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Antioxidant and Cytotoxic Activities of *Cuphea hyssopifolia* Kunth (Lythraceae) Cultivated in Egypt

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Cuphea hyssopifolia Kunth (Family Lythraceae) is a herb, newly cultivated in Egypt**.** Its richness in phenolics had led to test for its antioxidant and cytotoxic activities and isolation of its components. Also it urges us to check for its genotyping. Isolation of some phenolics was done using usual chromatographic techniques. The genetic fingerprinting of the aerial parts of *C. hyssopifolia* Kunth was determined using RAPD-PCR (Random Amplified Polymorphic DNA) technique which has been widely used in plants for the construction of genetic maps using 10 primers. The antioxidant activity of the extract was measured using the stable free radical DPPH assay while its cytotoxic property was tested using different cell lines *viz* MCF7 (breast carcinoma cell line), HEP2 (larynx carcinoma cell line), HCT116 (colon carcinoma cell line) and HEPG2 (liver carcinoma cell line) adopting SRB method. Several compounds had been isolated from the aqueous methanolic extract of the aerial parts of *C. hyssopifolia* Kunth namely valoneic acid dilactone **(1)**, 1,3−*O*−digalloyl-4-,6-hexahydroxydiphenoyl- β-*D*−$^4C_1$-glucopyarnose **(2)**, gallic acid **(3)**, genistein-7-*O*-β-*D*-$^4C_1$-glucopyranoside **(4)**, myricetin−3 − *O*− β-*D*−$^4C_1$-glucopyarnoside **(5)**, 3, 4, 5-trimethoxy benzoic acid **(6)**, vanilice acid **(7)** and quercetin **(8)**. Primer ***OPP-01*** was the best sequence for dominating *C. hyssopifolia* Kunth cultivated in Egypt. The antioxidant activity of the extract showed an inhibition 95.5% compared to that of ascorbic acid 98.35%. The extract showed moderate cytotoxic activity with IC_{50} ranging from 73.4 to 92.5 ug/ml for the tested cell lines. The study suggests that *C. hyssopifolia* Kunth may be the potential rich source of natural antioxidant.

Keyword: Antioxidant, *Cuphea Hyssopifolia*, Cytotoxic, Lythraceae, Phenolic Compounds, RAPD-PCR.

1. Introduction

Cuphea hyssopifolia Kunth (Lythraceae), a small shrub native to Mexico and Guatemala, is cultivated in Egypt as a horticultural plant[1].

Cuphea is the largest of the 32 genera of Lythraceae with about 260 species of herbaceous perennials and small shrubs[2-3]. A wide range of phytochemical constituents have been reported from genus *Cuphea viz.* tannins, flavonoids, phenolic acids, triterpenes, sterols, carbohydrates and unsaturated fatty acids. This genus is biologically studied for its cytotoxic, antiviral, antimicrobial, anti-*Helicobacter pylori*, antiprotozoal, cardiovascular, antioxidant and diuretic activities[4]. Some species of the genus *Cuphea* have been used for treating stomach disorders, syphilis, gonorrhea as well as being used as an oral contraceptive in folk medicines from South and Central Americas[1,5-7]. Little could be traced in literature concerning the *C. hyssopifolia* Kunth cultivated in Egypt so that urged to investigate its extract and to test for its activity.

2. Materials and Methods

2.1 Plant extraction, Isolation and Purification

Fresh samples of *Cuphea hyssopifolia* Kunth were purchased from Nasr City, Cairo, Egypt in December 2009 and the plant was kindly identified by agricultural engineer Teresa Labib, El-Orman Botanical Garden and Agricultural Consultant Dr. Mohamed El-Gebaly, National Research Center. A voucher specimen had been deposited in the herbarium, Faculty of Pharmacy, Egyptian Russian University.

