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# Secondary Metabolites Isolated from *Iris germanica*

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**Abstract:** Phytochemical investigations of methanol extract of rhizome of *Iris germanica* L. resulted in the isolation of one new compound, 6,6-ditetradecyl-6,7-dihydrooxepin-2(3H)-one **(1)** and five known compounds, 1-(2-(6′-hydroxy-2′-methylcyclohex-1′-enyloxy)-5-methoxyphenyl)ethanone **(2)** 4-hydroxy-3-methoxyacetophenone **(3),** irisolone **(4)** irisolidone **(5)** and 2-acetoxy-3,6-dimethoxy-1,4-benzoquinone **(6)**. The structures of the compounds were determined on the basis of spectroscopic techniques. The antifungal activity of different soluble portions was measured. The hexane soluble portion of the methanol extract showed significant antifungal activity where as the ethyl acetate and chloroform soluble portions showed moderate activity. The methanol extract showed no antifungal activity.

**Keywords:** *Iris germanica*; Iridaceae; secondary metabolite; antifungal activity.

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Isolated compounds from *Iris germanica*

## 1. Introduction

Iris germanica L. (Iridaceae) is widely distributed in most parts of the world and also found in Northern regions of Pakistan [1]. The essential oils of the plant find use in perfumes and cosmetics [2]. Different parts of plants are used to cure various diseases in the folk medicine. The rhizome of the plant is used in the traditional preparations. The juice of rhizome is applied to sores and for removal of freckles of skin. Decoction of the roots of the plant is used in dropsy and as anti-spasmodic, emmenagogue, stimulants, diuretic, aperient and in gall bladder diseases.
The rhizomes contain iridals which exhibited a potent pesticidal activity at a concentration less than 1 µg/mL and also showed potent anti-cancer activity [3, 4]. It is also employed as an ingredient for blood purifier and remedy for venereal diseases [2]. Its leaves are rich source of ascorbic acid and vitamins [4]. A number of secondary metabolites have been reported from the plant [5-11]. Our recent investigations on the methanolic extract of root and rhizome resulted in the isolation of one new compound and five known compounds. The structure of the new compound was determined on the basis of spectroscopic techniques where as the structures of the known compound were identifed on the basis of spectroscopic studies and comparision with literature values.

## 2. Materials and Methods

### *2.1. Extraction Procedure*

The dried plant material (6 kg) was extracted with methanol (30 L). The extract was concentrated on a rotary evaporator under reduced pressure. A portion of the extract (55g) was subjected to the silica gel column chromatography. The column was eluted with increasing polarities of petroleum ether/dichloromethane. This afforded 100 fractions. The fractions of similar Rf values were combined which resulted 3 main fractions, F1 (7g), F2 (3g) and F3 (4g). The same column was also eluted with 100% ethyl acetate. This resulted 50 fractions. The fractions of similar Rf values were combined to give two main fractions F4 (7g) and F5 (2g).

### *2.2. Instrumentation*

The UV spectra were recorded on a Shimadzu UV-240 spectrophotometer in MeOH while the IR spectra were recorded on a JASCO A-302 spectrophotometer in $CHCl_3$. High resolution mass spectra were recorded on a Varian MAT-312 mass spectrometer connected to a PDP 11/34 (DEC) computer system. The $^{1}H$- NMR spectra were recorded at 300 MHz on a Bruker AM-300 NMR spectrometer. The $^{13}C$-NMR spectra were recorded at 75 MHz on the same instrument. TLC experiments were performed on silica gel precoated plates (GF-254, 0.2 mm, E.Merck).

*2.3. Isolation and identification of chemical constituents*

**6,6-ditetradecyl-6,7-dihydrooxepin-2(3H)-one (1):** The fraction F1 was dried and subjected to the flash silica gel column chromatography. The column was eluted with chloroform/ pet. ether (2.0:8.0) as the solvent system to afford two fractions, F1-1 and F1-2. The fraction F1-1 was rechromatographed on the precoated silica-gel (GF-254) plates using chloroform/ pet. ether (5.0:5.0) as the solvent system which resulted in the isolation of the pure compound **1** as an amorphous material (10 mg, $R_f$ = 0.3).

*Spectral Data:* m.p.= 107-108 °C UV (MeOH) $\lambda_{max}$, nm: 199, 223 and 389; IR ($CHCl_3$) $\upsilon_{max}$, $cm^{-1}$: 2925 (C-H), 1741 (lactone C=O), 1458 (C=C) and 1259 (C-O), 1031; HRMS m/z: 504.0331 ($C_{34}H_{64}O_2$), 503, 429, 350, 151 and 60; $^1$H-NMR ($CDCl_3$, 300MHz) δ: Table-5; $^{13}$C-NMR ($CDCl_3$, 75MHz) δ: Table 6.

