.

### 2.3.3. Compound 3 ( $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranoside)

Compound 3 was obtained as crystalline solid while eluting the column with chloroform:methanol (95:5) from fraction no. 6 as white solid. It was crystallized in chloroform:methanol (90:10). Yield 27.0 mg.  $R_f$  0.56 (chloroform:methanol 88:12), m.p. 248 °C (d), IR<sub>vmax</sub>: 3410 (OH), 2961, 1634 (C=C), 1450, 1256, 1197, 1168, 1070, 973 cm<sup>-1</sup>; <sup>1</sup>H NMR (Py-*d*<sub>5</sub>,  $\delta$ ): 0.75, 0.78, 0.86, 0.93, 1.00, 1.19, (3H each, s, -CH<sub>3</sub>  $\times$  6), 3.45–4.25 (6H, m, -CH and CH<sub>2</sub> of glucose), 5.07 (1H, d,  $J$  = 7.5 Hz, H-1'), 5.37 (1H, m, H-6), <sup>13</sup>C NMR (py-*d*<sub>5</sub>,  $\delta$ ): 37.5 (C-1), 30.6 (C-2), 78.7 (C-3), 40.0 (C-4), 141.0 (C-5), 122.0 (C-6), 32.2 (C-7), 32.1 (C-8), 50.4 (C-9), 37.0 (C-10), 21.3 (C-11), 28.6 (C-12), 42.5 (C-13), 56.9 (C-14), 24.5 (C-15), 39.4 (C-16), 56.3 (C-17), 12.0 (C-18), 19.5 (C-19), 36.4 (C-20), 19.3 (C-21), 36.4 (C-22), 34.2 (C-23), 46.1 (C-24), 29.5 (C-25), 19.1 (C-26), 20.0 (C-27), 26.4 (C-28), 12.2 (C-29), Glc moiety: 102.6 (C-1'), 75.4 (C-2'), 78.5 (C-3'), 76.6 (C-4'), 78.1 (C-5'), 62.9 (C-6'), EIMS:  $m/z$  414 [M-gluc]<sup>+</sup>, 399 [M-CH<sub>3</sub>]<sup>+</sup>, 396 [M-H<sub>2</sub>O]<sup>+</sup>, 381, 271, 373, 255, 231, 213, 187, 161, 150, 134, 107, 92, 79, 43.

### 2.3.4. Hydrolysis of compound 3

Compound 3 (10 mg) was refluxed with 5% methanolic HCl (10 ml) for 4 h. After the completion of the reaction, the mixture was diluted with water (10 ml) and extracted with chloroform (3  $\times$  15 ml) and dried over anhydrous sodium sulphate. It was then filtered and the solvent was evaporated to furnish the aglycone part identified as  $\beta$ -sitosterol m.p. 136 °C [M]<sup>+</sup>  $m/z$  414. Aqueous mother liquor was neutralized with barium carbonate and filtered. The filtrate was then concentrated and identified as D-glucose (Co-PC with authentic sample).

## 3. Results and discussion

### 3.1. Compound 1 (curcumin)

Compound 1 was obtained as orange prisms by crystallization with chloroform, m.p. 184–186 °C. The IR spectrum indicated the presence of a hydroxyl group (3448 cm<sup>-1</sup>), a carbonyl group at 1631 cm<sup>-1</sup> and a band at 1524 cm<sup>-1</sup> for double bonds. The compound showed UV absorption at 240, 420 nm for the presence of an  $\alpha$ ,  $\beta$ -unsaturated carbonyl group. The EIMS at  $m/z$  368 corresponded to the molecular formula C<sub>21</sub>H<sub>20</sub>O<sub>6</sub>. A broad singlet at  $\delta$  6.52 (2H) in <sup>1</sup>H NMR was assigned for C-1 proton. Two broad singlets at  $\delta$  6.52 and 6.90 were assigned for magnetically equivalent H-3/H-3' and H-4/H-4' protons, respectively. The ortho-coupled protons of the aromatic ring appeared as doublets at  $\delta$  7.14 and 7.65 ( $J$  = 10.5 Hz each) assigned for H-9/H-9' and H-10/H-10', respectively, present in identical environment. The methoxyl protons appeared at  $\delta$  3.30 (6H) as a singlet. The compound was characterized as 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (curcumin) on comparison with the authentic sample and reported data (Govindarajan, 1980; Ali et al., 1995).

