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Chemical constituents of *Zingiber officinale* rhizome

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Abstract
Phytochemical investigation of chlorform extract of *Zingiber officinale* rhizome afforded characterized as [6]- Dehydrogingerol (1), N-methyl-2-pyrolidinone (2), [6]- gingerol (3), [8]-ginge rol (4), [6]- shogaol (5), tetrahydrocurcumin (6), hexahydrocurcumin (7) and Meso–3,5–diacetoxy–1,7– bis(4'–hydroxy–3'–methoxyphenyl)heptane (8). The compounds 1 and 2 are reported for the first time from this plant. The structures were elucidated by analysis of their spectroscopic data.

Keywords: *Zingiber officinale*, zingiberaceae, gingerol

1. Introduction
Ginger (*Zingiber officinale* Roscoe) belongs to family Zingiberaceae. This family species are fibrous rooted perennial which are cultivated in many tropical and subtropical area [1]. The early Greeks and Romans made extensive use of ginger as a spice and as a medicine [2]. In addition to its most common use as a flavoring, ginger root has been used in conventional medicine for countless centuries. Ginger is described in Ayurvedic and Tibb systems of medicine to be useful in inflammation and rheumatism [3]. It has been used to treat stomach ache, diarrhea, stroke, diabetes, asthma, toothache and arthritis [4, 5]. Numerous chemical investigations of this plant material have led to the isolation and identification of a large number of biologically active compounds, such as sesquiterpenes, steroids, paradol, gingerols, gingerones and shogaols ([6]-shogaol, [10]-shogaol) [6, 7, 8, 9, 10]. Several diarylheptanoids were reported from the ethanolic extract of the rhizomes of *Z. Officinale*. (3S,5S)-3,5-diacetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl) Heptanes and 5- hydroxy - 1- (3,4 - dihydroxy - 5 - methoxyphenyl) - 7 - (4 -hydroxy-3-methoxyphenyl)heptan-3-one, monoterpene, 10-O-b-D-glucopyranosyl-hydroxy cineole [11].

In the present study, we report the isolation and characterization of eight compounds shown in (Figure 1) were as follow [6]- Dehydrogingerol (1), N-methyl-2-pyrolidinone (2), [6]- gingerol (3), [8]-gingerol (4), [6]- shogaol (5), tetrahydrocurcumin (6), hexahydrocurcumin (7) and Meso–3,5–diacetoxy–1,7– bis(4'–hydroxy–3'–methoxyphenyl)heptane (8) from the chloroform soluble fraction of the ethanol extract of the rhizome of *Zingiber officinale*. The compounds 1 and 2 are reported for the first time from this plant. The structures were elucidated by analysis of their spectroscopic data.

2. Materials and Methods
2.1 General
Optical rotations were measured on a Perkin Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and 1cm micro cell. UV spectra were obtained from a Shimadzu 1601PC spectrophotometer. IR spectra were taken using a Hitachi 270-30 spectrophotometer. All the NMR spectral data were recorded on Bruker 200 and 300 MHz NMR spectrometer. TLC was carried out using Kieselgel 60 F_{254} (0.25mm thick, Merck, Darmstadt, Germany).

2.2 Plant Material
The rhizome of *Z. officinale* purchased from local market of Varanasi, Uttar Pradesh, India in the month of January 2016. The authentication were made by Prof V.K. Joshi, Department of Dravya Guna, Institute of Medical Sciences, Banaras Hindu University, Varanasi- 221005, Voucher specimen (MC006/2016) is kept in the herbarium of the investigators Department.

2.3 Extraction
Plant material (15 Kg) was percolated with ethanol (40 liters) and was allowed to stand at room temperature for 48 hrs. The percolate was collected. This process of extraction was repeated for five times, till the plant material was extracted exhaustively. The total extract was collected and filtered and concentrated under vacuum using rotavapour at 45–50 °C and weighed. The extract weighed (530 g, 15% yield).

