



Journal of Advanced Pharmacy Research

A Novel Compound and Biological Evaluation of Phytoconstituents Isolated from *Erythrina corallodendron* L. Flowers

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Submitted on: 25-05-2018; Revised on: 24-06-2018; Accepted on: 27-06-2018

ABSTRACT

Objective: This study aimed at phytochemical investigation of the 70% alcoholic extract of *Erythrina corallodendron* L. flowers and biological evaluation of the isolated compounds for their activity as antiprotozoal drugs also evaluation the binding affinity to opioid and cannabinoid receptors as well as the inhibition activity against monoamine oxidase (MAO) enzymes. **Method:** The 70% alcoholic extract was subjected to successive column chromatographic (CC) separations using silica gel normal phase, reversed phase RP-18, Diaion HP-20, and Sephadex LH-20. The structural elucidation of the isolated compounds was achieved using HR-ESI-MS, UV, 1D and 2D NMR spectroscopic analysis. The isolated compounds were screened *in vitro* for the binding affinity to opioid and cannabinoid receptors using receptor binding assay as well as the inhibition activity against MAO enzymes using kynuramine deamination assay, while their antiprotozoal activity was evaluated using parasite lactate dehydrogenase serum assay (pLDH). **Results:** The phytochemical evaluation of the alcoholic extract of *E. corallodendron* flowers, afforded the isolation of an indole alkaloid Hypaphorine **1**, a new flavonoid glucoside; Kaempferol-3-O- α -sophoroside **2** and three known flavonoid C-glycosides *vis.*; Neoschaftoside **3**, Isoschaftoside **4** and Vicenin-II **5**. Compounds **3** and **4** are reported for the first time from genus *Erythrina*. Compounds **4** and **5** showed significant antimalarial activity both with IC₅₀ value 1.7 μ g/mL against (D6) strain and with IC₅₀ 1.4 and 1.1 μ g/mL against (W2) strain, respectively. Compound **3** showed selective inhibition to MAO-B with IC₅₀ value of 32.08 μ M and selective index (SI) > 3.12. **Conclusion:** The significant antiplasmodial activity of compounds **4** and **5** correlated the known antimalarial activity of different *Erythrina* species to flavonoid C-glycosides, Also compounds **3** and **4** are position isomers but exhibited different response against MAO-B which gives indication about the selectivity pattern of the flavonoid C-glycosides with MAO-B receptor subtype.

Keywords: Antiplasmodium; Antiprotozoal evaluation; *Erythrina corallodendron*; Kaempferol-3-O- α -sophoroside; MAO inhibition

INTRODUCTION

Genus *Erythrina* (Fabaceae) comprises about 110 species which are distributed in tropical and subtropical areas, characterized with red/or orange flowers, and represented by trees, shrubs and herbaceous plants. In Africa there are about 30

Erythrina species and subspecies found in tropical regions¹. The genus *Erythrina* is well-known for its neurological properties where the alcoholic extracts of *E. velutina* and *E. mulungu* displayed anticonvulsant², tranquilizer³ and anxiolytic activities^{4,5}.

Also different *Erythrina* species were reported to exhibit wide range of biological activities such: as

antimicrobial (*E. caffra*)⁶, anticancer (*E. variegata*)⁷, antioxidant (*E. lysistemon*)⁸, anti-inflammatory (*E. crista-galli*)⁹ and anti HIV (*E. abyssinica*)¹⁰ activities. In many African countries different *Erythrina* species comprise a potential component of the folk remedies, for example in Sudan the bark of *E. abyssinica* is used for the treatment of coughs, ulcers and abdominal pain, the flowers are used for the treatment of dysentery, the leaves are used for peptic ulcers, arthralgia, the roots for epilepsy, malaria and syphilis, and the fruits for asthma¹¹. Several *Erythrina* species showed strong antiplasmodial activity against *Plasmodium falciparum* both chloroquine sensitive and resistant strains (*E. fusca*^{12,13}, *E. burttii*¹⁴, *E. abyssinica*^{15,16}, *E. subumbrans*^{17,18}, *E. crista-galli*¹⁹). *E. corallodendron* is known for its seeds lectins, which are carbohydrate recognizing molecules with a useful application in biomedicine and glycobiological systems²⁰. Very few literature was traced about *E. corallodendron* where only three studies have been conducted concerning the leaves and flowers^{21,22}.