The intact air dried plant material (600 g) was boiled in distilled water for 2 hours then filtered while hot. The filtrate was completely evaporated *in vacuo* till dryness. The solid residue was then extracted with methanol and the extract was completely evaporated to give 25 gm solid residue which was applied on a glass column (70 cm L x 5 cm D) packed with polyamide 6S (250 g, Riedel-de Haen AG, Seelze-Hannover, Germany) as adsorbent and eluted with distilled water and methanol of decreasing polarity. Fractions (200 mL) were collected and monitored by co-chromatograms using paper chromatography Whatman No.1mm using 2 solvents S_1 (*n*-BuOH:AcOH: H_2O, 4:1:5, v/v, upper layer) and S_2 (6% AcOH). The PC was examined under UV light, subjected to ammonia vapors and then sprayed with 1% methanolic ferric chloride. Similar fractions were pooled together and dried at *vacuo* at 45 ^{0}C, to give main 12 fractions (**I-XII**) [8-11]. Fraction **IV** (eluted with 40% Methanol, 0.75 g) was fractioned and purified on preparative TLC *Cellulose* PEI-F Sheets 200 x 200 mm using S_1 to afford compound **(1)** (11 mg). Fraction **V** (eluted with 40% Methanol, 2 gm) was re-fractionated over a glass column (30 cm L x 1 cm D) packed with sephadex LH 20 (50 gm, Sigma-Aldrich, USA) as adsorbent using water and methanol with decreasing polarity to afford compounds **(2-5).** They were purified on PPC using S_1. Compound **(2)** (20 mg) was eluted with 100% water while compounds **(3)** (12 mg) and **(4)** (16mg) were eluted with 50% methanol whereas compound **(5)** (11mg) was eluted was 100% methanol. Fraction **VIII** (eluted with 80% Methanol, 0.22 gm) was fractioned and purified on PPC using S_1 to afford compounds **(6)** (17 mg) and **(7)** (50 mg). Fraction **IX** (eluted with 80% Methanol, 0.95 g) was fractioned and purified on PPC using S_1 to give compound **(8)** (14 mg). ^{1}H-NMR spectra were measured by a Jeol ECA 500MHz NMR spectrometer (JEOL, Tokyo, Japan) at 500 MHz using (DMSO)-d_6. ^{1}H chemical shifts (δ) were measured in ppm, relative to tetramethylsilane (TMS). Ultraviolet (UV) spectra were measured with UV-1601 PC, UV-visible spectrophotometer, Shimadzu, Japan.

Compounds **(1-5)** were isolated from 40% methanol fraction while compounds **(6-8)** were isolated from the 80% methanolic fraction. Chromatographic and UV data of the isolated compounds are listed in Table (2) and their structures are shown in Figure (2). **Compound (1)** white amorphous powder, ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.48 (1H, *s,* H-4), 6.78 (1H, *s*, H-9), 6.92 (1H, *s*, H-6'); **Compound (2)**: white amorphous powder,^{1}H-NMR (DMSO-d_6) δ (ppm): 7.8 (2H, *s*, H-2" & H-6"), 7.6 (2H, *s*, H-2"' & H-6"'), 6.95 (1H, *s*, H-2), 6.87 (1H, *s*, H-2'), 5.14 (1H,*d*, *J*= 7.4 Hz, H-1""-glucose); **Compound (3):** off white amorphous powder, ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.87 (2H, *s*, H-2 & H-6); **Compound (4):** pale yellow crystals, ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.20 (1H, *s*, H-2), 7.61 (2H, *d*, *J*=8 Hz, H-2', H-6'), 7.50 (2H, *d*, *J*=8 Hz, H-3', H-5'), 6.84 (1H, *d*, *J*=1.2 Hz, H-8), 6.64 (1H, *d*, *J*=1.2 Hz, H-6), 5.04 (1H, *d*, *J*=7.4 Hz, H-1"-glucose); **Compound (5)**: off white amorphous powder, ^{1}H-NMR (DMSO-*d6*) δ (ppm): 7.43 (2H, *d*, *J*=8 Hz, H-2', H-6'), 6.34 (1H, *d*, *J*=1.2 Hz, H-8), 6.17 (1H, *d*, *J*=1.2 Hz, H-6), 5.10 (1H, *d*, *J*=7.4 Hz, H-1"-glucose), **Compound (6)**: off white amorphous powder, ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.82 (*s*, H-2 and H-6), 3.60(*s*, 6H, OCH3 at C-3 and C-5), 3.46 (*s*, 3H, OCH3 at C-4); **Compound(7)**: off white amorphous powder, ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.67 (1H, *dd*, *J*=8.1 and 1.8 Hz, H-6), 7.45 (1H, *d*, *J*=1.8 Hz, H-2), 7.05 (1H, *d*, *J*=1.8 Hz, H-5), 4.09 (3H, *s*, OCH3) and **Compound (8)**: yellow needles, ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.60 (1H, *dd*, *J*=2.1, 8.5 Hz, H-6'), 6.86 (1H, *d*, *J*=2.1 Hz, H-2'), 6.50 (1H, *d*, *J*=8.5 Hz, H-5'),

6.20 (1H, *d*, *J*=2.1Hz, H-8), 6.00 (1H, *d*, *J*= 2.1 Hz, H-6).

