# ISOLATION AND IDENTIFICATION OF SOME PHYTOCHEMICAL COMPOUNDS FROM DIFFERENT PARTS OF *IRIS NIGRICANS*

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**Abstract**

*Iris genus* (Iridaceae) is an elegant endemic plant species, and considered threatened to Jordan due to habitat degradation. Rhizomes of some Iris species contain flavonoids such as iridin, iriline A, irisone B. Iris leaves contain many flavonoids such as irilin, iristectorigenins, tectorigenin, and coumaronochromones. Iris flowers contain also flavonoids and proanthocyanidin while *Iris nigricans* have no previous studies conducted on leaves and flowers. In this study some phytochemical compounds were separated from different parts of *Iris nigricans* (rhizomes, leaves and flowers) by methanol. Isolation is carried out by using chromatographic technique and identified by ferric chloride reagent and UV light λmax254-365nm. The phenolic composition of methanolic extracts of *Iris nigricans* obtained from rhizomes, leaves and flowers were elucidated by MS, $^{1}$H-NMR and $^{13}$C-NMR.

**Keywords:** Methanolic extracts of *Iris nigricans* (different parts), chromatographic technique, MS, $^{1}$H-NMR, $^{13}$C-NMR, mangeferin, iristectoreginin A, proanthocyanidin

**Footnotes:** MS= Mass Spectrometry, $^{1}$H-NMR= Proton Nuclear Magnetic Resonance, $^{13}$C-NMR= Carbon 13 Nuclear Magnetic Resonance**.**

## Introduction

Medicinal plants have an essential role in the drug discovery and many modern drugs have their origin in traditional medicines of different cultures. Although the synthetic and combinatorial chemistry as well as molecular modeling are beneficial, medicinal plants

remain an essential source of new drugs, new drug leads and new chemical entities (NCEs). (Rana Abu-Dahab, et al., 2007)

Iris species which have different chemical constituents (glycosides, phenolic compounds and xanthones) which related to different biological activities such as anti-inflammatory, antioxidant activity, antiseptic, pain relief, antibacterial and antifungal. (Das DK, et al., 1999, Sajee' Huwaitat, et al., 2013)

Iris leaves contain many isoflavones particularily iristectorigenin A which has different biological activities such as estrogenic activity. (Eva Miadokova', 2009)

Other constituent like xanthone is found in Iris genus used as an insecticide (Jian Zhao, et al., 2005). Proanthocyanidins compounds are found in this genus which related to different biological activities. (Das DK, et al., 1999, Sajee' Huwaitat, et al., 2013)

**Methodology**

**Plant Material**

The rhizomes, leaves and flowers of *Iris nigricans* grown in Jordan were collected during the months of March and April (2012). The rhizomes and leaves were dried at room temperature in the shade for about 15 days and then weighed. The flowers were cut into small pieces and weighed.

**Extraction Methods**

**Extraction of rhizomes**

Dried rhizomes (12.800 gm) were grinded into powder by a mixer followed by extraction with 80% methanol in soxhlet apparatus for 48 hours at temperature between 60 - 70$^{o}$C. The evaporated methanolic extract (1.629gm) was collected in amber glass containers to avoid any oxidation. (Nighat Nazir, et al., 2008, Syeda F., et al., 2009).

**Extraction of leaves and flowers**

Fresh leaves (12.200gm) and fresh flowers (10gm) were macerated in 70% v/v and 95% v/ of methanol for one week in refrigerator. The evaporated ectracts were kept in amber glass containers to avoid any oxidation. (V. H. Booth, et al., 1960, E. Nicole Bridgers, et al., 2010).

**Chromatographic technique**

All extracts (rhizomes, leaves and flowers) were subjected to thin-layer chromatography using the mobile phases (chloroform 68 ml: methanol 32 ml), (chloroform 68 ml: methanol 32 ml) and (chloroform 50 ml: methanol 50 ml) respectively. All the chromatograms were visualized by UV light ($\lambda_{254}$ nm – $\lambda_{365}$ nm) and then spraying with ferric chloride solution.

**Isolation and Identification Methods**

One band from each extracts of *Iris nigricans* (rhizomes, leaves and flowers) were isolated by using preparative TLC (20 x 20 cm) coated with silica gel $GF_{254}$ layers of 1 mm thickness and using the same mobile phases as mentioned before. These bands were selected according to the presence of orange color and clear fluorescence under UV light and to the width of the bands. The isolated compounds out of the silica gel were subjected for further spectral analysis (MS, $^{1}$H-NMR and $^{13}$C-NMR).

**Results and Discussion**

**Extraction Method**

All the extracts from the rhizomes, leaves and flowers were gave dark blue color due to the formation of colored complex $Fe(OAr)_3$. (Ranju Pal, et al., 2012).

