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Evaluation of chemical composition, biological properties and biomedical effectiveness of Aloe vera

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Aloe vera (*Aloe barbadensis*) is one of the herbal remedies with a longstanding history for its curative properties for various ailments and it is one of the richest natural sources of health for human beings. The chemistry of the plant has revealed the presence of many different biologically active substances such as vitamins, enzymes, minerals, polysaccharides, and amino acids etc. According to the World Health Organisation (WHO), medicinal plants would be the best source for obtaining a variety of drugs. Numerous studies worldwide indicate that aloe vera is helping to fight illness of all kinds. Aloe vera is one such plant being studied extensively for cancer prevention. The goal of this study is to evaluate the chemical composition of aloe vera gel as well as its antioxidant, antimicrobial and antitumor activities. The obtained results revealed that the moisture content, crude protein, crude fiber, pH and TSS of gel were 97.25,0.09 ,0.36, 3.74 and 1.00 %, respectively. The results obtained by (HPLC) of aloe Vera extracts identified and quantified 26 phenolic and 12 flavonoid compounds. Epi-catechin, catechin and catechol were the highest phenolic compounds in all extracts while hesperidin and naringin were the highest flavonoid compounds in the methanol extract of aloe Vera. The antimicrobial activities showed high effect against both gram positive and gram negative bacteria. The aloe vera gel significantly inhibited the growth of all tested fungi and yeast. The effect of aloe Vera was studied against HepG2, Caco, Hela and MCF7 cells. The results of cytotoxicity revealed significant difference between all the tested compounds. The drug A (aloe Vera gel) showed the highest toxicity in all cell lines used in the experiment and showed very high toxicity on HepG2 (1.2±0.2). The lowest toxicity was observed on hela cell line when treated by all tested compound. It could be concluded from the results of this research that aloe vera is one of the most promising plants with high antioxidant, antimicrobial, anti-cancer properties.

Keywords: *Aloe vera*, chemical composition , antioxidant, antimicrobial, anticancer activities.

INTRODUCTION

Aloe Vera is a tropical or subtropical plant characterized by lance shaped leaves with jagged edges and sharp points. Aloe Vera (*Aloe barbadensis* Miller), botanically is a member of the family *liliaceae*. It is a short stemmed succulent, perennial herb with more than 600 species (Pulok et al., 2014). It is one of the oldest known plant and its first documented use by humans dates back to an Egyptian papyrus from 3500 BC. The natural source of aloe Vera is unclear as the species has been widely cultivated throughout the world , rather originating in Africa. It was grown in South Africa and Latin America, then it was introduced to China, India and various parts of Southern Europe in the 17th century (Efterpi and

Panagiota, 2010).

The aloe Vera gel is colorless and has a bitter taste (Karina et al., 2013). The gel consists primarily of water (95- 98%) and the pH is (4.4-4.7). Aloe Vera contains over 75 biologically active and naturally-occurring compounds and active constituents, such as vitamins, enzymes, sugars, lignin, saponins, salicylic acid, and amino acids and essential fatty acids. Aloe Vera is one of the only known natural vegetarian sources of vitamin B12, and it contains many minerals and vitamins vital to the growth process and healthy function of all the body's systems. Aloe Vera contains calcium, magnesium, zinc and vitamins A, B, C, E. Numerous studies worldwide indicate that aloe vera is a general tonic for the immune system, helping it to fight diseases of all kinds (Ahlawat and Khatkar, 2011; Thiruppathi et al., 2010). It also carries compounds responsible for antidiabetic, antioxidant, antimicrobial and wound healing activities. Concentrated extracts of aloe leaves are used as laxative and as a hemorrhoid treatment (Vidic et al., 2014). The use of plant product for pharmaceutical purpose has been gradually increased. According to World Health Organization, medicinal plants would be the best source for obtaining a variety of drugs (Rodríguez et al., 2010; Ghada et al., 2014).

Natural products are known to play an important role in Pharmaceutical biology. Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional medicines. In fact, many of the current drugs either mimic naturally occurring molecules or have structures that are fully or in part derived from natural motifs (Adesuyi et al., 2011). Natural antimicrobials can be derived from barks, stems, leaves, flowers and fruits of plants, various animal tissues or from microorganisms (Gordon, 2001). The medicinal use of natural products compounds that are derived from natural sources such as plants, animals or microorganisms precedes recorded human history probably by thousands of years (Hong et al., 2009). Aloe vera is well known for its considerable medicinal properties. This plant could be one of the richest natural sources of health for human beings in the coming years. The chemistry of the plant has revealed the presence of more than 200 different biologically active substances. Many biological properties associated with aloe species are contributed by the inner gel of the leaves (Maharjan et al., 2015).