**1-(2-(6′-hydroxy-2′-methylcyclohex-1′-enyloxy)- 4-methoxyphenyl)ethanone (2):** The main fraction F5 was dried and subjected to the flash silica gel column chromatography. The column was eluted with dichloromethane/ethyl acetate (3.0:7.0) to afford two fractions, F5-1 and F5-2. The fraction F5-2 was rechromatographed on the precoated silica-gel (GF-254) plates eluting with ethyl acetate/acetone (8.0:2.0) solvent system which resulted in the isolation of the pure compound **2** as an amorphous material (15mg, $R_f$ = 0.6).

*Spectral Data*: UV (MeOH) λmax, nm: 228, 275, 303 and 389; IR ($CHCl_3$) $\upsilon_{max}$, $cm^{-1}$: 3321(O-H), 2925 (aromatic C-H), 1711 (C=O), 1591(C=C) and 1031 (C-O); HRMS *m/z:* 276.0361 ($C_{16}H_{20}O_4$), 275, 261, 199, 166, 151 and 60; $^1$H-NMR ($CDCl_3$, 300MHz) δ: Table-5; $^{13}$C-NMR (CDCl3, 75MHz) δ: Table 6.

**4-hydroxy-3-methoxyacetophenone (3):** The main fraction F4 was dried and subjected to flash silica gel column chromatography to afford two fractions F4-1 and F4-2. The column was eluted with petroleum ether/dichloromethane (6.5:3.5). The fraction F4-2 was rechromatographed on PTLC precoated silica gel (GF-254) plates using dichloromethane/acetone (9.5:0.5) as the solvent system. This resulted in the isolation of the pure compound **3** as an amorphous material (15mg, $R_f$ = 0.7).

*Spectral Data:* UV (MeOH) $\lambda_{max}$, nm: 204, 230, 275 and 383; IR ($CHCl_3$) $\upsilon_{max}$, $cm^{-1}$: 3400 (O-H), 2915 (C-H), 1665 (C=O), 1589 (C=C) and 1027 (C-O); HRMS *m/z:*166.0529 ($C_9H_{10}O_3$), 165, 151,123, 107, 77 and 52; $^1$H-NMR ($CDCl_3$, 300MHz) δ: Table-5; $^{13}$C-NMR ($CDCl_3$, 75MHz) δ: Table 6.

**Irisolone (4):** The fraction F4-1 was dried and again subjected to the flash silica gel column chromatography. The column was eluted with dichloromethane/ ethyl acetate (2.0: 8.0) to afford two fractions F4-1a and F4-1b. The fraction F4-1b was rechromatographed on the precoated silica gel (GF-254) plates eluting with ethyl acetate/ methanol (9.0:1.0) which resulted in the isolation of the pure compound **4** as a crystalline material (30mg, $R_f$ =0.5).

*Spectral Data*: m.p.= 267-268°C UV (MeOH) λmax, nm: 203, 263 and 323; IR ($CHCl_3$) υmax, $cm^{-1}$: 3434 (O-H), 2952 (aromatic C-H), 1658 (C=O), 1065 (C-O); HRMS *m/z*: 312.0733

($C_{17}H_{12}O_6$), 311, 295, 294, 281 and 266; $^1$H-NMR ($CD_3OD$, 300 MHz) δ: Table-5; $^{13}$C-NMR ($CDCl_3$, 100MHz) δ: Table-6.

**Irisolidone (5):** The main fraction F3 was subjected to the PTLC silica gel (GF-254) plates and eluted with dichloromethane/acetone (9.5:0.5) which resulted in the isolation of a pure compound **5** as a crystalline material (5mg, $R_f$ =0.8).

*Spectral Data:* m.p.= 191°C UV (MeOH) λmax, nm: 201, 265 and 323; IR ($CHCl_3$) υmax, $cm^{-1}$: 3436 (O-H), 2952 (aromatic C-H), 1656 (C=O), 1065 (C-O); HRMS, *m/z*: 314.0690 ($C_{17}H_{14}O_6$), 313, 299, 298, 297, 296, 271, 139, 89 and 69; $^1$H-NMR ($CDCl_3$, 300MHz) δ: Table-5.

**2-acetoxy-3,6-dimethoxy-1,4-benzoquinone (6):** The fraction F1 was again subjected to flash silica gel column chromatography. The column was eluted with ethyl acetate/pet. ether (3.0:7.0) as the solvent system to afford two fractions F1-3 and F1-4. The fraction F1-3 was rechromatographed on PTLC precoated silica gel (GF-254) plates and eluted with ethyl acetate/ pet. ether (4.0:6.0). This resulted in the isolation of the pure compound **6** as an amorphous powder (15mg, $R_f$ = 0.6).

*Spectral Data:* m.p.= 149°C UV (MeOH) $\lambda_{max}$, nm: 195, 199, 206 and 292; IR ($CHCl_3$) $\upsilon_{max}$, $cm^{-1}$: 2946 (C-H), 1727 (quinone C=O), 1608 (quinone C=O), 1425 (C=O acetate) 1508 (C=C) and 1118 (C-O); HRMS *m/z*: 226.0361 ($C_{10}H_{10}O_6$), 225, 183, 166, 151 and 60; $^1$H-NMR ($CDCl_3$, 300MHz) δ: Table-5

### *2.4. Antifungal activity*

The antifungal activities of the different fractions of methanolic extract were studied through applying agar tube dilution protocol, which is one of the most precise and reliable methods. The hexane fraction showed significant activity against *Fusarium solani* while the ethyl acetate and chloroform fraction exhibited moderate activity. The methanolic fraction showed no activity (Tables 1-4).

