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Original Research Article

Antioxidant potentials of five flavonoids compounds isolated from Varthemia iphionoids

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Abstract

Purpose: *To evaluate the in vitro antioxidant activity of some flavonoids isolated from Varthemia iphionoids aqueous methanol extract.*
Methods: *A portion (330 g) of the aqueous methanol extract of Varthemia iphionoids was adsorbed onto 200 g of S silica gel (70 - 230 mesh) and then loaded onto a column of S silica gel (70 - 230 mesh, 100 g). The column was packed with chloroform and eluted with chloroform-methanol mixtures of increasing polarity. Column chromatography purification (silica gel, 400 mesh) and thin-layer chromatography (TLC) or recrystallization yielded 5 compounds subsequently identified by nuclear magnetic resonance (NMR) and mass spectroscopy (MS).*
Results: *The antioxidant activities of five identified flavonoids: Kumatakillin (1), Penduletin (2), Jeceidine (3), 6-Methoxy isokaemfride (4), and 3,3'-Di-O-methyl quercetin (5) were determined. The results indicated that compound 3 exhibited the best scavenging activity against DPPH radical and H_2O_2 comparable to that of quercetin as standard. All compounds had the ability to prevent the oxidation of β-carotene. In anti-hemolytic assay, the five compounds gave HT_{50} higher than vitamin C. The best protection activity of erythrocytes membrane was recorded with compound 2 (HT_{50} = 60.14 ± 0.72 min). Moreover, all compounds had significantly increased blood clotting time, and among them, compounds 1, 2, and 3 exhibited significantly increased thrombolytic activity compared to the control (***p < 0.001).*
Conclusion: *These results indicate that Varthemia iphionoids has good antioxidant activities but further studies on the plant extract are required.*

Keywords: *Varthemia iphionoids, Flavonoids, NMR, Free radicals, Antioxidant, Antihemolytic, Anticoagulant*

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INTRODUCTION

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drugs, because of the unmatched availability of chemical diversity. Many beneficial biological activities such as anticancer, antimicrobial,

antioxidant, antidiarrheal, analgesic, and wound healing potentials have been reported [1].

In many cases, claims of the beneficial effects of certain natural or herbal products have been made. However, clinical trials are necessary to verify the traditional effectiveness of a bioactive compound. Free radicals and reactive oxygen species (ROS) which are formed under normal physiological conditions, become deleterious when they are not eliminated by antioxidants defenses, thus creating harmful molecules. These reactive molecules contribute to degenerative processes related to aging and diseases and damage the structure of red blood cells membrane thereby impairing their function [2,3]. Antioxidants inhibit bimolecular oxidation, acting through their atom donation to form a new radical more stable than the initial one and as chain-breaking agents in many states such as lipid peroxidation. Therefore, in recent years, the search for natural and safe plant-based antioxidants has increased.

Phenolic compounds are considered as most important natural antioxidants and are reported to have positive effects on human health, such as anti-inflammatory and anti-carcinogenic effects. There is an increasing trend in correlating the phytochemical components of plants with their therapeutic activities. Plant materials contain many bioactive compounds which have been investigated and proven to be effective against diseases [1].

Varthemia iphionoids (Boiss. and Bblanche), Family Compositae (Asteraceae), is a common aromatic herb in Jordan that possesses a high content of essential oils. The water extract of the plant is used in Jordanian folk medicine for treatment of kidney and gastrointestinal disorders [4]. Previous studies on the plant resulted in the isolation of flavonoids in addition to two new monoterpenes [5,6]. In this present study, five compounds were isolated and identified from the plant. Their antioxidant activity was investigated through *in vitro* established methods that deal with free radicals, and metal ions in addition to coagulation factors.

EXPERIMENTAL

Instruments

Mass spectra were obtained using Finnigan MAT TSQ-70 spectrometer at 70 eV; ion source temperature = 200 °C. Nuclear magnetic resonance ^{1}H-NMR spectra were recorded on a Bruker DPX-300 MHz or 250 MHz spectrometer with TMS as internal standard, while ^{13}C-NMR spectra were recorded at 75.4767 MHz. All optical densities were recorded on SHIMADZO (UV- 1800) and DRAWELL (DV-8000) spectrophotometers.

