Antioxidant and anti-proliferative activities of *Acalypha fruticosa:* Possible elucidated mechanism

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Abstract: This study aimed to investigate the potential anti-oxidant activity of methanol (Aca-M) extract and *n*-hexane (Aca-H), chloroform (Aca-Ch), ethyl acetate (Aca-E), *n*-butanol (Aca-B) and aqueous (Aca-A) fractions obtained from the aerial parts of *Acalypha fruticosa (*Aca*)* using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Data obtained revealed that *A. fruticosa* methanol extract and different fractions inhibited the DPPH radicals in the following descending order: Aca-E >Aca-B >Aca-M >Aca-A >Aca-Ch >Aca-H compared to ascorbic acid. Additionally, *in vitro* 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium (MTT) assay against MCF-7, HCT-116, HepG-2 and non-cancerous MRC-5 cell lines was performed to determine their selective anti-cancer activity. The Aca-Ch fraction exhibited remarkable cytotoxic activity against all tested cancerous cell lines with IC_{50} 4.81- 12.2µg/mL, while both Aca-Ch and Aca-H fractions possessed potent cytotoxic activities on HCT-116 (IC_{50} 4.81 and 10.1, respectively) with negligible harm but selective effect on non-cancerous MRC-5 cells (IC_{50} 20.4 and 85.2, respectively).

Keywords: *Acalypha fruticosa*, antioxidant**,** cell lines, cytotoxicity, antiproliferative, apoptosis.

INTRODUCTION

Cancer is a global public health problem with increased number of patients and deaths each year. At present, there are more than 100 types of cancer, treated with different approaches, but mostly with chemotherapeutic drugs **(**Sarkar and Mandal, 2011). The major problems with currently used chemotherapeutic treatments are their non-selective cytotoxicity resulting in many adverse effects such as myelosuppression and anemia, besides both specific and non-specific multi-drug resistance, frequently, acquired by cancer cells (Arriagada et *al*., 2008; Chai *et al.,* 2010). Therefore, the current, limited success of clinical therapies in treating cancer demands the continuous search for new treatments. On the other hand, the preservation of tissue structure and functions requires a healthy balance between the harmful oxidizing effect of free radicals and the protective power of antioxidants as excessive free radicals lead to cellular stress and subsequent progression of cancer **(**Valko *et al*., 2006). Medicinal herbs are rich source of bioactive components (flavonoids, glycosides, alkaloids, terpenoids, steroids, etc.) with multiple pharmacological activities including antioxidant or anti-proliferative or both (Aravindaram and Yang, 2010). Many currently used anticancer drugs such as taxol, vinblastine, etoposide, camptothecin, and irinotecan are derived from plants (Choi *et al.,* 2008).

The genus *Acalypha* L. (family Euphorbiaceae) comprises around 500 species distributed in America, east and southern Africa, tropical Arabia, Yemen and parts of the Indian subcontinent (Cardiel *et al.,* 2013). A literature review was performed which confirmed its antiepileptic effect (Govindu and Adikay, 2014), the ability to treat stomachache, digestive disorders, dyspepsia, colic, fever, jaundice, dermatitis, diarrhea, skin diseases and poisonous bites (Paulsamy *et al.,* 2010). The aerial parts have been used, in Yemen, to treat wounds, skin diseases and malaria (Fleurentin and Pelt, 1982). *A. fruticosa* was also found to possess anti-microbial properties (Vinoth, 2013), anti-inflammatory (Gupta *et al.,* 2003), antioxidant, cytotoxic and anti-tumor activities (Rajkumar *et al.,* 2010; Thambiraj *et al.,* 2012).

The present study has aimed to investigate the potential anti-oxidant and anti-proliferative activities of the methanolic extract and different organic solvents fractions obtained from the aerial parts of *A. fruticosa*. To the best of our knowledge, this is the first scientific study conducted to evaluate the antitumor activity of the aforementioned plant against the cancer cell lines MCF-7, HCT-116, HepG-2 and the non-cancerous MRC-5 cells using the *in vitro* MTT assay. We also conducted a detailed discussion to elucidate the possible anticancer mechanism of *A. fruticose* aerial parts.

MATERIALS AND METHODS

Materials

Culture media

The mammalian cell lines MCF-7 cells (breast carcinoma), HCT-116 (colon carcinoma), HepG-2 (hepatocellular carcinoma), in addition to the lung fibroblast non-cancerous (MRC-5) cell lines were purchased from the American type culture collection

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(ATCC, Rockville, MD), Roswell Park Memorial Institute medium (RPMI-1640), Dulbecco's Modified Eagle Medium (DMEM); Heat-inactivated foetal bovine serum (FBS) and L-glutamine were purchased from PAA laboratories (GmbH), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, amphotericin B and gentamycin, 0.25 % trypsin-EDTA and MTT ([3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium]) were bought from Sigma, St. Louis, MO, USA. Doxorubicin hydrochloride (Adriamycin®) was obtained from Ebewe Pharma (Austria).

Apparatus and instruments

GFL 3107 shaker, Germany; Blinder; Rotary Evaporator (evaporation (Buchi Rota-Vapour R-200) equipped with Buchi heating bath B-490; Buchi vacuum controller V-800, Germany); BI Barnstead Electro thermal, UK; UV-visible spectrophotometer (Milton Roy, Spectronic 1201) and ELISA plate reader (Benchmark Microplate reader, BioRad Hercules, CA, USA),

Methods

Plant collection

The aerial parts of *A. fruticosa* were collected, in November 2014, from Al-Mahweet area, Yemen. The plant was, kindly, identified by Dr. A. Al-Ajami, Faculty of Science, Dhamar University, Yemen. A voucher specimen (#115) was placed at the herbarium of the department of pharmacognosy, faculty of pharmacy, Sana'a University, Sana'a, Yemen.

