

## Effects of *Chorisia crispiflora* Ethyl Acetate Extract on p21 and NF- $\kappa$ B in Breast Cancer Cells

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**Abstract: Purpose:** The current study addressed the evaluation of the *in-vitro* cytotoxic activity of *Chorisia Crispiflora* (Bombaceae) different extracts on MCF-7 breast cancer cell line, then in parallel phytochemical and molecular investigations to the most cytotoxic extract. **Materials and Methods:** The most cytotoxic extract against -7 breast cancer cell line was investigated for its effect on NF- $\kappa$ B, p21 and DNA fragmentation. The compounds isolated were identified using different spectroscopic techniques. **Results:** The most active extract was the ethyl acetate, where it exhibited cytotoxic activity at IC<sub>50</sub>; 5.2 and 4.2  $\mu$ g/ml compared with doxorubicin for 48 & 72 hours. Further investigations on the extract were achieved in parallel for detection the active principles responsible for that effect and for assessing the molecular mechanisms underlying extract may interfere with several cell signaling pathways. Protocatechuic acid, apigenin 7-rhamnoside, apigenin 7-glucoside, kaempferol 3- rutinoside and apigenin 7-neohesperidoside were isolated and identified through different spectroscopic methods. The anticancer effect on MCF-7 breast cancer cell line was produced as result of down regulation of NF- $\kappa$ B and up regulation of p21 levels at time and concentration dependent manner. **Conclusions:** *Chorisia crispiflora* extract may down regulate NF- $\kappa$ B and up regulate p21 levels at time and concentration dependent manner and also may assume that activated NF- $\kappa$ B antagonizes P53induces P21 function, possibly through the cross-competition for transcriptional coactivators. The phenolics isolated from the extract may responsible for the anticancer effect produced.

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**Key Words:** *Chorisia crispiflora*, phenolics, MCF-7, NF- $\kappa$ B and p21.

### 1. Introduction

Breast cancer is one of the most prevalent diseases affecting women. <sup>[1]</sup> In Egypt, breast cancer represents 18.9% of total cancer cases among the Egypt National Cancer Institute during the year 2001.<sup>[2]</sup> Breast cancer is the most common cause of cancer related deaths among women worldwide. <sup>[3]</sup> The aetiology of breast cancer involves environmental factors, inherited genetic susceptibility, genetic changes during progression and interaction among these factors, with the relative importance of each ranging from strongly genetic or strongly environmental. <sup>[4]</sup> In the process associated with the development of breast cancer, it is known that malignant transformation involves genetic and epigenetic changes that result in uncontrolled cellular proliferation and/or abnormal programmed cell death or apoptosis. NF- $\kappa$ B is widely used by eukaryotic cells as a regulator of genes that control cell proliferation and cell survival. Defects in NF- $\kappa$ B results in increased susceptibility to apoptosis leading to increased cell death. Thus, NF- $\kappa$ B is the subject of much active research among pharmaceutical companies as a target for anti-cancer therapy. <sup>[5]</sup> Many natural products (including anti-oxidants) that have been promoted to have anti-cancer and anti-inflammatory activity have also been shown to inhibit NF- $\kappa$ B.

Recent work by **Pikarsky, 2006 and Karin, 2008** <sup>[6-7]</sup> and others has highlighted the importance of the connection between NF- $\kappa$ B, inflammation, and cancer, and underscored the value of therapies that regulate the activity of NF- $\kappa$ B. <sup>[8]</sup> Extracts from a number of herbs and dietary plants are efficient inhibitors of NF-kappa B activation *in-vitro*. <sup>[9]</sup> Several reports published within the last decade showed that activation of NF- $\kappa$ B promotes cell survival and proliferation and down-regulation of NF- $\kappa$ B sensitizes the cells to apoptosis induction. <sup>[10]</sup> Several phytochemicals that are known to inhibit NF- $\kappa$ B activation can significantly suppress cell proliferation and sensitize cells to apoptosis induction. <sup>[11-12]</sup> Several micronutrients present in fruits and vegetables exhibit anticancer activity as a result of their actions on molecular targets involved in carcinogenesis and tumor progression. Curcumin, a phenolic phytochemical derived from the rhizome of *Curcuma longa*, exhibits both cancer-preventative activity and growth inhibitory effects on neoplastic cells. Several studies report that curcumin inhibits cancer cell proliferation and induces apoptosis in cancer cells through p21-mediated cell cycle arrest. Cancer cells that are deficient in p21 are also reported to be more prone to undergo apoptosis in response to a variety of cytotoxic agent. <sup>[13]</sup>

