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Anti-HIV-1 and cytotoxicity of the alkaloids of *Erythrina abyssinica* Lam. growing in Sudan

Magdy M.D. Mohammed[a]*, Nabaweya A. Ibrahim[a], Nagwa E. Awad[a], Azza A. Matloub[a], Ahmed G. Mohamed-Ali[b], Ezzieldeen E. Barakat[b], Ahmed E. Mohamed[b] and Paolo L. Colla[c]

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Erythrina abyssinica Lam. is an important medicinal plant growing in Sudan; its seeds were investigated for the first time for their alkaloidal constituents and biological activity. The *in vitro* cytotoxicity of the crude alkaloidal fraction (CAF) against the cell lines HeLa, Hep-G2, HEP-2, HCT116, MCF-7 and HFB4 showed promising activity, with IC_{50} values of 13.8, 10.1, 8.16, 13.9, 11.4 and 12.2 $\mu g\,mL^{-1}$, respectively. Doxorubicin (positive control) showed *in vitro* cytotoxic activity with IC_{50} values 3.64, 4.57, 4.89, 3.74, 2.97 and 3.96 $\mu g\,mL^{-1}$, respectively. Bioassay-guided fractionation and isolation of the CAF led to the isolation of five *Erythrina* alkaloids, identified as erythraline, erysodine, erysotrine, 8-oxoerythraline and 11-methoxyerysodine. These were evaluated for their *in vitro* cytotoxic activity against Hep-G2 which resulted in IC_{50} values 17.60, 11.80, 15.80, 3.89 and 11.40 $\mu g\,mL^{-1}$, respectively. Furthermore, *in vitro* cytotoxic activity against HEP-2 was evaluated, which resulted in IC_{50} values 15.90, 19.90, 21.60, 18.50 and 11.50 $\mu g\,mL^{-1}$, respectively. The CAF caused a reduction in the viability of mock-infected MT-4 cells with a CC_{50} of 53 μM and a 50% protection of MT-4 cells against HIV-1 induced cytopathogeneticy with a EC_{50} of >53 μM, compared with EFV as a positive control, which had a CC_{50} of 45 μM and an EC_{50} of 0.003 μM. We concluded that the isolated alkaloids were responsible for the carcinogenic actions of the plant extract previously reported in the literature.

Keywords: *Erythrina abyssinica* Lam.; Fabaceae (Leguminosae); *Erythrina* alkaloids; anti-HIV-1; cytotoxicity; SAR studies

1. Introduction

Erythrina abyssinica Lam., belonging to the family Fabaceae (Leguminosae), is one of the largest families containing alkaloids, comprising 643 genera/18,000

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species (Michael, 2006). The genus *Erythrina* comprises over 110 currently recognised species of orange and/or red flowered trees, shrubs and herbaceous plants and is found throughout the tropical and subtropical regions of the world except Europe and Antarctica. There are 30 species and subspecies in tropical Africa and six species in Southern Africa (Fabian & Germishhuizen, 1997). *Erythrina* species have been used in traditional medicine for the treatment of various diseases, especially microbial infections (Mitscher, Drake, Gollapudi, & Okwute, 1987; Pillay, Jager, Mulholland, & van Staden, 2001). These plants are known to be a rich source of bioactive alkaloids (Barakat, Jackson, & Abdulla, 1977; Cordell, 1981) and flavonoids, mainly isoflavones, pterocarpans, flavanones and isoflavanones (Chacha, Bojase-Moleta, & Majinda, 2005). Some of these flavonoids have been found to display a variety of biological activities, such as antimicrobial (Chacha et al., 2005; Mitscher et al., 1987), anti-HIV-1 (Mckee et al., 1997), antibacterial (Tanaka et al., 2002), anti-inflammatory (Njamen et al., 2004) and anti-plasmodial activities (Andayi et al., 2006).

