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Research Article

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Genotoxic and Antitumor Activity of Pollen Grains against Prostate Cancer Cell Line

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Abstract: Since the use of engineered antioxidants and antitumor is under investigation, inferable from its likely poisonousness, scientists have deflected their thoughtfulness regarding the quest for characteristic sources to meet the human medication and diet requests. Therefore the study aimed to evaluate the antitumor and antioxidant activities of maize pollen grains against the Prostate Cancer Cell (Pc3) line. Maize pollen grains were collected by Bee through a pollen trap, and then subjected for flavonoids and alkaloids analysis by HPLC method. an *in vitro* assays, were used to test the antitumor properties, against Pc3 cells. Furthermore, its antioxidant potential was also evaluated by DPPH. The detected flavonoids were identified to be quercetin, luteolin kaempferol, rutin, apigenin and naringin and the alkaloids were quinolone, hydroxyindolenine and conofoline. The antitumor efficacy of pollen grains extract increased with concentration and reached to 94.92 % that similar to the toxicity % of adriamycin at 1000 µg/mL, however, the IC_{50} (339.81 µg) of pollen grains extract was highest than IC_{50} (58.07 µg) of adriamycin. At 500 µg/mL of pollen grains extract, morphological changes of Pc3 were recorded. These changes deformed more at 1000 µg/mL. DPPH scavenging activity was found to be 92.26 % at 1280 µg/mL of pollen grains extracted with IC_{50} 425.4 µg/mL compared with IC_{50} (13.9 µg/mL) of the ascorbic acid. DNA fragmentation and quantitative RT-PCR examinations of *Bax* and *Bcl-2* genes demonstrated that pollen grains extract induced cellular apoptosis of Pc3 cells. This study concluded that the maize pollen grains may applied as natural safe source for inhibit Pc3 Cells proliferation as well as applied as antioxidant.

Keywords: Antitumor, Prostate Cancer Cell, Antioxidant, Pollen grains, Apitherapeutic.

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1. INTRODUCTION

Worldwide, cancer has become a main reason of mortality, more 9.5 million deaths from approximately 18 million as new cancer cases were predictable. Even if numerous reasons were reported for the appearance of cancer disease but the reasons of some cases still mysterious. Cancer invades the most of humans as well as animal cells. Cancer of prostate is one of the largely predominant malignancies and the subsequent driving reason for cancer-related deaths in men among human beings. Rate of prostate cancer has raised strikingly in numerous Asian nations in the previous twenty years [1.] Under stress conditions either biotic or abiotic, adverse molecules were formed inside animals as well as human cells. For example, reactive oxygen species (ROS) are synthesized continuously in cells which encompass free radicals remove comma, like hydroxyl ion and the superoxide anions and non-radical species, as H_2O_2 and singlet oxygen. As indicated by Campos et., al. [2] these components add to maturate and establishment of chronic degenerative illnesses, for example, malignancy, cardiovascular illnesses sickness, joint pain, gastric ulcer and so forth through harm of practical biomolecules, for example, proteins as well as amino acids, lipids, sugars, nucleic acids. Ever since the exposure to different poisons in climate is unavoidable, it gets important to investigate the molecules of natural sources for its antitumor potential. Through the pollen basket of the forager's hind legs, the pollen grains are transported as a little pellet to the beehive where it is put away and continuously utilized as a nutriment hotspot for the honey Bee hatchlings. Beside the nutritional and physiological characteristics of pollen grains [3.] it has additionally picked up consideration due to their various bioactive properties [4,5.] Therefore, therapeutic activities were attracted towards all types of pollen grains in many studies. In earlier study, pollen grains contained different compounds comprise gallic acid, naringenin, quercetin 3-o-neohesperidoside, vanillic acid, protocatechuic acid, *p*-coumaric acid, hesperidin, isorhamnetin, kaempherol, quercetin, isorhamnetin 3-o-rutinoside, rhamnetin 3-o-neohesperidoride, 3-o-rutinoside, rutin, apigenin and luteolin [6.] With respect to healthful properties, Bee pollen has been described as hormone regulator, liver protector, antiatheroscleorotic, antiallergic, anticarcinogenic, antioxidant, antimutagenicity, antimicrobial including fungi and bacteria, antianemic, tonic and restorative, intestinal regulator, vasoprotector [7-9.] Anticancer potential of some pollen grains was reported by Kaur et al. [10.] where pollen extract of *Bauhinia variegata, Cassia glauca, C. biflora* and *C. siamea* had antimutagenic activity against many mutagens *viz.,* mutagenic activity of sodium azide for *Salmonella typhimurium* TA 100, furthermore mutagenic potential of 4 nitro-o-phenylenediamine for TA 98 .Leja et al.[11] reveals the existence of bio components in pollen grains such as steroids, lipids, carbohydrates, proteins, amino acids, vitamins carotenoids, flavonoids, polyphenols and terpenes which reflect its bioactivity. Analysis of Bee corn pollen by Chantarudee et al. [12] reveal its contained a reasonably miscellaneous array of nutritive molecules, comprising invert sugar (19.9 g/100 g), biotin (56.7 µg/100 g), vitamin A and β carotene (1.53 mg/100 g) These components were detected previously with an average protein content about 23.8% and fatty acids are 3% [13.] Polysaccharides such as starch and sugars represent the main component of carbohydrates ranging among 13 and 55 g/100 g of pollen grains. Generally, Carpes et al.[14] found that the contents of pollen grains of phenolic contents existed in the range 19.28 to 48.90 mg GAE/ g. Not only levels and constituents of pollen components are influenced by botanical origin [13,14], they also depend on climatic conditions, geographical origin, and plant status [15.] Alicic et al. [16] and Leja et al. [11] reported the action mechanism responsible for antioxidant and antibacterial potential of pollen grains that accompanying with the existence of flavonoids, phenolic acids, and pigments like as β-carotene. Lately, Bleha et al. [17] evidenced the antioxidant potential of Bee pollen extracts correlated with high content of polyphenols. The high contents of flavonoids and phenols of *Apis mellifera* pollen grains represent the main reasons antioxidant activity [18.] Furthermore, the antiproliferative activities of Bee pollen due to the existence of polyphenols, can control cell proliferation and encourage apoptosis [19.] The current study was assigned to explore the therapeutic potential of maize pollen grains as antioxidant and antitumor natural sources.