### *2.4.1. Extraction procedure of plant for bioassay*

The powder plant material 250 g was extracted with methanol (5 liter) for ten days. This extract was concentrated on low pressure. The distilled water (250 mL) was added in methanolic extract. This was successively extracted with hexane, chloroform and ethyl acetate to get hexane, chloroform and ethyle acetate extracts respectively.

### *2.4.2. Method for Fungicidal assay*

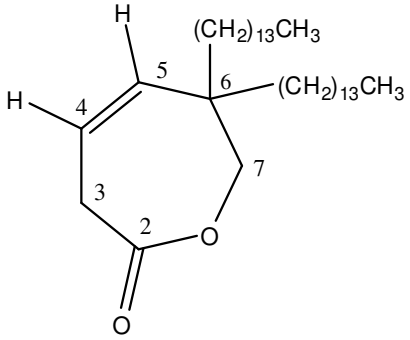
In the fungicidal test, human pathogens, *Trichophyton longifusus, Candida albicus, Asperillus flavus,* animal pathogens, *Micosporum canis* and plant pathogens, *Fusarium solani a*nd *Candida glaberata* were used. The agar tube dilution method was applied for the determination of the fungicidal activity (Rahman et. Al. 2001). Miconazole and Amphotericin B for human, animal and plant pathogens respectively were used as standard drugs. The crude extract at 300µg/media concentration was applied in the test and was incubated for 7 days at 29

$^0$C. After incubation, the percentage inhibition was measured with the help of the following formula:

% Inhibition = 100-Growth in sample ×100 / Growth in control

## 3. Results and Discussion

The mass spectrum of the compound **1** showed the molecular ion peak at *m/z* 504.0331, corresponding to the molecular formula $C_{34}H_{64}O_2$, indicating three degrees of unsaturation in the molecule. Other prominent peaks were found to occur at *m/z* 503, 429, 355, 281, 221, 147, 95 and base peak 57. The peak at *m/z* 503 indicated the loss of hydrogen atom while the peak at *m/z* 429 showed the loss of hexenyl group from the $[M-1]^+$.




**1**

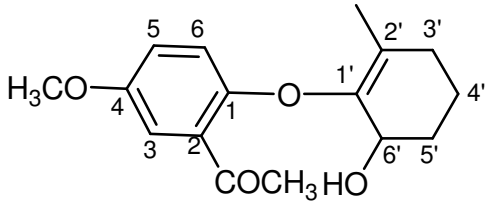
The UV spectrum (MeOH) showed the $\lambda_{max}$ absorptions at 199, 223 and 389 nm, suggesting a skeleton similar to the unsaturated lactone. The IR spectrum ($CHCl_3$) showed intense $\upsilon_{max}$ absorptions at 2925 $cm^{-1}$, 1741 $cm^{-1}$, 1458 $cm^{-1}$, 1259 $cm^{-1}$ and 1031 $cm^{-1}$, indicating the presence of C-H, lactone C=O, C=C and C-O functions in the molecule.

The $^1$H-NMR spectrum ($CDCl_3$, 300MHz) of **1** showed the presence of 64 protons resonance in the molecule. The spectrum showed 1H doublet at δ 5.33 (*J* = 7.4 Hz) which was assigned to H-5. The multiplet at δ 5.31 (1H ) was assigned to H-4. The singlet of 2H at δ 3.93 was assigned to a pair of methylene protons of $CH_2$-7, and multiplet at δ 2.83 of 2H was assigned to $CH_2$ -3. Another multiplet of 52 H between δ 1.23-1.29 was assigned for $CH_2$ –(8-20) and $CH_2$-(8′-20′). A multiplet of 6H between δ 0.83-0.85 was assigned to the $CH_3$-21 / $CH_3$-21′ methyl protons. The $^1$H-NMR chemical shift assignments are presented in Table 5.

The $^{13}$C-NMR spectrum ($CDCl_3$, 75 MHz) of the compound **1** showed the presence of thirty four carbon atoms in the molecule. The $^{13}$C-NMR chemical shift assignments were made by DEPT pulse sequences and are presented in Table 6. On the basis of spectral data, structure **1** was assigned to 6,6-di-tetradecyl-6,7-dihydrooxepine-2(3)H-one.

The mass spectrum of the compound **2** showed the molecular ion peak at *m/z* 276.0361, corresponding to the molecular formula $C_{16}H_{20}O_4$ indicating seven degrees of unsaturation in the molecule. Other prominent peaks were found to occur at *m/z* 275, 255, 239,227and base peak at

183. The peak at *m/z* 275 indicated the loss of hydrogen atom while the peak at m/z 261 showed the loss of methyl group from the molecular ion. The important peak at *m/z* 166 showed the cleavage of the molecule across the R-O bond.