Malaria is one of the most dangerous infectious diseases affecting tropical and subtropical areas. The incidence rate of malarial infection in Africa is about 300-500 million cases per year with 1.5–2.7 million deaths and up to 90% occurrence in children under the age of five²³. The development of drug-resistant malaria with this increased incidence²⁴ creates an urgent need for more discovering and exploring of new drugs from natural sources.

One of the important metabolizing enzyme systems is monoamine oxidases (MAOs). MAOs are involved in the pathogenesis of many neurodegenerative diseases. They are responsible for the oxidative deamination of the monoamine neurotransmitters. Recent studies clarify the important role of MAO-B inhibition in the treatment of both Alzheimer's²⁵ and Parkinson's diseases²⁶.

MATERIALS AND METHODS

General

The UV spectra were recorded on Cary-50 Bio-spectrophotometer, Agilent Technologies, USA. The ¹H and ¹³C NMR spectra were recorded on Varian Spectrometer 400 MHz; The ESIMS was obtained from Bruker Bioapex-FTESIMS with electrospray ionization. The chromatographic separation was carried out using normal phase Silica gel (70-230 mesh, Merck), Diaion-HP-20, ion exchange resin styrene adsorbent, (particle size >250 μm, Sorbent Technologies) and Sephadex LH-20 (Mitsubishi Kagaku, Tokyo, Japan). Open column chromatography technique was applied. Precoated thin layer chromatography (TLC) (Silica gel 60G F₂₅₄, Merck) used for monitoring. Vanillin sulfuric spraying agent (10% v/v conc. sulphuric acid in ethanol) used for visualization spots on TLC plates.

Recombinant Human MAO-A and MAO-B were obtained from BD Biosciences (Bedford, MA, USA), drug control, Clorgyline, Deprenyl, Phenelzine, (positive control) and DMSO (negative control) were purchased from Sigma Chemical (St. Louis, MO, USA). The MAO inhibition assay was carried on Spectra-Max M5 fluorescence plate reader (Molecular Devices, Sunnyvale, CA, USA). **Antiprotozoal Assay:** *Plasmodium falciparum* D6, *P. falciparum* W2 and Vero cells were used in the antiplasmodial assay. All organisms used were obtained from NCNPR, University of Mississippi, USA. The antiplasmodial drug control Chloroquine was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Plant material

The flowers of *Erythrina corallodendron* L. were collected from El-Zohria Botanical Garden in March 2012 and it was identified by Prof. Dr. Salwa Al Katuchi, Department of Systematic Plant Taxonomy, The National Research Centre (NRC), Cairo, Egypt. A voucher specimen was preserved in the herbarium of The National Research Centre (# M-125).

Extraction and isolation

The fresh flowers (1.5 kg) were extracted by maceration in methanol (70%, 8 L.) at room temperature, and the combined methanolic extracts were concentrated under reduce pressure to yield 101g. A hundred grams of the methanolic extracts were mixed with 50 g Celite and fractionated on Silica gel 550 g using vacuum liquid chromatography (VLC) eluted with dichloromethane (DCM): MeOH mixture, in a manner of increasing polarities (5%, 10%,...100%) fractions of (500 mL each), were collected and screened on TLC silica using systems S₁ and S₂, DCM: MeOH 9:1 and 8:2, respectively. TLC were visualized under UV lamp then sprayed with vanillin sulfuric, similar fractions were combined and evaporated resulting in five main fractions (A-E)

Fraction (D) (4.8g) was subjected to Diaion HP-20 CC (100g), eluted with 100% H₂O then with MeOH 100% to remove salts and sugars, (Fr. 1-20) were collected, screened on TLC silica gel using system S₁. Sub-Fraction (8) (800 mg) was further chromatographed on Sephadex LH-20 CC using MeOH 100% as eluent, (Fr. 1-100) were collected and screened on TLC silica using system S₃ (EtOAc: CH₃Cl: MeOH: H₂O) (20:10:11:5). Sub-fraction (48) (160 mg) showed precipitation it was filtered (20 mg ppt) and the supernatant was further purified using silica gel RP C-18 CC (5g) eluted with mixture of H₂O: MeOH of decreasing polarities H₂O: MeOH (10:0 to 9:1, 8:2, ...0:10). The resulted fractions (Fr. 1-10) were screened on TLC silica gel using system S₃. Sub-fraction (4) (60.6 mg) was subjected to further purification on