In this study we aimed to examine the molecular mechanisms underlying the most cytotoxic extract of *Chorisia crispiflora* which may interfere with several cell signaling pathways and insert anti-cancer effects through the suppression of NF- $\kappa$ B or activation of p21, as apoptosis regulatory gene on breast cancer cell lines MCF-7. At the same time to investigate the extract phytochemically to isolate and identify the active ingredients responsible for that effect.

## 2. Materials And Methods

### Phytochemical investigation

#### Plant source

*Chorisia* leaves were collected from Zoo Garden in Giza, Egypt, 2010 and were authenticated by Prof. Dr Abdel Salam El Noyehy, Prof. of Taxonomy, Faculty of Science, Ain Shams University, Cairo, Egypt. Voucher specimen was deposited at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. The leaves were dried in shade and reduced to a fine powder.

#### Extraction and isolation

Powder of air dried leaves of *Chorisia crispiflora* (1 kg) was extracted with 70 % ethanol on cold. The extract was completely dried and dissolved in a small amount of water and partitioned with *n*-hexane (extract 1), ethyl acetate (extract 2) butanol (extract 3) and finally with methanol (extract 4) successively.

Seven grams of ethyl acetate extract were chromatographed on top of a column made of silica gel (210 g). Elution started with chloroform and methanol mixture of increasing polarity.

Fifty fractions were collected and monitored by TLC and PC. Similar fractions were pooled together to give five groups. Fractions 25-27 (9 chloroform /1 methanol) yielded compound **1** and purified by preparative paper chromatography. Fractions 38 (8 chloroform /2 methanol) yielded two compounds (**2** and **3**) by using preparative paper chromatography and BAW as solvent system. Fractions 39-44 (7 chloroform /3 methanol) afforded compound **4** and **5** after application on preparative paper chromatography and acetic acid 6 % as solvent system.

#### Chemicals

Kaempferol, quercetin, apigenin and luteolin were obtained from NMR department, NRC, Cairo. Glucose, rhamnose and glucuronic acid were purchased from E.Merk, Darmstadt Germany. Sheets of Whatman paper No.1 and No.3 MM Paper Chromatography (PC) (Whatman Ltd. Maidstone, Kent, England) were used. Silica gel 60 for column chromatography (E-Merk), particle size (70-230 mesh), precoated silica gel 60 F 254 sheets for TLC (Riedel-De Haem AG, Germany) were also used.

### Ultraviolet spectrophotometric analysis

Chromatographically pure materials 1 mg each were dissolved in analytically pure methanol then subjected to UV spectroscopic investigation in 4 ml capacity quartz cells 1 cm thick using a Carl Zeiss spectrophotometer PMQ II. AlCl<sub>3</sub>, AlCl<sub>3</sub>/HCl, fused NaOAc/ H<sub>3</sub>BO<sub>3</sub> and NaOMe reagents were separately added to the methanolic solution of investigated material and UV measurements were then carried out.

### Nuclear magnetic resonance spectroscopic analysis

The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. <sup>1</sup>H- spectra run at 500 MHz and <sup>13</sup>C- spectra were run at 75.46 MHz in deuterated dimethylsulphoxide (DMSO-*d*6). Chemical shifts are quoted in  $\delta$  and were related to that of the solvents.

### Cytotoxic activity

#### Cell line

Breast cancer cell line (MCF-7) were obtained from the National Cancer Institute, Cairo University, maintained in RPMI medium (SIGMA ALORICH#R8758) supplemented with 10% fetal bovine serum (SIGMA, USA#F2442) in the presence of penicillin (100 units/ml) and streptomycin (100  $\mu$ g/ml). These cells were cultured in 5% CO<sub>2</sub> incubator in a humidified incubator at 37 °C.