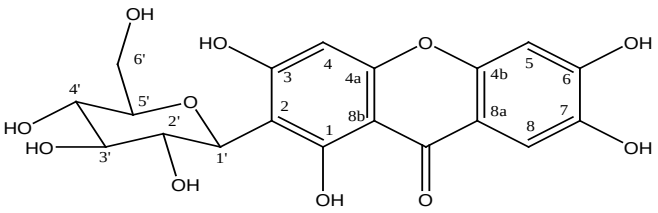
According to the Preparative Thin-Layer Chromatography of the rhizomes, among the bands one yellow band in daylight gave the clear fluorescent band and named by band 1 for rhizomes which subjected for further spectral analysis (MS, $^{1}$H-NMR and $^{13}$C-NMR) and gave the following results.

EI-mass spectrum of band 1 from yellow band 2 of rhizomes was measured on high resolution mass spectrometer using ESI technique (electron spray ionization) and methanol and chloroform as solvent. The spectrum showed the following bands at *m/z*: 422 ($M^{+\bullet}$), 423 ($M^{+\bullet}$ +1), 424 ($M^{+\bullet}$ +2) due to the existence C isotope.

$^{1}$H-NMR spectrum (MeOH-$d_4$, 500 Hz) of this fraction showed the appearance of the following bands (δ ppm): 7.32 (1H,s, H-8); 6.39 (1H,s, H-5); 6.19(1H,S, H-4); (5.00-4.96) (free $OH_s$ exchangeable with $D_2O$); (4.15-3.95) (2H, m, H-1' and H-5'); (3.75-3.65) (3H, m, H-2', H-3', H-4'); (3.55- 3.45) (2H, m, H-6').

$^{13}$C-NMR spectrum (MeOH-$d_4$, 500 Hz) showed the following chemical shifts δ (ppm): 182.20 (C=O); 162.10 (C-3); 160.10 (C-1); 155.70 (C-4a); 150.70 (C-6); 149.70 (C-4b); 140.10 (C-7); 117.20 (C-8a); 115.00 (C-8); 106.30 (C-2); 105.40 (C-5), 102.40 (C-8b); 97.30 (C-4); 85.00 (C-5'); 80.70 (C-3'); 77.20 (C-1'); 72.90 (C-2'); 70.50 (C-4'); 65.30 (C-6').

The above spectral data (MS, $^{1}$H-NMR and $^{13}$C-NMR) for this band is consistent with the structure of mangiferin presented below. (Nathalie Wauthoz, et al., 2007 and Watson, 2001)




**Mangiferin ($C_{20}H_{22}O_{10}$)**

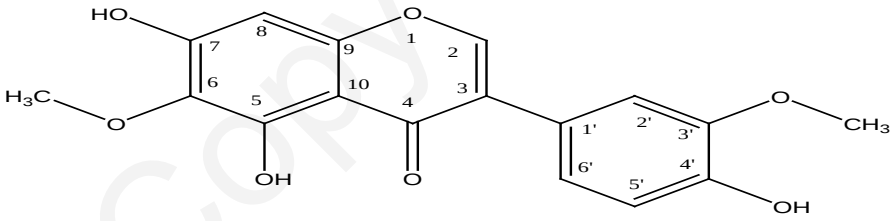
According to the Preparative Thin-Layer Chromatography of the leaves, orange band in daylight and under UV light was appeared clearly and then subjected to spectral analysis by MS, $^{1}$H-NMR and $^{13}$C-NMR. Orange band gave the following results.

EI-mass spectrum of orange band was measured on high resolution mass spectrometer using ESI technique (electron spray ionization). The spectrum showed peaks at *m/z*: 330 ($M^{+\bullet}$), 331 ($M^{+\bullet}$ +1) and 332 ($M^{+\bullet}$ +2) due to the existence C isotope.

$^{1}$H-NMR spectrum (MeOH-$d_4$, 500 Hz) of this fraction showed the appearance of the following bands δ (ppm): 7.75 (1H, m, H-2); 7.15 (1H, m, H-5'); 6.95 (1H, m, H-6'); 6.55 (1H, m, H-2'); 6.25 (1H, s, H-8); 5.5- 5.25 (3H, br.s, free OHs exchangeable with D2O); 3.85 (6H, s, $OCH_3$-6 and $OCH_3$-3').

$^{13}$C-NMR using MeOH-$d_4$ as solvent and high resolution 500 Hz. Spectrum showed the following chemical shifts δ (ppm): 175.40 (C-4); 153.60 (C-9); 153.20 (C-2); 152.40 (C-7); 147.20 (C-5); 146.50 (C-4'); 137.10 (C-6 and C-3'); 126.60 (C-1'); 123.50 (C-3); 120.40 (C-6'); 117.20 (C-2'); 113.60 (C-5'); 106.50 (C-10); 99.00 (C-8); 56.50 ($OCH_3$-6 and $OCH_3$-3').