2.4 Fractionation of ethanolic extract
The aqueous ethanolic extract (500 g) was taken and fractionated with *n*–hexane (500ml x15), the *n*–hexane soluble fraction was then concentrated under reduced pressure at 40 °C; weight of extract obtained was (200 g). Residue obtained was fractionated with chloroform (500ml x15), chloroform soluble fraction was concentrated under reduced pressure; weighed (40 g).

2.5 Gross column chromatography of chloroform fraction
Chloroform soluble fraction (40 g) was taken up for the isolation of compounds. An aliquot (35.0 g) was dissolved in MeOH, adsorbed over silica gel and subjected to column chromatography over silica gel (100–200 mesh), 190 g. The column was eluted with a mixture of *n*–hexane: EtOAc and then with EtOAc: MeOH to afford 125 fractions (250 ml each), which were further pooled on the basis of their TLC profile, making them to ten fractions.

2.6 Isolation of compound 1
Fractions from *n*–hexane: EtOAc (90:10) showed one major spot along with minor impurities. They were pooled according to their TLC profile and chromatographed over silica gel (230–400) mesh. Elution of column with mixture of *n*–hexane: EtOA. A colorless compound was obtained from *n*–hexane: EtOAc (92:08), which was washed with hexane to remove wax impurity. The solvent was evaporated, dried under vacuum and yielded white solid, which showed a single spot on TLC and it was designated as compound 1(15 mg); mp: 134–137 °C; $[a]_D^{31}$: –25.1° (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max}: 3455, 1680 cm^{-1}; ESI–MS: *m/z* 293 $[M+H]^+$.

2.7 Isolation of compound 2
Fractions from *n*–hexane: EtOAc (80:20) was chromatographed over silica gel (230–400 mesh) eluted with mixture of *n*–hexane: EtOAc. The solvent polarity was gradually increased and finally the fraction eluted with *n*–hexane: EtOAc (75:25) were collected, concentrated and TLC was checked for each fraction. One major spot was observed along with minor impurity. They were pooled and evaporated to dryness. A tan colored compound precipitated out while dissolved in MeOH, washed carefully with MeOH and dried under vacuum. It was designated as compound 2 (11 mg); obtained as light brown coloured solid; IR (KBr)ν_{max}: 3307, 2943, 1659 cm^{-1}; UV (MeOH)λ_{max}: 203, 223, 283 nm; ESI–MS: *m/z* 100 $[M+H]^+$.

2.8 Isolation of compound 3 & 4
Fractions eluted from *n*–hexane: EtOAc (70:30) was re–chromatographed over silica gel (230–400 mesh). Fractions were collected, concentrated and TLC was checked for alternate fractions, pooled according to their TLC profile. Fractions showed one major blue colored spot on TLC along with many impurities. Its reverse phase TLC (RP–18) was checked using solvent system MeOH: water (50:50), which showed clearly separated spots. Fraction was concentrated and then dissolved in minimum amount of $CHCl_3$. It was then adsorbed at celite and packed at reverse phase column (RP–18) using solvent system MeOH: water (10:90). The solvent polarity was gradually decreased to MeOH: water (15:85) to (50:50). Fractions were collected, reduced and TLC was checked for alternate fractions, pooled according to their TLC profile. The fraction eluted with MeOH: H_2O (35: 65) showed single blue color spot. It was then concentrated and named as compound 3 (18mg); obtained as light blue solid; $[a]_D^{28}$: +31.2° (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max}: 3420, 2920, 2850, 1710, 1610, 1520, 1270, 1040 and 820 cm^{-1}; ESI–MS: *m/z* 295 $[M+H]^+$.
And another spot eluted with MeOH:H_2O (30: 70) was coded as compound 4(10 mg); obtained as colourless oil; $[a]_D^{26}$: +26.0° (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max}: 3425, 2920, 2850, 1710, 1520, 1270, 1040 and 820 cm^{-1}; ESI–MS: *m/z* 323 $[M+H]^+$.