#### Cell cytotoxicity measurements

Sulforhodamine B assays were conducted to measure the effects of *Chorisia crispiflora* extract on the proliferation of MCF-7 cells. Cells were seeded at a concentration of  $2.5 \times 10^4$  cells/ml in 96-well plates, and allowed to recover for 24 hrs prior to drug addition. The extract was prepared in a stock solution in DMSO, and then serially diluted to multiwell plates to a final concentration of 5, 12.5, 25 or 50  $\mu$ g/ml. Control wells received diluted vehicle only, corresponding to the amount present in the 50  $\mu$ g/ml extract well (0.1% DMSO solution). Following 48 and 72 hrs of treatment, the 96 well plates was fixed with absolute methanol containing 1% acetic acid (at -10°C) for 30 minutes, the methanol decanted, and the plate again air-dried. Sulforhodamine B (0.5% in 1% acetic acid) was added to each well, and the plate incubated at 35°C for 1 hour. Plates were rinsed with 1% acetic acid, air-dried, and the bound dye eluted with 1 ml of 10 mM Tris buffer, pH 10. The absorbance was measured in a spectrophotometer at 540 nm; the amount of dye released is proportional to the number of cells present in the dish, and is a reliable indicator of cell cytotoxicity.

### Molecular biology

#### DNA fragmentation

MCF-7 cells were collected after incubation with different concentrations of *Chorisia crispiflora* extract and different time intervals. Total DNA was extracted and purified from the treated and control samples with Genomic DNA Purification kit (Gentra,

Minnapolis, Minnesota, USA) in accordance with the manufacturer's instructions. The required amount of DNA was separated on 1% agarose gel with 0.5 X TBE buffer (44.5 mM Tris base, 44.5 mM boric acid, 1 mM EDTA, pH 8.0). Ethidium bromide-stained DNA in the gel was visualized under UV light and photographed.

#### Nuclear factor Kappa Bp65

Nuclear factor Kappa Bp65 was determined by ELISA using kits supplied from Glory Science Co., Ltd, USA according to Adams, 2009<sup>[14]</sup>. This assay employs the quantitative sandwich immunoassay technique. An antibody specific for NF-κBp65 has been coated onto the wells of the microtiter strips provided. Samples, including a standard containing NF-κBp65, unknowns, and NF-κBp65 antibodies labeled with biotin, and combined with streptavidin-HRP are pipetted into these wells. During the incubation NF-κBp65 antibodies labeled with biotin, and combined with streptavidin-HRP react with the NF-κBp65 antigen bound to the immobilized (capture) antibody. After washing, tetra-methylbenzidine (TMB) substrate solution is added, which is acted upon by the bound enzyme to produce blue color which turn into yellow color after addition of stopping reagent. The intensity of this colored product is directly proportional to the concentration of NF-κB p65 present in the original specimen. The colored reaction product is quantified using a spectrophotometer.

#### Quantitative determination of human P21 in cell lysates

P21 was determined qualitatively in cell lysate using enzyme immunometric assay (EIA) Kit supplied from Assay designs company (Catalog#900-161) according to El-diery *et al.*, 1993 and; Macleod *et al.*, 1995.<sup>[15,16]</sup>

### 3. Results

Phytochemical investigation of *Chorisia crispiflora* lead to the isolation of five phenolic compounds (Tab.1), Protocatechuic acid **1**, apigenin 7-rhamnoside **2**, apigenin 7-glucoside **3**, kaempferol 3-rutinoside **4**, apigenin 7-neohesperidoside **5**.

The structures of these compounds (Fig. 1) were unambiguously determined by their

chromatographic behaviors as well as spectroscopic analysis via UV (Tab. 1), <sup>1</sup>H-NMR (Tab. 2) and <sup>13</sup>C-NMR (Tab. 3).

#### Cell growth inhibition by chorisia extracts treatment

SRB assay showed that the treatment of MCF-7 breast cancer cells with chorisia extracts resulted in a dose- and time-dependent inhibition of cell proliferation (Tab. 4 & Fig. 2). The proliferation of MCF-7 cells was significantly inhibited by chorisia ethyl acetate extract treatment (median inhibitory concentration IC<sub>50</sub> of 5.2 and 4.2 μg/ml) compared with doxorubicin treatment for 48 & 72 hours (IC<sub>50</sub> of 3.2 and 4.1 μg/ml), respectively. Furthermore, SRB assay revealed that the median inhibitory concentration IC<sub>50</sub> values for cells treated with extracts 1, 3 & 4 for 48 hrs were 7, 17.85 and 17 μg/ml (Fig. 2c), respectively.