2.2 DNA Profiling

Sample of fresh leaves was stored at -70 ^{0}C, freeze dried and ground under liquid nitrogen to fine powder prior to DNA isolation[8]. The polymerase chain reactions were carried out with 100 ng of genomic DNA template following a thermal cyclic program. Amplified products were analyzed by electrophoresis on 1.4% agarose gel & finally stained with ethidium bromide. A molecular size marker was used as standard marker. Ten oligonucleotide primers were used for RAPD analysis in this study namely:

OPB-06 (5'-TGCTCTGCCC-3'),
OPB-12 (5'-CCTTGACGCA-3'),
OPE-05 (5'-TCAGGGAGGT-3'),
OPE-08 (5'-TCACCACGGT-3'),
OPE-04 (5'-GTGACATGCC-3'),
OPB-18 (5'-CCACAGCAGT-3'),
OPE-02(5'-GGTGCGGGAA-3'),
OPE-06 (5'-AAGACCCCTC-3'),
OPO-02 (5'-ACGTAGCGTC-3'),
OPP-01 (5'-GTAGCACTCC-3').

2.3 Antioxidant Activity (in-vitro assay)

Diphenyl picryl hydrazyl (DPPH, Sigma-Aldrich Chemie, Steinheim, Germany) which is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color [9-11]. The solution of DPPH in methanol (6 x 10^{-5} M) was freshly prepared before UV measurements. Extract solution was prepared by dissolving 0.025 g of dry extract in 10 ml of methanol. Three ml of the DPPH solution were mixed with different volumes of the prepared sample extract solution in 1 cm path length cuvette. Similarly, three ml of the DPPH solution were mixed with the same volumes of the standard (positive control, vitamin C (ascorbic acid, Sigma chemical Co., Egypt). The samples were kept in the dark for 30 minutes at room temperature and then the decrease in the absorption was measured. Absorption of the blank sample containing the same amount of methanol and DPPH solution was prepared and measured (negative control)[12-13]. The experiment was carried out in triplicate and the radical scavenging activity (Inhibition %) was calculated according to the following equation:

$$\%Inhibition = [(A_B - A_A)/A_B] \times 100$$

Where A_B= the absorption of blank (t=0); A_A= the absorption of sample (t=30 minutes)

To calculate IC_{50}, the concentration of the substrate that causes 50% loss of DPPH activity (color), different concentrations of the tested sample and standards were calculated from the graph plotted for the % of inhibition against concentration in µg/ml.

2.4 Cytotoxic screening

The different cancer cell lines *viz.* MCF7 (breast carcinoma cell line), HEP2 (larynx carcinoma cell line), HCT116 (colon carcinoma cell line) and HEPG2 (liver carcinoma cell line) were obtained frozen in liquid nitrogen (-180 °C) from the American Type Culture Collection and were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. The sensitivity of the human tumor cell lines to the total aqueous methanolic extract of *C. hyssopifolia* Kunth was determined by the SRB assay using doxorubicin as a positive control. SRB is a bright pink aminoanthracene dye with two sulphonic groups. It is a protein stain that binds to the amino groups of intracellular proteins under mildly acidic conditions to provide a sensitive index of cellular protein content[14]. The optical density (O.D.) of each well was measured spectrophotometrically at 564 nm with an ELIZA microplate reader (Meter tech.Σ 960, U.S.A.). The mean background absorbance was automatically subtracted and mean values of each drug concentration were calculated. The percentage of cell survival was calculated according to the following equation:

$$\text{SurvivingFraction} = \frac{\text{O. D. (treatedcells)}}{\text{O. D. (controlcells)}}$$

Where O.D.; optical density

The IC_{50} values (the concentrations of drug required to produce 50% inhibition of cell growth) were measured. The experiment was repeated 3 times for each cell line.

3. Results and Discussion

3.1 Phytochemical Isolation

The chromatographic and UV data of the isolated compounds were recorded in Table (1). Compound **(1)**; valoneic acid dilactone showed an intense violet spot on PC under UV light. It showed one intense peak at λ_{max} 270 nm. The ^{1}H-NMR revealed two signals at δ ppm 7.48 (1H, *s,* H-4) and 6.78 (1H, *s*, H-9) representing the ellagic acid moiety and 6.92 (1H, *s*, H-6') representing the galloyl moiety [15]. Chromatographic analysis of compound **(2),** 1, 3 -*O*-digalloyl-4, 6-hexahydroxydiphenoyl-*β*-*D*-$^4C_1$-glucopyranose, revealed an intense violet spot under UV light. When treated with $FeCl_3$ in methanol, it gave blue violet color. Similarly it showed one intense peak at λmax 270 nm. The ^{1}H-NMR spectrum revealed five signals; there were two peaks representing two galloyl moiety 7.8 (2H, *s*, H-2" & H-6") and 7.6 (2H, *s*, H-2"' & H-6"') while the hexahydroxydiphenic acid moiety is represented by 6.95 (1H, *s*, H-2) and 6.87 (1H, *s*, H-2'). The anomeric proton of *β* -glucose is identified by 5.14 (1H, *d*, *J*= 7.4 Hz, H-1""-glucose) [16]. Compound **(3)**; gallic acid was identified as gallic acid with its one signal 6.87 (2H, *s*, H-2 & H-6) [16]. Compounds **(1-3)** were firstly reported in this genus.