Cancer is a generic term for a large group of diseases that can be chronic and are responsible for a large number of deaths worldwide (Jemal et al., 2007). Choice of cancer treatment is influenced by several factors, including the specific characteristics of the tumor, patient`s overall condition and whether the goal of treatment is to cure cancer, keep it from spreading, or to relieve the symptoms caused by cancer. Depending on these factors, patient can receive one or more of the following clinical traditional therapies such as surgery, chemotherapy, radiation therapy, hormonal therapy, targeted therapy and /or biological therapy, but these types of treatments has limited success on treating cancer and have many side effects for patients (Ferlay et al., 2012; Abdullaev, 2001).

There is some evidence that crude extracts from aloe Vera can act synergistically with chemotherapeutic drugs reducing cytotoxicity. Aloe emodin, an anthraquinone compound present in the leaves of aloe vera, has exhibited anticancer biological function in esophageal, colon, pancreatic, and many other types of cancers. Aloe emodin seems to target cancerous cells by down regulating many key cancer promoting molecules without cytotoxic effects (Kai-Yuan Lin and Yih-Huei Uen, 2010; Hamman, 2008).

Aloe Vera is the most commercial aloe species and processing of the leaf pulp has become a large worldwide industry. In the food industry, it has been used as a source of functional foods and as an ingredient in other food products, for the production of gel-containing health drinks and beverages. In the cosmetic and toiletry industry, it has been used as base material for the production of creams, lotions, soaps, shampoos, facial cleansers and other products. In the pharmaceutical industry, it has been used for the manufacture of topical products such as ointments and gel preparations, as well as in the production of tablets and capsules (Haque et al., 2012; Kulveer and Bhupender, 2011).

The aim of this study is to determine the chemical composition of aloe vera and highlight on its antioxidant properties and evaluate its antimicrobial and anti-tumor activities.

MATERIALS AND METHODS

Plant Material: Aloe vera

(*Aloe barbadensis Miller*) was obtained from Orman Botanical Garden, Ministry of Agriculture. Fresh whole leaves are between 50 and 70 cm

length corresponding to 3-years old plant, they were washed by distilled water, their margins were removed and the spikes placed along before slicing the leaf to separate the parenchyma cells (gelatinous layer) from the epidermis (skin).The filets were extensively washed with distilled water to remove the exudates from their surfaces. Further, the filets were cut into cubes, blended in a food processor and squeezed through a 200 mesh screen then it was analyzed (Boudreau and Beland, 2006).

Physicochemical analysis:

TSS, TS, pH value, acidity (% malic acid), moisture, ash, crude protein, crude lipid, crude fiber, total sugars, reducing sugars and non-reducing sugars, of aloe vera were determined according to the methods described in AOAC (2005). Total phenols were determined according to the methods described in Singleton and Rossi (1965). and total antioxidant capacity was carried out according to the methods described by Hu et al. (2003). Total flavonoids were measured using colorimetric assay developed by Zhishen et al. (1999). All analysis was done in triplicates and results were expressed on average basis.

Preparation of aloe vera extracts

Aloe Vera gel was extracted by 4 different solvents (hexane, ethyl acetate, methanol and distilled water) according to the method of Sonia and Mohamed (2008).

Analysis of total phenol and total flavonoid contents

Analysis of total phenol and total flavonoid contents was carried out on aloe Vera gel and the for mentioned extracts by the method of Muthu et al., (2018).

Determination of free radical scavenging activity in aloe vera

The free radical scavenging effect of each extract was assessed by the discoloration of a methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (violet color) according to the method of (Brand-Williams, 1995).

Fractionation and identification of phenolic and flavonoid compounds of the aloe vera by HPLC

Identification and quantitative analysis of phenolic and flavonoid compounds in all aloe vera tested samples were carried out by HPLC according to the method described by Park et al., 1998). A HPLC Agilent 1200 series was equipped with quaternary pump, auto sampler, column compartments ET at 35°C, multi wavelength detector set at 330, 280nm for detection of flavonoid compounds and phenolic compounds respectively degasser. The column used for fractionation Zorbax OD. 4.6x250 mm and the flow rate phenolic and flavonoid phase during run was 1 ml/min.