#### Effect on DNA fragmentation and apoptosis

Fig. 3b showed that incubation of *Chorisia crispiflora* extract-2 with MCF-7 cells for 5.2 μg/ml and 4.2 μg/ml at both time intervals (48 and 72 hrs) respectively, there was a significant DNA fragmentation compared to control untreated group. Fig (3a)

#### Detection of NF-κB and p21

*Chorisia crispiflora* diminished the level of NF-κB in MCF-7 cells at dose (5.2 μg/ml and 4.2 μg/ml) at both time intervals (48 and 72 hrs) respectively. Significant decrease in NF-κB level was detected compared to control (untreated MCF-7 cells) and standard samples treated with doxorubicin at dose (3.3 μg/ml and 4μg/ml) after 48hr incubation ( $P < 0.05$ ), (Fig 4; a) but after 72 hr incubation there was a significant decrease in NF-κB level observed in standard samples and treated ones compared to the control group ( $P < 0.05$ ), with no difference between the standard and treated group (Fig 4; b).

#### Detection of p21

There was no significant difference was observed in p21 level among the different studied groups after 48 hrs incubation, but after 72 hrs incubation there was significant increase in p21 level in the treated group compared to standard and control ones ( $P < 0.05$ ). (Fig 5; a & b)

**Table 1: UV-Spectral data for the phenolics of *Chorisia crispiflora***

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
MeOH	258, 294	268	266, 336	266, 350	266, 336
+NaOAc,		256,267	265, 350	377, 305, 274	265, 350
NaOAc + H <sub>2</sub> BO <sub>3</sub> ,		267	266, 341	353, 277	266, 341
+NaOMe,		245,269		401, 325, 274	
+AlCl <sub>3</sub> ,		276,300	274, 297, 343, 383	398, 274	274, 297, 343, 383
AlCl <sub>3</sub> + HCl		277,299	275, 297, 340, 381.	395, 274	275, 297, 340, 381.

**Table 2: <sup>1</sup>H-NMR data for the phenolics of *Chorisia crispiflora* ethyl acetate extract**

	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
1	12.32, s, COOH				
2	7.33, dd				
3	9.68, s, OH	6.78, s	6.8, s		
4	9.29, s, OH				
5	6.79, d				
6		6.39, d, <i>J</i> =1.9	6.38, d, <i>J</i> = 2.0	6.2, d, <i>J</i> =1.9	
8		6.8, d, <i>J</i> =1.9	6.77, d, <i>J</i> =2.0	6.3, d, <i>J</i> =1.9	6.84, d, <i>J</i> = 2.0
2'		7.87, d, <i>J</i> =8.8	7.9, d, <i>J</i> =8.4	8.05, d, <i>J</i> =8.8	7.91, d, <i>J</i> =8.8
3'		6.93, d, <i>J</i> =8.8	6.8, d, <i>J</i> = 8.8	6.8, d, <i>J</i> =8.8	6.92, d, <i>J</i> = 8.
5'		6.93, d, <i>J</i> =8.8	6.8, d, <i>J</i> = 8.8	6.8, d, <i>J</i> =8.8	6.92, d, <i>J</i> = 8.
6'		7.87, d, <i>J</i> =8.8	7.9, d, <i>J</i> =8.4	8.05, d, <i>J</i> =8.8	7.9, d, <i>J</i> =8.8
1''		5.19,d, <i>J</i> =1.911q	5.02, d, <i>J</i> = 7.2	5.12, d, <i>J</i> =7.4	5.20, singlet like
6''		1.1, d, <i>J</i> =6.2	6.8, s		
1'''				4.5, d, <i>J</i> =1.5	5.08, d, <i>J</i> = 7.3
6'''				1.1, d, <i>J</i> =6.1	1.16, d, <i>J</i> =6.3