Chemicals

Silica gel S (Riedel de-Haen 70-130 mesh), DF_{254} (Riedel de-Haen 400 mesh), or HF_{254} (Merck 400 mesh) were used for column chromatography (CC). In some cases, the first step of purification was performed on TLC using TLC glass plates which were manually prepared using silica gel DGF_{254}, silica gel DG (Riedel de-Haen), or HF_{254} (Merck). Final purification of compounds was performed on commercial silica gel glass plates GF_{254}, 0.5 mm thickness (Merck). Ascorbic acid, 1,1-Diphenyl-2-picryl-hydrazil (DPPH), 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH), quercetin, β-carotene, Linoleic acid, trichloroacetic acid, hydrogen peroxide, iron trichloride/ferrous chloride, potassium ferricyanide, and Folin-Ciocalteau reagent were purchased from Sigma-Aldrich and Fluka (Germany).

Solvents

Several types of solvents were used, throughout the work, which in most cases were re-distilled prior to use. Detection of the compounds was achieved using short UV radiation, then spraying with anisaldehyde spray reagent followed by heating on a hot plate.

Plant material, extraction, and fractionation

Varthemia iphionoids (Boiss and Blanche) was collected near Al-Mustaba 30 km north of Amman along the highway to Irbid during the flowering period in July 2002. The plant was identified by Prof. Musa Abu Zarga at the Chemistry Department, University of Jordan. A voucher specimen (NoJU/02/CV/1001) was deposited at the Natural Products Laboratory, University of Jordan.

The aerial part of the plant was dried at room temperature in dark, ground to fine powder (19 kg), and defatted via extraction with petroleum ether 40 - 60 ^{o}C (50 L) at room temperature for one week. The residual defatted plant material was then extracted with ethanol at room temperature (50 L, 3 times, 7 days each).

The combined ethanol extract was evaporated under vacuum and the resulting crude residue (1 kg) was partitioned between 10 % aqueous methanol as one phase and n-hexane as the other phase. The aqueous methanol layer was

evaporated to yield a gummy residue that weighed 660 g.

Chromatography of aqueous methanol extract

Part of the aqueous methanol extract (330 g) was adsorbed on 200 g of silica gel S (70 - 230 mesh) and loaded on a silica gel column S (70 - 230 mesh particle size, 100 g). The column was packed in chloroform and eluted with chloroform-methanol mixtures of increasing polarity to give 131 fractions (5000 mL each) which were combined into eleven groups (I - XI) according to their TLC behavior. Each group was further purified via column chromatography (silica gel, 400 mesh particle size) and TLC or recrystallization.

Fraction I (47 g) gave kumatakillin **(1)** (820 mg) and penduletin **(2)** (450 mg). The combined fractions II and III (23 g) resulted in jeceidine **(3)** (600 mg). Fractions IV and V were combined (40 g) to give quercetin-3,3'-di-O-methyl ether **(4)** (170 mg. Fraction VI (25 g) gave 6-methoxyisokaemferide **(5)** (160 mg). The other fractions did not give any identifiable products.

Radical DPPH scavenging assay

The DPPH bleaching assay was conducted according to the method of Aouachria *et al* [7]. A volume of 50 µL of various dilutions of compounds or standard (quercetin) was mixed in a test tube containing 1250 µL of a 0.004 % methanol solution of DPPH. After an incubation of 30 min in the dark at room temperature, the absorbance of each sample was measured at 517 nm. Lower absorbance values indicated higher free radical-scavenging activity. The percentage of DPPH scavenging activity was determined using Eq 1.

$$\text{Scavenging activity (\%)} = \{(A_c - A_s)/A_c\}100 \quad \ldots (1)$$

where A_c is the control absorbance and A_s is the absorbance in the presence of sample. The data are presented as the mean of triplicates and the concentration (IC_{50}) was determined graphically as the amount of the tested compound or standard necessary brings about a 50 % reduction of DPPH radical.

Hydrogen peroxide scavenging

The capacity of the compounds to scavenge hydrogen peroxide (H_2O_2) was carried out according to Guemmaz *et al* [8] with slight modifications. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4), and 3.4 mL of the extract in phosphate buffer was added to 0.6 mL of H_2O_2. After 10 min of incubation, absorbance was determined at 230 nm against a blank solution containing phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging was calculated as in Eq 1.