Compound **(4)**; genistein-7-*O*- *β* –*D* -$^4C_1$-glucopyranose (genistin) appeared as a dark purple spot on PC that gave dirty green color upon spraying with $FeCl_3$ in methanol. The UV absorption showed a peak at λ_{max} 275 nm and 380 *sh* indicating the isoflavone nature. By adding NaOAc, no shift in band II was observed indicating that the OH group at position 7 is occupied. Further addition of H_3BO_3 showed no significant bathochromic shift indicating the absence of ortho dihydroxy in ring A or B (Table 1). Confirmation of the structure was achieved through ^{1}H-NMR. The spectrum showed singlet at δ ppm 8.20 assigned for H-2. Also the spectrum displayed a pair of ortho coupled protons of ring B as doublet at 7.61 for H-2', H-6'and 7.50 for H-3', H-5'). The protons of ring A resonated at δ ppm 6.64 and 6.84 assigned for H- & H-8 respectively. Their downfield shift revealed that the glycosylation was at 7-hydroxy position. The anomeric proton at δ ppm 5.04 assigned for the sugar moiety (glucose) with *J*=7.4 Hz coupling constant indicating with β configuration[17].

Compound **(5)**; myricetin -3-*O*- *β* -*D*-$^4C_1$-glucopyranose showed absorbance at λ max 271 nm and 370 indicating the flavonol nucleus. The ^{1}H-NMR spectrum displayed a peak of ortho coupled protons of ring B as doublet at δ ppm 7.4 for H-2' and H-6'. The protons of ring A resonated at δ ppm 6.17 and 6.34 for H-6 and H-8 respectively. The anomeric proton at δ ppm 5.10 assigned for the sugar moiety (glucose) with *J*=7.4 Hz coupling constant indicating with β configuration[17].

Compound **(6)**; 3, 4, 5-trimethoxy benzoic acid showed an intense violet spot under UV light and one intense peak at λ max 261 nm. The ^{1}H-NMR spectrum revealed one signal at δ ppm 6.82 (*s*, H-2 and H-6) and two distinct signals at δ ppm 3.60 and 3.46 revealing the presence of methoxy groups at positions 3, 4 and 5[18].

Compound **(7)**; vanillic acid appeared as a violet spot on PC showing two absorption peaks at λ_{max} 265 and 280 nm. The ^{1}H-NMR spectrum revealed a *dd* peak at 7.67 assigned for H-6. Similarly H-2 and H-5 appeared a doublet at δ ppm 7.45 and 7.05 respectively. A distinct signal appeared at δ ppm 4.09 indicating the presence of methoxy group at position 3[19].

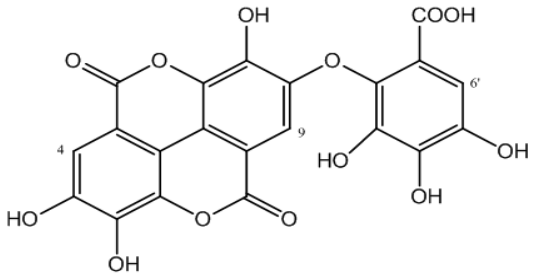
Compound **(8)**; quercetin was identified as a yellow spot on PC that turned into dirty green apon spraying with $FeCl_3$ in methanol. The UV absorption at λ_{max} 268 and 330 nm indicating flavonol nucleus. The ^{1}H-NMR spectrum

displayed five peaks at δ ppm 7.60 for H-6', 6.86 for H-2' and 6.50 for H-5', while H-6 and H-8 resonated at δ ppm 6.00 and 6.20 respectively [17].

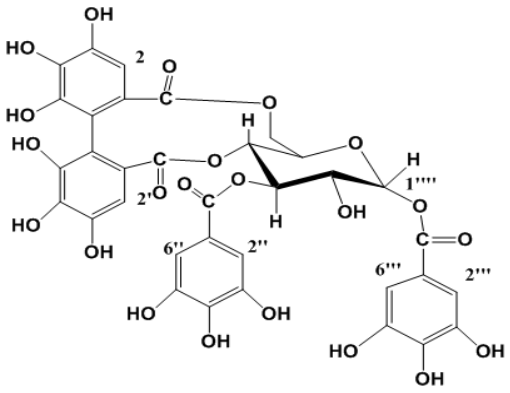
Table 1: Chromatographic and UV data of compounds (1-8) 1998.