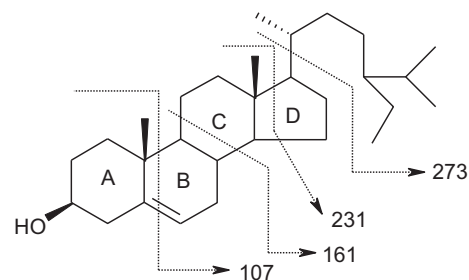
### 3.2. Compound 2 (demethoxycurcumin)

Compound **2** was obtained as orange yellow powder by preparative TLC in very low yield (3.0 mg), m.p. 168–170 °C. The IR spectrum of the compound showed the presence of O–H stretching at  $3448\text{ cm}^{-1}$  and a band at  $1631\text{ cm}^{-1}$  for the presence of a double bond. Compound also showed positive UV bands at 251 and 423 nm for the presence of conjugated aromatic system present in it. The EIMS at  $m/z$  338 suggested the molecular formula  $\text{C}_{20}\text{H}_{18}\text{O}_5$ . The  $^1\text{H}$  NMR of the compound showed similar shift values as for compound **1** but it showed the presence of only one methoxy group placed at  $\delta$  3.93 as a singlet. The spectral values were in good agreement with those reported in the literature (Ali et al., 1995). The molecular ion peak at  $m/z$  338 was inconsistent with the present structure having deficiency of 30 mass units (methoxy group) from compound **1**. This further authenticated the proposed structure. Thus, the compound was characterized as 1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (demethoxycurcumin) in comparison with the authentic sample and reported values (Govidarajan, 1980).

### 3.3. Compound 3 ( $\beta$ -sitosterol-3-O- $\beta$ -D-glucopyranoside)

Compound **3** was obtained while eluting the column with chloroform:methanol (95:5) as crystalline solid, m.p. 248 °C (d). It showed positive Liebermann Buchard (LB) and positive Feigl's test, suggesting for the presence of a steroidal glycoside. The IR spectrum of **3** exhibited a broad band at  $3410\text{ cm}^{-1}$  for the presence of a hydroxyl group and a band at  $1634\text{ cm}^{-1}$  for the double bond. The other bands obtained were at 2961, 1450, 1256, 1197, 1168, 1070, and  $973\text{ cm}^{-1}$ . The molecular ion peak were not observed in the EIMS of this compound as the molecule provides an ion at  $m/z$  414 in consistent with the aglycone moiety. The  $^1\text{H}$  NMR showed six singlets at  $\delta$  0.75, 0.78, 0.86, 0.93, 1.00 and 1.19 (3H each) for the presence of six methyl groups in the compound. The spectrum further revealed broad signals between  $\delta$  3.45 and 4.25 (6H, m) for the presence of –CH and  $\text{CH}_2$  groups of the sugar moiety. A doublet at 5.07 ( $J = 7.5\text{ Hz}$ ) was observed for the anomeric proton. A multiplet at  $\delta$  5.37 was observed for H-6 proton. The  $^{13}\text{C}$  NMR of the compound showed the presence of 35 intense carbon signals. The DEPT experiment indicated the presence of 14 methine, 12 methylene, 6 methyl and 3 quaternary carbons. The lesser number of methyl and higher number of methylenes suggested that it is not a triterpene and may be a steroid.

Acid hydrolysis of the compound afforded aglycone, m.p. 136 °C.  $[\text{M}]^+ m/z$  414 identified as  $\beta$ -sitosterol and the glycone moiety identified as D-glucose by Co-PC with the authentic sample. The presence of four methane signals at  $\delta$  75.4, 76.6, 78.1, and 78.5 ppm, one methylene at  $\delta$  62.9 ppm and a methine signal at  $\delta$  102.6 ppm in  $^{13}\text{C}$  NMR further confirmed the presence of glucose in the molecule. The EIMS of aglycone moiety showed fragments (Fig. 2) at  $m/z$  273 [M-side chain] $^+$ , 23 [273–42 (ring D)] $^+$ , 161 [273–70 (ring C)] $^+$ , 107 [161–54 (ring B)] $^+$ , 92 [107–15 ( $\text{CH}_3$ )] $^+$  which are characteristic for sitosterols (Aizawa et al., 1974). Since the aglycone moiety is sitosterol and glycone is D-glucose, therefore, the compound **3** must be a 3-O-glucoside of sitosterol and the linkage of C-3 hydroxyl group to anomeric carbon of glucose moiety is  $\beta$ - which was confirmed from the coupling constant of anomeric proton at



**Figure 2** EI-MS fragmentation pattern of aglycone moiety ( $\beta$ -sitosterol) of  $\beta$ -sitosterol-3-O- $\beta$ -D-glucopyranoside (**3**).

$\delta$  5.07 ( $J = 7.5\text{ Hz}$ ) in its  $^1\text{H}$  NMR spectrum. On the basis of above evidences and comparison of spectral data with those reported in the literature (Srivastava, 1998) structure of compound **3** was characterized as  $\beta$ -sitosterol-3-O- $\beta$ -D-glucopyranoside. This compound is being reported for the first time from *C. aromatica*.

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