2.9 Isolation of compound 5
Fractions eluted with *n*–hexane: EtOAc (75:25) was re–chromatographed over silica gel (230–400 mesh) using hexane as the initial solvent. The solvent polarity was gradually increased and finally *n*–hexane: EtOAc (85:15) showed one major spot on TLC along with minor impurity. They were pooled and evaporated to dryness. A white colored compound precipitated out while dissolved in MeOH, washed carefully with MeOH and dried under vacuum, which was designated as compound 5(13 mg), obtained as pale yellow viscous solid, IR (KBr)ν_{max}: 3430, 1665 cm^{-1}; ESI–MS: *m/z* 276 $[M+H]^+$.

2.10 Isolation of compound 6
Fractions from *n*–hexane: EtOAc (65:35) was loaded on a silica gel column (230–400 mesh). Elution was carried out first with n–hexane and then with mixture of *n*–hexane: EtOAc. The fraction obtained with *n*–hexane: EtOAc (70:30) was again loaded on a silica gel column and eluted. The last fractions eluted with *n*–hexane: EtOAc (50:50) got purified and coded as compound 6(14 mg); obtained as yellow coloured solid; mp: 95–96 °C; ESI–MS: *m/z* 372 $[M]^+$.

2.11 Isolation of compound 7 & 8
Fractions from *n*–hexane: EtOAc (60:40) was re–chromatographed over silica gel (230–400 mesh) using *n*–hexane: EtOAc (95:30) as the initial eluent. The fractions showing similar spots on TLC were pooled together and evaporated to dryness. Sub–fractions eluted with *n*–hexane: EtOAc (40:60) showed one major and one minor spot in TLC. Repeated column chromatography using mixture of *n*–hexane: EtOAc (60:40) yielded compound 7(8 mg); mp: 81–82 °C; $[\alpha]_D^{20}$: +6.0° (*c* 1.68, $CHCl_3$); IR (KBr)ν_{max}: 3435, 1702, 1603, 1516, 1033 cm^{-1};UV (MeOH)λ_{max}: 227, 281 nm; ESI–MS: *m/z* 374 $[M+H]^+$.
Last ten fractions of this column were pooled, concentrated and again chromatographed using *n*–hexane: acetone. Early fractions of *n*–hexane: acetone (80:20) elution showed single spot on TLC. They were pooled, concentrated and vacuum dried, coded as compound 8(13 mg); colourless oil; $[\alpha]_D^{25}$: 0° (*c* 0.95, $CHCl_3$); IR (KBr)ν_{max}: 3420, 1720, 1600 and 1025 cm^{-1}; UV (EtOH)λ_{max}: 227, 281 nm; ESI–MS: *m/z* 460 $[M]^+$.

3. Result and Discussion
3.1 Characterization of compound 1
Compound 1 was obtained as white solid. The ESI–MS exhibited a protonated molecular ion peak at *m/z* 293 $[M+H]^+$. The molecular formula was derived as $C_{17}H_{24}O_4$ by Mass, ^1H NMR and ^{13}C NMR spectral data. The IR spectrum suggested the presence of carbonyl (ν_{max} 1680 cm^{-1}) and hydroxyl (ν_{max}

3455 cm^{-1}) absorptions.

The ^1H NMR spectrum (Table–01) exhibited three aromatic proton signals [δ_H, 7.12 (1H, *d*, *J*= 8 Hz), 7.07 (1H, *s*) and 6.94 (1H, *d*, *J*= 8 Hz)], one methine proton at δ_H 4.14 (1H, *m*) and one methoxy group at δ_H 3.95 (3H, *s*). Multiplet at δ_H 1.50–1.20 integrated for eight protons, showed correlations with four methylene carbons in the HSQC spectrum. ^1H NMR spectrum exhibited a three proton triplet δ_H 0.90, owing to a terminal methyl group.