Compound	Chromatographic properties					UV Spectral data λ_{max}(nm)		
	R_fs (x100)		Color of Spots			MeOH	NaOAc	NaOAC-H_3BO_3
	BAW	6%AcOH	UV[1]	NH_3[1]	$FeCl_3$[1]			
(1)valoneic acid dilactone	**53**	**55**	**v**	**int.**	**b**	**270**		
(2) **1,3-*O*-digalloyl-4-,6-hexahydroxydiphenoyl – *β*-*D*-$^4C_1$-glucopyarnose**	**45**	**51**	**v**	**int.**	**b**	**270**		
(3)**gallic acid**	**53**	**56**	**v**	**int.**	**b**	**267**		
(4) **genistein-*7-O-β-D*-$^4C_1$-glucopyranoside**	**81**	**20**	**dp**	**Y**	**dg**	**275, 380sh**	**273, 380sh**	**277, 380sh**
(5) **myricetin-3-*O-β-D*-$^4C_1$-glucopyranoside**	**52**	**45**	**dp**	**int.**	**dg**	**271, 370**	**290, 380**	**277,390**
(6) **3,4,5-trimethoxy benzoic acid**	**77**	**59**	**v**	**int.**	**-**	**261**		
(7)**vanilice acid**	**90**	**11**	**v**	**int.**	**bu**	**265, 280**		
(8)**quercetin**	**95**	**11**	**y**	**int.**	**dg**	**268, 330**	**273, 380sh**	**268, 350**

[1]v=violet, int.=intensified, b=blue, dp=dark purple, y=yellow, dg=dirty green, bu=buff



(1)



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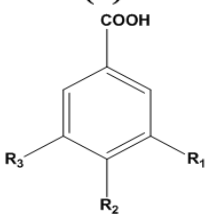

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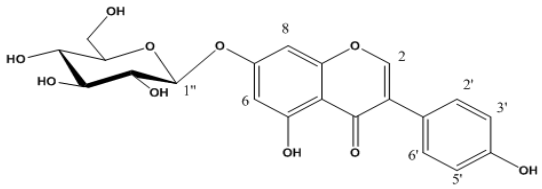
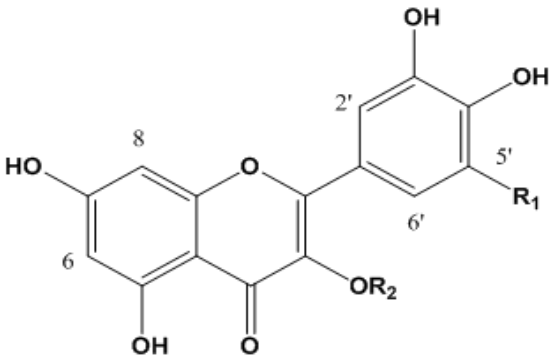


(4)

(3): R_1=OH, R_2=OH, R_3=OH

(6): R_1=OCH_3, R_2=OCH_3, R_3=OCH_3

(7): R_1=OCH_3, R_2=OH, R_3=H



(5): R_1=OH, R_2=β-glucose

Fig 1: Structures of compounds (1-8)

3.2 DNA Profiling

The extracted DNA of *C. hyssopifolia* Kunth was amplified using ten decamer primers to detect their genetic pattern. The ten primers had successfully directed the amplification of a genome-specific fingerprint of DNA fragments. The ten primers (***OPB-06, OPB-12, OPE-05, OPE-08, OPE-04, OPB-18, OPE-02, OPE-06, OPO-02***and ***OPP-01***) of arbitrary sequences generated 87 fragments in *C. hyssopifolia* Kunth Table (2). The obtained RAPD-PCR products *C. hyssopifolia* Kunth using ten decamer primers are represented in Figure (2).

The ten primers had produced multiple band profiles with a number of amplified DNA fragments ranging from 13 when ***OPP-01*** was used. Whereas, the least number of fragments was 6 being produced by ***OPB-18***. Primers ***OPE-08*** and ***OPE-02*** showed good dominating for *C. hyssopifolia* Kunth producing 11 amplified DNA fragments and primer ***OPB-06*** produced 9 amplified DNA fragments. Primers ***OPE-05*** and ***OPE-06*** showed moderate dominating producing 8 amplified DNA fragments. Whereas primers ***OPB-12, OPE-04 and OPO-02*** produced only 7 amplified DNA fragments. Therefore primer ***OPP-01*** was the best sequence for dominating *C. hyssopifolia* Kunth cultivated in Egypt.

Table 2: Total Numbers of RAPD-PCR Fragments in *C. hyssopifolia* Kunth.