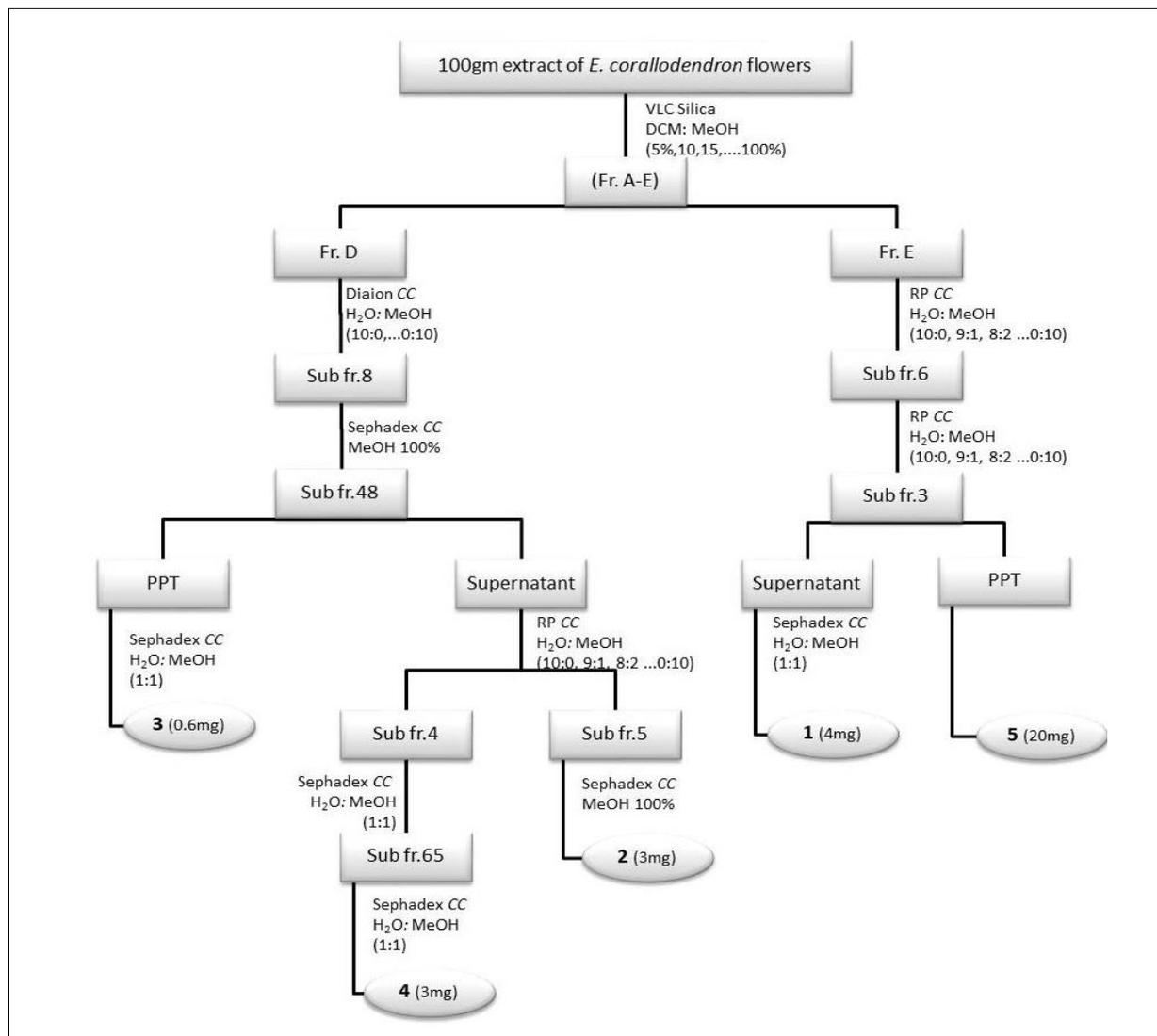


Figure 1. Flow chart of fractionation and isolation of compounds from 70% methanolic extract of *E. corallodendron* flowers.

successive Sephadex LH-20 CC eluted with MeOH: H₂O (1:1) resulted in compound **4** (Isoschaftoside) (3 mg). Sub-fraction (5) (41mg) was subjected to further purification using Sephadex LH-20 CC eluted with MeOH 100% resulted in compound **2** (Kaempferol 3-O- α -D-sophoroside) (3 mg). The sub-fraction (48) (Precipitate left after filtration) (20 mg) was purified on two successive Sephadex LH-20 CC eluted with MeOH 100% then MeOH: H₂O (1:1) affording compound **3** (Neoschaftoside) (0.6mg).