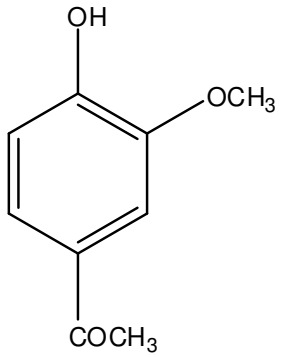

**2**

The UV spectrum (MeOH) showed the $\lambda_{max}$ absorptions at 228, 275, 303 and 389 nm, suggesting a skeleton similar to the benzene derivatives. The IR spectrum ($CHCl_3$) showed intense $\upsilon_{max}$ absorptions at 3321 $cm^{-1}$, 2925 $cm^{-1}$, 1711 $cm^{-1}$, 1591 $cm^{-1}$ and 1031 $cm^{-1}$, indicating the presence of O-H, aromatic C-H, α, β-unsaturated carbonyl, C=C and C-O functions in the molecule.

The $^1$H-NMR spectrum ($CDCl_3$, 300MHz) of **2** showed the presence of 20 protons resonance in the molecule. The spectrum showed three aromatic protons signals. A doublet proton signal at δ 7.52 with meta coupling ($J$ = 1.9 Hz) was assigned to H-6 while a double doublet at δ 7.50 with ortho and meta couplings ($J_1$ = 8.7, $J_2$ = 1.9 Hz) was assigned to H-4. A doublet at δ 6.92 with ortho coupling ($J$=8.7 Hz) was assigned to H-3 [12]. The three singlets (each 3H) at δ 3.93, δ 2.53 and δ 1.61 were assigned to the methoxy, acetyl and methyl protons, respectively. A broad singlet at δ 6.02 and a triplet at δ 3.66 ($J$ = 7.4, Hz) were assigned to the 6′-OH and 6′-H respectively, suggesting β-stereochemistry of the latter proton [13]. Being attached on a carbon which contain an oxygen H- 6′ should have been appeared well above δ 3.00. However, in the preferred conformation of the hexene ring, it falls in the shielding zone of the aromatic ring. This suggests β-stereochemistry of the proton besides its upfield chemical shift also. Its placement at 6′-C may be attributed due to the presence of the same chemical shift of the six aliphatic protons between δ 1.59-1.64 as a multiplet. If it would have been elsewhere in the cyclohexene ring, the six aliphatic protons would have not been equivalent and their resonance values have not been the same. The $^1$H-NMR chemical shift assignments are presented in Table 5.

The $^{13}$C-NMR spectrum ($CDCl_3$, 75MHz) of the compound **2** showed the presence of sixteen carbon atoms in the molecule. The $^{13}$C-NMR chemical shift assignments made by DEPT pulse sequences are presented in Table 6. On the basis of spectral data structure **2** was assigned to 1-(6′-hydroxy-2′-methylcyclohex-1′-enyloxy)-4-methoxy,2-acetophenon.

The mass spectrum of the compound **3** showed the molecular ion peak at *m/z* 166.0529, corresponding to the molecular formula $C_9H_{10}O_3$ indicating five degrees of unsaturation in the molecule. Other prominent peaks were found to occur at m/z 165, 151, 123, 107, 77 and 52. The peak at *m/z* 165 indicated the loss of hydrogen atom while the peaks at *m/z* 151 and 123 showed the loss of methyl group and an acetyl group, respectively from the molecular ion. The UV spectrum (MeOH) showed the $\lambda_{max}$ absorptions at 204, 230, 275 and 383 nm suggesting a benzene derivative skeleton. The IR spectrum ($CHCl_3$) showed intense $\upsilon_{max}$ absorptions at 3400, 2915, 1665, 1589 and 1027 $cm^{-1}$, indicating O-H, aromatic C-H, C=O, C=C and C-O functions in the molecule.

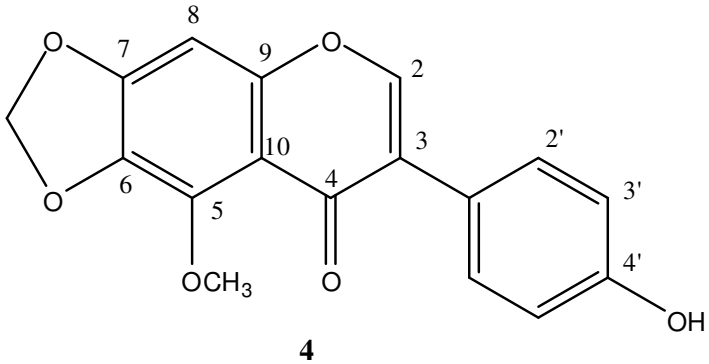



**3**

The $^{1}$H-NMR spectrum ($CDCl_3$, 300 MHz) of the compound **3** indicated the presence of 10 protons. The spectrum showed two 3H singlets at δ 3.93 and δ 2.52 assigned to the methoxy and acetyl methyl protons respectively. The spectrum showed three aromtic protons signals. The double doublet at δ 7.43 with ($J_1$=8.5, $J_2$=2.1 Hz) was assigned to H-6 while 1H doublet at δ 7.36 (*J* = 2.1Hz) which was assigned to H-2. The doublet δ 6.82 (*J* = 8.5 Hz) was assigned to H-5 [14]. The $^{1}$H-NMR chemical shift assignments are presented in the Table 5.