Primer Code	RAPD Fragments
OPB-06	**9**
OPB-12	**7**
OPE-05	**8**
OPE-08	**11**
OPE-04	**7**
OPB-18	**6**
OPE-02	**11**
OPE-06	**8**
OPO-02	**7**
OPP-01	**13**
Total	**87**

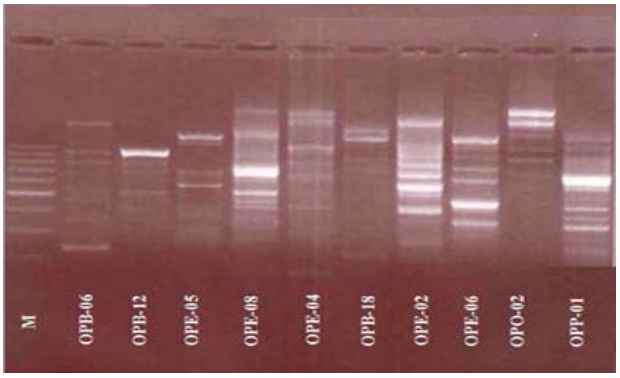


Fig 2: RAPD-PCR Products for *C. hyssopifolia* Kunth Using Ten Decamer Primers

3.3 Antioxidant Activity

The extract showed a very strong antioxidant effect with an inhibition 95.5% compared to the inhibition of the ascorbic acid 98.35% against the stable free radical DPPH. The calculated IC_{50} of the extract is 12.34 µg/ml compared to that of ascorbic acid 1.82 µg/ml. This may be contributed to its richness with polyphenolics Table (3) Figure (3).

Table 3: Antioxidant Activity of The Aqueous Methanolic Extract of The Aerial Parts of *C. hyssopifolia* Kunth Cultivated in Egypt.

µl of the extract added to 3ml DPPH	Concentration of the sample (µg/ml)	%Inhibition	
		Ascorbic acid	Extract
0	0	0	0
1.75	1.456	39.7	2.19
2.3	1.916	53.5	2.42
3.5	2.91	74.5	7.61
7	5.83	88.2	30.3
10	8.33	94.8	39.2
14	11.6	97.9	47
19	15.83	98.17	65.29
38	**31.6**	98.27	93.43
77	**64.16**	98.35	95.5
IC_{50} (µg/ml)		1.82	12.34
Potency of 77 µl of the sample		100	97.1

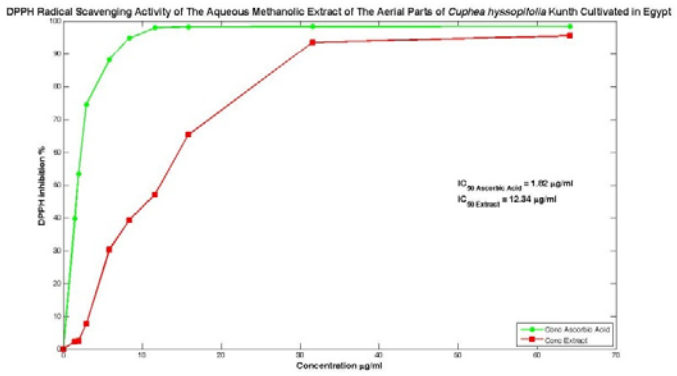


Fig 3: DPPH Radical Scavenging Activity of The Aqueous Methanolic Extract of The Aerial Parts of *C. hyssopifolia* Kunth cultivated in Egypt.

3.4 Cytotoxic Screening

The aqueous methanolic extract of the aerial parts of *C. hyssopifolia* Kunth was screened for its cytotoxic activity using SRB method against MCF7 (breast carcinoma cell line), HEP2 (larynx carcinoma cell line), HCT_{116} (colon carcinoma cell line) and HEPG2 (liver carcinoma cell line) with IC_{50} 92.5, 84.9, 81 and 73.4 ug/ml respectively using doxorubicin as a positive control[13]. Results are represented as mean surviving fractions of carcinoma cells Tables (4-7) Figures (4-7). The extract showed weak cytotoxic activity compared to the IC_{50} of doxorubicin 3.7 to 5 ug/ml. This may be attributed to the hydrophilic character of the extract that may hamper its interaction with cell membrane where all the signal transduction pathways occur[20-21].

Table 4: Cytotoxic activity of the aqueous methanolic extract of the aerial parts of *C. hyssopifolia* Kunth against MCF7 (Breast Carcinoma Cell Line).

MCF7 (breast carcinoma cells)			
Extract Conc. µg/ml	**Surviving Fractions (Mean ± S.E.M.)**	**Doxorubicin Conc. µg/ml**	**Surviving Fractions (Mean ± S.E.M.)**
0	1.000 ± 0.000	0	1.000 ± 0.000
125	0.340 ± 0.010	5	0.478 ± 0.031
250	0.318 ± 0.008	12.5	0.411 ± 0.031
375	0.309 ± 0.018	25	0.256 ± 0.021
500	0.310 ± 0.010	50	0.309 ± 0.023

Table 5: Cytotoxic activity of the aqueous methanolic extract of the aerial parts of *C. hyssopifolia* Kunth against HEP2 (Larynx carcinoma cells).