Fraction E (19 g) was chromatographed on RP C-18 silica CC eluted with mixture of water and MeOH of decreasing polarities H₂O: MeOH (10:0, 9:1, 8:2,...0:10). The resulting fractions (Fr. 1-10) were screened on TLC silica RP using system S₄ MeOH: H₂O (6.5: 3.5). Sub-fraction (6) (10 g) was subjected to

more fractionation on RP C-18 silica CC eluted with mixture of H₂O: MeOH of decreasing polarities H₂O: MeOH (10:0, 9:1, 8:2, ...0:10). The resulted fractions (Fr. 1-10) were screened on TLC silica RP C-18 using system S₄. Sub-fraction (3) showed precipitation which was filtered and dried to yield compound **5** (Vicenin-II) (20mg). The supernatant was purified on successive Sephadex LH-20 CC eluted with mixture MeOH: H₂O (1:1) to afford compound **1** (Hypaphorine) (4mg). (**Figure 1**)

Anti-malarial Assay

In vitro antimalarial activity was determined using pLDH serum assay, against chloroquine-sensitive (D6, Sierra Leone) and chloroquine-resistant (W2, Indo China) strains of *P. falciparum* by measuring

plasmodial LDH activity as previously reported²⁷. Tested compounds were dissolved in DMSO (2 mg/ml). A 200 μ l suspension of *P. falciparum* culture (2% parasitemia and 2% hematocrit in RPMI 1640 medium supplemented with 10% human serum and 60 μ g/ml amikacin) was added to the wells of a 96-well plate containing 10 μ l of serially diluted samples. The plate was flushed with a gas mixture of 90% N₂, 5% O₂, and 5% CO₂ and incubated at 37°C for 72 h in a modular incubation chamber. Plasmodial LDH activity was determined by using Malstat™ reagent (Flow Inc., Portland, OR). In brief, 20 μ l of the incubation mixture was mixed with 100 μ l of the Malstat reagent and incubated for 30 min. Then, 20 μ l of a 1:1 mixture of NBT/PES (Sigma, St. Louis, MO) was added and the plate is further incubated for 1 h in dark. The reaction was stopped by adding 100 μ l of a 5% acetic acid solution. The plate was read at 650 nm using the EL-340 Biokinetics Reader (Bio-Tek Instruments, Vermont). IC₅₀ values were obtained from the dose-response curves generated by plotting percent growth versus drug concentration. All IC₅₀ values are calculated using the XLFit curve fitting software. Chloroquine was included in each assay as positive control. DMSO (0.25%) was used as a vehicle control. The selectivity indices (SI) – ratio of VERO IC₅₀ to D6 or W2 IC₅₀ – were calculated.

Monoamine oxidase inhibitor assay

The inhibitory effect of the isolated constituents was investigated on human recombinant MAO-A and MAO-B. The 96-well plates kynuramine deamination assay was performed as previously reported²⁸. A fixed concentration of substrate (Kynuramine) for each of MAO-A and -B were 80 and 50 μ M, respectively and five concentrations for constituents from 0.0001 to 1 μ g/mL were used to determine the IC₅₀ values (concentration where 50% inhibition of the enzyme catalytic activity occurred). Reactions were performed in 200 μ L of 0.1 M potassium phosphate buffer, pH 7.4. The inhibitors and compounds were dissolved in DMSO, diluted in the buffer solution, and pre-incubated at 37°C for 10 min (1.0% of DMSO final). Reactions were initiated by the addition of 50 μ L of MAO-A (5 μ g/mL) and -B (10 μ g/mL), incubated for 20 min at 37°C, and terminated immediately by the addition of 75 μ L of 2 N NaOH. The enzyme product formation (4-hydroxyquinoline) was recorded fluorometrically using a Spectra- Max M5 fluorescence plate reader (Molecular Devices, Sunnyvale, CA, USA) with an excitation (320 nm) and emission (380 nm) wavelength, using the Soft Max Pro program. The assays were calculated as percent of product formation compared to the corresponding control (enzyme-substrate reaction) without inhibitors. Controls including samples where the enzyme or the

substrate was added after stopping the reaction were checked simultaneously to determine the interference with the fluorescence measurements. The determination of IC₅₀ values for MAO-A and -B inhibition by the selected samples was performed using varying concentrations of the inhibitor and fixed concentration of the substrate. The IC₅₀ values were calculated from the concentration dependent inhibition curves using XLFit software