Reducing power assay

Reducing power assay of compounds was assessed according to Guemmaz *et al* [9]. To 400 µL of extract or standard (ascorbic acid), 400 µL phosphate buffer (0.2 M, pH 6.6), and 400 µL of a 1 % potassium ferricyanide ($K_3Fe(CN)_6$) were added. Then, the mixture was incubated at 50 °C for 20 min. About 400 µL (10 %) of trichloroacetic acid (TCA) was added to the mixture and centrifuged for 10 min (3000 rpm). Finally, 400 µL of the supernatant solution was mixed with 400 µL of distilled water and 80 µL $FeCl_3$ (0.1 %), and the absorbance was recorded at 700 nm. An increase in the absorbance of the reaction mixture indicates higher reducing power.

β-carotene/ linoleic acid assay

Antioxidant capacity of the compounds was further assessed according to the procedure of Aouchria *et al* [7]. This was achieved by measuring the inhibition of the conjugated diene hydroperoxides arising from linoleic acid oxidation. Briefly, 0.5 mg of β-carotene was dissolved in 1 mL of chloroform, and 25 µL of linoleic acid and 200 mg of Tween 40 were added. Chloroform was removed under reduced pressure at 40 °C, and 100 mL of oxygenated distilled water was added to the mixture with vigorous agitation, to form an emulsion. To 2.5 mL aliquot of this emulsion, 350 µL portions of the antioxidant (extracts and BHT) prepared in methanol at 2 mg/mL concentrations were added. The absorbance was monitored spectrophotometrically for 48 h at 490 nm. The antioxidant activity (Aat, %) of the sample extracts and standard was expressed in terms of β-carotene bleaching, using Eq 2.

$$\text{AAt (\%)} = (A_0/A_t)100 \quad \ldots\ldots\ldots\ldots (2)$$

where A_0 is absorbance in presence of the sample and A_t is control absorbance.

Anti-hemolytic activity

Anti-hemolytic activity was measured as the capacity of red blood cells (RBCs) to withstand free radical-induced hemolysis, according to Zerargui *et al* [10] with slight modifications. Mice erythrocytes were isolated by centrifugation of blood at 3000 rpm for 10 min and washed three

times with phosphate buffer (10 mM, pH 7.4). The AAPH was used in order to induce free-radical chain oxidation in, red blood cell suspensions (2 % hematocrit). Briefly, 80 µL of RBCs was added to 20 µL of extract (0.1 mg/mL) and the mixture was treated with 136 µL of AAPH (300 mM). The whole blood hemolysis was determined using a 96-well microplate reader (BioTek ELx800, USA). The kinetics of RBCs' resistance to hemolysis was determined at 37 °C by continuously monitoring the rate of decrease of absorbance at 630 nm.

Blood resistance to free radical attack is expressed by the time needed to reach 50 % hemolysis of RBCs (half-time hemolysis, HT_{50} in min).

Effect on clotting time

The anticoagulant effect of compounds was conducted as outlined by Zerargui *et al* [11]. A solution of 74 µL of each compound (0.5 mg/mL) and negative control (distilled water) were placed in the different test tubes, respectively. Fresh blood from healthy volunteers was collected and 370 µL of blood was immediately added to the test tubes. The time required for complete blood clotting was recorded using a stopwatch.

Thrombolytic activity

A 2 mg/mL solution of compounds was prepared. Venous blood drawn from a healthy volunteer was distributed (370 µL/tube) to each previously weighed sterile microtube and incubated at 37 °C for 45 min to form the clot. After that, the serum was totally removed without disturbing the clot, and then all the tubes were again weighed to determine the clot's weight. A volume of 70 µL of test solution was added to each microtube containing a pre-weighed clot (W_1). Distilled water was used as negative control. All the tubes were then incubated at 37 °C for 90 min and observed for clot lysis. Fluid released was removed after incubation and tubes were again weighed to observe the difference in the weight of blood clot (Wc) [11]. Percentage clot lysis was calculated using Eq 3.

$$\text{Clot lysis (\%)} = (Wc/W_1)100 \quad \ldots\ldots\ldots (3)$$

where the weight of released clot = weight of clot before lysis – weight of clot after lysis.