Microbiological examination

Antimicrobial activity of aloe vera extract included total bacterial count, yeast and mold at different concentrations: 30, 40, 50,100,200,400,600 and 800 µg/ml. It was tested against 5 types of bacteria (*Salmonella typhimurium* (733), *Bacillus cereus* NCIM 2106, *Pseudomonas aeruginosa*, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923) and three fungal cultures of *Fusarium spp*, *Penicillium spp* and *Aspergillus niger*, it was also tested against 3 strains of yeasts Candida *tropicalis ,Candida albicans* and *Saccharomyces cerevisiae* by the disc diffusion methods (Drummond and Waigh, 2000). The tested microbes were obtained from Al-azhar University Center for Biotechnology Fermentation and Applied Microbiology .While the tested fungi were kindly provided from Mycology laboratory of the faculty of science, Cairo University.

Screening of aloe Vera extracts for antitumor activity:

***In vitro* study**

Plant extraction:

Aloe Vera was prepared in three extract as follows:-

A- Aloe Vera extract :Fresh whole leaves were washed, their margins were removed and the spikes placed along before slicing the leaf to separate the parenchyma cells (gelatinous layer) from the epidermis (skin).The filet was extensively washed with distilled water to remove the exudates from their surfaces. Further, the filets were cut into cubes, blended in food processor and squeezed through a 200-mesh screen then it was analyzed (Boudreau and Beland, 2006).

B-Aloe vera gel water extract: a water extract of aloe vera was prepared by the method described by Taiwo et al., (2005).

C- Whole aloe vera extract: Fresh whole leaves were washed and cut into cubes, blended in food

processor and squeezed through a 200 mesh screen (Boudreau and Beland, 2006).

Doses: every extract has been diluted by distilled water to the following concentrations 6.25, 12.5 ,25 ,100 ,200 µg/ml**.**

Cell lines

A human hepatocellular carcinoma cell line Hep-G2 (Liver carcinoma cell line), Caco (colon carcinoma cell line), Hela (cervical cancer cells line), human breast cancer cell line (MCF7) were preserved in the National Cancer Institute (NCI), Cairo, Egypt laboratory. Cells were cultivated in RPMI-1640 culture medium containing 10% fetal bovine serum and penicillin / streptomycin at 37 ºC in a 5% CO_2 incubator.

MTT assay:

HepG2, Caco, Hela and MCF7 cells were treated by aloe vera using the colorimetric methyl tetrazolium test (MTT) as described and modified by (Kai-Yuan Lin and Yih-Huei Uen, 2010). Percentage of relative viability and the half maximal inhibitory concentration (IC50) were calculated by the prism program.

Statistical analysis:

Data were analyzed using SPSS version 22 (IBM SPSS, Armonk, NY, USA). Numerical data were summarized as means and standard deviations (SD). Comparison between variables was performed by using T independent test, and analysis of variance (ANOVA). Probability (*p*-value) equal or less than 0.05 was considered significant. To ascertain the significant among means of different samples, LSD test was applied (Steel et al., 1997).

RESULTS AND DISCUSSION

Physicochemical composition of aloe vera gel .

The chemical composition of aloe gel was determined and the obtained results are shown in Table (1).The results showed that the moisture content of gel was 97.25%. The present data are in agreement with the results of (Muñoz et al., 2015; Ghada et al., 2015).

The percentages of protein, crude fiber and lipid contents were 3.27,13.09 and 2.90%, respectively. From the same table, it could be noticed that the reducing sugars and the non-reducing sugars were 13.45% and 10.54% respectively. The present data were in agreement with (Ghada et al., 2015; Haque et al., 2014).

The ash content (20.14%) showed similar values to those reported by Ghada et al. (2015) and Haque et al. (2014).Those results disagreed with Karina et al. (2013) and Ghada et al. (2014), who found that the ash was (0,20 and 0.12%) ,respectively on fresh weight basis. The pH and titrimetric acidity values were 3.74 and 0.75, respectively. Those results were in agreement with the results reported by Ghada et al. (2014); Muaz and Hussain (2013), and disagreed with Karina et al. (2013), who found that pH and acidity values were 4.74 and 0.06, respectively.

It is well known that The antioxidant activity is in correlation with the content of their phenolic and flavonoid compounds. Aloe vera had high contents of phenolic and flavonoids being 31.91 and 20.00 %, respectively, while the antioxidant activity was 57.82 %. The present data were in agreement with the results of Karina et al., (2013); Vidic et al., (2014); Muñoz et al., (2015).

Table 1; Physicochemical composition of Aloe Vera gel.