The ^{13}C NMR spectrum indicated presence of total 17 carbons in the molecule. The ^{13}C NMR and DEPT spectra (Table–3.2) indicated the presence of one methyl (δ_C 14.3), five methylenes, one methoxy (δ_C 56.2), one oxymethine (δ_C 68.2), five methines and four quaternary carbons (including one carbonyl, δ_C 201.2). Two signals at δ_C 144.0 (δ_H 7.52, 1H, *d*, *J*=16 Hz) and 124.4 (δ_H 6.59, 1H, *d*, *J*=16 Hz) account for a trans double bond. In the ^1H–^1H COSY experiment, two methylene protons at δ_H 2.78 (2H, *m*, H–4) were coupled with the signal at δ_H 4.14 (1H, *m*, H–5). Moreover, the correlation of COSY and the coupling constants of the aromatic protons suggested the presence of one 1,3,4–trisubstituted benzene. In HMBC experiment, the olefinic proton at δ_H 7.52 (H–1) correlated with the signal at δ_C 109.7 (C–2'), 123.9 (C–6') and the carbonyl carbon at δ_C 201.2 (C–3), which determined the position of double bond as well as that of carbonyl group. From these HSQC and HMBC correlations, the overall structure was established. Compound 1 was deduced as, (*E*)–1–(4'–hydroxy–3'–methoxyphenyl)–5–hydroxy–dec–1–en–3–one). The data are in agreement with the earlier reported compound, i.e., (*S, E*)–1–(4'–hydroxy–3'–methoxyphenyl)–5–hydroxy–dec–1–en–3–one) had $[\alpha]_D^{26}$ +38.4° (*c* 1.0, $CHCl_3$). But compound 1 showed specific rotation at $[\alpha]_D^{31}$ –25.1 (*c* 0.1, $CHCl_3$). Therefore, the stereochemistry at C–5 is assigned '*R*'. Finally compound 1 was characterized as (*R, E*)–1–(4'–hydroxy–3'–methoxyphenyl)–5–hydroxy–dec–1–en–3–one), trivially known as, (*R, E*)–[6]–dehydrogingerol [12].

The 'S' form of this compound is synthetically known (no ^{13}C and 2D NMR were reported so far). We are reporting its ^{13}C and 2D NMR data for the first time, and also this is the first report of isolation of compound 1 from nature.

Table 1: ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) data of compound 1 in $CDCl_3$

Position	δ_H (mult., *J* in Hz)	δ_C
1	7.52 (1H, *d*, *J*= 16)	144.0
2	6.59 (1H, *d*, *J*= 16)	124.4
3	–	201.2
4	2.78 (2H, *m*)	46.8
5	4.14 (1H, *m*)	68.2
6	1.50–1.20 (2H, *m*)	36.8
7	1.50–1.20 (2H, *m*)	25.4
8	1.50–1.20 (2H, *m*)	32.0
9	1.50–1.20 (2H, *m*)	22.8
10	0.90 (3H, *t*, *J*= 6.8)	14.3
1'	–	133.3
2'	7.07 (1H, *s*)	109.7
3'	–	147.1
4'	–	144.0
5'	6.94 (1H, *d*, *J*= 8)	115.1
6'	7.12 (1H, *d*, *J*= 8)	124.0
–OCH_3	3.95 (3H, *s*)	56.2

3.2 Characterization of compound 2

Compound 2 was obtained as light brown coloured solid compound. The ESI–MS spectrum exhibited protonated molecular ion peak at *m/z* 100 $[M+H]^+$, corresponding to its molecular formula C_5H_9NO. The UV absorption bands at λ_{max} (MeOH) 203, 223, 283 nm and IR (in $CHCl_3$) absorption bands at 3307, 2943, 1659 cm^{-1}, clearly indicated the presence of nitrogen attached alkyl system and carbonyl group in the molecule.

^1H NMR spectrum (Table–02) showed two proton quintet at δ_H 1.95 (2H, *qn*, *J*= 7.5 Hz), which showed COSY correlation with peaks at δ_H 2.30 (2H, *t*, *J*= 7.9 Hz) and δ_H 3.30 (2H, *t*, *J*= 7.5 Hz). Three proton singlet at δ_H 2.80, corresponds to N–methyl group.

^{13}C NMR and DEPT (135 & 90) spectra showed three aliphatic methylenes at δ_C 31.8 (C–3) 18.7 (C–4), 50.9 (C–5), one methyl signal appeared at δ_C 29.9 and a quaternary carbon at δ_C 176.0. The most downfield signal at δ_C 176.0 was assigned to the carbonyl group.