Statistical analysis

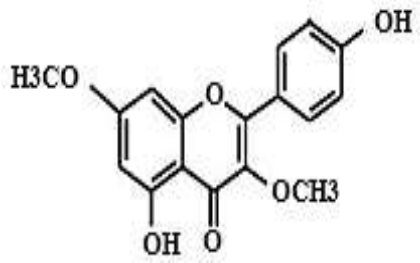
The results are expressed as mean ± standard deviation (SD) of triplicates. Data were analyzed using the Tukey test to determine statistical significance. *P*-value < 0.05 was considered indicative of significance. All statistical analyses and graphs were performed using GraphPad Prism 5.0 software.

RESULTS

Chromatography of the aqueous methanol extract

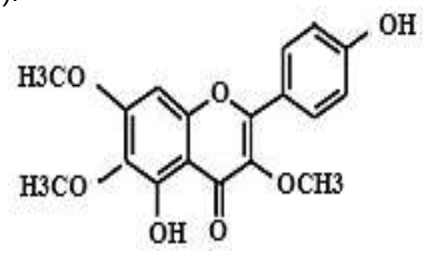
Chromatography of the aqueous methanolic extract of *V. iphionoids* resulted in 5 known flavonoid compounds. These compounds were identified via their spectral data. The kumatakillin **(1)** [12], penduletin **(2)** [12], jeceidine **(3)** [13], 6-methoxy isokaemferide **(4)** [14] and 3,3'-di-O-methyl quercetin **(5)** [12].

The NMR and MS data of the five flavonoids for which the biological activity was studied are here reported.



Scheme 1: Kumatakillin (1)

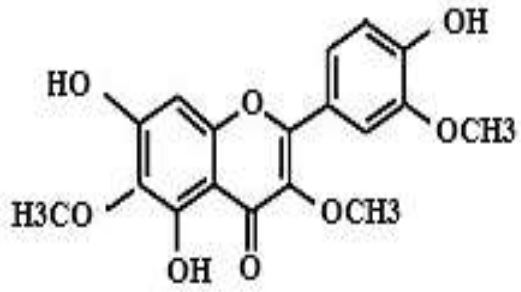
^{1}H-NMR(DMSO-d_6): 3.77, 3.83 (each 3H, s, 2xOCH_3), 6.33 (1H, d, J=2Hz, H-6), 6.69 (1H, d, J=2Hz, H-8), 6.94 (2H, d, J=8.8 Hz, H-3' + H-5'), 7.95(2H, d, J=8.8 Hz, H-2' + H-6'), 12.64 (1H, bs, 5-6H). ^{13}C-NMR (DMSO-d_6): 178.4 (C-4), 165.4 (C-7), 161.2 (C-5), 160.6 (C-4'), 156.6 (C-9), 138.1 (C-3), 130.5 (C-2' + C-6'), 120.8 (C-5), 116.0 (C-3' + C-5'), 10.5 (C-10), 98.0 (C-6), 92.6 (C-8), 60.0, 56.4 (2xOCH_3). EIMS m/z (%): 314 $[M]^+$ ($C_{17}H_{14}O_6$) (87), 313 (100) $[M-H]^+$, 295 (25), 271 (36), 187 (14), 167 (21), 143 (34), 121 (33), 93 (16).



Scheme 2: Penduletin (2)

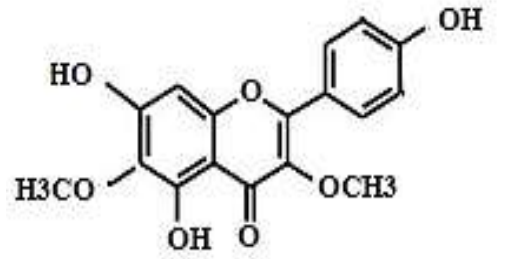
^{1}H-NMR (DMSO-d_6) δppm = 3.69, 3.75, 3.86 (each 3H, s, 3xOMe), 6.33 (1H, s, H-8), 6.91 (2H, d, J=8.9 Hz, H-3' + H-5'), 7.92 (2H, d, J=8.9 Hz,

H-2' + H-6'),10.27 (1H, bs, 4'-OH), 123.58 (1H, s, 5-OH). ^{13}C-NMR (DMSO- d6) δppm: 56.9, 60.5 (3xOCH$_3$) , 91.8(C-8) , 106.0 (C-10), 116.1 (C-3' + C-5'), 121.0 (C-1'), 130.7 (C-2' and C-6'), 132.0 (C-6), 138.1 (C-3), 152.2 (C-3), 152.2 (C-5), 156.4 (C-9), 159.1 (C-7), 160.8 (C-4'), 178.7 (C-4'). EIMS m/z (%): 344[M]$^+$ ($C_{18}H_{16}O_7$) (100), 329 (58), 300 (11), 181 (10), 153 (10), 134 (10), 121 (20).