HEP2 (larynx carcinoma cells)			
Extract Conc. µg/ml	**Surviving Fractions (Mean ± S.E.M.)**	**Doxorubicin Conc. µg/ml**	**Surviving Fractions (Mean ± S.E.M.)**
0	1.000 ± 0.000	0	1.000 ± 0.000
125	0.260 ± 0.006	5	0.316 ± 0.023
250	0.202 ± 0.009	12.5	0.255 ± 0.026
375	0.200 ± 0.015	25	0.242 ± 0.025
500	0.182 ± 0.010	50	0.263 ± 0.029

Table 6: Cytotoxic activity of the aqueous methanolic extract of the aerial parts of *C. hyssopifolia* Kunth against HCT_{116} (Colon carcinoma cells).

HCT_{116} (colon carcinoma cells)			
Extract Conc. µg/ml	**Surviving Fractions (Mean ± S.E.M.)**	**Doxorubicin Conc. µg/ml**	**Surviving Fractions (Mean ± S.E.M.)**
0	1.000 ± 0.000	0	1.000 ± 0.000
125	0.233 ± 0.018	5	0.447 ± 0.056
250	0.189 ± 0.011	12.5	0.343 ± 0.041
375	0.206 ± 0.010	25	0.296 ± 0.026
500	0.222 ± 0.019	50	0.281 ± 0.020

Table 7: Cytotoxic activity of the aqueous methanolic extract of the aerial parts of *C. hyssopifolia* Kunth against HEPG2 (Liver carcinoma cells).

HEPG2 (liver carcinoma cells)			
Extract Conc. µg/ml	**Surviving Fractions (Mean ± S.E.M.)**	**Doxorubicin Conc. µg/ml**	**Surviving Fractions (Mean ± S.E.M.)**
0	1.000 ± 0.000	0	1.000 ± 0.000
125	0.152 ± 0.021	5	0.392 ± 0.037
250	0.169 ± 0.016	12.5	0.246 ± 0.028
375	0.199 ± 0.008	25	0.219± 0.017
500	0.162 ± 0.008	50	0.261 ± 0.022

Fig 4: Cytotoxic activity of the aqueous methanolic extract of the aerial parts of *C. hyssopifolia* Kunth against MCF7 (Breast Carcinoma Cell Line).

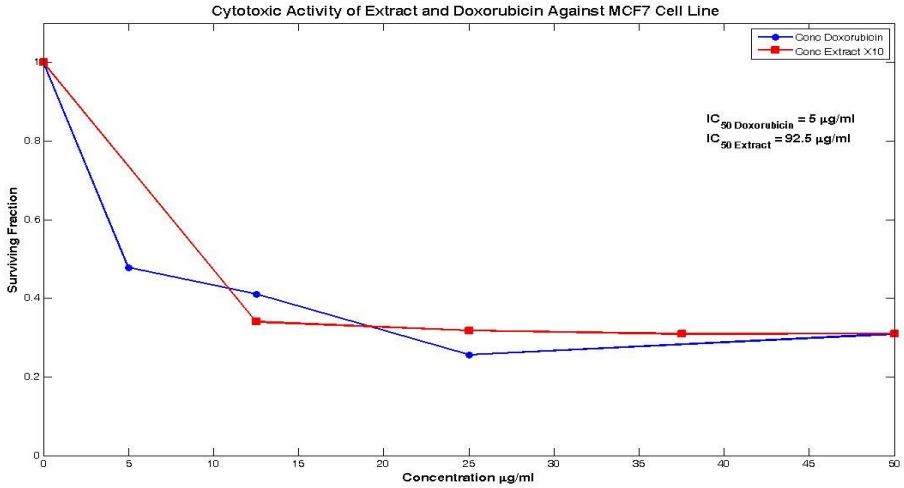


Fig 5: Cytotoxic activity of the aqueous methanolic extract of the aerial parts of *C. hyssopifolia* Kunth against HEP2 (Larynx carcinoma cells).