RESULTS AND DISCUSSION

The phytochemical evaluation of the 70% methanolic extract of *E. corallodendron* flowers resulted in the isolation and identification of one indole alkaloid **1**, one new flavonoid glucoside **2** and three known flavonoid C-glycosides **3-5** (Figure 2). Flavonoids **3** and **4** are reported for the first time from genus *Erythrina*. All the isolated compounds were screened for their antiprotozoal activity against *Plasmodium falciparum*. Compounds (**1-3**) did not show antiplasmodial activity. While compounds **4** and **5** exhibited significant antiplasmodial activity. Both compounds displayed more activity against chloroquine-resistant (W2) than chloroquine-sensitive (D6) strains with IC₅₀ values 1.1, 1.4 and 1.7, 1.7 μ g/mL, respectively. For the chloroquine-resistant strain (W2) assay compound **4** showed better activity than **5** with SI= 4.3 and 3.4, respectively. The selectivity index is directly related to the activity.

The results of MAO inhibitory assay revealed that the indole alkaloid **1** did not show any activity towards both enzyme subtypes. Flavonoids **2**, **4** and **5** exhibited nonselective MAO inhibitory activity against both enzymes MAO-A and MAO-B (Table 2). Compound **3** displayed selective inhibitory activity against MAO-B subtype with IC₅₀ 32.08 μ M and SI > 3.2. The aglycone part is responsible mainly for the activity in this assay. The C-glycoside nature of compounds **3-5** makes the molecules geometrically rigid and the presence of pentose at position C-8 in compound **3** instead of hexose increase the selectivity towards MAO-B and demolish any activity against MAO-A, which highlight clearly the important role of the substituent size in directing the activity and also the selectivity of the flavonoids in the MAO assay.

Furthermore, compounds **1**, **4** and **5** were investigated for their binding affinity against human opioid and cannabinoid receptors subtypes (δ , k , and μ) and (CB1 and CB2), respectively attest concentration 10 μ M, which in turn evaluate the analgesic potency of the tested compounds towards neuropathic pain²⁹.

All the evaluated compounds did not exhibit activity at the concentrations ranges of the opioid and cannabinoid binding affinity assay.

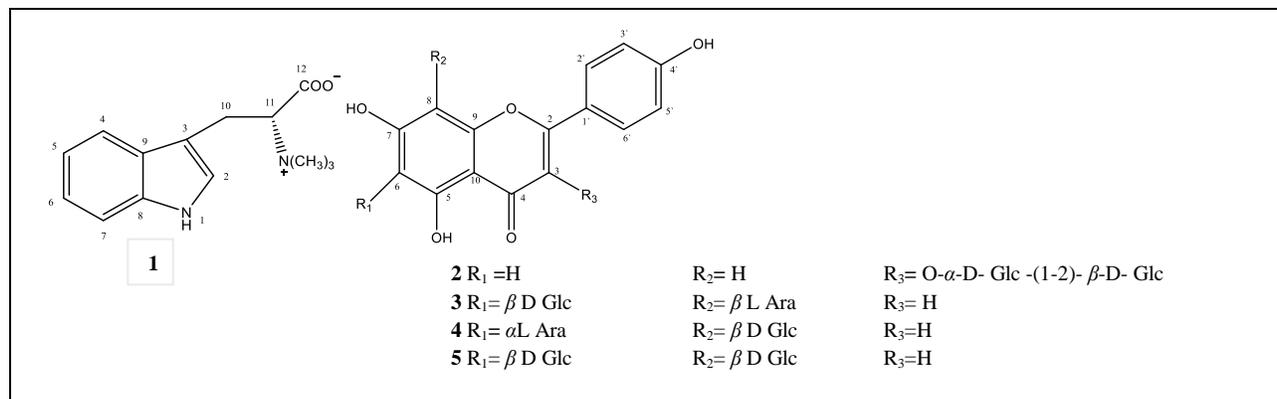


Figure 2. Structures of isolated compounds (1-5) from *E.corallo dendron* flowers

Furthermore, compounds **1**, **4** and **5** were investigated for their binding affinity against human opioid and cannabinoid receptors subtypes (δ , k , and μ) and (CB1 and CB2), respectively attest concentration 10 μ M, which in turn evaluate the analgesic potency of the tested compounds towards neuropathic pain²⁹.

All the evaluated compounds did not exhibit activity at the concentrations ranges of the opioid and cannabinoid binding affinity assay.