Preparation of extract and fractions

The finely-ground dried plant material (500 g) was extracted, until exhaustion, by cold maceration with 85% methanol. The combined methanol extract was dried under vacuum till dryness to give a dark crude residue (Aca-M, 75 g); 60 grams of which were suspended in water and consecutively extracted with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. The obtained fractions were evaporated till dryness to yield fractions Aca-H (*n*-hexane, 9.5 g), Aca-Ch (chloroform, 15g), Aca-E (ethyl acetate, 4.8 g), Aca-B (*n*-butanol, 9.5g) and the remaining aqueous fraction (Aca-A, 20 g).

Antioxidant activity using DPPH radical scavenging assay

The antioxidant activities of the methanol extract and obtained fractions were determined using the DPPH free radical scavenging assay. The free radical scavenging activity was carried based on the scavenging activity of stable DPPH, as described by Braca *et al*. (2001). The freshly prepared solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in 95% methanol (0.004% w/v) was stored at 10oC. To prepare the stock solutions, the reference standard, ascorbic acid, was dissolved in distilled water (5 mg/mL), while the stock solutions of *A. fruticosa* fractions were prepared by mixing with 95 % methanol (5mg/mL). Finally serial dilutions of the methanol extract and obtained fractions were prepared (5-160µg/ml). *A. fruticosa* fractions were added to the freshly prepared DPPH solution in test tubes and the final volume was adjusted to 3mL. The mixtures were left in dark at room temperature for 30 min. The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). All the measurements were carried out in three replicates where 95 % methanol served as blank. The formula:

% of radical scavenging activity = $([(A_{control}-A_{test})/A_{control}]) \times 100$, was used to calculate the radical scavenging activity, where $A_{control}$ is the absorbance of the control sample and A_{test} is the absorbance of the test sample.

Cytotoxic activity using MTT assay

The methanol extract and the organic solvents fractions of *A. fruticosa* were diluted in dimethyl sulfoxide (DMSO) (80 mg/mL). Final dilutions were made in DMEM containing 5% FBS. No cell damage was produced by using DMSO in concentrations ranging from 0.06 to 2.0% in the cell cultures**.**

Cell culture & cell lines

Three human cancer cell lines (MCF-7, HCT-116 and HepG-2) and one human normal cell line (MRC-5) were used for cytotoxicity assays. The cell lines were purchased from the American type culture collection (ATCC, Rockville, MD). HCT-116, and MCF-7 cells were cultured in RPMI-1640 while MRC-5, while HepG2 cells were grown in DMEM, 5% (v/v) heat-inactivated FBS (Sigma, St. Louis, MO, USA), 2mM L-glutamine, amphotericin B (10 ml/l) and 0.1% gentamycin were added to both media., 1mM of sodium pyruvate was further added to the medium containing MCF-7 cells. Cells were grown in a humidified incubator with 5% CO_2 at 37°C and were subcultured two times a week. After reaching 80% confluences, cells were detached using a solution of 0.25 % trypsin-EDTA.

Antiproliferative MTT assay

Cell viability was evaluated by the MTT reduction assay [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium] **(**Mosmann, 1983) with some modifications as described by **(**Valencia *et al*., 2012). Doxorubicin was used as a reference drug. The percentage cell viability was calculated using the Microsoft Excel®. Percentage cell viability was calculated as follows: % Cell viability = $([(A_{control}-A_{test})/A_{control}]) \times 100$, where: A: absorbance at 570 nm, $A_{control}$ and A_{test} are the absorbances of the control sample and the test sample, respectively. The anti-proliferative activities of methanolic extract and fractions were reported as IC_{50} values (IC_{50} was defined as the concentration of extract required to inhibit cell proliferation by 50 %). Graphpad Prism software program (version5) was used for the dose response curve drawing in order to calculate IC_{50}

Table 1: Antioxidant activity (% DPPH scavenging) of *A. fruticosa* fractions

Sample concentration (µg/ml)	(Mean of scavenging fractions ± S.D.[#])						
	Ascorbic acid	Aca-B	Aca-E	Aca-H	Aca-Ch	Aca-A	Aca-M
160	99.96±0.05	80.60±1.57	82.05±0.92	54.12±0.51	58.41±2.0	67.71±2.86	70.48±0.46
80	98.7±1.02	77.10±1.29	78.07±1.63	38.47±1.63	42.54±4.73	48.92±1.85	55.75±0.85
40	93.05±1.13	73.52±2.71	75.78±1.26	29.54±2.28	29.85±1.39	37.46±2.99	21.55±0.67
20	70.3±2.00	45.70±2.24	61.38±1.29	21.55±1.23	22.90±2.29	25.52±1.51	13.00±1.67
10	21.18±0.32	27.99±2.61	42.16±1.48	16.04±0.62	19.16±4.06	20.66±3.25	7.25±1.11
5	13.03±0.94	21.58±0.57	25.77±0.36	11.64±1.10	13.92±0.14	16.57±2.21	3.51±0.67
IC_{50} (µg/ml)	14.2±0.6	23±1.1[c]	14±0.8	138.9±6.8[a]	117.6±3.5 [a]	84.5±1.9 [a]	75.6±1.3 [a]

DPPH, 2,2-diphenyl-1-picrylhydrazyl; [a]p value <0.001, [c]p value <0.05; compared to reference drug[^]. [#]Mean of variability %± standard deviation (n = 3).