Erythrina abyssinica Lam. is an important medicinal plant in Sudan; it has been used in folk remedies by the Sudanese habitants for the treatment of various ailments; its bark was used for the treatment of coughs, skin ailments, ulcers, abdominal pain, vomiting, liver inflammation, colic, trachoma and elephantiasis, flowers for the treatment of dysentery and abortifacient, leaves for the treatment of peptic ulcers, arthralgia and diarrhea, roots for the treatment of epilepsy, malaria and syphilis, and fruits for the treatment of asthma (Neuwinger, 2000). Nakanishi and co-workers isolated pterocarpans, flavanones and a chalcone from the roots, of which, some possessed antimicrobial activity and inhibited platelet aggregation (Kamat, Chuo, Kubo, & Nakanishi, 1981). In a previous report, abyssinins I, II and III, together with four flavanones, have been isolated and identified in the stem bark of *E. abyssinica* Lam., (Ichimaru et al., 1996; Moriyasu et al., 1998). Although, folkloric uses of the seeds of *E. abyssinica* Lam. exist for the treatment of different types of tumours for instance, in stomach, nose, epidermis, pharynx cancers and hepatitis (Duke, Bogenschutz-Godwin, Ducellier, & Duke, 2002; Hartwell, 1982), there has been less concern regarding its alkaloidal content, which prompted us to initiate the phytochemical and biological investigation of the alkaloidal content of the seeds growing in Sudan.

The crude alkaloidal content was isolated according to the 'acid-base shakeout' method. After that, its cytotoxicity was *in vitro* evaluated against HeLa, Hep-G2, HEP-2, HCT116, MCF-7 and HFB4, which resulted in $IC_{50} = 13.8$, 10.1, 8.16, 13.9, 11.4 and 12.2 $\mu g\,mL^{-1}$, respectively. Further chromatographic fractionation of the crude alkaloidal fraction (CAF) led to the isolation of erythraline (**1**), erysodine (**2**), erysotrine (**3**), 8-oxoerythraline (**4**) and 11-methoxyerysodine (**5**). The isolated alkaloids showed *in vitro* cytotoxic activity against Hep-G2 with $IC_{50} = 17.6$, 11.8, 15.8, 3.89 and 11.4 $\mu g\,mL^{-1}$, respectively, and against HEP-2 with $IC_{50} = 15.90$, 19.90, 21.60, 18.50 and 11.50 $\mu g\,mL^{-1}$, respectively. Furthermore, the alkaloidal fraction caused a reduction for the viability of mock-infected MT-4 cells by $CC_{50} = 53\,\mu M$ and 50% protection of MT-4 cells from the HIV-1 induced cytopathogeneticy by $EC_{50} = > 53\,\mu M$, compared with EFV as positive control which showed $CC_{50} = 45\,\mu M$ and $EC_{50} = 0.003\,\mu M$, as determined by the methyl thiazol tetrazolium (MTT) method.

2. Results and discussion

Plants, micro-organisms and more recently, marine organisms of various types have traditionally represented a main source of cytotoxic anticancer agents since the beginning of chemotherapy. Even if the new technologies of combinatorial chemistry and high-throughput screening represent an important step in drug discovery, the role of natural sources in providing new cytotoxics should not be disregarded for the future. The number of microbial species studied in this regard is still very low, and the marine ecosystem is largely unexplored (Cozzi, Mongelli, & Suarato, 2004). Around half of the drugs currently in clinical use as anticancer drugs are of natural product origin, and it has been estimated that about 60% of new chemical entities introduced in the 1981–2002 period in this field were natural products or were derived from a natural lead compound (Newman & Cragg, 2007). In line with the above-mentioned facts, our group started the phytochemical and biological evaluation of the crude alkaloidal content of the seeds of the Sudanese medicinal plant *E. abyssinica* Lam.