Parameters	Fresh weight basis	Dry weight basis
pH	3.74±0.01	--
TS%	2.72±0.06	--
TSS%	1.00±0.00	--
Moisture%	97.25±0.05	--
Ash%	0.554±0.18	20.14
Crude protein%	0.09±0.17	3.27
Crude lipid%	0.08±0.02	2.91
Crude fiber%	0.36±0.07	13.09
Total sugar%	0.66±0.01	23.99
Reducing sugar%	0.37±0.02	13.45
Non-reducing sugar%	0.29±0.01	10.54
Acidity (% malic acid)	0.021±0.00	0.76
Total phenolic content (mg/g)	0.85 ±2.31	31.04
total flavonoid content	0.55 ±0.83	20.10
Antioxidant activity	1.59	57.82±1.65

Mean ± standard error of three independent determinations

Total Phenolic and flavonoid contents of aloe vera gel and its extracts

Data in Table (2) showed the phenolic and flavonoid compounds (mg/100g) of different aloe vera samples (aloe vera gel and the four extracts).

The results showed that the highest content of the total phenolic compounds was in the aqueous extract and the aloe vera gel being 8.42 and 7.10 mg/100g, respectively. The methanol extract recorded 6.88 mg/100g and the lowest was in the ethyl acetate extract (5.01 mg/100g). The flavonoid compounds were 9.67, 9.55 and 8.11mg/100g in aloe vera gel, aqueous extract and hexane extract respectively. The present data was in agreement with the results of Vidic et al.,

2014; Muthu et al., 2018.

Table 2; Total phenolic and flavonoids compounds of *aloe vera* gel and its extracts.

Content mg/100g (dry weight)	phenolic content as Gallic acid	flavonoids content as qercetine	P value	T
Aloe vera gel	7.10 ±0.01*	9.67 ±0.02	<0.001	-199.07
Hexane extract	6.54±0.03	8.11±0.11	<0.001	-28.37
Ethyl acetate extract	5.01±1.1	6.12 ±0.12	0.129	-1.909
Methanol extract	6.88 ±0.08	8.25 ±0.10	<0.001	-15.66
Aqueous extract	8.42 ±0.4	9.55±0.3	0.017	-3.914

*Data were expressed as mean ± SD, P <0.05 is significant

Table 3; Fractionation and identification of Phenolic contents of aloe vera gel and its extracts by (HPLC).

Constituents mg/100g	Aloe vera gel	Methanol extract	Aqueous extract	ethyl acetate extract	Hexane extract
Pyrogallol	7.81	7.23	7.43	6.76	3.76
Gallic acid	25.80	20.65	23.51	11.9	13.7
4-Amino benzoic acid	16.7	11.9	12.3	1.3	---
Protocatchuic	32.6	31.2	30.9	27.5	25.2
Oleuropein	44.3	38.8	40.5	32.9	34.7
3-Hydroxy tyrosol	40.5	38.7	37.0	28.3	32.1
Chlorogenic acid	77.7	67.2	70.3	57.4	61.1
Epi- catechin	97.8	94.3	96.1	91.6	90.8
Catechin	91.6	88.7	87.4	87.2	86.9
Catechol	87.3	77.7	84.1	78.4	80.8
Caffeine	12.8	8.6	9.3	----	---
P-OH-Benzoic acid	23.8	22.3	20.3	18.2	18.8
Vanillic acid	32.6	31.9	28.4	29.4	27.9
Caffeic acid	26.3	25.5	23.9	19.8	20.5
p-Coumaric acid	33.8	31.6	28.7	28.4	28.6
Ferulic acid	36.1	35.8	32.4	33.9	34.7
Iso Ferulic acid	29.1	27.1	19.3	18.4	15.3
Resveratrol	9.9	8.2	6.4	2.3	4.6
Ellagic acid	43.3	32.8	23.3	22.4	20.4
E-vanillic acid	37.4	29.4	26.3	22.4	25.3
Alpha Coumaric acid	25.6	22.5	28.4	11.3	16.3
Benzoic acid	28.9	23.5	20.4	15.3	19.3
3,4,5methoxy-cinnamic acid	54,7	36.0	50.1	35.9	40.5
Coumarin	7.82	5.1	3.0	---	---
Salycilic acid	34.7	30.3	29.4	25.3	26.6
cinnamic acid	32.2	26.9	25.5	9.2	14.2

Fractionation and identification of phenolic compounds in aloe vera gel and its extracts by HPLC