The $^{13}$C-NMR spectrum ($CDCl_3$, 75 MHz) of the compound **3** showed the presence of 9 carbon atoms in the molecule. The $^{13}$C-NMR chemical shift assignments determined through DEPT experiments are presented in Table-6. On the basis of above spectral data the compound **3** was identified as 4-hydroxy-3-methoxyacetophenone [15].

The mass spectrum of the compound **4** showed the molecular ion peak at *m/z* 312.0733, corresponding the molecular formula $C_{17}H_{12}O_6$ indicating twelve degrees of unsaturation in the molecule. Other major peaks were found to occur at *m/z* 311, 295, 294, 281 and 266. The peak at *m/z* 311 indicated the loss of hydrogen atom while the peak at *m/z* 281 showed the loss of methoxy group. The peak at *m/z* 266 showed the loss of O-$CH_2$-O from the molecular ion. The peak at *m/z* 294 and 295 suggested the loss of water molecule and hydroxyl group from the molecule, respectively.




**4**

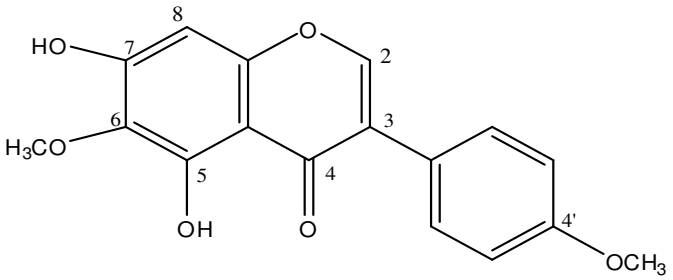
The UV spectrum (MeOH) of the compound **4** showed λmax absorptions at 203 263 and 323 nm, suggesting the isoflavone skeleton. The IR spectrum ($CHCl_3$) showed intense absorptions at 3434, 2952, 1658 and 1065 $cm^{-1}$, indicating that O-H, aromatic C-H, α, β-unsaturated carbonyl and C-O functions in the molecule.

The $^{1}$H NMR spectrum (300 MHz, $CD_3OD$) of the compound **4** showed 12 proton

resonances in the molecule. The aromatic proton appeared at δ 6.76 as a singlet was assigned to H-8. The other singlet at δ 8.01 was assigned to the H-2 indicating the isoflavone skeleton. Two peaks at δ 7.31 (d, *J*= 8.5 Hz, 2H) and 6.79 (d, *J*= 8.5 Hz, 2H) showed the presence of four aromatic protons attached to C-2′, 6′ and C-3′, 5′ respectively. The position of hydroxyl group on B-ring was determined by the $^{1}$H-NMR. The splitting pattern of four aromatic protons on the B-ring was characteristic of para disubstitution. The 3H singlet at δ 4.03 was assigned to the 5-$OCH_3$ protons, while the 2H singlet appeared at δ 6.12 was assigned to the methylenedioxy protons [16]. The $^{1}$H-NMR shifts are given in Table 5.

The $^{13}$C-NMR spectrum ($CDCl_3$, 100 MHz) of the compound **4** showed the presence of 17 carbon atoms in the molecule. The $^{13}$C-NMR chemical shift assignments determined through DEPT experiments are presented in Table 7. On the basis of above spectral data, the compound **4** was identified as irisolone [16-19].

The mass spectrum of the compound **5** showed the molecular ion peak at *m/z* 314.0690, corresponding to the molecular formula $C_{17}H_{14}O_6$, indicating 11 degrees of unsaturation in the molecule, Other prominent peaks were found to occur at *m/z* 313, 299, 298, 297, 296, 271, 139, 132, 89 and 69. The peak at *m/z* 313 indicated the loss of hydrogen atom while the peak at *m/z* 299 showed the loss of methyl group from the molecular ion. The peak at *m/z* 298, 297 and 296 suggested the loss of oxygen atom, hydroxyl group and water molecule from the molecule, respectively.