The CAF of the seeds of *E. abyssinica* Lam. was tested *in vitro* for its cytotoxicity against HeLa, Hep-G2, HEP-2, HCT116, MCF-7 and HFB4 cell lines, which resulted with $IC_{50} = 13.8$, 10.1, 8.16, 13.9, 11.4 and 12.2 $\mu g\,mL^{-1}$, respectively, while Doxorubicin (+ve control) showed *in vitro* cytotoxicity with $IC_{50} = 3.64$, 4.57, 4.89, 3.74, 2.97 and 3.96 $\mu g\,mL^{-1}$, respectively (Table 1). The obtained results revealed that the CAF was more potent against Hep-G2 and HEP-2 with $IC_{50} = 10.1$ and 8.16 $\mu g\,mL^{-1}$, respectively, compared with Doxorubicin's cytotoxicity with $IC_{50} = 4.57$ and 4.89 $\mu g\,mL^{-1}$, respectively, which showed a moderate cytotoxicity against the other cells. This prompted us to follow-up the bioassay-guided fractionation and isolation of the CAF to find out the cytotoxic principles. Further chromatographic separation using neutral alumina column chromatography led to the isolation of five main fractions, which all gave (+ve) Dragendorff's and Mayer's detection, suggesting the presence of alkaloids. The isolated alkaloids were purified using neutral alumina-oxide TLC eluted with different volumes of the solvent system e.g., benzene: ethyl acetate (9:1), (8:2) and (7:3).

All the isolated alkaloids (**1–5**) belong to the 1,6-diene skeleton of the *Erythrina*-type alkaloids (Figure 1). This was confirmed by the IR absorbance at 1610 cm^{-1} and UV absorbance bands at 230 and 235 nm, which are characteristics for the diene-system (Alfred & Kenneth, 1965; Dyke & Quessy, 1981; Pretsch, Bühlmann, & Affolter, 2000). All the isolated alkaloids gave a characteristic band at 285 nm, which is characteristic for the di-oxygenated aromatic ring (**Ring A**) (Dyke & Quessy, 1981; Pretsch et al., 2000). Furthermore, all the 1,6-diene *Erythrina*-type alkaloids showed a simple mass fragmentation pattern, summarised in (Supplementary Scheme S1 – online only), in which the main pathway involves loss of the allylic substituent at C-3 (Dyke & Quessy, 1981). ^{1}H-NMR spectra of the isolated alkaloids (**1–5**) were similar to the *Erythrina* type alkaloids (Masouda, Maurice, & Alan 1991) and can be summarised as follows.

Compounds (**1** & **4**) showed the presence of the characteristic methylene-dioxy group at δ 5.90 − 5.95 (2H, br*s*, –O–CH_2–O–), a singlet methoxyl at δ 3.30 (3H, *s*, 3−OCH_3), two singlets corresponding to the *para*-aromatic protons (**Ring A**) at δ 6.63 (1H, *s*, H–17) and δ 6.77 (1H, *s*, H–14) for compound (**1**), and at δ 6.72 (1H, *s*, H–17) and δ 6.74 (1H, *s*, H–14) for compound (**4**). The main spectral difference

Table 1. The *in vitro* cytotoxicity of the CAF of the seeds of *E. abyssinica* Lam., and that of the Doxorubicin.