Data in Table (3) showed the phenolic compounds (mg/100g) of aloe vera gel and extracts by high performance liquid chromatography (HPLC). In the present study 26 phenolic compounds were detected confirming that the aloe vera is a natural source of The major components (mg/100g) of aloe gel were epi-catechin (97.8), catechin (91.6), catechol (87.3), chlorogenic (77.7), 3,4,5-methoxy-cinnamic acid (54.7), ellagic (43.3), p-coumaric (33.8), vanillic (32.6). While in the aqueous extract epi-catechin, catechin and catechol were 96.1, 87.4 and 84.1 mg/100g, respectively. The results were in agreement with Park et al., 1998; Genovese et al., 2010; Aroa et al., 2013.

4- Fractionation and identification of flavonoid compounds in aloe vera gel and its extracts by HPLC.

The results in Table (4) revealed the presence of a total of 11 flavonoid compounds (mg/100g) while 7-Hydroxy flavone was not detected in all tested samples. Aloe vera gel contained rosmarinic (54.2), hesperidin (26.54), rutin (19.4), quercetin (2.54) and hesperetin (1.61), luteolin (1.86).

In ethyl acetate extract hesperidin (27.67), luteolin (23.64), rutin (4.06) , quercetin (3.54), kaempferol (1.18) were detected while Naringin was not found. From the same table it could be observed that hesperidin (65.71) was the highest compound in methanolic extract followed by naringin (51.09), luteolin (21.54) and rutin (8.15).

Hesperidin was the highest compound in hexane and aqueous extracts being 7.60 and 7.08, respectively. The results were in agreement with Aroa et al., 2013; Ilham, 2018. Genovese et al., 2010.

The DPPH free radical scavenging activity of aloe Vera

Data in Table (5) showed the DPPH free radical scavenging activity of aloe vera gel and its four extracts. It could be observed that aloe vera gel, methanol extract and aqueous extract recorded the highest value of free radical scavenging activity while hexane extract and ethyl acetate gave the lowest values. These results are in agreement with Brand-Williams e al., (1995), who found that the highest antioxidants were found in polar solvent extracts aqueous> methanol > ethyl acetate > acetone**.** From the same table it could be noted that the highest value of free radical scavenging activity was 69.42% in aloe vera gel (800 µg/ml),while the lowest value was 31.85% in the hexane extract (50 µg/ml).

Aloe vera gel had the highest free radical activity because it contains all the biologically active substances which are more than 200 substances as cited by Maharjan et al., 2015 and also it may be due to synergestic effect with other components in aloe gel.

Table 4; Fractionation and identification of Flavonoid compounds of aloe vera gel and its extracts by (HPLC).

constituents mg/100g	Aloe Vera gel	Methanol extract	Aqueous extract	ethyl acetate extract	Hexane extract
Luteolin	1.86	21.54	1.79	23.64	0.32
Naringin	0.98	51.09	1.92	----	0.42
Rutin	19.4	8.15	0.71	4.06	0.08
Hesperidin	26.54	65.71	7.08	27.67	7.60
Rosmarinic	54.2	2.78	0.93	0.87	0.06
Quercetrin	0.73	0.36	0.13	0.80	0.09
Quercetin	2.54	0.00	0.41	3.54	1.02
Hesperetin	1.61	1.08	0.08	0.71	0.35
Kaempferol	0.56	0.32	0.18	1.18	0.11
Apigenin	0.21	0.11	0.14	0.39	0.08
7-Hydroxy flavone	-----	-----	-----	-----	-----
Naringenin	-----	0.35	0.20	1.79	0.12

Table 5. The DPPH free radical scavenging activity (%) of aloe vera gel and its extracts

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S.No. C	Concentration of extract (µg/ml.	Aloe vera gel	Methanol	Aqueous	Ethyl Acetate	n-Hexane	P Value	F
1	50	42.13 c	38.26 a	41.23 c	32.63 b	31.85 b	<0.001	48.417
2	100	57.24 d	46.14 a	55.01 d	40.45 b	33.42 c	<0.001	715.334
3	200	55.87 a	52.11 a	54.33 a	43.98 b	30.33 c	<0.001	100.702
4	400	51.64 b	57.69 a	49.31 b	47.71 b	37.31 c	<0.001	200.159
5	600	46.03 d	60.5 a	45.22 d	52.57 b	36.68 c	<0.001	137.395
6	800	69.42 a	67.23 a	66.77 a	56.66 b	41.1 c	<0.001	873.488