**Table 3: <sup>13</sup>C-NMR data for the phenolics of *Chorisia crispiflora* ethyl acetate extract**

	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
1	121.62	-	-	-	-
2	116.51	164.0	165.8	164.4	158.52
3	144.86	102.8	103.8	103.2	135.62
4	149.98	181.0	182.5	182.1	179.44
5	115.11	161.0	161.9	161.1	162.98
6	121.85	99.7	100.0	99.4	99.95
7	167.28	162.7	163.5	162.6	166.01
8		94.6	95.4	94.6	94.87
9		156.6	157.5	157.1	159.35
10		105.1	105.8	105.5	105.66
1'		120.7	121.5	120.9	123.15
2'		128.3	129.2	128.7	117.69
3'		115.7	116.5	116.2	145.84
4'		160.8	161.6	161.7	149.81
5'		115.7	116.5	116.2	116.06
6'		128.3	129.2	128.7	123.55
1''		99.3	105.2	98.2	104.69
2''		70.1	73.6	77.6	75.74
3''		69.9	76.9	77.4	78.20
4''		71.8	69.9	70.1	71.42
5''		69.9	77.7	76.8	77.25
6''		17.8	61.1	60.9	68.56
1'''				101.01	102.42
2'''				71.0	72.12
3'''				70.8	72.26
4'''				72.3	73.94
5'''				68.8	69.71
-CH3				18.5	17.87

**Tab. 4 (a): Cytotoxic effect of *Chorisia crispiflora* ethyl acetate extract in MCF-7 cell line following 48 hr of treatment in comparison to doxorubicin**

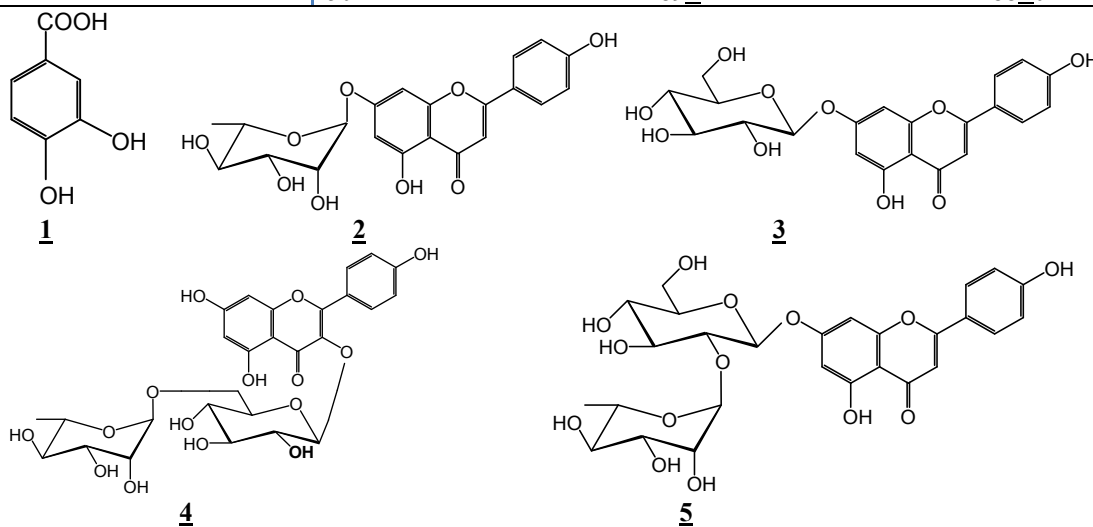
Cell line		Doxorubicin	Extract
48 hrs	Concentration ( $\mu\text{g/ml}$ )	Survival Fraction	Survival Fraction
	0	1.00 $\pm$ 0.00	0.99 $\pm$ 0.01
	5	0.19 <sup>a</sup> $\pm$ 0.01	0.56 <sup>a</sup> $\pm$ 0.04
	12.5	0.17 <sup>a</sup> $\pm$ 0.01	0.24 <sup>a</sup> $\pm$ 0.02
	25	0.19 <sup>a</sup> $\pm$ 0.00	0.29 <sup>a</sup> $\pm$ 0.06
	50	0.20 <sup>a</sup> $\pm$ 0.01	0.29 <sup>a</sup> $\pm$ 0.06
72 hrs	0	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	5	0.21 <sup>a</sup> $\pm$ 0.01	0.28 <sup>a</sup> $\pm$ 0.01
	12.5	0.17 <sup>a</sup> $\pm$ 0.01	0.22 <sup>a</sup> $\pm$ 0.01
	25	0.14 <sup>a</sup> $\pm$ 0.01	0.18 <sup>a</sup> $\pm$ 0.01
	50	0.11 <sup>a</sup> $\pm$ 0.01	0.12 <sup>a</sup> $\pm$ 0.00

Each point is the mean  $\pm$  SEM of 3 separate experiments performed in triplicate.