Table 2: *In Vitro* cytotoxic activities of *A. fruticosa* fractions on MCF-7 cell line

Sample concentration (µg/ml)	(Mean of surviving fractions ± S.D.[#])						
	Doxorubicin	Aca-B	Aca-E	Aca-H	Aca-Ch	Aca-A	Aca-M
100	3.33±0.38	89.60±2.82[a]	91.47±2.51[a]	21.76±2.93[b]	17.00±2.47[c]	36.67±2.7 [b]	43.12±2.44 [b]
50	4.22±0.78	96.44±1.21[a]	97.24±0.96[a]	34.66±2.64[a]	27.82±2.51[a]	54.50±2.99[a]	65.83±2.36 [a]
25	7.88±0.59	99.26±0.66[a]	99.72±0.48[a]	46.38±2.63[a]	39.50±5.34[b]	75.27±2.66[a]	83.97±4.82 [b]
12.5	13.35±0.97	100.0±0.0[a]	100.0±0.0[a]	69.68±4.68[a]	48.74±4.49[a]	91.11±1.61[a]	94.29±2.56 [a]
6.25	18.86±0.96	100.0±0.0[a]	100.0±0.0[a]	87.08±2.37[a]	76.03±2.14[a]	97.21±1.78[a]	99.06±0.57 [a]
3.125	24.34±1.54	100.0±0.0[a]	100.0±0.0[a]	92.76±1.45[a]	88.94±2.71[a]	99.59±0.72[a]	100.0±0.0 [a]
IC_{50} (µg/ml)	0.46±0.6	>100	>100	23.1±1.2 [a]	12.2±0.6 [a]	62.6±3.2 [a]	84.9±1.55 [a]

Table 3: *In Vitro* cytotoxic activities of *A. fruticosa* fractions on HCT-116 cell line

Sample concentration (µg/ml)	(Mean of surviving fractions ± S.D.[#])						
	Doxorubicin	Aca-B	Aca-E	Aca-H	Aca-Ch	Aca-A	Aca-M
100	3.89±0.25	64.14±6.84[b]	74.03±3.13[a]	15.03±2.22[c]	8.44±0.73[a]	35.36±0.61[a]	26.25±2.28[b]
50	5.43±0.48	83.88±2.86[a]	89.37±1.99[a]	26.32±1.52[a]	16.60±1.31[a]	49.00±2.84[a]	40.29±2.67[a]
25	8.97±0.66	91.85±2.34[a]	96.07±1.59[a]	37.72±2.76[a]	24.78±1.33[a]	67.36±3.74[a]	59.83±2.97[a]
12.5	15.20±0.80	96.42±2.42[a]	98.96±0.96[a]	46.20±3.17[a]	35.55±0.79[a]	82.93±2.84[a]	73.66±4.38[a]
6.25	19.11±1.02	99.06±0.94[a]	100.0±0.0[a]	55.95±3.68[a]	43.04±1.47[a]	90.19±1.67[a]	87.85±2.43[a]
3.125	24.57±1.22	100.0±0.0[a]	100.0±0.0[a]	69.46±4.35[a]	58.10±3.10[a]	95.83±1.36[a]	95.47±1.23[a]
IC_{50} (µg/ml)	0.46±0.8	>100	>100	10.1±0.8 [a]	4.81±0.4 [a]	48.6±2.3 [a]	37.6±2.1[a]

Table 4: *In Vitro* cytotoxic activities of *A. fruticosa* fractions on HepG-2 cell line

Sample concentration (µg)	(Mean of surviving fractions ± S.D.[#])						
	Doxorubicin	Aca-B	Aca-E	Aca-H	Aca-Ch	Aca-A	Aca-M
100	3.31±0.38	69.57±4.32[b]	81.54±2.72[a]	15.89±3.17[c]	9.44±0.88[a]	39.35±3.73[b]	38.85±1.21[a]
50	4.34±0.53	86.83±2.78[a]	90.53±0.98[a]	28.81±2.47[a]	18.25±1.30[a]	63.28±2.59[a]	60.19±1.51[a]
25	9.50±0.81	94.65±1.25[a]	97.27±0.85[a]	40.43±3.68[a]	26.87±1.14[a]	82.49±2.16[a]	75.80±2.03[a]
12.5	14.81±0.92	98.05±0.50[a]	99.42±0.72[a]	52.84±4.80[a]	35.20±2.83[a]	91.81±1.70[a]	88.87±1.71[a]
6.25	17.16±1.07	100.0±0.0[a]	100.0±0.0[a]	68.15±3.50[a]	43.25±2.36[a]	98.00±0.78[a]	97.11±1.69[a]
3.125	25.48±1.46	100.0±0.0[a]	100.0±0.0[a]	77.45±2.92[a]	63.52±4.28[a]	100.0±0.0[a]	99.39±1.06[a]
IC_{50} (µg/ml)	0.51±0.9	>100	>100	15.4±0.8 [a]	5.21±0.7 [a]	77.7±3.4 [a]	73.9±1.3 [a]

[a]p value <0.001, [b]p value <0.01, [c]p value <0.05 compared to reference drug. [#]Mean of variability % ±standard deviation (n = 3).