Antimicrobial activity of aloe vera aqueous extract

Bacteria

The present study carried out on the aloe vera aqueous extract investigated antimicrobial activity against different food borne pathogens by the disc diffusion method. The results presented in Table (6) revealed that antibacterial activities of aloe vera extract showed high activity against both gram positive and gram negative bacteria. It exhibited maximum zone of inhibition against *Salmonella Typhimurium* and E. *coli* (66.87 mm and 63.76 mm), respectively for the (conc.800 µg/disc). This observation was in conformity with Fatemeh, 2013, While the lowest activity for the same concentration was for *Staphylococcus aureus* (49.98 mm)

Another observation was found by Shahzad (2009), who stated that antibacterial susceptibility testing of aloe vera gel showed greatest inhibitory effects on the *Staphylococcus aureus*. This result could be responsible for the popular use of aloe vera gel and leaf to relieve many types of gastrointestinal irritations (Joshua, 2010).

Mold and Yeast

Data in Table (7) revealed that aloe vera had a high effect on mold and yeast. Antifungal activity of aloe vera gel was determined against three plant pathogens Fusarium *spp.* ,*Penicillium spp.* and *Aspergillus niger* and three yeasts *Candida tropicalis* ,*Candida albicans* and *Saccharomyces cerevisiae*. Result showed that aloe vera aqueous extract significantly inhibited the growth of all tested yeast and mold in all concentration .Results exhibited maximum zone of inhibition against *Penicillium spp.* and *Fusarium spp*. (47.94 mm and 45.78 mm), respectively for the (conc.800 µg/disc).These findings were in accordance with Agarry et al., (2005).

Table 6; Antibacterial activity in aqueous extract of aloe vera.

Concentration (µg/disc) and Zone of inhibition in mm									
Pathogenic bacteria	**30**	**40**	**50**	**100**	**200**	**400**	**600**	**800**	**P value**
Salmonella Typhimurium	22.35±1.2 f	26.72±1.0 e	28.15±1.15 e	43.81±1.1 d	45.63±2.9 cd	46.79±3.5 bc	48.43±2.5 b	66.87±2.07 a	<0.001
Bacillus cereus	20.55±1.55 e	22.69±1.1 e	27.46±2.06 d	39.65±2.5 c	41.23±3.2 c	43.65±1.2 b	45.07±1.02 b	58.65±1.5 a	<0.001
Pseudomonas aeruginosa	21.12±0.12 g	23.97±2.1 f	28.45±0.45 e	41.7±2.3 d	42.43±3.4 cd	43.98±2.5 c	46.65±1.2 b	57.24±2.4 a	<0.001
E. coli	25.82±1.3 h	28.75±1.25 g	31.55±1.7 f	50.54±4.2 e	53.73±3.2 d	55.94±.98 c	59.98±2 b	63.76±1.6 a	<0.001
Staphylococcus aureus	25.65±1.05 d	28.98±1.01 cd	29.68±3.4 bcd	43.65±2.4 bc	45.76±2.8 abc	47.77±2 ab	47.23±2.1 abc	49.98±3.5 a	<0.001

*Data were expressed as mean ± SD, P <0.05 is significant

data having the same letter in the same variable are statistically similar

Table 7;Anti-fungal and Yeast activity in aqueous extract of aloe vera.