2. MATERIALS AND METHODS

2.1 Pollen Grains Source

Samples of pollen grains were collected by Bees through Pollen trap that was fitted to the entrance of a hive of Bees, during the season of maize flowering period August 2020. The hive of Bee was located within 20 meters of maize fields, the main plant near the sampling site, Egypt. The newly collected pollen grains were dehydrated in an oven (40°C for 12 hours) and next it put away at 28°C for further examination.

2.2 Preparation of Pollen Extracts

The crude Pollen grains were prepared by extracting 10g of pollen grains in 25 mL of 100% methanol[7].

2.3 Cell Line

Cytotoxicity of pollen grains extract was evaluated using a human prostate cancer (Pc3) cell line. The source of Pc3 was the American Type Culture Collection (ATCC, USA). The origin of cells was from homo sapiens (human), tissue (prostate derived from metastatic site: bone), cell type epithelial and disease grade IV (adenocarcinoma)[20].

2.4 Estimation of Cytotoxicity Potential of Pollen Extracts Using MTT Protocol

To build up a total monolayer sheet, counted cells 1×10^5 cells/mL (100 µl/well) were insulated in 96 well tissue culture plates followed by incubation for 24 hours at 37 °C. From 96 well, medium growth was decanted from 96 well microtiter plates after intersecting sheets of cells were formed, cell monolayer was washed twice with wash media. Pollen grains extract and Adriamycin (two-fold dilutions) were made alone in RPMI medium supplemented with 2% serum. The tested compounds at each dilution (0.1 mL) were experienced in the wells with exit control containing three wells, receiving only maintenance medium. At 37 °C, the plate was incubated and then examined. The 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution was prepared in phosphate buffered saline (5 mg/mL) (Bio Basic Canada Inc). Next, 20 µl of MTT were added to each well, to thoroughly mix the MTT into the media, placing it on a

shaking table at 150 rpm for 5 minutes. At 37 °C, the plate was incubated in a humidified 5% (v/v) CO_2 for one to five hours to permit the MTT to be metabolized. Followed by re-suspend formazan (MTT metabolic product) in 200 µl DMSO on a shaking table at 150 rpm for five minutes, to thoroughly mix the formazan into the solvent. Finally, at 560 nm the optical density was read and the background was subtracted at 620 nm. The cell quantity corresponded with the optical density [20].

2.5 Morphological Observation Under Phase Contrast

Twelve-well flat bottom microtiter plates were cultivated by Pc3 cells and then treated with pollen grains extract and Adriamycin (positive control) at different concentrations (31.25-1000 µg/mL). Under a phase contrast microscope, the morphological features of Pc3 cells after 24 hours of treatment were examined.

2.6 HPLC analysis of flavonoids and alkaloids

Methanolic pollen grains extract (10 g of pollen grains in 25 mL of 100% methanol) was analyzed by HPLC, for flavonoids and alkaloids detection. The utilized HPLC-(Agilent 1100) consists of two LC pumps and a UV/V detector. Description of the C18 guard column was 125 mm × 4.60 mm and 5 µm particle size. Temperature of column was kept at 25 °C. The obtained chromatograms were analyzed using the Agilent ChemStation. The injection volume was 25 µL with flow rate 0.5 mL/minutes. Flavonoids and alkaloids were identified by comparing its retention times with standards[6].

2.7 Antioxidant Activity of Pollen Grains Extract

According to Barros et al. [21], the free radical scavenging ability of the pollen grains extract was carried out using DPPH (1,1-diphenyl, 2-picrylhydrazyl). DPPH was dissolved in 95% methanol at concentration (0.004% w/v), then it was added to the extract of pollen grains in the test tube, followed by making serial dilutions ranging from 1 µg to 500 µg. The mixture was homogenized strongly and permitted to stand in the dark for 10 minutes at room temperature 25 °C. The reduction of DPPH radicals was recorded by UV-visible spectrophotometer (Milton Roy, Spectronic 1201) at 515 nm. For comparing the antioxidant of pollen grains extract with synthetic antioxidants. Ascorbic acid was dissolved in distilled water to make the stock solution of a positive control. Furthermore, the same volume of the reaction mixture excluded any pollen grains extracted or ascorbic acid was functioned as blank. The inhibition percentage (IP) of the DPPH radical was recorded regarding the provided formula:

$$IP = \frac{(CA + TA)}{CA} \times 100$$

CA, meaning the absorbance of the control at t = 0 minutes, while TA meaning the treatment absorbance at t = 16 minutes.