<sup>a</sup>: Statistical significance as compared to the control value using one way analysis of variance (ANOVA) followed by Tukey test for post-hoc analysis where a has a P-value ( $P < 0.05$ ).

**Tab. 4 (b): Percentage inhibition of *Chorisia crispiflora* ethyl acetate extract in MCF-7 cell line following 48 hr of treatment in comparison to doxorubicin.**

Cell line		Doxorubicin	Extract
48 hrs	Concentration ( $\mu\text{g/ml}$ )	% Inhibition	% Inhibition
	0	0 $\pm$ 0	0 $\pm$ 0
	5	81 $\pm$ 1	44 $\pm$ 4
	12.5	83 $\pm$ 1	76 $\pm$ 2
	25	81 $\pm$ 0	71 $\pm$ 6
	50	80 $\pm$ 1	71 $\pm$ 6
72 hrs	0	0 $\pm$ 0	0 $\pm$ 0
	5	79 $\pm$ 1	72 $\pm$ 1
	12.5	83 $\pm$ 1	78 $\pm$ 1
	25	86 $\pm$ 1	82 $\pm$ 1
	50	89 $\pm$ 1	88 $\pm$ 0

**Fig. 1: Phenolics isolated from ethyl acetate extract of *Chorisia crispiflora***

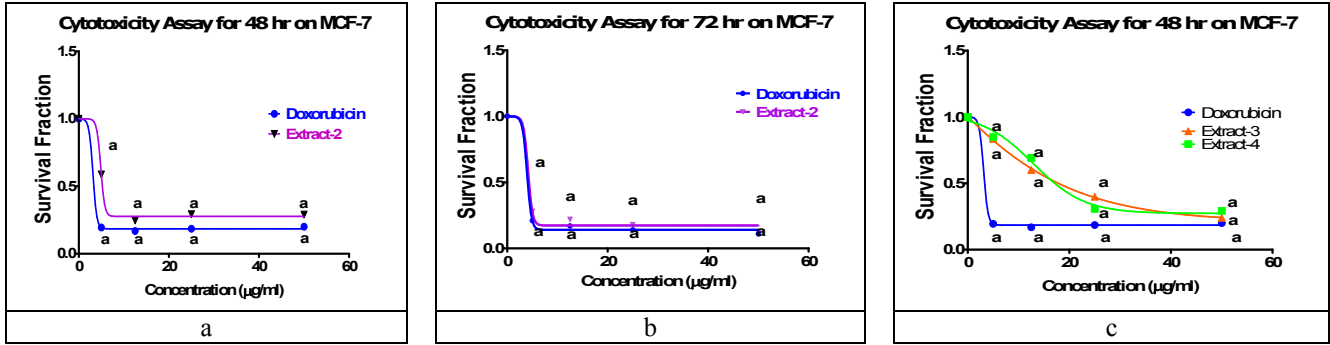


Fig. 2: (a & b): Cytotoxic effect of *Chorisia crispiflora* ethyl acetate extract in MCF-7 cell line following 48 & 72 hrs of treatment in comparison to doxorubicin. (c): Cytotoxic effect of *Chorisia crispiflora* extracts (3 & 4) in MCF-7 cell line following 48 hr of treatment in comparison to doxorubicin.

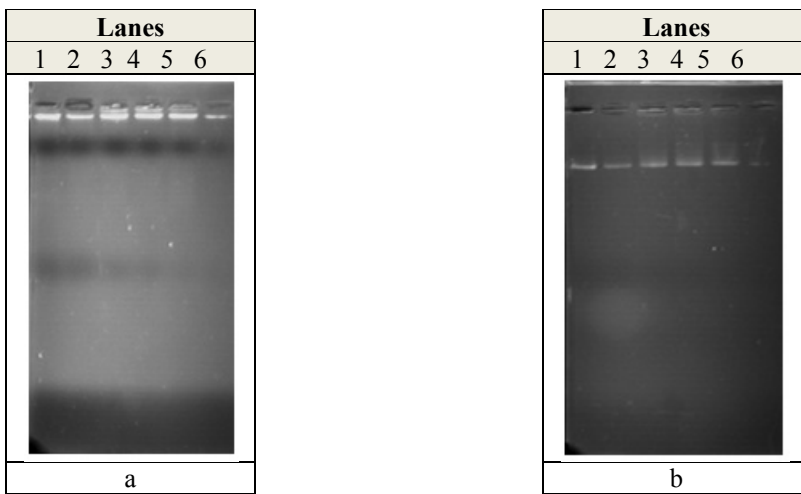


Fig. 3: *Chorisia crispiflora* ethyl acetate extract induced DNA fragmentation in MCF-7 cells