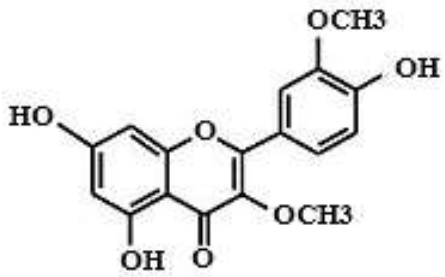
Scheme 3: Jeceidine (3)

^{1}H-NMR(DMSO-d$_6$) δppm: 3.71, 3.74, 3.78 (each 3H, s, 3xOCH$_3$), 6.49 (1H, s, H-8), 6.09 (1H,d , J= 8.4 Hz, H-5'), 7.49 (1H, dd, J= 1.8, 8.4 Hz, H-6'), 7.57 (1H,bs, H-2'), 12.76 1H, s, 5-OH). ^{13}C-NMR (DMSO-d$_6$) δppm: 178.6 (C-4), 157.7 (C-7),156.0 (C-2), 152.0 (C-9), 150.2 (C-4'), 147.9 (C-3'), 137.8 (C-3),131.5 (C-6), 122.6 (C-6'), 121.3 (C-1'),116.1 (C-5), 112.4 (C-2'), 105.1 (C-10), 94.5 (C-8), 60.4, 56.1 (3XOCH$_3$). EIMS m/z (%): 360 [M]$^+$ ($C_{18}H_{16}O_8$) (100), 345 (90), 317 (70), 299 (45), 274 (25), 246 (13), 167 (32), 151 (50), 69 (75).



Scheme 4: 6-Methoxy isokaemfride (4)

^{1}H-NMR (DMSO-d$_6$) δppm: 3.70, 3.72 (each3H, s, 2xOH$_3$), 6.49 (1H, S, H-8), 6.89 (2H, d, J=8.3 Hz, H-3' + H-5'), 7.88 (2H, d, J=8.3 Hz, H-2' and H-6'), 12.73 (1H, s, 5'-OH). ^{13}C-NMR (DMSO-d$_6$) δppm: 178.6 (C-4), 160.5 (C-4'), 157.9 (C-7), 156.2 (C-9), 152.9 (C-5), 152.0 (C-2), 137.7 (C-3), 131.6 (C-6), 130.6 (C-2' and C-6'), 121.1 (C-1'), 116.1 (C-3' and C-5'), 105.0 (C-10), 94.5 (C-8), 60.1, 60.4 (2XOH3). EIMS m/z (%): 330 [M]$^+$ ($C_{17}H_{14}O_7$) (100), 315 (27), 287 (34), 269 (20), 244 (10), 151 (10), 134 (14), 121 (23), 105 (8), 69 (11).



Scheme 5: 3,3'-Di-O-methyl quercetin (5)

^{1}H-NMR (DMSO-d$_6$) δppm: 3.75,3.81 (each 3H, s, 2xOCH$_3$), 6.14 (1H,d, J= 2.0 Hz, H-6), 6.40 (1H, d, J=2.0 Hz,H-6), 6.91 (1H, d, J= 8.4 Hz, H-5'), 7.51 (1H, dd, J=8.4 + 2.0 Hz, H-6'), 7.58 (1H, d, J=2.0 Hz,H-2'), 9.90, 10.75 (each 1H, bs, 2xOH),12.63 (1H, s, 5-OH). ^{13}C-NMR (DMSO-d$_6$) δppm: 178.2 (C-4), 164.5 (C-7), 161.5 (C-5), 156.6 (C-9), 155.6 (C-2), 150.0 (C-4),147.7 C-3'), 138.0 (C-3), 122.5 (C-1'), 121.1 (C-6'), 115.9 (C-5'), 112.3 (C-2'), 104.5 (C-10), 98.9 (C-6), 94.1 (C-8), 59.9, 55.9 (2xOCH$_3$). EIMS m/z (%): 330 [M]$^+$ ($C_{17}H_{17}O_7$) (100),315 (44), 287 (48), 269 (14), 244 (17), 217 (8), 153 (10), 151 (14), 135 (11), 121 (10), 108 (8), 69 (10).