Cell lines	Percentage of inhibition ± SE											
	HEP-2		HCT116		HEP-G2		HeLa		HFB-4		MCF-7	
Concentration ($\mu g\,mL^{-1}$)	CAF	Doxorubicin	CAF	Doxorubicin	CAF	Doxorubicin	CAF	Doxorubicin	CAF	Doxorubicin	CAF	Doxorubicin
5	36.2 ± 0.017	61.3 ± 0.007	25.4 ± 0.079	67.8 ± 0.054	10.9 ± 0.096	66.0 ± 0.019	24.7 ± 0.029	79.7 ± 0.022	9.7 ± 0.063	78.5 ± 0.013	30.0 ± 0.079	80.8 ± 0.029
	*	*	*	*	*	*	*		*	*	*	*
	**	**		**	**	**	**	*		**		**
	(59.05)***		(37.46)***		(16.51)***		(30.99)***	**	(12.36)***		(37.13)***	
12.5	67.7 ± 0.061	71.8 ± 0.01	46.0 ± 0.017	74.4 ± 0.010	67.8 ± 0.034	69.9 ± 0.008	45.5 ± 0.066	80.9 ± 0.012	50.2 ± 0.017	84.5 ± 0.061	53.9 ± 0.052	82.8 ± 0.019
	*	*	*	*	*	*	*	*	*	*	*	*
	**	**	**	**	**	**		**	**	**	**	**
	(94.29)***		(61.82)***		(96.99)***		(56.24)***		(59.41)***		(65.10)***	
25	88.8 ± 0.008	87.7 ± 0.01	83.3 ± 0.003	82.9 ± 0.031	78.4 ± 0.058*	79.6 ± 0.017	90.9 ± 0.037	91.9 ± 0.005	76.1 ± 0.008	87.1 ± 0.016	74.1 ± 0.053	81.4 ± 0.051
	*	*	*	*		*	*	*	*	*	*	*
	**	**	**	**	**	**	**	**	**	**	**	**
	(101.25)***		(100.48)***		(98.49)***		(98.91)***		(87.37)***		(91.03)***	
50	87.6 ± 0.009	89.8 ± 0.002	83.2 ± 0.028	81.5 ± 0.010	84.6 ± 0.039	84.4 ± 0.026	87.0 ± 0.063	90.3 ± 0.011	74.5 ± 0.010	85.9 ± 0.014	73.7 ± 0.024	79.9 ± 0.059
	*	*	*	*	*	*	*	*	*	*	*	*
	**	**	**	**	**	**	**	**	**	**	**	**
	(97.55)***		(102.08)***		(100.24)***		(96.35)***		(86.73)***		(92.24)***	
IC_{50} ($\mu g\,mL^{-1}$)	8.16	4.89	13.90	3.74	10.10	4.57	13.80	3.64	12.20	3.96	11.40	2.97

Notes: Each value represents the mean of percentage of inhibition cells of three replicates ± SEM (standard error of mean).
*, **Significantly different from control values at $p < 0.05$, $p < 0.005$, respectively, according to paired- samples *t*- test.
***Relative inhibition of crude alkaloid fraction of *E. abyssinica* Lam. related to Doxorubicin on the growth of different human cell lines.

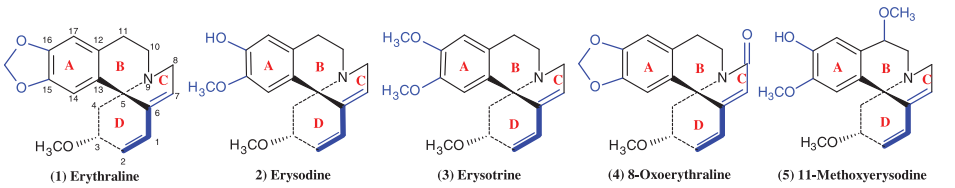

Figure 1. The structural features of the isolated alkaloids (**1–5**) with high multifunctional activities.

between compounds (**1** & **4**) is the presence of a carbonyl carbon at C-8 of compound (**4**) with a characteristic IR absorbance at 1665 cm^{-1}. This carbonyl group caused a down-field shift for the 1,6-diene system to appear at δ 6.67 (1H, br*s*, H–1), δ 6.30 (1H, *m*, H–2) and δ 6.00 (1H, *s*, H–7), compared with compound (**1**) in which the 1,6-diene system appeared at δ 6.54 (1H, br*s*, H–1), δ 5.96 (1H, *m*, H–2) and δ 5.73 (1H, *s*, H–7). A multiplet signal appeared at δ 3.50–3.76 (2H, *m*, 8-CH_2) corresponding to the methylene group of compound (**1**) (**Ring C**). Furthermore, overlapped multiplets appeared at δ 2.49 – 3.90 and at δ 2.80 – 3.70 for compounds (**1**) & (**4**) corresponding to (7H, br*m*, H–3, H–4, H–10 & H–11), respectively.