Table 5: *In Vitro* cytotoxic activities of *A. fruticosa* fractions on MRC-5 cell line

Sample concentration (µg/ml)	(Mean of surviving fractions ± S.D.[#])						
	Doxorubicin	Aca-B	Aca-E	Aca-H	Aca-Ch	Aca-A	Aca-M
100	4.04±0.22	85.43±1.55	93.22±1.55	45.06±4.06	23.48±1.45	72.00±3.77	64.10±3.44
50	6.52±1.32	91.22±2.66	95.31±2.11	59.33±3.58	37.28±3.27	86.03±1.75	82.33±4.11
25	10.84±0.95	98.11±3.23	99.10±3.02	67.04±3.61	44.66±3.39	94.06±2.84	92.43±2.55
12.5	18.09±1.31	99.93±1.35	100±0.00	82.22±1.82	59.21±2.22	99.28±0.65	96.55±1.77
6.25	25.02±1.65	100±0.00	100±.0.00	91.19±1.34	77.55±3.65	100±0.00	99.11±1.33
3.125	36.90±1.55	100±0.00	100±0.00	96.87±1.92	87.78±2.14	100±0.00	100±0.00
IC_{50} (µg/ml)	1.66±0.39	>100	>100	85.2±2.7 [a]	20.4±2.36 [a]	>100	>100

STATISTICAL ANALYSIS

IC_{50} (the concentration needed to scavenge DPPH radical or reduce the growth of cells by 50%) values were obtained by using Graphpad Prism software program (version5). DPPH scavenging activity (IC_{50}) of Aca fractions was expressed as means ± SD. One way ANOVA (followed by Dunnett posttest) was conducted using the GraphPad Prism software program (version 5) to compare IC_{50} of each Aca fraction with both ascorbic acid and doxorubicin (performed on individual cell line). The differences between means were considered significant at *p* values of less than 0.05. The growth inhibitory effect (IC_{50}) of Aca fractions was expressed as means ± SD. While, two-ways ANOVA test (followed by Bonferroni post-test) was used to compare IC_{50} of each Aca fraction on studied cancerous cell lines (all columns) *vs*. MCR-5 (control column) to evaluate its selective cytotoxicity effect using GraphPad Prism software program (version 5). Differences were considered significant at *p* values of less than 0.05.

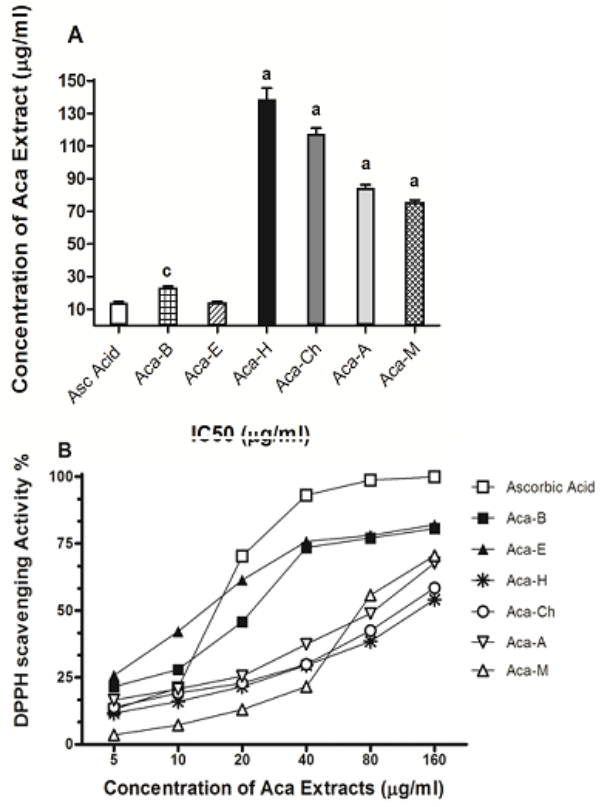

Fig. 1: Antioxidant activities of *A. fruticosa* fractions and ascorbic acid using DPPH scavenging method; type of extract vs. its IC_{50} (A) and concentration of extract vs. its DPPH scavenging % (B). ^{c}p value <0.05, ^{a}p value <0.001 compared to ascorbic acid. Data expressed in mean of variability % ± standard deviation (n = 3).

RESULTS

The current study was conducted to assess the potential antioxidant and anticancer activities of the aerial parts of *A. fruticosa*. Six different fractions were selected for this study, as these fractions, in many studies, were reported to have solubilizing ability for most of the present secondary metabolites.

DPPH scavenging activity

The free radical scavenging activity of *A. fruticosa* fractions was assessed by DPPH assay. As shown in table 1 and fig. 1, all the extracts demonstrated a dose dependent inhibition of the DPPH radicals with the following descending order: A-ca E > Aca-B > Aca-M > Aca-A > Aca-Ch > Aca-H. On the other hand, both Aca-E and Aca-B fractions revealed significant inhibitory activities against the DPPH radical (14 µg/ml & 23 µg/ml, respectively) compared to ascorbic acid (14.2 µg/ml).

Cytotoxic activities

As shown in table 2, 3 and 4, Aca fractions displayed a dose-dependent cytotoxic effect against the tested cancer cell lines. The chloroform fraction (Aca-Ch) exhibited remarkable cytotoxic activity against MCF-7, HCT-116 and HepG-2 cancer cell lines (IC_{50} 12.2±0.6, 4.81±0.4 and 5.21±0.7µg/mL, respectively) whereas, the *n*-Hexane (Aca-H) extract was slightly less active with IC_{50} 23.1±1.2, 10.1±0.8 and 15.4±0.8 µg/mL, respectively in comparison to doxorubicin (IC_{50} = 0.44–1.13µg/mL). The total methanol (Aca-M) and aqueous (Aca-A) fractions showed moderate to weak inhibitory activity on MCF-7 (IC_{50} = 62.6±3.2 and 84.9±4.1µg/ml), HCT-116 (IC_{50} = 48.6± 2.3 and 37.6± 1.6 µg/ml), and HepG-2 cells lines (IC_{50} = 77.7±3.4 and 73.9±3.1µg/ml), respectively. The *n*-butanol (Aca-B) and ethyl acetate (Aca-E) fractions were inactive against all the tested cell lines (IC_{50} >100µg/mL) compared to doxorubicin. Regarding sensitivity of cancer cells, the HCT-116 cells were the most sensitive towards the tested extracts, followed by HepG-2 and finally MCF-7 cancer cell lines. On the other hand, all Aca extracts showed conferring negligible harm to non-cancerous MRC-5 cells (except Aca-Ch and Aca-H extracts but selective effect) (tables 2-5; figs. 2 & 3).