Identification of isolated compounds:

Compound 2

Obtained as amorphous yellow precipitate (3 mg), the molecular formula C₂₇H₃₀O₁₆ was calculated from the ¹³C NMR and HR-ESI-MS which showed quasi-molecular ion peak at m/z 609.15580 [M-H]⁻ (calculated 609.14547).

The flavonoid nucleus was deduced from the color change to yellow upon exposure to ammonia vapor, also the UV spectrum of MeOH showed two major absorption peaks at 268, 337 which indicated the presence of flavone or 3-O-substituted flavonol nucleus³⁰. The diagnostic UV reagents revealed the presence of free OH function groups at position C-4' and C-7, where both shifting reagents NaOMe and NaOAc displayed a bathochromic shift characterized with increase in intensity in case of NaOMe. The ¹H NMR (DMSO, 400MHz) showed distinctive features of AA', BB' spinning system, δ_H 8 (2H, d, J=8.4Hz, H-2',6'), δ_H 6.9 (2H, d, J=8.4Hz, H-3',5') denoting a mono substituted B ring, it displayed also two singlet signals at δ_H 6.4 (1H, s, H-8) and δ_H 6.1 (1H, s, H-6), indicating the absence of any C-glycosidic linkage. The appearance of two anomeric protons resonating at δ_H 5.6 (1H, d, J = 2.8 Hz, H-1'') and δ_H 4.6 (1H, d, J=8Hz, H-1''') accompanied with absence of singlet signal characteristic of H-3 indicate that position C-3 is occupied with sugar moieties. The ¹³C NMR spectrum showed twelve carbon signals characteristic of two glucose units in the sugar region. It displayed also a

carbonyl carbon resonated at δ_C 177.8 (C-4) characteristic of flavonol moiety. In addition to the downfield shift of (C-2) at δ_C 156.6 and the small upfield shift of (C-3) at δ_C 133.2 which verify that glycosylation occurred at position C-3³¹.

The 2D spectrum HMQC showed two anomeric protons at δ_H 5.6 (H-1'') and δ_H 4.6 (H-1''') correlated with δ_C 98 (C-1'') and δ_C 104.1 (C-1'''), respectively. The HMBC spectrum revealed the connections between anomeric proton of the secondary glucose resonating at δ_H 4.6 (H-1''') with δ_C 82 (C-2''), which exhibited notable down field shift characteristic to the glycosylated position indicating the presence of sophoroside moiety³². The chemical shift values of the anomeric position of the secondary glucose δ_H 4.6 (1H, d, J=8Hz, H-1''') / δ_C 104.1 (C-1''') was very comparable to that of β glucopyranoside³³, while that of the primary glucose δ_H 5.6 (1H, d, J=1.6Hz, H-1'') / δ_C 98 (C-1'') indicated the α orientation of the glucose moiety³⁴.

The spectroscopic data was comparable to the previously published data of (Kaempferol-3-O- β -sophoroside)³³, except the coupling constant of the anomeric proton of the primary glucose δ_H 5.6 (1H, d, J=2.8 Hz, H-1'') / δ_C 98 (C-1'') which is typical for α configuration. Accordingly, compound 2 was identified as Kaempferol-3-O- α -D-glucopyransyl-(1 \rightarrow 2)- β -D-glucopyranoside (Kaempferol-3-O- α -sophoroside), which is isolated and identified for the first time in nature.

Compounds 3 (0.6mg) and **4** (3mg) are position isomers that showed the same ESI-MS molecular ion peaks (M-H) at 563 m/z and 563 m/z, respectively from which the molecular formula C₂₆H₂₈O₁₄ was concluded. UV spectral data λ_{max} (nm) (MeOH): 269, 334 and 273,334 nm, respectively, denoting the presence of flavone nucleus³¹. The Apigenin aglycone with free hydroxyl groups at C-4', C-7 and C-5 was deduced from the diagnostic UV reagents, bathochromic shift

Table 1. MAO inhibitory activity of the isolated compounds against human recombinant MAO-A and B enzymes

Compounds	MAO-A IC ₅₀	SD value	MAO-A IC ₉₀	MAO-B IC ₅₀	SD value	MAO-B IC ₉₀	SI ^a
1	>100	NA	>100	>100	NA ^c	>100	NS ^b
2	48.36	5.41	>100	39.14	1.27	>100	NS
3	>100	NA	>100	32.08	4.42	>100	>3.12
4	38.06	0.63	>100	29.61	2.53	>100	NS
5	44.78	3.09	>100	33.23	3.58	>100	NS
Clorgyline^d	0.0057	0.00	0.0344	-	-	-	-
Deprenyl^d	-	-	-	0.0524	0.0001	0.3586	-
Phenelzine^d	0.249	0.0172	>10	0.1528	0.0126	>1	-

^a SI: ratio of IC₅₀MAO-A/ IC₅₀MAO-B, IC₅₀ expressed as μM.