The molecular formulas of compounds (**1**) and (**4**) were determined to be $C_{18}H_{19}NO_3$ and $C_{18}H_{17}NO_4$, respectively, using HRESIMS (+ve), which showed a quasi-molecular ion peak at *m*/*z* 298.13333 $[M+H]^+$ (calcd 298.14377 for $C_{18}H_{20}NO_3$) for compound (**1**) and at *m*/*z* 312.2000 $[M+H]^+$ (calcd 312.12303 for $C_{18}H_{18}NO_4$) for compound (**4**). Both the collision-induced dissociation (CID) spectra of $[M+H]^+$ resulted in the loss of the allylic substituent at C–3 (**Ring D**), and the formation of ion peaks at *m*/*z* 266 and at *m*/*z* 280 corresponding to $[M-OCH_3]$ of compounds (**1**) and (**4**), respectively (Scheme S1). Hence, according to the above-mentioned data and by comparison with the literature (John, Keith, Andrew, & László, 2010) compounds (**1**) and (**4**) were established as depicted in Figure (1) and assigned to erythraline and 8-oxoerythraline, respectively.

Compounds (**2, 3** & **5**) appeared to have the same structure-skeleton, with compound (**2**) being the parent one and compounds (**3** & **5**) its derivatives. Their ^{1}H-NMR spectra look the same, with very slight differences in both **Ring A** & **B** depending on the substitutions.

Compound (**2**) showed one singlet methoxyl signal (**Ring A**) at δ 3.78 (3H, *s*, 15–OCH_3), two singlets corresponding to the *para*-aromatic protons (**Ring A**) at δ 6.70 (1H, *s*, H–17) and δ 6.80 (1H, *s*, H–14), for compound (**3**) two singlet methoxyl signals (**Ring A**) at δ 3.64 (3H, *s*, 15–OCH_3) and δ 3.74 (3H, *s*, 16–OCH_3), two singlets of (**Ring A**) at δ 6.71 (1H, *s*, H–14) and δ 6.48 (1H, *s*, H–17), for compound (**5**) one singlet methoxyl signal (**Ring A**) at δ 3.75 (3H, *s*, 15–OCH_3) and two singlets of (**Ring A**) at δ 6.71 (1H, *s*, H–17) and δ 6.79 (1H, *s*, H–14). An extra methoxyl singlet appeared at δ 3.33, δ 3.22 and δ 3.29 for compounds (**2, 3** & **5**), respectively, corresponding to (3H, *s*, 3–OCH_3) of (**Ring D**). Compound (**5**) showed an extra methoxyl singlet (**Ring B**) at δ 3.40 (3H, *s*, 11–OCH_3). The 1,6-diene system for compounds (**2, 3** & **5**) appeared at δ 6.58, δ 6.00 and δ 5.74 for compound (**2**), at δ 6.36, δ 5.85 and δ 5.58 for compound (**3**), and at δ 6.45, δ 5.90 and δ 5.60 for compound (**5**) corresponding to (1H, br*s*, H–1), (1H, *m*, H–2) and (1H, *s*, H–7), respectively.

Furthermore, overlapped multiplets appeared at δ 2.53–4.04 for compound (**2**), at δ 2.48–3.95 for compound (**3**), and at δ 2.50–3.90 for compound (**5**) corresponding to (9H, br*m*, H–3, H–4, H–8, H–10 & H–11).