2.8 DNA fragmentation

Effect of pollen grains extract on DNA fragmentation of Pc3 cells was examined after removal of the cultivated cells, then centrifuged for 5 minutes at 3000 rpm. The gathered pelts of Pc3 cells were mixed with 10 mM Tris-HCl as a hypotonic lysis buffer (pH 8.0) that comprised 0.5% Triton X-100 and 10 mM EDTA for its lyses. RNA of Pc3 cells was broken down via enzyme RNase and next via proteinase K action for two hours at 50 EC. The extraction of DNA was made via a combination of different solvents with a ratio 25:24:1 for phenol, chloroform and isoamyl alcohol, respectively. Followed by the addition a similar quantity of isopropanol for DNA precipitation, then stored for 12 hours at 20EC in dark, then centrifuged for 15 minutes at 12,000 rpm and 4EC. The collected pellet was desiccated in air, re-suspended in buffer (20 µL tris acetate EDTA) appended with 2 µL of 0.25% bromophenol blue, glyceric acid (30%) as a buffer, then separated electrophoretically on agarose gel comprising ethidium bromide (1 µg) and imagined via UV transillumination [22].

2.9 cDNA synthesis and real-time PCR (RT-PCR)

Detection of genes expression analysis of Pc3 cells treated by IC_{50} of pollen grains extract including *Bax* and *Bcl-2* were examined by real time PCR. All primer sequences of oligonucleotides were listed (Table 1). RNA of Pc3 treated by IC_{50} of pollen grains extracted for 24 hours was using Qiagen RNA extraction/BioRad SYBR®green PCR MMX kit (Quality Endorsed Company, Australia) and estimated by RT-PCR. Synthesis of cDNA was done followed by PCR amplification. Software Rotor-Gene 6000 Series Software 1.7 (Build 87) was applied for estimation of the melting curve of PCR products. One µL of primers, 2 µL cDNA, and 21 µL reaction buffers (SYBR Green involved) were prepared as reaction mixture. RT-PCR cycles were performed at 95 °C for 4 minutes, then at 95 °C for 35 second followed 58 °C for 30 second, finally at 72 °C for 30 second. β-actin was functional as a housekeeping gene, and the relative quantity of the genes was calculated via 2-ΔΔct[22].

3. STATISTICAL ANALYSIS

Three independent replicates of tests were recorded for determining the mean± standard error (S.E). SPSS ver. 22.0 software was carried out by computer for statistical analysis of the obtained data.

4. RESULTS

4.1 Analysis of Pollen Grains Contents and Antitumor Activity

From HPLC analysis, different and varied flavonoids and alkaloids were detected (Table 2 & Fig. 1) in the maize pollen grains. The detected flavonoids were rutin, quercetin, kampherol kaempferol, Apigenin, Naringin and luteolin in different concentrations. Pollen grains of maize had the highest content of quercetin (25 µg/mL) while naringin, rutin

and apigenin were noticed only in a delete and replace few with less few quantities (Table 2). Quinolone (19.30 µg/mL) represents the highest alkaloid in maize pollen grains followed by hydroxyindolenine (10.06 µg/mL) and conofoline (4.12 µg/mL) (Table 2).The antitumor potential of pollen grains extract was tested against Pc3 (Table 3 and Fig. 3) compared with Adriamycin as synthetic antitumor. The observed toxicity (%) of pollen grains extract was dependent on the concentrations. Although the percentage of toxicity (94.92) at pollen grains extract treatment was very similar to toxicity % (94.39) of Adriamycin at 1000 µg/mL, but surprisingly the IC_{50} (half inhibitory concentration) of Adriamycin was very more less than IC_{50} of pollen grains extract. The strongest antitumor activity of pollen grains appeared at 500 µg/mL with 77.09 % toxicity (Table3 and Fig.2). Under different concentrations of pollen grains extract, morphological profile of PC3 cells were examined compared with Adriamycin and control (Fig. 3a, b & c). As a result of pollen grains extract on Pc3 cells, substantial morphological changes were determined in distinction with the morphology of the untreated Pc3 cells. At 10, 50, 100, 500, and 1000 µg/mL dose, the cells were detached from their substrate and spiked in spindle form and cellular granulation was observed. By increasing the concentration, the rise in the quantity of damaged cells was noted. After addition of pollen grains extract (250 µg/mL concentration), no major change was witnessed, but at the concentrations of 500 µg/mL, changes in the morphology were observed. These changes became more intense with increasing concentration1000 µg/mL, where . Where the cells became rounded and shrunken, detaching from the surface of tissue culture flasks and floating in the tissue culture substrate, and lastly, they indicated clear cell expanding and burst; while unprocessed Pc3 cells remained in its natural form known as polygonal. On the other hand, the efficacy of Adriamycin on the morphology of Pc3 cells was more clearly till at low concentrations beginning from 62.5 µg/mL (Fig. 3c).