(a) The extracted DNA from untreated MCF-7 cells (control group) the DNA was contacted to the wells.

(b) The extracted DNA from control group (lanes 1, 6)

The extracted DNA from treated MCF-7 cells with standard doxorubicin (lanes 2, 5),

The extracted DNA from treated MCF-7 cells with *chorisia crispiflora* ethyl acetate extract (5.2 µg/ml and 4.2 µg/ml) at both time intervals (48 and 72 hrs) (lane 3, 4) respectively.

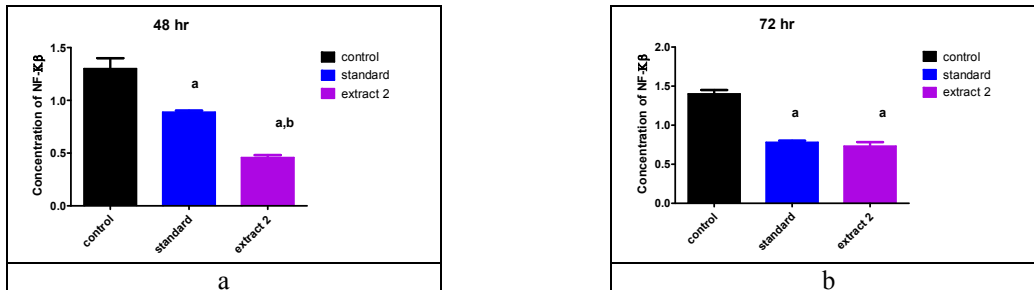
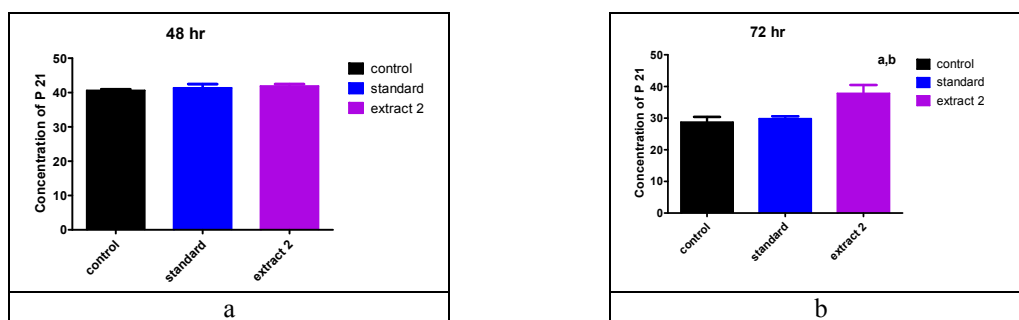


Fig.4 (a & b): NF-κB level of *Chorisia crispiflora* ethyl acetate extract in MCF-7 cell line following 48 & 72 hrs of treatment

<sup>a</sup>: Statistical significance as compared to the control value using one way analysis of variance (ANOVA) followed by Tukey test for post-hoc analysis where a has a P-value (P<0.05).

<sup>b</sup>: Statistical significance as compared to the standard (doxorubicin) value using one way analysis of variance (ANOVA) followed by Tukey test for post-hoc analysis where a has a P-value (P<0.05).



**Fig.5 (a & b): P21- level of *Chorisia crispiflora* ethyl acetate extract in MCF-7 cell line following 48 & 72 hrs of treatment**

Each point is the mean  $\pm$  SEM of 3 separate experiments.

<sup>a</sup>: Statistical significance as compared to the control value using one way analysis of variance (ANOVA) followed by Tukey test for post-hoc analysis where a has a P-value ( $P < 0.05$ ).

<sup>b</sup>: Statistical significance as compared to the standard (doxorubicin) value using one way analysis of variance (ANOVA) followed by Tukey test for post-hoc analysis where a has a P-value ( $P < 0.05$ ).