The molecular formulas of compounds (**2, 3** & **5**) were determined to be $C_{18}H_{21}NO_3$, $C_{19}H_{23}NO_3$ and $C_{19}H_{23}NO_4$, respectively, using HRESIMS (+ve), which showed a quasi-molecular ion peaks at *m/z* 300.06667 $[M+H]^+$ (calcd 300.15942 for $C_{18}H_{22}NO_3$) for compound (**2**), at *m/z* 314.18820 $[M+H]^+$ (calcd 314.17507 for $C_{19}H_{24}NO_3$) for compound (**3**), and at *m/z* 330.16988 $[M+H]^+$ (calcd 330.16998 for $C_{19}H_{24}NO_4$) for compound (**5**). The CID of $[M+H]^+$ resulted in the loss of the allylic substituent at C-3 (**Ring D**), and the formation of ion peaks at *m/z* 268, 282 and 298 corresponding to $[M-OCH_3]$ of compounds **2, 3** & **5** (Scheme S1). Hence, according to the above-mentioned data and by comparison with the literature (John et al., 2010), compounds (**2, 3** & **5**) were formulated as shown in Figure (1) and assigned to erysodine, erysotrine and 11-methoxyerysodine, respectively.

From the extract of *E. abyssinica* Lam., we have isolated five alkaloids (**1–5**). *In vitro* cytotoxic activity against Hep-G2 and HEP-2 cell lines of the isolated compounds was performed by MTT assay, resulting in $IC_{50} = 17.6$, 11.8, 15.8, 3.89 and 11.4 $\mu g\,mL^{-1}$, respectively, against Hep-G2 (Supplementary Table S1 and Figure S1 – available online only). Furthermore, the isolated alkaloids (**1–5**) exhibited moderate cytotoxicity against HEP-2 with $IC_{50} = 15.90$, 19.90, 21.60, 18.50 and 11.50 $\mu g\,mL^{-1}$, respectively, (Supplementary Table S2 and Figure S2 – available online only).

The study of the structure activity relationship (SAR) of the isolated alkaloids (**1–5**) revealed that for HEP-2 cell line of compounds (**1–3**), it could be discerned that methylene dioxy group at (Ring A) is optimal substituent as neither a hydrogen bond donor (Compound 2) nor dimethoxy substituent (Compound 3) in (Ring A) improves the HEP-2 cytotoxic activity exhibited by the alkaloids. A carbonyl group vicinal to the nitrogen atom in (Ring C) is detrimental to the HEP-2 cytotoxic activity. For Hep-G2 cell line, SAR analysis showed contradiction to the results observed with HEP-2 cytotoxic activity. Compound (4) with a keto group vicinal to the nitrogen atom in ring C showed the highest cytotoxic potency against Hep-G2 cell lines. In comparison to structurally constrained analogue (Compound 1), the unconstrained analogue (compound 3) showed better cytotoxic potency. Compound (2) with hydrogen donor (phenolic OH group) exhibits better potency than its corresponding methylated analogues (Compounds 1 & 3).

We can conclude that the presence of the substituted methoxyl groups may attribute to the cytotoxic activity of the isolated alkaloids. It is generally considered critical for efficient binding to a cysteine residue in tubulin polypeptide chain, thus preventing cell division. It has been suggested that for the antimitotic activity of these alkaloids, it should possess a methylene dioxy group and/or at least one methoxy group on (**Ring A**) together with the 1,6-diene system (Figure 1) (Gupta, 1994).

The presence of α, β-unsaturated carbonyl group (**Ring C**) in 8-oxoerythraline (**4**) was mainly attributed to its highest cytotoxicity against Hep-G2 with $IC_{50} = 3.89\,\mu g\,mL^{-1}$, and even more than Doxorubicin with $IC_{50} = 4.57\,\mu g\,mL^{-1}$. The unsaturated carbonyl compounds as potential drug candidates are a controversial topic since their potential Michael acceptor activity can lead to cell damage and cytotoxicity. Nevertheless, α, β-unsaturated carbonyl functionality can

be employed as a tool to fine tune biological activity by directly manipulating this entity. Depending on their electronic properties, α, β-unsaturated carbonyl functionalities display different reactivities, namely Michael addition, radical scavenging, oxidation or double bond isomerisation. Modifying the α-position of α, β-unsaturated carbonyl system, a concept that has not been widely explored, could produce new, very interesting derivatives. Currently in drug development, irreversible binding in active sites has proven to be one answer to drug resistance in cancer treatment. Overall, natural products containing α, β-unsaturated carbonyl unit possess multiple biological activities that could be transferred into novel pharmaceutical agents (Sabine, 2010).