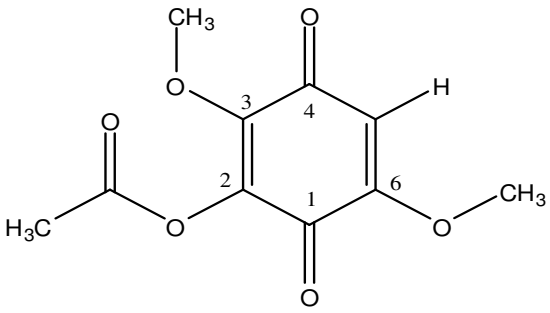

**5**

The UV spectrum (MeOH) of the compound **5** showed the λ max absorptions at 201, 265 and 323 nm, suggesting the isoflavone skeleton. The IR spectrum ($CHCl_3$) showed intense absorptions at 3434 $cm^{-1}$, 2952 $cm^{-1}$, 1658 $cm^{-1}$ and 1065 $cm^{-1}$, indicating the presence of O-H, aromatic C-H, α, β-unsaturated carbonyl and C-O functions in the molecule.

The $^{1}$H NMR spectrum (300 MHz, $CD_3OD$) of the compound **5** showed 14 proton resonances in the molecule. The aromatic proton appeared at δ 6.49 as a singlet was assigned to H-8. The upfield shift of the H-8 proton was due to the presence of OH substitution at adjacent carbon. The other singlet at δ 7.84 was assigned to the H-2 indicating the isoflavone skeleton. Two peaks at δ 7.44 (d, *J*= 8.2 Hz, 2H) and 6.97 (d, *J*= 8.2 Hz, 2H) showed the presence of four aromatic proton attached to C-2′, 6′ and C-3′, 5′ respectively [3]. The position of methoxy group on B-ring was determined by the $^{1}$H-NMR. The splitting pattern of four aromatic protons on the B-ring was characteristic of para disubstitution. The two singlets of 6H at δ 4.03 and δ 3.82 was assigned to the 6-$OCH_3$ and 4′-$OCH_3$ protons, as the irradiation of singlet at δ 6.97 (H-3′/H-5′) resulted in NOE at δ 3.82 [20].

The broad singlet at δ 13.10 was assigned to chelated 5-OH. On the basis of spectral data, the compound **5** was identified as irisolidone [20-22]. The $^{1}$H-NMR chemical shifts are presented in Table 5. The $^{13}$C-NMR chemical shift assignments determined through DEPT experiments are presented in Table 7.

The mass spectrum of the compound **6** showed the molecular ion peak at *m/z* 226.0361, corresponding to the molecular formula $C_{10}H_{10}O_{6,}$ indicating five degrees of unsaturation in the molecule. Other prominent peaks were found to occur at *m/z* 225, 183, 166, 151 and 60. The peak at *m/z* 225 indicated the loss of hydrogen atom while the peaks at *m/z* 183 showed the loss of $COCH_3$ group from the molecular ion. The important peak at *m/z* 166 showed the loss of $HCOOCH_3$ moiety from the molecule.




**6**

The UV spectrum (MeOH) showed the $\lambda_{max}$ absorptions at 195, 199, 206 and 292nm, suggesting a skeleton similar to the quinone. The IR spectrum ($CHCl_3$) showed intense absorptions at 2946, 1727, 1608, 1425, 1265 and 1118 $cm^{-1}$, indicating that C-H, C=O(quinone, 2 position), C=O(quinone, 5 position), acetate C=O, C=C and C-O functions in the molecule.

The $^1$H-NMR spectrum ($CDCl_{3,}$ 300 MHz) of the compound **6** indicated the presence of 10 protons. The spectrum showed 1H singlet at δ 5.98 assigned to the H-5. The two singlets of 6H each at δ 3.84, δ 3.44 were assigned to the two methoxy groups at position 3 and 6 respectively. The 3H singlet appeared at δ 1.93 was assigned to acetate methyl protons[23]. The $^1$H-NMR chemical shift assignments are presented in the Table 5. The $^{13}$C-NMR chemical shift assignments determined through DEPT experiments are presented in Table 7.

**Table 1.** In *Vitro* Antifungal Bioassay of Hexane Fraction

| Name of the Fungus | Linear growth (mm) | | % Inhibition | Std. Drugs MIC µg/mL | % Inhibition of Std. Drug |
|---|---|---|---|---|---|
| | Sample | Control | | | |
| *Trichophyton longifusus* | 50 | 100 | 50 | Miconazole | 70 |
| *Candida albicans* | 100 | 100 | 0 | Miconazole | 110.8 |
| *Aspergillus flavus* | 100 | 100 | 0 | Amphotericin B | 20 |
| *Microsporum canis* | 70 | 100 | 30 | Miconazole | 98.4 |
| *Fusarium solani* | 30 | 100 | 70 | Miconazole | 73.25 |
| *Candida glaberata* | 100 | 100 | 0 | Miconazole | 110.8 |

Key: Concentration of Sample 400 µg/mL of DMSO. Incubation Temp. 27($28^o \pm 1\ ^oC$)