DISCUSSION

Cancers of the breast, colon and liver are highly dominant diseases among people all over the world. They are, also, the leading cause of death in approximately half of the cancer-related deaths. Upon investigation, many ethnomedicinal plants were defined to have beneficial therapeutic potential and many clinically used drugs are derived from natural origin (Kim *et al.*, 2005).

The human body, through daily activities, is frequently exposed to the toxic effect of reactive oxygen species (ROS) and free radicals which accelerates the different oxidative degradation processes to biomolecules (nucleic acids) and enhance the pathogenesis of oxidative stress related contemporary diseases such as ulcer, cancer and heart failure (Droge, 2002).

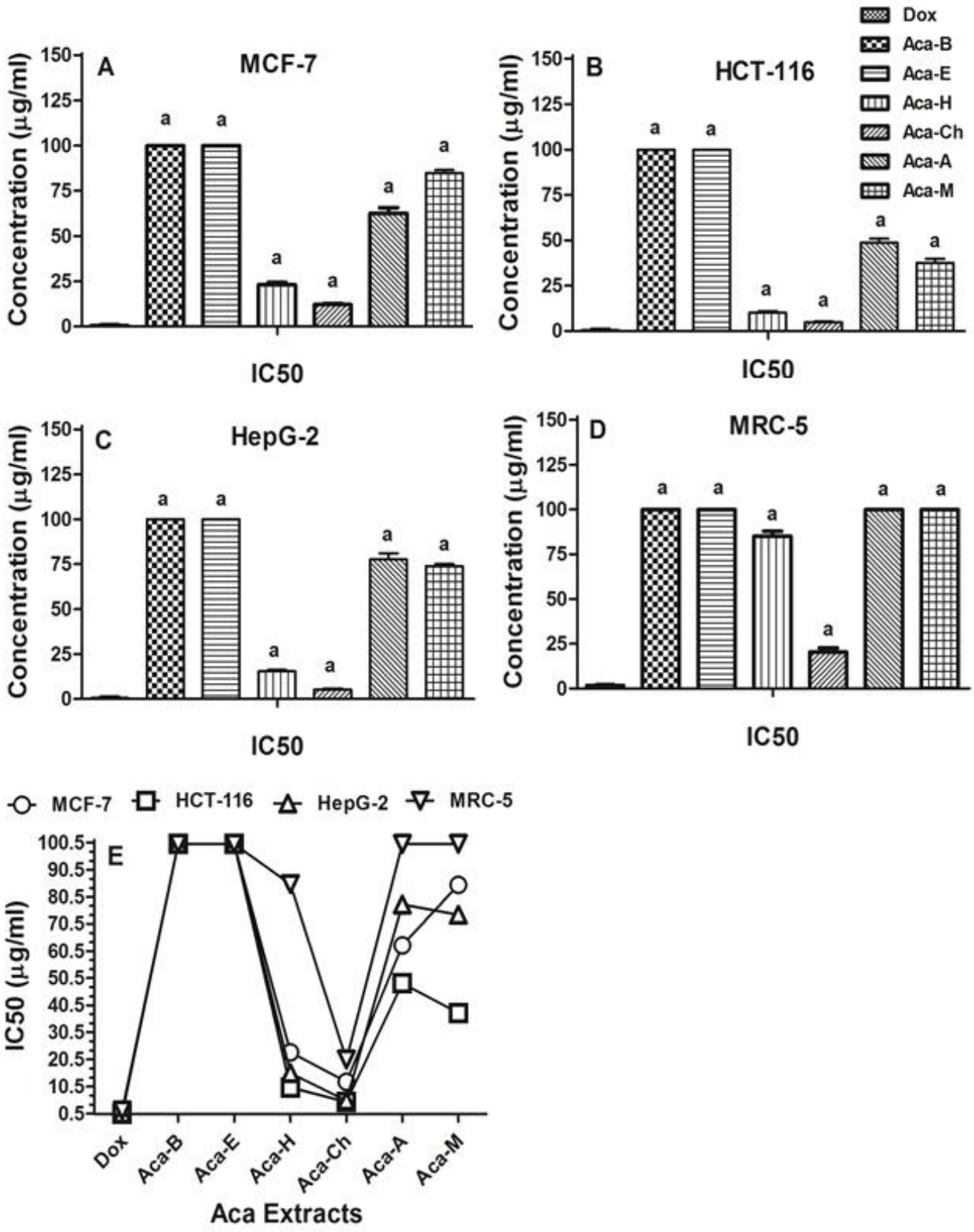

Fig. 2: Growth inhibitory effects of studied extracts of *Acalypha Fruticosa* (Aca) and Doxorubicin (Dox) on in form of IC_{50} vs. concentration (µg/ml) and Aca extracts vs. IC_{50} on all studied cell lines as a collective figure (E). ^{a}p value <0.001 compared to Doxorubicin. Data expressed as mean of variability % ± standard deviation (n = 3).