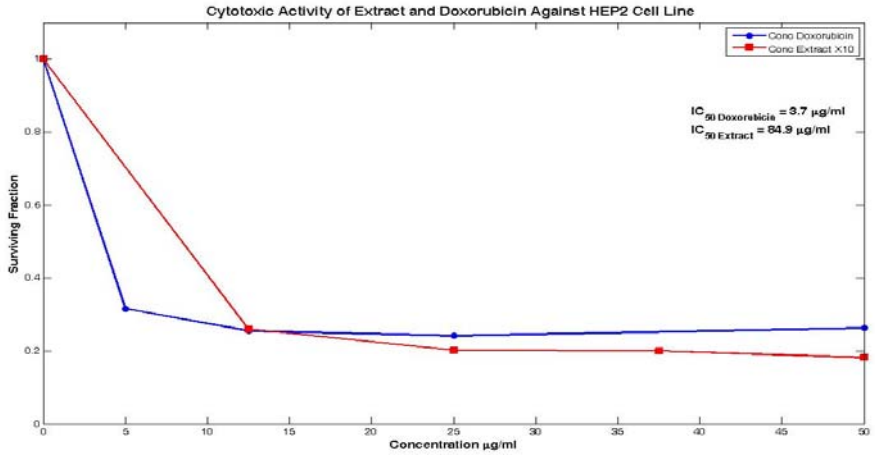


Fig 6: Cytotoxic activity of the aqueous methanolic extract of the aerial parts of *C. hyssopifolia* Kunth againstHCT$_{116}$ (colon carcinoma cells).

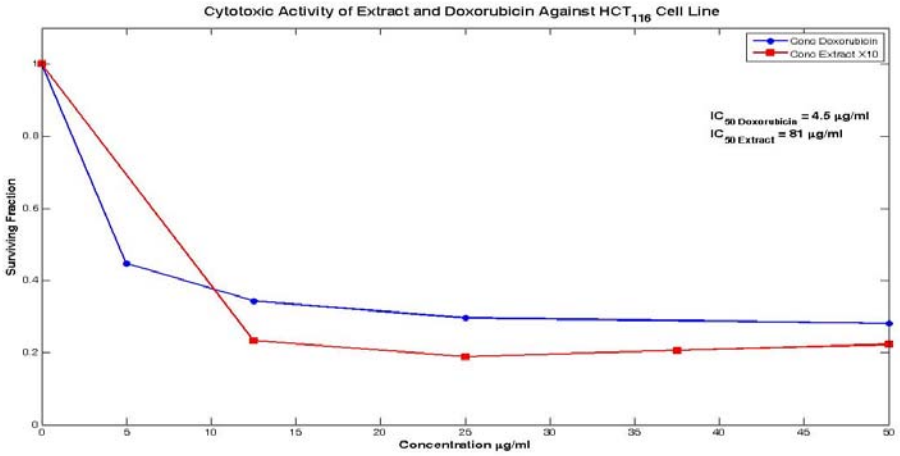
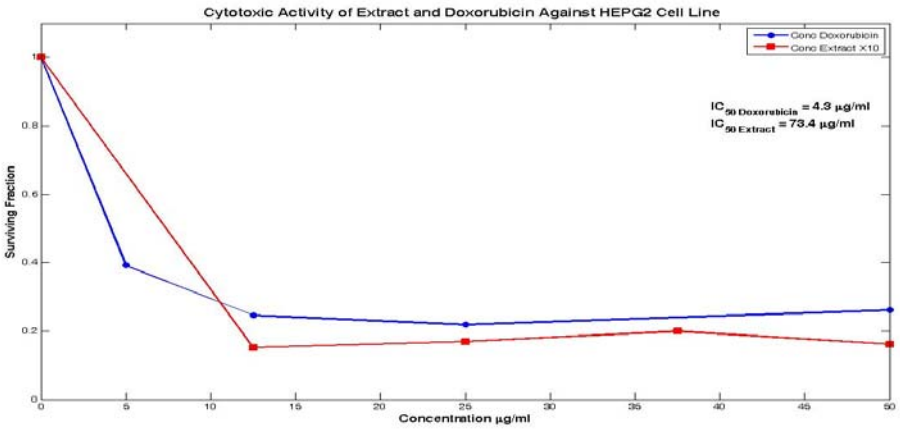


Fig 7 : Cytotoxic activity of the aqueous methanolic extract of the aerial parts of *C. hyssopifolia* Kunth against HEPG2 (liver carcinoma cells).



4. Conclusion

Isolation of 8 phenolic compounds from the aqueous methanolic extract of *C. hyssopifolia* Kunth namely valoneic acid dilactone **(1)**, 1,3-*O*-digalloyl-4-,6-hexahydroxydiphenoyl – *β*-*D*-$^4C_1$-glucopyarnose **(2)**, gallic acid **(3)**, genistein-*7*-*O*-*β*-*D*-glucopyranoside**(4)**, myricetin-3-*O*-*β*-*D*-glucopyranoside**(5)**, 3,4,5-trimethoxy benzoic acid **(6)**, vanilice acid **(7)** and quercetin **(8).** The study resulted in DNA Profiling of *C. hyssopifolia* Kunth using the random amplified polymorphic DNA (RAPD) PCR technique; where primer ***OPP-01*** was the best sequence for dominating *C. hyssopifolia* Kunth cultivated in Egypt producing 13 RAPD-PCR fragments. The aqueous methanolic extract showed strong antioxidant activity against DPPH using ascorbic acid as a positive control and weak cytotoxic activity against different carcinoma cell lines.

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