Isolated compounds from *Iris germanica*

**Table 2.** In *Vitro* Antifungal Bioassay of Ethyl acetate Fraction

| Name of the Fungus | Linear growth (mm) | | % Inhibition | Std. Drugs MIC µg/mL | % Inhibition of Std. Drug |
|---|---|---|---|---|---|
| | Sample | Control | | | |
| *Trichophyton longifusus* | 50 | 100 | 50 | Miconazole | 70 |
| *Candida albicans* | 100 | 100 | 0 | Miconazole | 110.8 |
| *Aspergillus flavus* | 30 | 100 | 40 | Amphotericin B | 20 |
| *Microsporum canis* | 70 | 100 | 30 | Miconazole | 98.4 |
| *Fusarium solani* | 40 | 100 | 50 | Miconazole | 73.25 |
| *Candida glaberata* | 100 | 100 | 0 | Miconazole | 110.8 |

Key: Concentration of Sample 400 µg/mL of DMSO. Incubation Temp. 27($28^{o}\pm1\ ^{o}C$)

**Table 3.** In *Vitro* Antifungal Bioassay of Chloroform Fraction

| Name of the Fungus | Linear growth (mm) | | % Inhibition | Std. Drugs MIC µg/mL | % Inhibition of Std. Drug |
|---|---|---|---|---|---|
| | Sample | Control | | | |
| *Trichophyton longifusus* | 80 | 100 | 20 | Miconazole | 70 |
| *Candida albicans* | 100 | 100 | 0 | Miconazole | 110.8 |
| *Aspergillus flavus* | 100 | 100 | 0 | Amphotericin B | 20 |
| *Microsporum canis* | 60 | 100 | 40 | Miconazole | 98.4 |
| *Fusarium solani* | 50 | 100 | 50 | Miconazole | 73.25 |
| *Candida glaberata* | 100 | 100 | 0 | Miconazole | 110.8 |

Key: Concentration of Sample 400 µg/mL of DMSO. Incubation Temp. 27($28^{o}\pm1\ ^{o}C$)

**Table 4.** In *Vitro* Antifungal Bioassay of Methanol Fraction

| Name of the Fungus | Linear growth (mm) | | % Inhibition | Std. Drugs MIC µg/ml | % Inhibition of Std. Drug |
|---|---|---|---|---|---|
| | Sample | Control | | | |
| *Trichophyton longifusus* | 50 | 100 | 0 | Miconazole | 70 |
| *Candida albicans* | 100 | 100 | 0 | Miconazole | 110.8 |
| *Aspergillus flavus* | 85 | 100 | 3 | Amphotericin B | 20 |
| *Microsporum canis* | 40 | 100 | 0 | Miconazole | 98.4 |
| *Fusarium solani* | 90 | 100 | 10 | Miconazole | 73.25 |
| *Candida glaberata* | 100 | 100 | 0 | Miconazole | 110.8 |

Key: Concentration of Sample 400 µg/mL of DMSO. Incubation Temp. 27($28^{o}\pm1\ ^{o}C$)

**Table 5.** $^{1}$H-NMR Chemical Shift Assignments for the Compounds, **1**, **2, 3, 4, 5** and **6**.

| Proton No. | Chemical Shift (δ) | Integration | Multiplicity | Coupling Constant (*J*) in Hz |
|---|---|---|---|---|
| **6,6-ditetradecyl-6,7-dihydrooxepin-2(3H)-one (1)** | | | | |
| H-3 | 2.79 | 2H | m | - |
| H-5 | 5.33 | 1H | d | 7.4 |
| H-4 | 5.31 | 1H | m | - |
| H-7 | 3.93 | 2H | s | - |
| H-(8-20)/(8′-20′) | 1.23-1.29 | 52H | m | - |
| $CH_3$-21/$CH_3$-21’ | 0.83-0.85 | 6H | m | - |
| **1-(2-(6′-hydroxy-2′-methylcyclohex-1′-enyloxy)-5-methoxyphenyl)ethanone (2)** | | | | |
| H-4 | 7.52 | 1H | dd | 8.7, 1.9 |
| H-6 | 7.50 | 1H | d | 1.9 |
| H-3 | 6.92 | 1H | d | 8.7 |
| 5-$OCH_3$ | 3.93 | 3H | s | - |
| 1- CO*$CH_3$* | 2.53 | 3H | s | - |
| 6′-OH | 6.02 | 1H | bs | - |
| 6′-H | 3.66 | 1H | t | 7.4 |
| 2′-$CH_3$ | 1.61 | 3H | s | - |
| -3′, 4′, 5′-$CH_2$ | 1.23-1.60 | 6H | m | - |
| **4-hydroxy-3-methoxyacetophenone (3)** | | | | |
| H-6 | 7.43 | 1H | dd | 8.5, 2.1 |
| H-2 | 7.36 | 1H | d | 2.1 |
| H-5 | 6.82 | 1H | d | 8.5 |
| 3-$OCH_3$ | 3.93 | 3H | s | - |
| $COCH_3$ | 2.52 | 3H | s | - |
| 4-OH | 1.60 | 1H | bs | - |
| **Irisolone (4)** | | | | |
| H-8 | 6.93 | 1H | s | - |
| H-2′,6′ | 7.31 | 2H | d | 8.5 |
| H-3′,5′ | 6.79 | 2H | d | 8.5 |
| H-2 | 8.01 | 1H | s | - |
| 5-$OCH_3$ | 4.03 | 3H | s | - |
| Methylenedioxy | 6.12 | 2H | s | - |
| 4′-OH | 9.49 | 1H | s | - |
| **Irisolidone (5)** | | | | |
| H-2 | 7.84 | 1H | s | |
| H-2′,6′ | 7.44 | 2H | d | 8.2 |
| H-3′,5′ | 6.97 | 2H | d | 8.2 |
| H-8 | 6.49 | 1H | s | - |
| 6-$OCH_3$ | 4.03 | 3H | s | - |
| 4′-$OCH_3$ | 3.82 | 3H | s | - |
| 5-OH | 13.10 | 1H | bs | - |
| **2-acetoxy-3,6-dimethoxy-1,4-benzoquinone (6)** | | | | |
| H-5 | 5.98 | 1H | s | - |
| 3-$OCH_3$ | 3.84 | 3H | s | - |
| $COCH_3$ | 1.93 | 3H | s | - |
| 6-$OCH_3$ | 3.44 | 3H | s | - |