The anti-HIV-1 activity of the alkaloidal fraction using MTT method was performed, which resulted in a reduction in the viability of mock-infected MT-4 cells by $CC_{50} = 53\,\mu M$ and achieved 50% protection of MT-4 cells from the HIV-1-induced cytopathogeneticy by $EC_{50} = > 53\,\mu M$, compared with EFV as positive control, which showed $CC_{50} = 45\,\mu M$ and $EC_{50} = 0.003\,\mu M$. The resulting cytotoxicity of mock-infected MT-4 cells by $CC_{50} = 53\,\mu M$ was mainly attributed to the presence of isoquinoline-type alkaloids, which are the biosynthetic precursors to *Erythrina* alkaloids (Ulrich & Meinhart, 1997), which inhibit the replication cycle of human immunodeficiency virus HIV-1 by virus adsorption or reverse transcription process (Vlietinck, De Bruyne, Apers, & Pieters, 1998). These isoquinoline-type alkaloids seem to cause enzyme inhibition by interacting with the template primers, particularly those of the adenine-thymine base pairs (Tan, Kinghorn, Hughes, & Pezutto, 1991). It was further shown that the inhibitory activity correlates well with the anti-leukemic effects in mice (Vlietinck et al., 1998). It is also generally accepted that classical anticancer and antiviral approaches, such as DNA interacting agents, will remain in the main therapeutic arsenal, and that natural products will continue to be an invaluable source of drug prototypes.

3. Experimental

3.1. *Plant material*

Erythrina abyssinica Lam. was obtained from Kadogli, Nuba Mountain; Southern Kordofan State, Sudan. About 900 km West to Khartoum Town; the plant material was authenticated by Dr Wael El-Sadig at the Medicinal and Aromatic Plants Research Institute, Ministry of Science and Technology, Khartoum, Sudan. A voucher specimen (No. 06-0001) was deposited at the Herbarium of the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan. The authentication of the plant sample was confirmed by Dr S.A. Kawashty at the Herbarium of National Research Center (NRC), Cairo, Egypt. A voucher specimen collection (No. 0001) was deposited at the Herbarium of the NRC, Cairo, Egypt. The seeds of *E. abyssinica* Lam. to be investigated were collected, dried at room temperature and then finely grounded into fine powder for further processing.

3.2. *General*

Melting points (uncorrected) were determined on a Koffler's melting point apparatus. Infra-red (IR) spectra were obtained (KBr-DISK and/or FILM/NaCl)

on a Mattson 5000 Infrared Fourier Transform (FTIR) spectrometer. Ultraviolet (UV) spectra were measured using SHIMADZU MPS-2000. HPLC–MS/MS analysis: The analytical system consisted of a Waters (Milford, MA, USA) chromatograph (model 600 E pumps and controller) interfaced to a Thermo Finnigan (Winsford, Cheshire, UK) LCQ classic quadrupole ion trap mass spectrometer *via* an atmospheric pressure chemical ionisation (APCI) source. The alkaloids were separated on a Supelco (Bellefonte, PA, USA) Discovery C_{18} column (250×4.6 mm i.d.; 5 μM) eluted at 1 mL min^{-1} with a linear gradient mobile phase programmed from three solvent reservoirs, A (0.1% ammonium acetate, pH 7.4), B (methanol) and C (acetonitrile), as follows: 0 min, 75:20:5 (A:B:C); 10 min, 50:45:5 (A:B:C); and 15 min, 50:45:5 (A:B:C). The vapouriser of the APCI source was set to 450°C with sheath and auxiliary nitrogen gas pressures 80 and 20 psi, respectively, and the needle current was 5 μA; the heated capillary temperature was 150 C. The MS was programmed to survey ions with m/z in the range 150–500 and to subject the most intense ion in the survey scan to MS/MS analysis, followed by MS/MS/MS analysis of the most intense product ion in the MS/MS scan. The collision energy was 40% using an ion isolation width of 3 amu'. The alkaloids were identified by comparison of their HRESI-MS, and CID spectrums with the previously published data. Further confirmation of the identity of the isolated compounds was done through ^{1}H-NMR (Joel EX, 300 & 270 MHz, Japan) recorded in $CDCl_3$; chemical shift values were in ppm, J values were in Hz, and TMS was used as internal standard.