These spectral data (MS, $^{1}$H-NMR and $^{13}$C-NMR) for this fraction are consistent with the structure of iristectorigenin A presented below. (Le Minh Ha, et al., 2009 and Watson, 2001)




**Iristectorigenin A ($C_{17}H_{14}O_7$)**

The results for the Preparative Thin-Layer Chromatography of the flowers, band 1 out of several bands was choosed for clear fluorescent band under UV light then this band was subjected to different spectral analysis including MS, $^{1}$H-NMR and $^{13}$C-NMR, the results were illustrated.

EI-mass spectrum of band 1 from total flowers extract was measured on high resolution mass spectrometer using ESI technique (electron spray ionization).

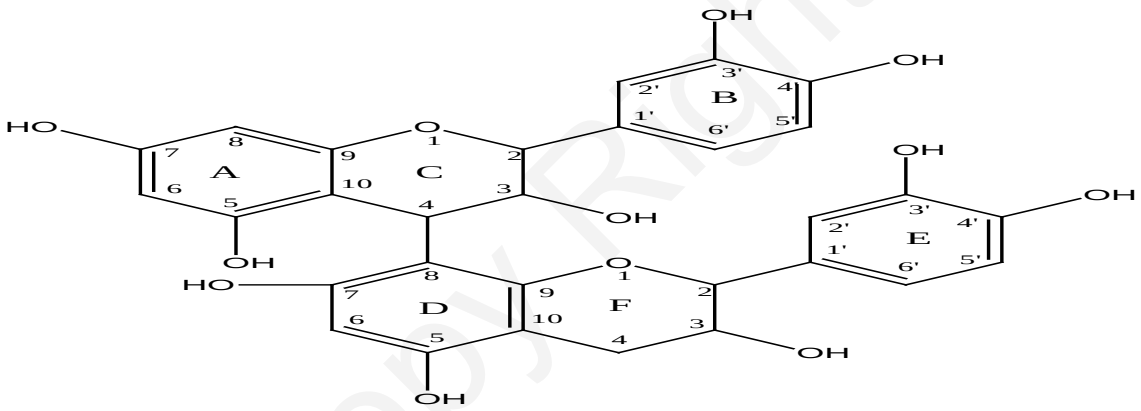
The spectrum showed the following peaks at *m/z*: 578 ($M^{+\bullet}$); 579 ($M^{+\bullet}$ +1) and 580 ($M^{+\bullet}$ +2) due to the existence C isotope.

$^{1}$H-NMR spectrum (MeOH-$d_4$, 500 Hz) of this fraction showed the appearance of the following bands δ (ppm): 7.65-7.55 (6H, m, H-2', H-5', H-6' in rings B and E); 7.25-7.15 (3H,

m, H-6 and H-8 in ring A and H-6' in ring D) ; 5.35- 5.15 (10H, br.s, free OHs exchangeable with $D_2O$); 4.95- 4.85 (2H, m, H-2 in rings C and F); 4.45- 4.25 (2H, m, H-3 protons in rings C and F); 2.95- 2.85 (1H, m, H-4 in ring C); 2.75-2.55 (2H, m, H-4 in ring F).

$^{13}$C-NMR spectrum (MeOH-$d_4$, 500 Hz) showed the appearance of the following chemical shifts δ (ppm): 158.80 (C-7 in ring A); 157.40 (C-5 in ring A); 155.70 (C-5 in ring D); 154.80 (C-9 in ring A and C-7, C-9 in ring D); 147.40 (C-4' in ring B and ring E); 144.60 (C-3' in ring B and ring E); 132.60 (C-1' in ring B and ring E); 122.20 (C-6' in ring B and ring E); 117.40 (C-5' in ring B and ring E); 115.20 (C-2' in ring B and ring E); 82.40 (C-2 in ring C); 79.50 (C-2 in ring F); 72.70 (C-3 in ring C); 67.80 (C-3 in ring F); 29.20 (C-4 in ring C) and 26.90 (C-4 in ring F).

These spectral data (MS, $^1$H-NMR and $^{13}$C-NMR) for this fraction are consistent with the structure of dimeric proanthocyanidin presented below. (Kohei Kamiya, et al., 2001 and Watson, 2001).




**Proanthocyanidin ($C_{30}H_{26}O_{12}$)**

## Conclusion

Since there is no previous studies on the chemical constituents of the leaves and flowers of *Iris nigricans* grown in Jordan, this research showed the isolation and identification of some of phenolic compounds (glycosides, flavonoids and xanthones). Mangiferin was elucidated in rhizomes, the isoflavonoid iristectorigenin A was documented in leaves and proanthocyanidin was determined in flowers.

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