Antioxidant potential of purified compounds

DPPH scavenging

The results showed that all compounds exhibited a good scavenging potential against DPPH radical in the following order **3** ≈ **4** ≈ **1** > **5** > **2** (Figure 1), and were considered as effective as the antioxidant reference quercetin. Thus, the highest activity was registered by compounds **3**, **4,** and **1** with comparable IC_{50} = 13.41 ± 0.21, 14.52 ± 0.32, and 15.24 ± 0.19 µg/mL, respectively. However, both compounds **2** and **5** showed slightly lower activity.

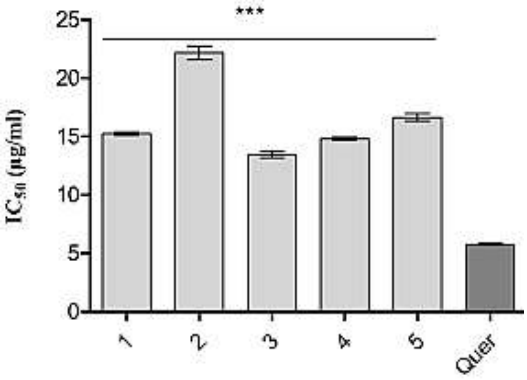


Figure 1: IC_{50} of compounds isolated from *V. iphionoids* and quercetin in DPPH scavenging assay. **1** (Kumatakillin), **2** (Penduletin), **3** (Jeceidine), **4** (6-Methoxy isokaemfride), and **5** (3,3'-Di-O-methyl quercetin), Quer: quercetin. ***P ≤ 0.001 versus quercetin

Hydrogen peroxide scavenging

The results of this assay are summarized in **Figure 2**. Compound **3** showed greater hydrogen peroxide (H_2O_2) scavenging with an IC_{50} of 15.86 ± 0.04 µg/mL, slightly lower than quercetin used as standard ($P \leq 0.05$). In contrast, compounds **1**, **2**, **4,** and **5** showed an H_2O_2 scavenging activity twice that of the standard ($P \leq 0.001$).

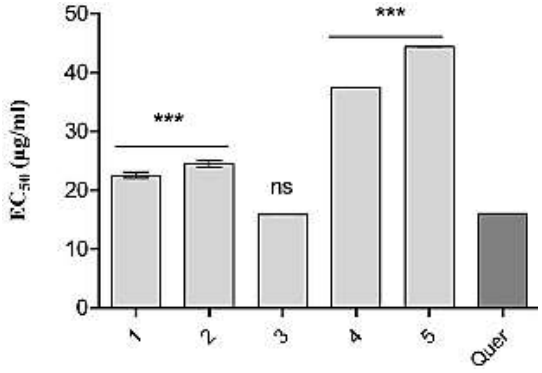


Figure 2: IC_{50} of compounds isolated from *V. iphionoids* and quercetin in hydrogen peroxide scavenging assay. **1** (Kumatakillin), **2** (Penduletin), **3** (Jeceidine), **4** (6-Methoxy isokaemfride), and **5** (3,3'-Di-O-methyl quercetin), Quer: quercetin. ***$P \leq 0.001$, ns: $p \leq 0.05$ versus quercetin

Reducing power

The absorbances obtained from ferric reducing assay are indicated in Figure 3. Absorbance (A) of all tested compounds reached from 0.147 to 0.326, and was nearly ten times lower than vitamin C (A = 1.436 ± 0.004) which has the highest reducing power ($P < 0.001$). All the analyzed compounds were able to reduce weakly the ferric ion in subsequent order: **2** > **3** > **4** ≈ **5** > **1**.