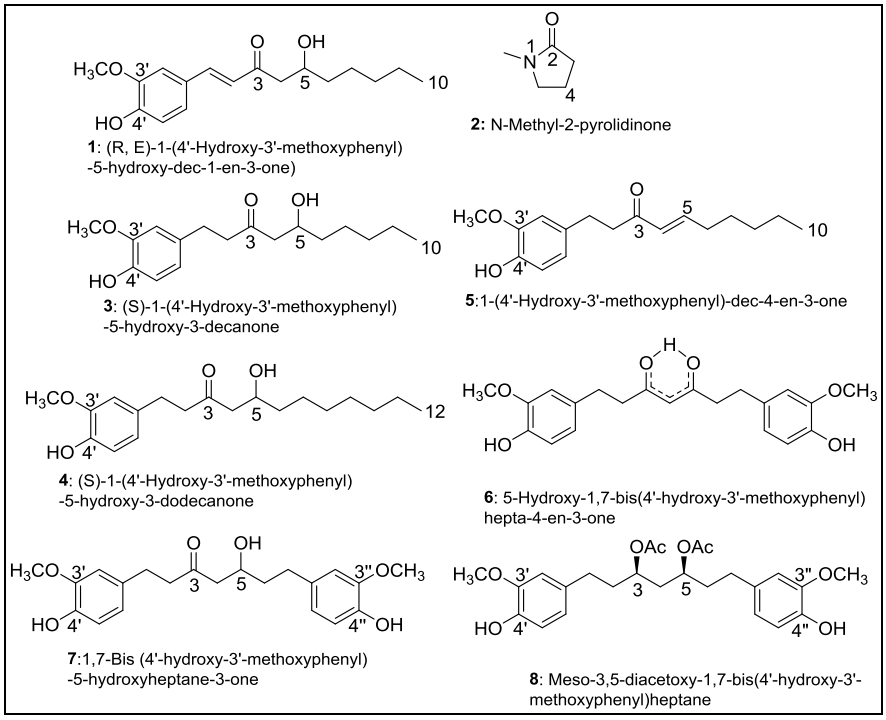

Fig 1: Structure of isolated compounds

The attachment of protons to the respective carbons was assigned with the help of HSQC experiment. The final structure was derived by analysing the HMBC spectrum of the molecule. From the analysis of above data compound **2** was characterized as *N–methyl–2–pyrolidinone*. It was further confirmed by comparison of its physicochemical data with that of the reported in the literature [13]. It has been isolated for the first time from this plant

Table 2: ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) data of compound 2 in $CDCl_3$

Position	δ_H (mult., *J* in Hz)	δ_C
2	–	176.0
3	2.30 (2H, *t*, *J*= 7.9)	31.8
4	1.95 (2H, *qn*, *J*= 7.5)	18.7
5	3.30 (2H, *t*, *J*= 7.5)	50.9
–N–CH_3	2.80 (3H, *s*)	29.9

The structures of other known compounds [6]- gingerol (3) [12], [8]-gingerol (4) [12], [6]- shogaol (5) [14], tetrahydrocurcumin (6) [15], hexahydrocurcumin (7) [16] and Meso–3,5–diacetoxy–1,7– bis(4'–hydroxy–3'–methoxyphenyl)heptane (8) [17] respectively on the basis of comparison od their sata with those reported in literature.

4. Conclusion

In conclusion, the present study involves chemical investigation of *Zingiber officinale* rhizome. Eight compounds [6]- Dehydrogingerol (1), N-methyl-2-pyrolidinone (2), [6]- gingerol (3), [8]-ginge rol (4), [6]-shogaol (5), tetrahydrocurcumin (6), hexahydrocurcumin (7) and Meso–3,5–diacetoxy–1,7– bis(4'–hydroxy–3'–methoxyphenyl)heptane (8) were isolated and characterized out of which compounds 1 and 2 are reported for the first time from this plant. The structures were elucidated by analysis of their spectroscopic data.

5. Acknowledgements

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