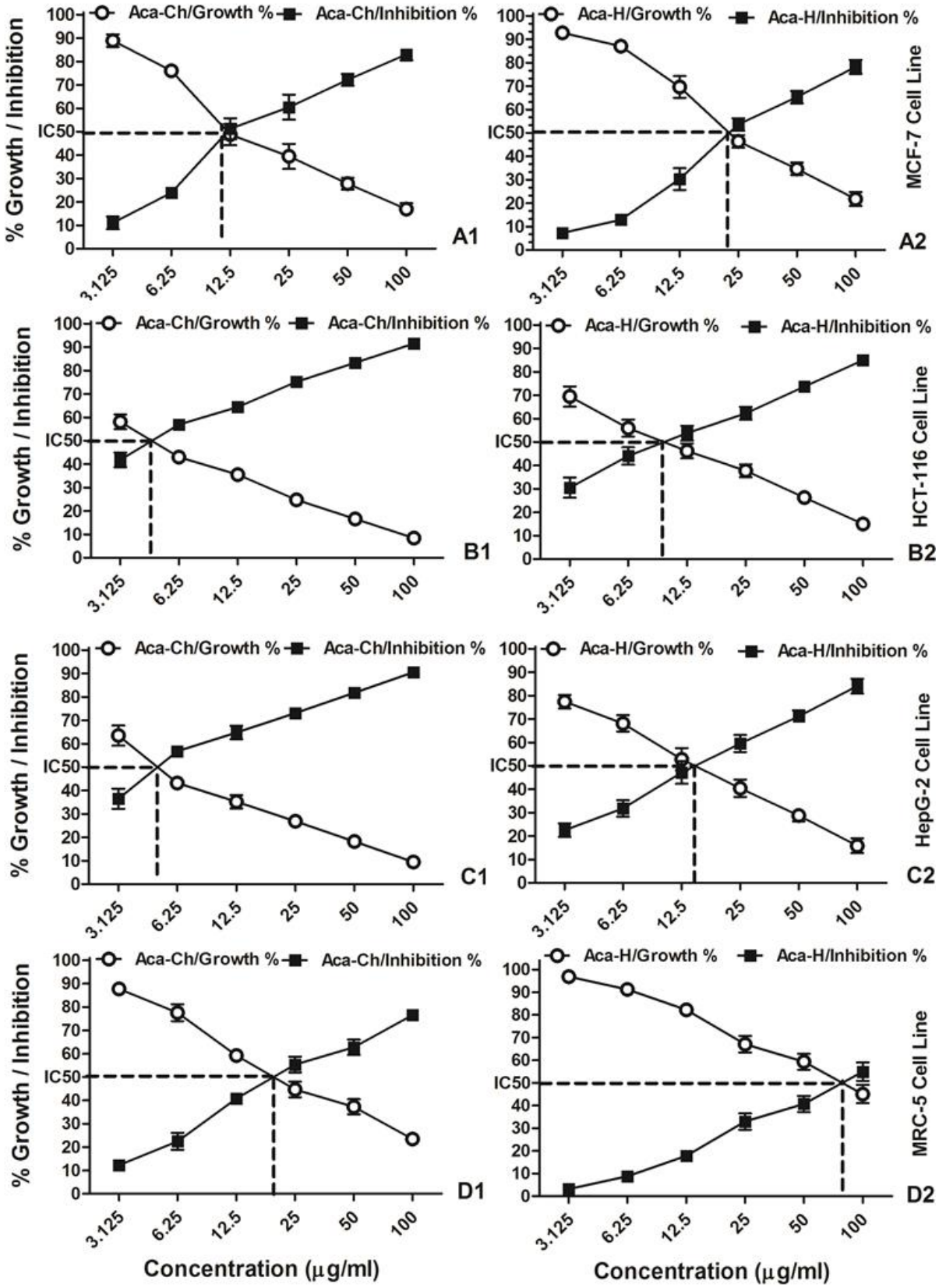

Fig. 3: Cytotoxic effects of chloroform (Ch) and n-hexane (H) extracts of *A. fruticosa* (Aca) on *MCF-7* (A1 & A2)*; HCT-166* (B1 & B2)*; HepG-2* (C1 & C2)*;MRC5 cells* (D1 & D2).