#### 4. Discussions and Conclusions

Many clinically successful anticancer drugs are themselves either natural products or have been developed from naturally occurring compounds. Great interest is currently being paid to flavonoids—one of the major classes of natural products with widespread distribution in fruits, and vegetable for their interesting pharmacological activities. The antiproliferative effects against breast cancer cells, anti-aromatase activity and binding affinities for the estrogen receptor of these compounds have drawn attention due to their role as potential anti-breast cancer agents.<sup>[17]</sup>

In this study, we investigated the anticancer effects of the *Chorisia crispiflora* different extracts; *n*-hexane, ethylacetate, butanol and methanol. We also sought to investigate the underlying molecular mechanisms of anticancer action and phytochemical investigation of the most cytotoxic extract.

SRB assay showed that the treatment of MCF-7 breast cancer cells with chorisia extracts resulted in a dose- and time-dependent inhibition of cell proliferation (Tab. 4 & Fig.2). The proliferation of MCF-7 cells was significantly inhibited by chorisia extract 2 (ethyl acetate) treatment compared with doxorubicin treatment for 48 & 72 hours. However in comparison, extract (2) produced more than 3 fold cytotoxic activity than extracts 1, 3 or 4. The data presented in this study indicated that the ethyl acetate extract is the most effective one in suppressing the growth of MCF-7 cells in culture. Thus, based on the significance of the cytotoxicity assay, extract 2 was chosen for further molecular characterization and an in-depth phytochemical investigation.

As flavonoids are potent bioactive molecules that possess anticarcinogenic effects since they can interfere with the initiation, development and progression of cancer by the modulation of cellular proliferation, differentiation, apoptosis<sup>[18]</sup>, so the

cytotoxic activity can be due to the presence of phenolic compounds contained in this extract. This is the first report of these phenolics from the leaves of *Chorisia crispiflora*.

We further evaluated the molecular mechanisms underlying the most cytotoxic extract of *Chorisia crispiflora* which may interfere with several cell signaling pathways and insert anti-cancer effects which showed a significant decrease in NF- $\kappa$ B level in samples treated with the extract compared to standard and control which may indicate that extract of *Chorisia crispiflora* has antitumor activity that is time- and concentration-dependent via down regulation of NF- $\kappa$ B.

In our study, we investigated the early cellular responses induced by extract of *Chorisia crispiflora* treatment in an attempt to define the events associated with cytotoxicity. To understand and confirm the cell death, DNA fragmentation was done; a significant dose-dependent DNA fragmentation was seen after treatment with extract of *Chorisia crispiflora* for 48 and 72 hrs. so we also estimated the p21 level at apoptotic regulatory gene and we found that there was significant effect on p21 level after 48 hrs incubation which may be due to that apoptosis can be controlled by other pathway rather than P21 in agreement with **Porath and Weinberg, 2005; Campisi and d'Adda di Fagagna, 2007; Adams, 2009**<sup>[19, 20, 14]</sup>.

On the other hand a significant increase in p21 level in the treated group compared to standard and control ones after 72 hrs incubation which indicated that *Chorisia crispiflora* may up regulate p21 level at concentration 4.2  $\mu$ g/ml at which Cellular senescence and apoptosis are a programme of irreversible cell cycle arrest that normal cells undergo after a finite number of divisions, the **Hayflick** limit.<sup>[21]</sup> Under other circumstances, the pRB pathway can be activated independently of p53, through upregulation of

p16INK4A, an inhibitor of the cyclinD/cdk4, 6 kinases that also phosphorylate the RB family of proteins.<sup>[18-20]</sup>

Our data also may conclude that *Chorisia crispiflora* extract may down regulate NF- $\kappa$ B and up regulate p21 levels at time and concentration dependent manner and also may assume that activated NF- $\kappa$ B antagonizes P53 induces P21 function, possibly through the cross-competition for transcriptional coactivators in accordance to **Chikashi and Masakazu, 2005**.<sup>[22]</sup>

Further studies should be done to improve that activation of NF- $\kappa$ B affects p21 level after treatment with the extract of chorisia.

### Conflict of Interest

All authors declare that they have no competing financial or personal interest or any kind of conflict of interest relevant to this study.

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