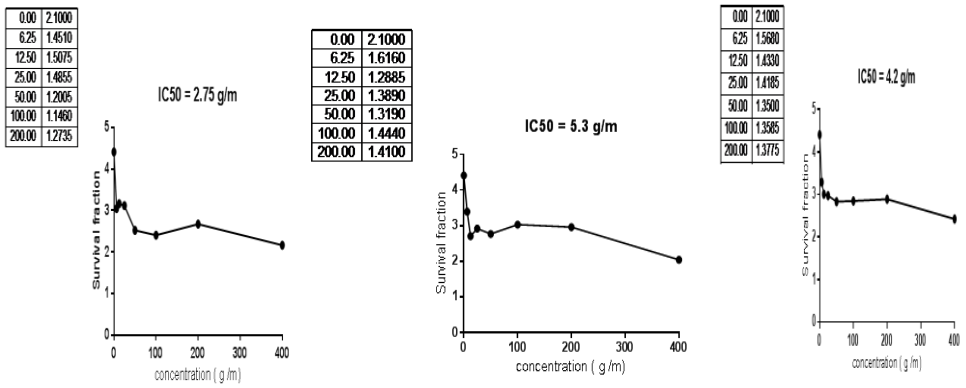
Concentration (µg/disc) & Zone of Inhibition in mm aqueous extract								
Test microorganism	**30**	**40**	**50**	**100**	**200**	**400**	**600**	**800**
***Fusarium* spp.**	20.45±3.56 [a]	24.77±2.15[a]	27.44±4.87[aA]	36.79±2.65[b]	39.33±2.49[b]	41.78±4.26[b]	43.56±1.33[b]	45.78±9.56[b]
***Penicillium* spp.**	18..23±3.87[a]	22.34±5.22[a]	26.12±2.14[a]	38.16±1.87[b]	40.55±8.66[b]	43.55±6.12[b]	45.78±1.26[bA]	47.94±2.12[bA]
Aspergillus niger	18.87±1.54[a]	21.58±9.41[a]	24.41±1.81[a]	31.44±1.61[b]	33.56±1.54[b]	35.12±1.71[b]	38.12±1.92[b]	40.98±2.32[b]
Candida tropicalis	57.65±6.46[c]	60.92±6.12[c]	62.81±1.43[c]	84.53±2.71[d]	86.71±6.31[d]	88.61±1.81[d]	89.59±1.22[d]	93.11±1.41[dA]
Candida albicans	18.18±1.43[a]	21.89±7.87[a]	24.23±1.12[a]	35.43±6.32[b]	37.44±1.33[b]	39.88±1.57[b]	41.55±4.66[b]	42.56±1.23[b]
Saccharomyces cerevisiae	19.11±1.23[b]	39.35±1.27[b]	38.87±1.25[b]	37.43±1.15[b]	35.45±7.12[b]	26.23±2.89[a]	22.54±1.56[a]	44.12±1.13[b]

Concerning the yeast experiments, maximum zone of inhibition was noticed for *Candida tropicalis* and *Saccharomyces cerevisiae* (93.11 and 44.12 mm), respectively for the (conc.800µg/disc) .The results are in agreement with the finding of Rosemary et al., 2013; Arunkumar and Muthuselvam, 2009; Atefl and ErdoUrul, 2003, who tested antifungal activity of natural aloe vera gel on four plant pathogens fungi and four yeasts.

The antitumor activities of Aloe Vera:

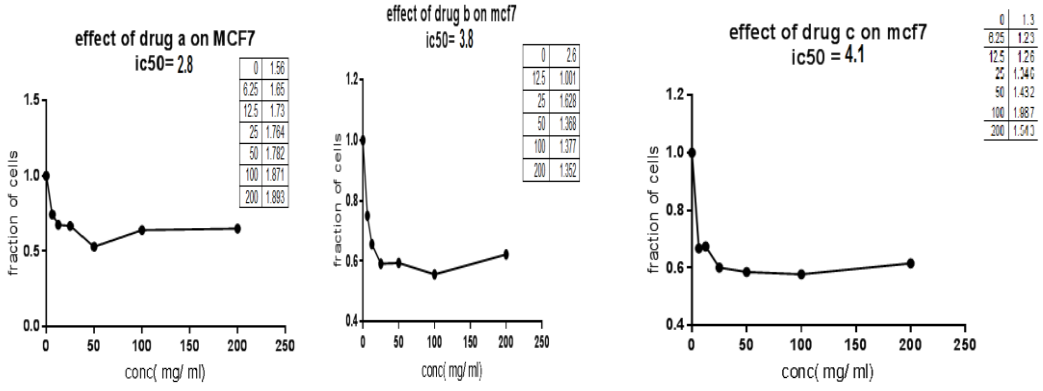
The results of cytotoxicity in fig (a,b,c,d) revealed significant difference between the three tested compounds against different tumor cell lines with P value (0.05) as shown in Table (8).The drug A showed the highest toxicity in all used cell lines in the experiment and showed very high toxicity on HepG2 (1.2±0.2). The results also revealed that the lowest toxicity on hela cell line when treated by all tested compound. Moderate toxic effect on CaCo and McF7 cells was noticed when treated by Adrug. Where the cells showed low toxicity when treated by B and C drugs. The data revealed that drug A was more promising and effective than B and C drugs. The results were in agreement with (Naveena et al., 2011; Pecere et al., 2000; Lee et al., 2001 Kuo et al., 2002; Maram et al., 2015; Winters et al., 1981) and disagreed with (El-Shemy et al., 2010; Niciforovic et al., 2007).