^bNS: nonselective

^cNA: not active

^dClorgyline: positive control selective for MAO-A, Deprenyl positive control selective for MAO-B, Phenelzine: nonselective positive control for MAO

induced by (NaOMe) (+65) accompanied with increase in intensity in band I, a bathochromic shift by (NaOAc) (+9) in band II. A bathochromic shift by (AlCl₃), which was stable even after adding (HCL), hypsochromic shift induced by (H₃BO₃) to (NaOAc) in band I³¹.

The ¹H NMR (DMSO, 400MHz) spectra of both compounds **3** and **4** provided the same features it showed AA' BB' spinning system 7.9 (2H, *d*, *J*=8Hz, H-2',6'), 6.9 (2H, *d*, *J*=8Hz, H-3',5') and 8.0 (2H, *d*, *J*=8.4Hz, H-2',6'), 6.9 (2H, *d*, *J*=7.6Hz, H-3',5'), respectively. Singlet proton signal resonated at 6.8 (1H,*s*,H-3) in both spectra. The absence of H-6 and H-8 proton signals accompanied with the appearance of two anomeric proton signals in **3** at 4.5 (*d*, *J*= 10Hz, H-1''), δ_H 5.4(*brs*, H-1''') and at δ_H 4.70 (*d*, *J*= 8.8Hz, H-1''), 4.76 (*d*, *J*= 8.8Hz, H-1''') in **4** suggested the presence of two sugar substitutions at C-6 and C-8 positions, respectively. The type of glycoside linkage is concluded to be C-glycoside rather than O-glycoside. Furthermore, the mass difference of both compounds with Vicenin-II referred to replacement of one glucose moiety with pentose sugar unit. The HMQC spectrum of **3** correlated the anomeric proton at δ_H 4.5 (H-1'') to δ_C 73.9 (C-1'') and the anomeric proton at δ_H 5.4 (H-1''') to δ_C 71.3 (C-1''') which is typical for C-β-D-glucopyranoside and β-L-arabinopyranoside, respectively³⁵. While in **4** both anomeric protons at δ_H4.70 (H-1'') and δ_H 4.76 (H-1''') were correlated to δ_C 74.0 (C-1'') and δ_C 74.3 (C-1'''), suggesting the presence of α-L-arabinopyranoside and C-β-D-glucopyranoside, respectively³⁵. The analysis of the HMBC spectral data of compound **3** displayed correlations between the anomeric proton of glucose (H-1'') with δ_C 110.1(C-6) and 160.4 (C-7) which pointed the attachment of glucose to C-6 and the anomeric proton of arabinose (H-1''') with δ_C103.9 (C-

8) and δ_C154 (C-9) assigning the arabinose to position C-8³⁵. While in case of **4** the anomeric proton at δ_H 4.70 (H-1'') exhibited correlation with δ_C 161.7 (C-7) and δ_C70.4 (C-5'') of the arabinose suggesting that δ_H 4.70 (H-1'') is the arabinose anomeric and assigning it to position C-6³⁶. In the same time the anomeric proton of the glucose at δ_H 4.76 (H-1''') displayed correlation with δ_C155.1 (C-9), accordingly, glucose was assigned to position C-8. The spectroscopic data of both compounds **3** and **4** are in a good accordance with the previously published data^{35,36}. From the above discussion compound **3** was identified as 6-C-β-D-glucopyranosyl-8-C-β-L-arabinopyranosylapigenin (Neoschaftoside), while compound **4** was identified as: 6-C-α-L-arabinopyranosyl-8-C-β-D-glucopyranosylapigenin(Isoschaftoside). It is worth mentioning that Neoschaftoside and Isoschaftoside are isolated for the first time from genus *Erythrina*.