4.2 *Antioxidant Activity of Pollen Grains*

DPPH The radical scavenging ability of pollen grains extract was estimated by DPPH method. (Table 4 and Fig. 4) is estimated (Table 4 and Fig. 4). By incrementing the pollen grains extract concentration, it was accompanied by an increase in the activity. High concentration (1280 µg/mL) of pollen grains extract showed DPPH radical scavenging of about 92.26% with IC50 value of 425.4 µg/mL, whereas the positive control ascorbic acid exhibit DPPH radical scavenging with IC50 value of 13.9 µg/mL, which is a well-known antioxidant. create DPPH scavenging reached to 92.26 % but unfortunately, the IC_{50} of the extract was 425.4 µg/mL, as contrasting to that of the positive control ascorbic acid (IC_{50}, 13.9 µg/mL) as a reference standard, which is a well-known antioxidant.

4.3 *DNA Fragmentation and Genes Expression*

As illustrated in Fig. (5), Pc3 cells exposed to IC_{50} dose of pollen grains extract showed breakdown of DNA which appeared fragmented. The clear alterations in the DNA content among the control and treated cells point to that the pollen grains extract have caused obvious cell fatality. The apoptotic effects of Pc3 cells treated by IC_{50} of pollen grains extract was evaluated through the analyzing of mRNA levels of two genes including *Bax* and *Bcl-2*. The obtained finding indicated an increase in *Bax* gene expression (2.93589-fold) and sharply reduction in the expression of Bcl-2 (0.21004-fold) as comparison with the control (Table 5 and Fig.6). IC_{50} of pollen grains exert a difference among gene expression of *Bax* and *Bcl-2*.

Table 1. Real Time PCR Primers Sequences

Gen name	Sequences of Primer (5'- 3')	
Bax	Forward (F)	5'-ATGTTTTCTGACGGCAACTTC-3'
	Reverse (R)	5'-AGTCCAATGTCCAGCCCAT-3'
Bcl-2	F	5'-ATGTGTGTGGAGACCGTCAA-3'
	R	5'-GCCGTACAGTTCCACAAAGG-3'

Table 2. Flavonoids and Alkaloids detection in Maze pollen grains by HPLC

Compound		Chemical formula	Concentration mg/mL	RT
Flavonoids	Rutin	$C_{27}H_{30}O_{16}$	8.22	7.01
	Kampherol	$C_{15}H_{10}O_6$	9.31	7.55
	Quercetin	$C_{15}H_{10}O_7$	25.00	11.0
	Apigenin	$C_{15}H_{10}O_5$	7.56	12.0
	Naringin	$C_{27}H_{32}O_{14}$	4.12	14.0
	Luteolin	$C_{15}H_{10}O_6$	14.59	15.0
Alkaloids	Conofoline	$C_{43}H_{52}N_4O_7$	4.12	5.0
	Hydroxyindolenine	C_8H_7NO	10.06	5.66
	Quinolone	C_9H_7N	19.30	10.12

Table 3. Anticancer activity of pollen grains extracts and adriamycin against Pc3 cells

Concentration (µg/mL)	Pollen grains				Adriamycin			
	Mean O.D	Standard Error	Viability %	Toxicity %	Mean O.D	Standard Error	Viability %	Toxicity %
1000	0.374	0.004	5.08	94.92	0.021	0.002	5.61	94.39
500	0.019	0.001	22.90	77.09	0.033	0.005	8.82	91.18
250	0.087	0.006	60.52	39.48	0.050	0.002	13.37	86.63
125	0.226	0.006	94.74	5.25	0.074	0.006	19.88	80.12
62.5	0.354	0.004	99.73	0.27	0.164	0.006	43.85	56.15
31.25	0.373	0.004	99.38	0.62	0.326	0.009	87.25	12.75
IC_{50} (µg)	339.81				58.07			

Table 4. Antioxidant capacity of pollen grains extract and ascorbic acid.

Pollen grains			Ascorbic acid		
Concentration (µg/mL)	DPPH Scavenging %	S.D.*	Concentration (µg/mL)	DPPH Scavenging %	S.D.
1280	92.26	1.48	40	93.48	0.74
640	73.16	4.52	35	87.53	1.39
320	38.63	2.91	30	80.65	1.21
160	23.06	1.22	25	77.41	0.87
80	17.74	0.84	20	70.94	1.38
40	11.47	0.31	15	54.86	2.96
20	9.74	0.46	10	17.45	2.39
10	8.68	0.34	5	11.78	0.64
0	0	S.D.	0	0	0
IC_{50} = 425.4 ±10.4 µg/mL			IC_{50} =13.9 ± 1.5 µg/mL.		

Values are the average of triplicate tests and represented as mean± standard deviation.

Table 5. *Bax* and *Bcl-2* genes expression Pc3 cells treated with IC_{50} concentrations of pollen grains extract

Sample	*Bax*							
	Control cells			Test cells				FLD
	B Actin	*Bax*	*ΔCTC*	*B Actin*	*Bax*	*ΔCTE*	*ΔΔ CT*	*2^ ΔΔCT*
	HC	TC	TC-HC	HE	TE	TE-HE	ΔCTE-ΔCTC	Eamp =1.844
Treated PC3	24.65	32.76	8.11	24.83	31.18	6.35	-1.76	2.93589
Control Pc3	24.65	32.76	8.11	24.65	32.76	8.11	0	1
				Bcl-2				
Treated PC3	24.65	29.82	5.17	24.83	32.55	7.72	2.55	0.21004
Control Pc3	24.65	29.82	5.17	24.65	29.82	5.17	0	1