The anti-tumor activities of aloe Vera:



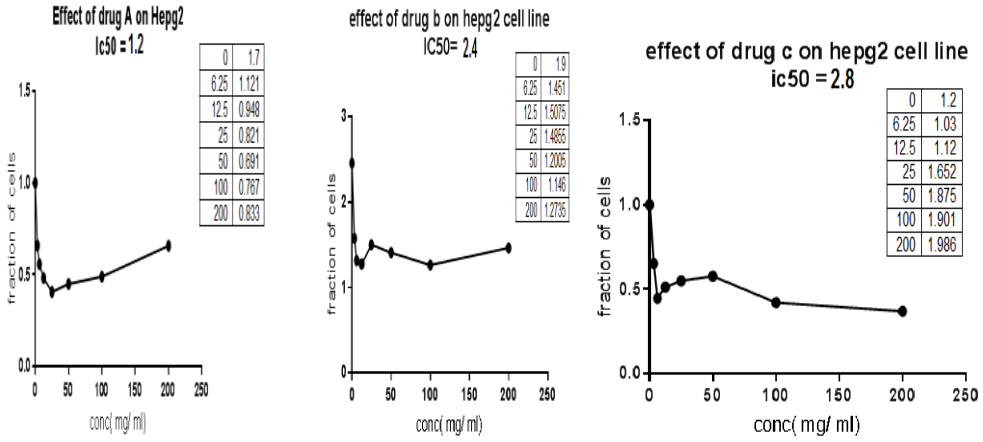
1 2 3

Figure. A: Effect of drug A on Caco cell line Effect of Drug B on Caco cell line Effect of drug C on Caco cell line



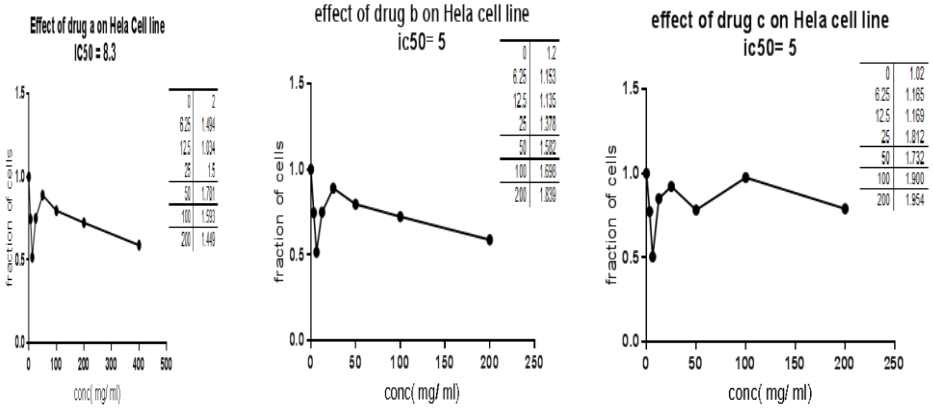
4 5 6

Fig. B: Effect of drug A on MCF7 cell line Effect of Drug B onMCF7 cell line Effect of drug C on MCF7 cell line



7 8 9

Figure. C: Effect of drug A on HepG2 cell line Effect of Drug B on HepG2 cell line Effect of drug C on HepG2 cell line



10 12 11

Figure. D. Effect of drug A on Hela cell line Effect of Drug B on Hela cell line Effect of drug C on Hela cell line

Table 8; Comparison between IC50 of Aloe vera

	Drug A	Drug B	Drug C	F	P value
CaCo	2.7 5±0.25*a#	4.2±0.15 b	5.3±0.2 c	117.78	<0.001
HepG2	1.2±0.2 a	2.4±0.35 b	2.8±0.1 b	36.17	<0.001
Hela	3.8±0.1 a	5±0.1 b	5±0.05 b	192.0	<0.001
MCF7	2.8±0.05 a	3.8±0.2 b	4.1±0.1 c	79.43	<0.001

IC50 (the half maximal inhibitory concentration)
*Data were expressed as mean ± SD, P <0.05 is significant
data having the same letter in the same variable are statistically similar
DrugA: Aloe vera extract.
Drug B:Aloe vera gel water extract .
DrugC: Whole aloe vera extract

CONCLUSION

The drug A (aloe Vera gel) showed the highest toxicity in all cell lines used in the experiment and showed very high toxicity on HepG2 (1.2±0.2). The lowest toxicity was observed on hela cell line when treated by all tested compound. It could be concluded from the results of this research that aloe Vera is one of the most promising plants with high antioxidant, antimicrobial, anti-cancer properties.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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