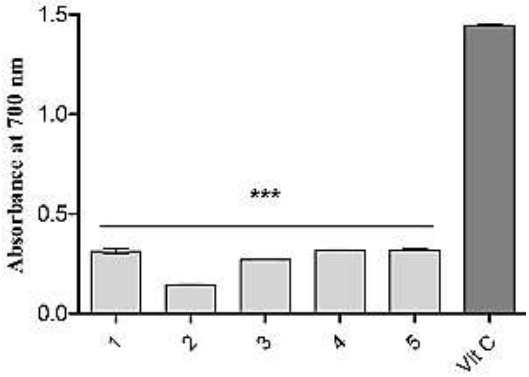


Figure 3: The reducing power of compounds isolated from *V. iphionoids* and ascorbic acid (Vit C). **1** (Kumatakillin), **2** (Penduletin), **3** (Jeceidine), **4** (6-Methoxy isokaemfride) and **5** (3,3'-Di-O-methyl quercetin). ***$P \leq 0.001$ versus vitamin C

β-carotene/ linoleic acid bleaching assay

The antioxidant capacity of the purified compounds and the controls (BHT, methanol and water) are illustrated in Figure 4. All the compounds have the capacity to inhibit β-carotene bleaching. The highest inhibition activity was observed with compound **4** (88.92 ± 0.62 %), which was comparable to BHT, followed by **5** and **3** (81.79 ± 1.83 and 75.53 ± 1.07 %, respectively) whereas, **1** and **2** possessed moderate activities.

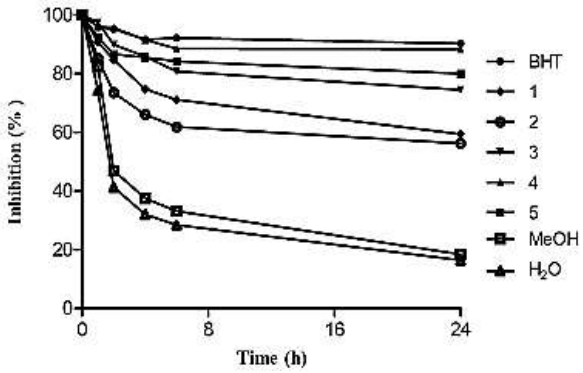


Figure 4: Antioxidant activities of compounds isolated from *V. iphionoids*, positive control (BHT) and negative controls (MeOH and H_2O) in β-carotene bleaching assay for 24 h. **1** (Kumatakillin), **2** (Penduletin), **3** (Jeceidine), **4** (6-Methoxy isokaefefride) and **5** (3,3'-Di-O-methyl quercetin).

Antihemolytic activity

The results showed that compounds **2**, **3**, **4,** and **5** increased significantly the erythrocytes half time hemolysis compared to ascorbic acid (HT_{50} = 83.38 ± 4.30 min) ($p \leq 0.001$), with the exception of compound **1** which exhibited an extension time almost equal to that of ascorbic acid (ns) (Figure 5).

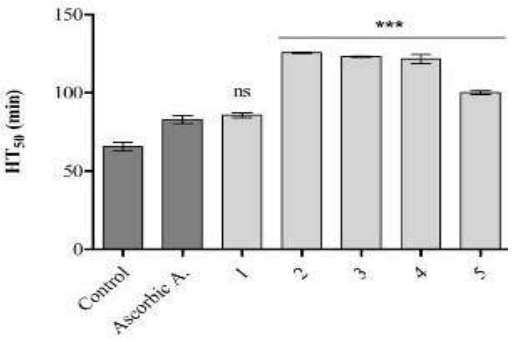


Figure 5: Antihemolytic activity expressed by the half-hemolysis time (HT_{50}), of compounds **1** (Kumatakillin), **2** (Penduletin), **3** (Jeceidine), **4** (6-Methoxy isokaemfride), **5** (3,3'-Di-O-methyl quercetin). Ns: $p > 0.05$, versus control, ***$p \leq 0.001$ versus ascorbic acid

Effect on clotting time

The present study shows that all the compounds had increased blood clotting time significantly (***p < 0.001) when compared to control as depicted in (Table 1).

Table 1: Effect of different compounds: **1** (Kumatakillin), **2** (Penduletin), **3** (Jeceidine), **4** (6-Methoxy isokaemfride), and **5** (3,3'-Di-O-methyl quercetin), on *in vitro* clotting time of human blood

Compound (0.5 mg/mL)	Clotting time (min)
1 (kumatakillin)	5.00
2 (penduletin)	5.82±0.31
3 (jeceidine)	6.55±0.03
4 (6-methoxy isokaemfride)	5.41±0.01
5 (3,3'-di-o-methyl quercetin)	5.90±0.39
H_2O	2

Thrombolytic activity

The results showed that compounds **1**, **2,** and **3** presented an effective increase in the lysis of the human blood clot (***p < 0.001) compared to the control, compound **5** was the least effective (*p < 0.05) (Figure 6).