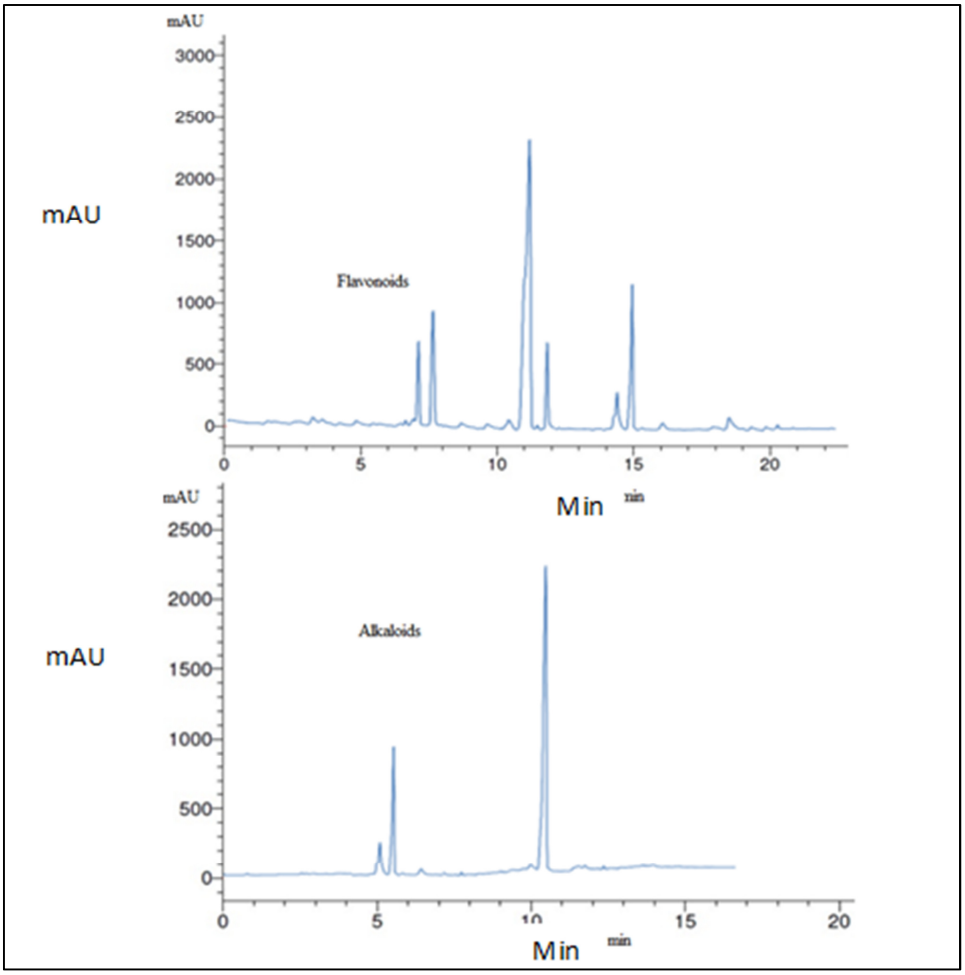

Fig.1. HPLC Chromatogram of Flavonoids and Alkaloids Detected In Pollen Grains Extract.

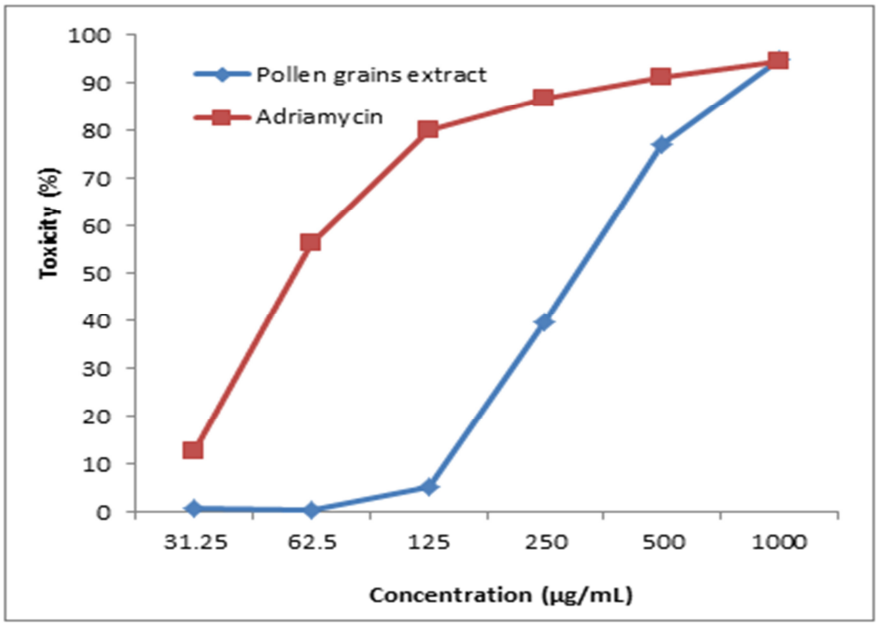

Fig. 2. Cytotoxicity of pollen grains extract and Adriamycin against Pc3 cells.