Isolated compounds from *Iris germanica*

**Table 6.** $^{13}$C- NMR Shift Assignments for the Compounds **1, 2,** and **3**.

| **Compound 1** | | **Compound 2** | | **Compound 3** | |
|---|---|---|---|---|---|
| Carbon No. | Chemical Shift (δ) | Carbon No. | Chemical Shift (δ) | Carbon No. | Chemical Shift (δ) |
| 2-C | 174.28 | 1′-C | 150.3 | 4-C | 150.43 |
| 3-C | 34.11 | 2′-C | 77.2 | 3-C | 146.65 |
| 4-C | 127.91 | 3′-C | 29.0 | 2-C | 113.79 |
| 5-C | 130.19 | 4´-C | 24.7 | 1-C | 130.36 |
| 6-C | 25.52 | 5´-C | 33.3 | 5-C | 109.83 |
| 7-C | 71.37 | 6′-C | 113.7 | 6-C | 123.98 |
| 8-C/8′-C | 31.91 | 1-C | 130.3 | *CO*$CH_3$ | 196.65 |
| 9-C/9′-C | 27.19 | 2-C | 151.4 | 3-O*$CH_3$* | 56.11 |
| 10-C/10′-C | 29.68 | 3-C | 122.3 | *$CH_3$*CO | 26.12 |
| 11-C/11′-C | 29.58 | 4-C | 124.7 | - | - |
| 12-C/12′-C | 29.44 | 5-C | 156.4 | - | - |
| 13-C/13′-C | 29.34 | 6-C | 115.1 | - | - |
| 14-C/14′-C | 29.24 | 2-CO*$CH_3$* | 27.1 | - | - |
| 15-C/15′-C | 29.14 | *CO* | 196.7 | - | - |
| 16-C/16′-C | 29.14 | 5-O$CH_3$ | 56.0 | - | - |
| 17-C/17′-C | 29.14 | 6´-$CH_3$ | 15.7 | - | - |
| 18-C/18′-C | 29.14 | - | - | - | - |
| 19-C/19′-C | 24.96 | - | - | - | - |
| 20-C/20′-C | 22.68 | - | - | - | |
| 21-C/21′-C | 14.08 | - | - | - | - |

**Table 7.** $^{13}C$- NMR Shift Assignments for the Compounds **4, 5,** and **6.**

| **Compound 4** | | **Compound 5** | | **Compound 6** | |
|---|---|---|---|---|---|
| Carbon No. | Chemical Shift (δ) | Carbon No. | Chemical Shift (δ) | Carbon No. | Chemical Shift (δ) |
| 2-C | 153.1 | 2-C | 151.3 | 1-CO | 178.8 |
| 3-C | 127.0 | 3-C | 122.9 | 2-C | 132.3 |
| 4-C | 171.3 | 4-C | 179.9 | 3-C | 155.6 |
| 5-C | 137.0 | 5-C | 152.3 | 4-CO | 180.5 |
| 6-C | 131.2 | 6-C | 131.1 | 5-C | 105.3 |
| 7-C | 152.0 | 7-C | 156.0 | 6-C | 160.7 |
| 8-C | 94.0 | 8-C | 94.1 | *COO* | 169.2 |
| 9-C | 152.0 | 9-C | 153.1 | 3-$OCH_3$ | 51.9 |
| 10-C | 124.0 | 10-C | 107.0 | 6-$OCH_3$ | 52.1 |
| 1′-C | 122.1 | 1′-C | 121.9 | *$CH_3$*COO | 21.3 |
| 2′-C/6′-C | 131.5 | 2′-C/6′-C | 129.7 | - | |
| 3′-C/5′-C | 116.1 | 3′-C/5′-C | 113.3 | - | |
| 4′-C | 165.1 | 4′-C | 158.7 | - | |
| $OCH_2O$ | 104.0 | 4′-$OCH_3$ | 55.7 | - | |
| 5-$OCH_3$- | 61.3 | 7′-$OCH_3$ | 59.8 | - | |

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