3.3. *Extraction and isolation*

The seeds of *E. abyssinica* Lam. (100 g) were grounded, and then defatted by maceration with 250 mL petroleum ether (60–80°C) several times to remove all waxes. The residue was extracted with 250 mL chloroform; the combined $CHCl_3$ extracts were evaporated to dryness and the residue extracted with 5% HCL to isolate all *N*-containing compounds in the form of its salt. The aqueous acidic solution was basified with 25% ammonia solution to pH = 8.5 and then extracted several times with chloroform (3×100 mL), which got concentrated under reduced pressure and it was dried over anhydrous sodium sulphate to afford 1.08 g of the CAF. In this study, 5 mL aliquots of the fraction were treated with the Dragendorff's, Wagner's and Mayer's for the presence of alkaloids, and resulted with orange, reddish orange- and cream-coloured precipitates, respectively (Cordell, 1981). The resulting 1.08 g of the CAF was chromatographed over aluminum oxide column chromatography (500×20 mm, Neutral Aluminum Oxide, Brookmann activity Grade-1, Sisco Research Laboratories, Mumbai, India), eluted with benzene 100% gradually increasing the polarity with ethyl acetate till 100% and finally washing with methanol 100%. Also, 50 mL of each fraction was collected, evaporated till dryness and tested over preparative TLC (neutral aluminum oxide 60 GF_{254} grade 3, Merck) using solvent system; benzene – ethyl acetate (7:3). Further separation was achieved by subsequent TLC, which resulted in the isolation of the alkaloidal compounds. Dragendorff's and Mayer's reagents were used for the detection *via* dipping method (Cordell, 1981).

3.4. *Cytotoxic assay procedures*

3.4.1. *Cells*

Authentic culture, HeLa, Hep-G2, HEP-2, HCT116, MCF-7 and HFB4 were obtained in a frozen-phase under liquid nitrogen (−180°C) from The American Type Culture Collection, USA. The tumour cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing.

3.4.2. *Culture media*

The carcinoma cells were suspended in Roswell Park Memorial Institute 1640 medium supplemented with 10% foetal calf serum, 1% antibiotic-antimycotic mixture (10.000 U mL^{-1} K-penicillin, 10.000 µg mL^{-1} streptomycin sulphate and 25 µg mL^{-1} amphotericin B) and 1% L-glutamine (all purchased from Lonza, Belgium).

3.4.3. *Assay method for cytotoxic activity*

The cytotoxicity against HeLa, Hep-G2, HEP-2, HCT116, MCF-7 and HFB4 were performed in the National Cancer Institute, according to the sulforhodamine B assay method by Skehan et al. (1990). Adriamycin® (Doxorubicin) 10 mg vials (Pharmacia, Sweden) was used as the reference drug.

3.4.4. *Statistical analysis of data*

All values were expressed as the mean with five replicates for each treatment. Data were subjected to paired-samples *t*-test using SPSS Statistical Software Package (version 9.0). $P < 0.005$ and 0.05 were regarded as significant.

3.5. *Antiviral assay procedures*

It was carried out according to the detailed reported method (Mohammed et al., 2009).

Supplementary material

Tables S1 and S2, Figures S1 and S2 and Scheme S1 relating to this paper are available online.

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