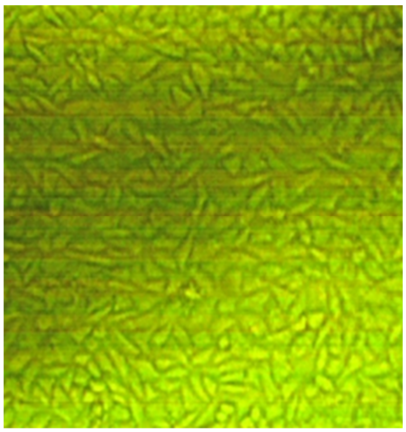

Fig. 3a. Pc3 cells without any treatment (Control).

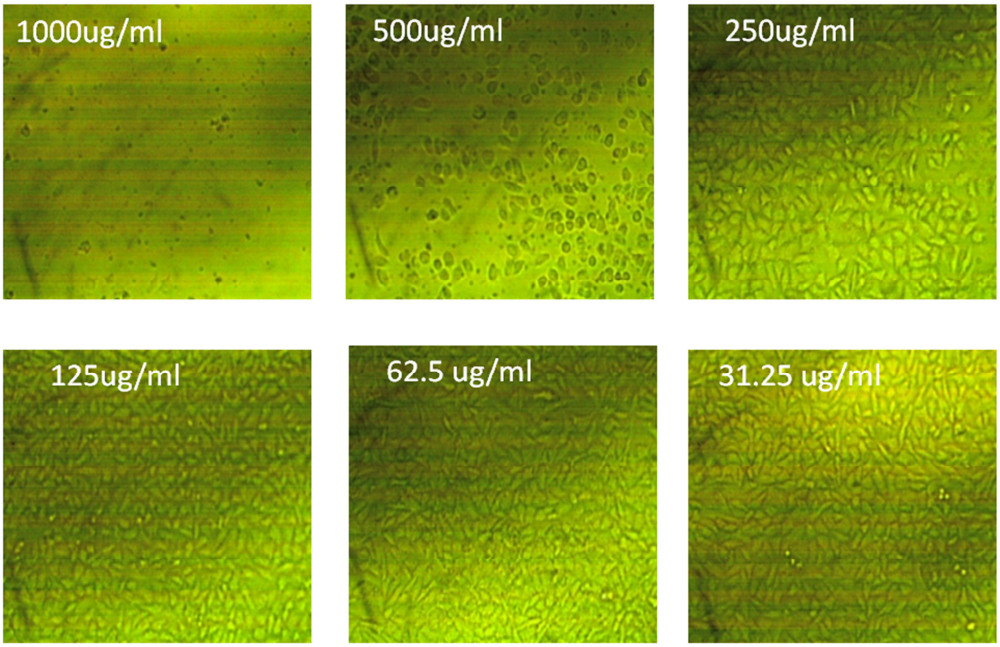

Fig. 3b. Image of Pc3 cells at different concentrations of pollen grains extract.

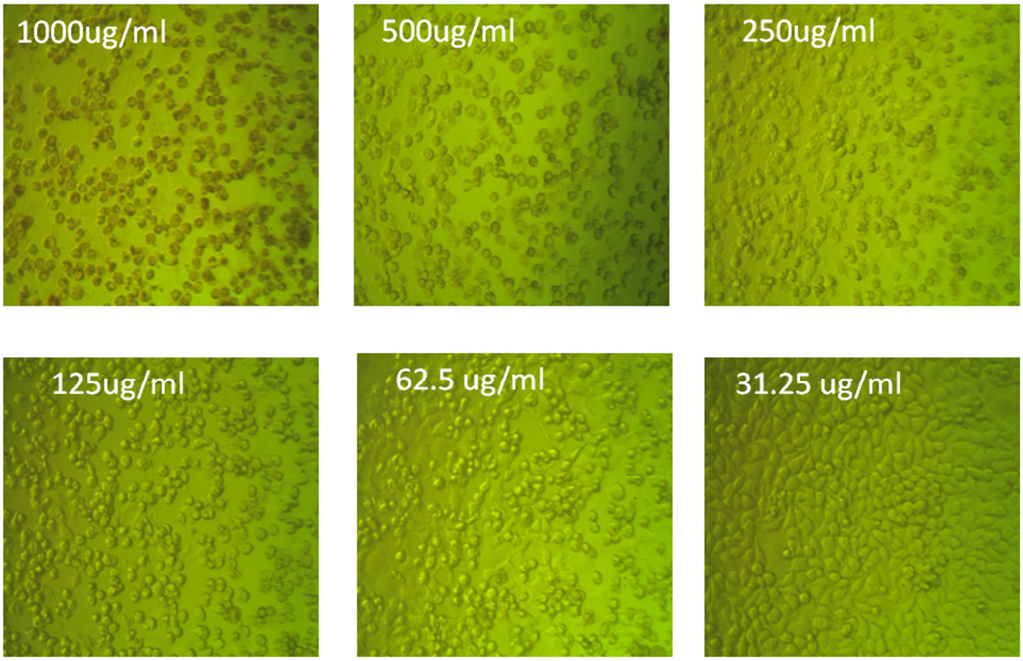

Fig. 3c. Image of Pc3 cells at different concentrations of Adriamycin.