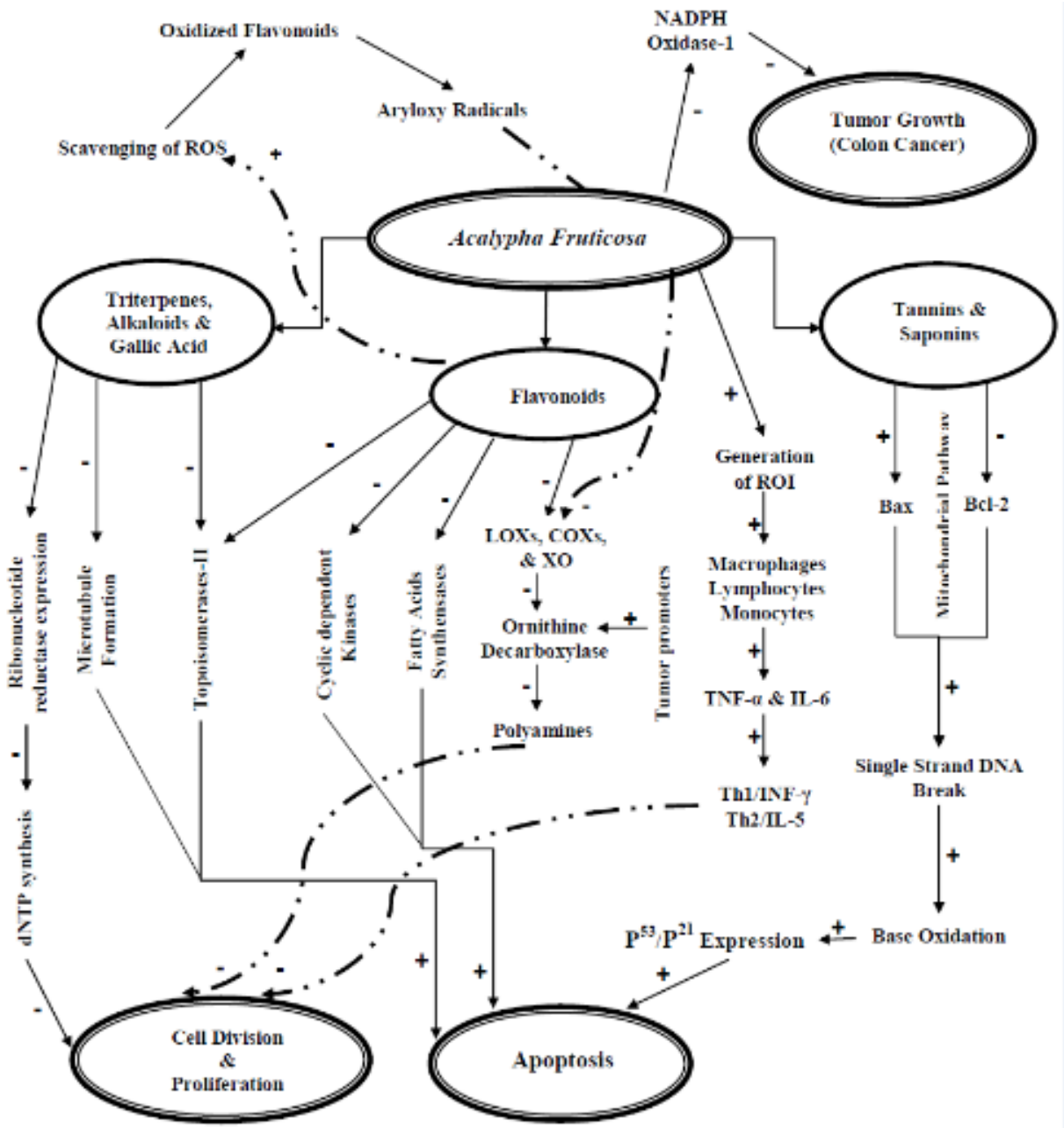

Fig. 4: The possible elucidated mechanism for anti-proliferative effects of extracts from the aerial parts of *A. Fruticosa* on studied cell lines.(-), decrease, inhibits or downregulate; (+), increase, activate, or upregulate; IL, interleukin; TNF-α, tumor necrosis factor alpha; INF-γ, interferon-gamma; P^{53}/P^{21}, tumor suppressor genes; ROS, reactive oxygen species; ROI, reactive oxygen intermediates; dNTP, deoxy nucleotide triphosphate; Bax, pro-apoptotic gene; Bcl-2, anti-apoptotic gene; XO, xanthine oxidase; LOXs, lipoxygenases; COXs, cyclooxygenases; Th1/Th2, T helper lymphocyte subsets.

The formation of ROS is a major step in the human tumor progression, a stage leading to the damage of DNA and division of cells with unrepaired harm causing mutations (Valko *et al.*, 2006). Antioxidants intervene with protective effect by quenching with ROS or chelating the catalytic metal ions, and thus prevent the damage caused to cellular macromolecules (Russo *et al.*, 2001) thus consequently hindering degenerative diseases.

In the current study, the ethyl acetate and *n*-butanol extracts of *A. fruticose* displayed a powerful free radical scavenging effect in the DPPH assay (IC_{50} 14, 23µg/ml respectively) compared to ascorbic acid (IC_{50} 14.2). On the other hand, the total methanol, the aqueous, the chloroform and the *n*-hexane extracts showed less effective scavenging activity with IC_{50} of 75.6, 84.5, 117.7 and 138.9µg/ml, respectively (table 1; fig. 1).

Variable antioxidant activities for various *Acalypha* species, *A. wilkesiana, A. guatemalensis, A. platyphilla, A. siamensis*, were reported in previous studies (Mothana *et al.,* 2008).

The various phenolic compounds, previously identified in *Acalypha* species as for example the polyhydroxyphenol gallic acid and flavonoids, are known to have high radical scavenging activity (Park *et al.,* 2004) which rationalize the powerful antioxidant effect of *A. fruticosa* extracts observed in the current study.

In addition, all extracts revealed a dose-dependent cytotoxicity towards the studied cell lines. Both Aca (Ch & H) fractions demonstrated the strongest growth inhibitory effect on MCF-7 (IC_{50} =12.2 ±0.6 and 23.1±1.2 µg/ml), HCT-116 (IC_{50} =4.81±0.4 and 10.1±0.8µg/ml), and HepG-2 (IC_{50} =5.21±0.7 and 15.4±0.8µg/ml) cell lines, respectively (Aca-Ch have stronger effect than Aca-H). On the other hand, Aca (A & M) fractions showed mild to moderate cytotoxic activity on MCF-7, HCT-116 and HepG-2 cell lines with IC_{50} 37.6-84.9µg/ml. Meanwhile, Aca-M fraction had stronger cytotoxic effects on both HCT-116 and HepG-2 cell lines than Aca-A fraction, while Aca-A extract exhibited stronger cytotoxic effect on MCF-7 cells than Aca-M fraction.