Hypaphorine 1: was isolated as amorphous yellow residue (3 mg) the molecular formula was deduced to be C₁₄H₁₈N₂O₂ from the ESI-MS which exhibited pseudomolecular ion peak at m/z (M+H)⁺ 247. ¹H NMR (DMSO, 400 MHz): indolic NH δ_H 10.92 (*brs* NH), olefinic proton 7.15 (1H, *s*, H-2), 7.32 (1H, *d*, *J*=7.6, H-7), 7.60 (1H, *d*, *J*=7.2, H-4), 7.06 (1H, *t*, *J*=7.6, 6.4, H-6), 6.98 (1H, *t*, *J*=7.2, 7.2, H-5), 3.19 (9H, *s*, N(CH₃)₃) and 3.22 (2H, *s*, H-10). ¹³C NMR (DMSO, 100 MHz) δ_C 167 (C-12), 136 (C-8), 127 (C-9), 123.9 (C-2), 120 (C-6), 118 (C-4), 118 (C-5), 111(C-7), 109 (C-3), 78 (C-11), 23(C-10), 51(N-CH₃)₃, the 2D spectra analysis HMBC, HMQC provided further confirmation for the structure, the spectral data are in a good agreement with the previously published one⁶, Hypaphorine was previously reported from this species²².

Table 2. Antiplasmodial activity of some isolated compounds against *P. falciparum* sensitive (D6) and resistant (W2) strains

Compounds	<i>P. falciparum</i> D6 IC ₅₀	<i>P. falciparum</i> D6 SI ^b	<i>P. falciparum</i> W2 IC ₅₀	<i>P. falciparum</i> W2 SI
1	>4.760	NS ^a	>4.760	NS
2	>4.760	NS	>4.760	NS
4	1.702	>2.8	1.1049	>4.3
5	1.744.2	>2.7	1.4099	>3.4
Chloroquine	< 0.0264	>9	0.2085	>1.1

^aNS: non selective

^bSI: ratio of IC₅₀in of VERO cell / IC₅₀of D6 or W2, IC₅₀ expressed as µg/mL.

Compound **5**: was obtained as amorphous yellow precipitate (20 mg), the molecular formula C₂₇H₃₀O₁₅ was calculated from the ESI-MS which showed quasi-molecular ion peak at m/z 593 [M-H]⁻. ¹H NMR (DMSO, 400MHz): δ_H 7.91 (2H, d, J=8, H-2',6'), 6.88 (2H, d, J=8, H-3',5'), 6.78 (1H, overlapped, H-3), 4.7 (1H overlapped, H-1''), 4.6 (1H, overlapped, H-1'''), 3.3-3.75 (10H, m, H-2''-H-6'' and H-2'''-H-6'''), ¹³C NMR (DMSO, 100 MHz): δ_C 183.4 (C-4), 165.6 (C-2), 165 (C-7), 161.9(C-4'), 159.6 (C-5), 156.4 (C-9), 130.6 (C-2',C-6'), 122.3 (C-1'), 116.9, (C-3',C-5'), 107.8 (C-6),104.9 (C-8),104.2 (C-10), 103.4 (C-3), 81.6 (C-5''), 81.2 (C-5'''), 79.6 (C-3''), 78.2 (C-3'''), 75.1(C-1''), 75.0 (C-1'''), 71.3(C-2''), 70.1(C-2'''), 69.5 (C-4''), 69.4 (C-4'''), 62.0 (C-6''), 60.8 (C-6''')

the analysis of the 2D spectra HMBC and HMQC provide further confirmation of the structure. The spectral data are in a good accordance with the previously published data³⁷, Vicenin-II was isolated from the same species²¹.

CONCLUSION

The phytochemical investigation of *E. corallodendron* flowers resulted in isolation of new flavonoid glycoside compound **2** in addition to two flavonoid C-glycosides compounds **3** and **4**, which were isolated for the first time from genus *Erythrina*. The isolated flavonoid C-glycosides compounds **4** and **5** showed significant antimalarial activity over the flavonoid O-glycoside compound **2**, which could correlate the antiplasmodial activity of *Erythrina* plants to flavonoid C-glycosides. All the isolated flavonoids compounds (**2-5**) exhibited inhibition activity against both MAO enzymes which in turn confirm the folk medicinal uses of *Erythrina* in treatment of the neurological disorders.

The selective inhibition of compound **3** against MAO-B provides important conclusion about the significant role of the substituent's size in orienting the structure selectivity.

Acknowledgment

Authors are grateful to the Egyptian government, the National Research Centre as well as to the School of Pharmacy- University of Mississippi for their financial support through the Scientific Channel Program.

Conflict of Interest

The authors declare no conflict of interest.

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