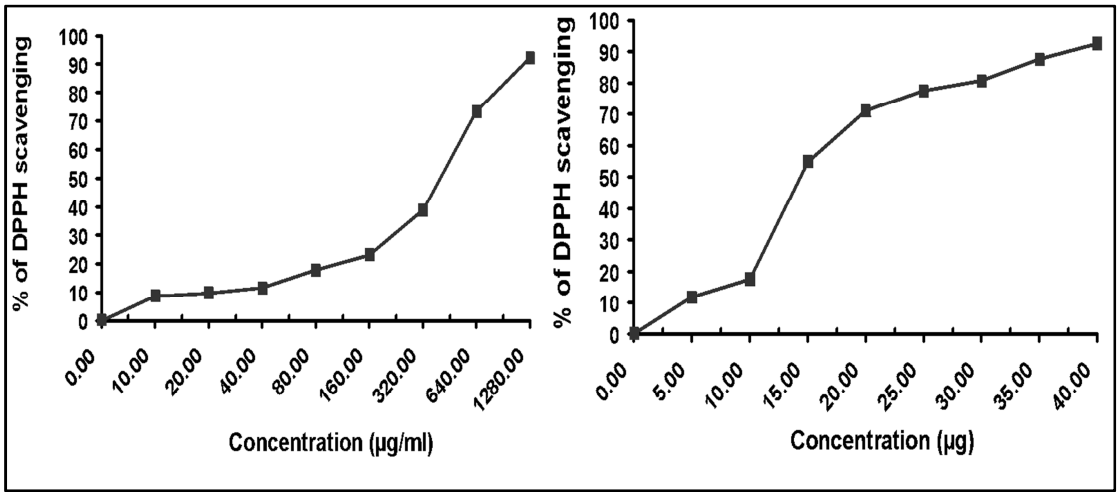

Fig. 4. DPPH radical scavenging activity of pollen grains (left) extract and Ascorbic acid as Reference standard (right).

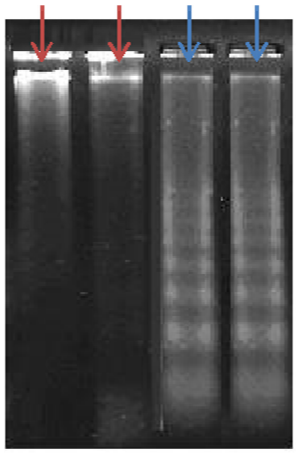

Fig. 5. DNA fragmentation assay of Pc3 cells treated with IC_{50} concentration of pollen grains extract (blue arrow) compared with control (red arrow).

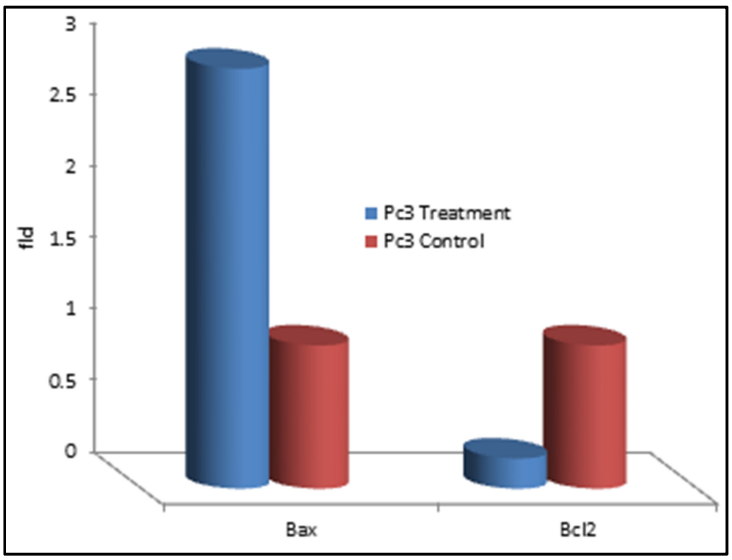

Fig 6. *Bax* and *Bcl-2* genes expression of Pc3 exposed to pollen grains extract.