The mild to moderate cytotoxic effects of Aca-A fraction on studied cell lines possibly due to lack of steroids and triterpenes from its components as reported from previous phytochemical screening results (El-Shaibany *et al.*, 2015). However, a weak cytotoxic activity of Aca fractions (B & E) has been detected against the tested cancer cell lines (IC_{50} >100 µg/ml); possibly due to lack of phenols and tannins in these fractions (tables 2-5; figs. 2 & 3).

The strong cytotoxic effect of Aca (Ch & H) fractions might be attributed to the rich constituents of flavonoids, phenols, tannins, steroids and triterpenes. The observed strong effect of Aca-Ch and Aca-H fractions is in the same line with Nandhakumar *et al.* (2009) who reported the strong α-amylase inhibition effect of the chloroform and *n*-hexane extracts of *A. indica* against porcine pancreatic amylase. Lim *et al.* (2011) found that the *n*-hexane and ethyl acetate extracts of *Acalypha wilkesiana* possess cytotoxic effects through activation of DNA-damage-mediated apoptosis in A549 and U87MG cancer cell lines. On the other hand, the selectively stronger effect of Aca (Ch &H) fractions on HCT-116 cells could be due to the ability of these fractions to down regulate the over expressed NADPH oxidase I (NOX 1), in colon and prostate cancer cell lines, and subsequently counters the tumor growth (Fukuyama *et al.*, 2005; Lim *et al*., 2011).

The data obtained, in the current study, is consistent with those previously, reported by Madlener *et al.* **(**2009) and Ramzi *et al.* (2010) confirming the cytotoxic potential of *A. fruticosa, A. platyphylla, A. siamensis* and *A. guatemalensis* against different cancer cell lines. In addition, Rajkumar *et al.* (2010) investigated the protective effect of *A. fruticosa* extracts against DNA breakage and concluded that it is a very promising candidate for cancer prevention. Moreover, some species of the genus *Acalypha* have shown antiproliferative activity, such as extracts of *A. wilkesiana* seeds as stated by Bussing *et al.* (1999) as a result of the cytotoxic and immunomodulating effects of its saponins content.

Collectively, the possible elucidated mechanism of anti-proliferative activity induced by species of Acalypha (including A. fruticosa) on cancerous cell lines have not been previous reported. We, here, describe the possible mechanism of *A. fruticosa* cytotoxic effect depending on both results of the current and previous studies (fig. 4).

In the current study, the differential antiproliferative activity of *A. fruticosa* fractions was observed between non-cancerous (MRC-5) and cancerous cell lines. These observations are in agreement with Bussing *et al.* (1999) demonstrating the seeds of *Acalypha wilkesiana* encourage apoptosis through the generation of reactive oxygen intermediates (ROI) and the release of lymphocytes, monocyte or macrophage-associated cytokines and increased number of granulocytes. Owing to their high metabolic stress–response rate, plant extracts possess greater activity to the more susceptible to apoptosis, by ROI, cancer cells than from the non-cancerous MRC5 cells (Singh *et al.,* 2007).

Secondary metabolites isolated from plants such as alkaloids, triterpenes and lignans usually induce apoptosis through topoisomerase II inhibition and antimicrotubule formation (Huang *et al.,* 2004; Wada and Tanaka, 2006). In addition, tannins and saponins cause apoptosis via initiation of the mitochondrial pathway involving inhibition of Bcl-2 (Tin *et al.,* 2006; Kim *et al.,* 2008).

Moreover, an enormous number of scientific studies have confirmed the protective effect of flavonoids against cancer**.** Induction of cancer apoptosis by flavonoids is thought to be through several mechanisms including detoxification of mutagenic xenobiotics (Myhrstad *et al*., 2002), radical scavenging (Ross and Kasum, 2002), inhibition of cyclic-dependent kinases (Choi *et al.,* 2001), fatty acid synthase activity (Brusselmans *et al.,* 2005) and topoisomerases (Birt *et al*., 2001).

Compounds isolated from various members of the genus *Acalypha* (such as cycloartane-type triterpenoids, gallic acid and geraniin) have shown a diversity of biological activities (Adesina *et al.,* 2000). Gallic acid is an effective inhibitor of the enzyme ribonucleotide reductase (RR; EC1.17.4.1), which is often over expressed in cancer cells and catalyzes the rate-limiting step for cell division

(Madlener *et al*., 2007). Finally, Aca (E & B) extracts of *A. fruticosa* had significant antioxidant but weaker cytotoxic effect than other extracts. In contrast, Aca (Ch & H) extracts possess valuable cytotoxic effects on studied cell lines and weaker antioxidant effect than other extracts. Thereby, we predict that the mechanism due which the anticancer effect of *Acalypha fruticosa* is any of the above mentioned mechanisms and illustrated in fig. 4 other than antioxidant mechanism.

CONCLUSION

We conclude that both Aca (E & B) fractions of *A. fruticosa* revealed the best antioxidant effects while, only Aca (Ch & H) fractions possessed potent cytotoxic activities on HCT-116. All extracts exhibited negligible harm to non-cancerous MRC-5 cell line (except Ch and H extracts but selective effect). Data obtained in this study encourage further investigations to reveal the bioactive compounds responsible for these activities. Further investigations will be conducted to assess the effects of the plant on expression of apoptotic, pro-apoptotic and tumor suppressor genes, and on cell cycle analysis to understand its selective anti-tumor activity.

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