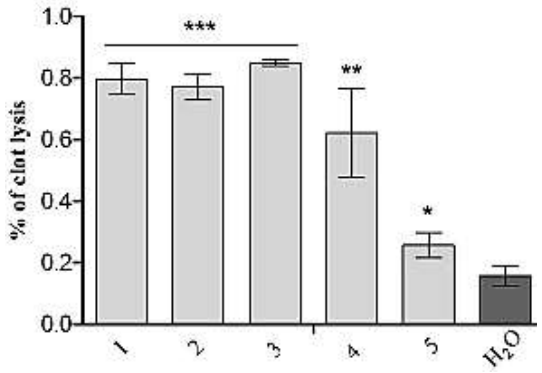


Figure 6: Effect of five compounds: Kumatakillin **(1)**, Penduletin **(2)**, Jeceidine **(3)**, 6-Methoxy isokaemfride **(4)** and 3,3'-Di-O-methyl quercetin **(5)** on *in vitro* thrombolytic activity. ***P < 0.001, **p < 0.01 and *p < 0.05 versus water

DISCUSSION

Stable DPPH radical and hydrogen peroxide (H_2O_2) could be reduced by accepting an electron donated by an antioxidant compound thereby neutralizing their effect. From the current results, jeceidine **(3)** was the most potent in scavenging DPPH and H_2O_2. The degree of antioxidant activity depends mainly on the ability of compounds to provide protons to neutralize free radicals [15]. The study of Lin *et al* [16] revealed the presence of a relationship between antioxidant activity and 5 flavonoids' structure, in which the activity depends on the configuration and the pattern of hydroxyl groups, things which may be comparable to the present results.

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [17]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes so that they can act as primary and secondary antioxidants.

β-carotene and linoleic acid are considered as a model for lipid peroxidation. Cell membranes are rich in unsaturated fatty acids particularly linoleic acid and arachidonic acid, which are the most susceptible fatty acids to oxidation by ROS, and the targets of lipid peroxidation. Phenolic compounds seem to be responsible for the antioxidant activity by their `chain-breaking` property, which can counteract peroxyl and alkoxyl radicals generated during lipid peroxidation to prevent continual hydrogen abstraction and thus inhibit the chain propagation step [18].

Free radicals attack erythrocyte membrane components, leading to the alteration of the structure and function of the membrane, resulting in hemolysis. The AAPH was used to imitate the *in vivo* condition of oxidative stress, mediating the oxidation of erythrocytes, leading to hemolysis. The results demonstrated that the peroxyl radicals were initiated by AAPH-induced hemolysis in a time-dependent manner, and the presence of the studied flavonoids leads to high protection of blood cell membranes. These results are in agreement with previous findings mentioned by Joujeh *et al* [19].

Blood clotting is one of the major problems during blood circulation. Lodge formation by thrombi into blood vessels, block the flow of blood, deprive tissue in that location, and cause lacking oxygen supply to different parts of the body resulting in tissue necrosis. Clots are generally formed to fibrinogen or thrombin and are lysed by plasmin that activates plasminogen. The present investigation showed a significant delay in clotting time that may be due to the decrease of any clotting factor activity involved in the intrinsic pathway [11].

Thrombolytic activity showed that the compounds tested possess notable ability to break down blood clots thus strengthening its anticoagulant potential. In addition, many previous studies on medicinal plants advocate the presence of flavonoids (quercetin, quercitrin, myricetin, and rutin), tannins, and phenolic compounds with

antithrombotic thrombolytic activities [20,21]. Quercetin, a well-known flavonoid blocks the glycoprotein pathway and inhibits the response of platelets to collagen [22]. It has been also shown that flavonoids could inhibit platelet aggregation by binding to the A2 receptors for thromboxane [23,24].

CONCLUSION

The present work reports for the first time the antioxidant screening of five compounds isolated from *V. iphionoids* extract. These natural compounds have shown promising *in vitro* antioxidant properties that need to be further explored to justify their possible use in pharmacological applications. This investigation paves way for the possible development of natural antioxidants as substitutes for chemical therapeutics that have various and severe adverse effects.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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