5. DISCUSSION

Although different plants and its parts comprising aerial parts such as stem or leaves as well as roots and bark have been discovered for its biological activity like as antimutagenicity, antigenotoxicity, antitumor, antioxidant activities and etc., but only a few scientific studies are offered on the medicinal potential of plant pollen grains particularly maize. Currently, pollen grains are used as nutritious food and studied for its potential therapeutic properties due to its contents of various compounds. The detected flavonoids by HPLC such as quercetin, kampherol and luteolin may not be specific to Maize pollen grains, because it is detected in other plant pollen grains *Eucalyptus* and *Cecropia* [23]. Quercetin and kaempferol[24] were previously identified in the species *Ricinus communis* L. According to some literatures on the content of pollen grains, the phenolic compounds specifically quercetin, kaempferol, caffeic acid [25] and naringenin[26] represent the main effective compounds in biological activities. Rutin and Kampferol were detected in pollen grains of *Phoenix canariensis* [27]. Earlier in 1984 Ceska and Styles [28], recognize the chemical flavonoids ingredients of pollen grains of corn, such as flavanol glycoside, quercetin, isorhamnetin and kaempferol, although they did not explore the apigenin, naringin and luteolin that were reported in the present study. However, Chantarudee et al. [12] detected the flavone 7-O-R-apigenin and phenolic hydroquinone as a bioactive compound in corn pollen grains. In the present study, a highest content of quercetin (25 µg/mL) was recorded in Pollen grains of maize. From prior research [29], the yellow color of maize pollen grains is associated with the existence of the quercetin as a flavonoid pigment beside its derivatives. The obtained finding is in well accordance with these data. Alkaloids are considered as an important group exhibiting diverse biological activities. Surprisingly, the existence of quinolone in the pollen grains is extracted under study, and records the highest level. These contents of quinolone reflect the therapeutic potential of pollen grains extract. Currently, studies revealing a widespread array of biotic and pharmacological applications for quinoline including antimalarial, bactericidal, fungicidal, anthelmintic, cardiotonic, anticonvulsant, anti-inflammatory and antioxidant activity [30], beside anticancer and analgesic activity [31]. Therefore it has attracted scholars' attention for more recent development of quinolone and its derivatives. From HPLC analysis the detected phytochemicals in pollen grains are excellent alternatives for chemical drugs. The finding cytotoxicity of maize pollen grains may due to presence of rutin and quercetin, where the validities of anticancer properties for 3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside (Rutin) against different cell lines was reported including leukemia [32], colon cancer and neuroblastoma [33]. The current results are partly in agreement with the finding of some prior reports concerning the vital role of natural separated compounds from pollen grains of Secale cereale as secalosides on S180 sarcoma by Jaton et al. [34]. The authors observed that secalosides had been very effective for suppressing the tumor of S180 sarcoma. Observation about the antitumor activity was previously confirmed, where pollen grains of some plants such as *Rosa rugosa* [35], as well as other plants such as *Cassia biflora, C. glauca*, *C. siamea* and *Bauhinia variegata* [36] showed antitumor activities. It was earlier reported that derivatives of polysaccharides of *Brassica napus* L. pollen grains have appeared antitumor potential against Sarcoma 180-bearing mice and B16 melanoma bearing mice [37]. Depending on the morphological examination in the provided research, it was exhibited that the extract of pollen grains encouraged apoptosis in Pc3 cells but not as observed in case adriamycin, where morphological deformation of Pc3 cells was more obviously at its lowest concentrations. However, adriamycin caused extra noticeable morphological changes compared to those observed with pollen grains extract, these alterations were mostly confined to apoptotic and cytotoxic changes to the cells. But Adriamycin causes side effects such as failure of kidney functions [38]. The antioxidant properties of the obtained pollen grains were observed with DPPH scavenging up to 92.26 %. This observation was previously confirmed by Chantarudee et al. [12] where pollen grains derived from maize (*Zea mays*), provided a good free radical scavenging activity. As mentioned above, these pollen grains extract contain numerous flavonoids. For example quercetin and rutin have been recognized to be concomitant to antioxidative action in living organizations. Furthermore, represses protein kinases, restrains DNA topoisomerases and manages gene expression correlated to oxidative stress and the antioxidant defense system [29, 39-41]. Another explanation of the antioxidative effects of pollen grains extract is perhaps coupled to the efficiency of antioxidant

enzymes. Antioxidant activity was reported for many pollen grains but varied significantly among different pollen types as mentioned earlier [11,] where antioxidant activity more than 60% was recorded for pollen of numerous plants such as *Robinia pseudoacacia*, *Malus domestica, Pyrus communis, Sinapis alba, Taraxacum officinale* and *Phacelia tanacetifolia*.To understand the mechanism of cells death that is exposed to pollen grains extract, DNA fragmentation was achieved on Pc3 cells in the current study. Although, no documents were available on the effect of pollen grains extracted on DNA fragmentation in Pc3 cells but effects of other plant products were detected, therefore the finding here is in line with that of Salim et al. [42] who recorded fragments in DNA of Pc3 treated by propolis. Increases in *Bax* gene expression (2.93589-fold) while decreasing in Bcl-2 gene expression (0.21004-fold) were observed compared with the control. The finding expression established that intrinsic and extrinsic apoptotic pathways were effective. Replace the word Risen with increase in Risen in *Bax* gene expression and decline the expression of *Bcl-2* were observed in Pc3 but treated by Baneh extract. [43.] Increase in the sensitivity of apoptosis according to Yao et al. [44] was due to increasing expression of *Bax* gene, which is parallel to the finding of the present study. Previously, Begnini et al. [45] suggested that *Bax* and *Bcl-2* associated with apoptotic actions. Immunoblotting tests by Ma et al. [46] exhibited that cytochrome c was liberated accompanied with increment *Bax* expression; and *Bcl-2* was down-regulated from HeLa cells because of its exposure to *Pinus massoniana* bark extract. Herein the results suggested that promoting of Pc3 apoptosis as a result pollen grains extract may be due to the active role of mitochondrial death signaling pathway. Furthermore, the reasons for the apoptosis process may relate to proapoptotic *Bcl-2* family members.

6. CONCLUSION

From the referenced outcomes and its discussion it can be concluded that the extract pollen grains replace with pollen grains extract has the potent anticancer and antioxidant molecules which might be liable for its mitigating and chemoprotective as well as validate the origin of utilizing this natural extract as folkloric cures and represents a safe alternative in medical practice. Modulation of *Bax* and *Bcl-2* genes expression indicated apoptosis induction in Pc3 cells treated by pollen grains extract. Furthermore, the action mode mechanism of the promising compounds in the pollen grains extract against cancer should be investigated more.

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8. CONFLICT OF INTEREST

Conflict of interest declared none.

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