ISSN : 0974 - 7508 Volume 9 Issue 6

Natural Products

An Indian Journal

Full Paper

NPAIJ, 9(6), 2013 [251-257]

Phenolic composition and biological activities of methanolic extract of *strawberry* leaves *(Fragaria ananassa)*

Amani M.D.El-Mesallamy[1], Sahar A.M.Hussein[2], Mohamed El.Gerby[1], Mohamed H.M.Abd El Azim[1]*

[1]Department of Chemistry, Faculty of Science, Zagazig Universiy, Zagazig, 44511, (EGYPT)

[2]Department of Phytochemistry and Plants systematic, Division of Pharmaceutical Chemistry, National Research Center, Dokki, Cairo, (EGYPT)

E-mail: mhmsm01213@yahoo.com

ABSTRACT

Our study aimed to evaluate antimicrobial, antioxidant and antitumor activities and currently phenolic of polar *extract of Strawberry (Fragaria ananassa) leaves. Seventeen* phenolic compounds were isolated from methanolic extract of *Strawberry* leaves and identified as hydrolysable tannins, flavanol, flavonol glycosides and phenolic acids.

The antioxidant activity was measured by 2, 2' - Diphenyl Picrylhydrazyl (DPPH) radical scavenging method. The methanolic extract completely inhibited DPPH at three different concentrations 19, 38, 77 µl which showed very high antioxidant capacity, which was close to ascorbic acid standard used. Antimicrobial activities of plant extracts were studied against five bacterial strains and five fungal species, 0.1 ml of plant extract (10 mg / 1 ml) had inhibitory effect for most bacterial spp. and showed no effect against all fungal spp., Clearly inhibitory effect was obtained against all of the tested bacterial and fungal strains at 0.3 ml (10 mg /1 ml) of the extract. The methanolic extract of *strawberry* leaves was tested against three human cell lines; the extract has activity against all cell lines tested.

KEYWORDS

Strawberry (Fragaria ananassa) leaves;
Hydrolysable tannins;
Flavanoids;
Phenolic acids;
UV;
MS;
NMR;
Antimicrobial;
Antitumor and antioxidant activities.

INTRODUCTION

Berry fruits are reported to contain a wide variety of phenolics including hydroxybenzoic and hydroxycinnamic acid derivatives, anthocyanins, flavonols, flavanols, condensed tannins (proanthocyanidins) and hydrolyzable tannins[10]. These phenolic acids are bioactive compounds and they are widely distributed in the vegetal world. They take part in the diary diet and they influence the health. It has been demonstrated that they act as antioxidants, anti-inflammatories, antihistaminics and anti-tumorals. Many of these beneficial effects are attributed to phenolic acids present in vegetables and fruits[11]. *Strawberries* are a good source of ascorbic acid (AA), anthocyanins and flavonols and, among the fruits, they have one of the highest antioxidant activities evaluated by oxygen radical absorbance capacity[5,18]. Ellagic acid was the main phenolic compound in the berries of the genus Rubus *(red raspberry, Arctic bramble and cloudberry)* and genus *Fragaria (strawberry)*; the data of this study suggested that berries have potential as good dietary

Full Paper

sources of quercetin or ellagic acid[7]. *Strawberry (Fragaria x ananassa Duch.)* fruits contain phenolic compounds that have antioxidant, anticancer, antiatherosclerotic and anti-neurodegenerative properties. Phenolics were identified as ellagic acid (EA), EA-glycosides, ellagitannins, gallotannins, anthocyanins, flavonols, flavanols and coumaroyl glycosides. The anthocyanidins were pelargonidin and cyanidin, found predominantly as their glucosides and rutinosides[13]. *Strawberry cv. Elsanta* fruit and flowers contain preformed antifungal compounds which differ markedly in number and activity during flower and fruit development[15]. Using GC-MS, phenolic and non-volatile acids and soluble sugars were identified and quantized in *strawberry* tree (*Arbutus unedo* L.) fruits. The phenolic acids quantized were gallic (10.7 mg/g dry weight), gentisic (1.9 mg/g), protocatechuic (0.6 mg/g), *p*-hydroxybenzoic (0.3 mg/g), vanillic (0.12 mg/g) and *m*-anisic (0.05 mg/g). Fructose and glucose among the sugars, fumaric and malic acids among the non-volatile acids and gallic acid among the phenolic acids were found to be major compounds contributing the taste of the fruits[2]. Significant increases in the plasma total antioxidant capacity (TAC) have already been reported after acute intake of *strawberries*[16].The aim of the present study was to identify the different phenolic classes of the polar extract of leaves of the Egyptian *strawberry (Fragaria ananassa)* and to investigate the antimicrobial, antioxidant and antitumor activities of polar extracts from leaves of *strawberry*, for which a limited data have been previously published.

EXPERIMENTAL

Plant materials

Fresh leaves of *strawberry* was collected on December (2007) from Sharkiaa, Egypt. And identified by Botany Department, Faculty of Science, Zagazig University.

Test micro-organisms

The bacterial and fungal strains were personally obtained from the microbiology Lab., Botany Department, Faculty of Science, Zagazig University. Bacterial species tested were *Psudomonas areuginosa, Kelbseilla* sp., *Salmonella typhi*, *Staphyllococcus aureus* and *E. coli.* and fungal species were *Fusarium oxysporum, Aspergillus flavus, Aspergillus niger, Cladosporium* sp. *and Penicillium* sp.

Materials for cytotoxic activity

Human tumor cell lines: HEPG2 (liver carcinoma cell line), MCF7 (breast carcinoma cell line) and HCT116 (colon carcinoma cell line).

Chromatography

Paper chromatography

Sheets of Whatman paper No 1 or 3 MM were used for two- dimensional, comparative or preparative paper chromatography.

Column chromatography

The separation of the phenolic and flavonoid components was performed by column over Polyamide powder, polyamide 6-S for CC, Riedel-De Haen AG, seelze- Hannover, Germany.

Physical tests

Ultra-violet spectrophotometric analysis

Chromatographically, pure materials dissolved in analytically pure methanol were subjected to UV spectrophotometric investigation in 4 ml capacity quartz cells Zeiss spectrometer PMQ -II. In case of flavonoids, $AlCl_3$, $AlCl_3$/HCl, fused NaOAc/H_3BO_3 and NaOMe reagents were separately added to methanolic solution of the investigated material and UV measurements were then carried out[8,12].

Nuclear magnetic resonance spectroscopic analysis

NMR spectra were measured on Jeol ECA 500 MHz NMR Spectrometer at National Research Center, Dokki, Cairo, Egypt. 1H chemical shifts (δ) were measured in ppm, relative to dmso-d6 and converted to TMS scale.

Mass spectrometric analysis

The isolated pure compounds were subjected, in most cases to Fast Atom Bombardment (positive and negative) mass spectroscopic analysis (FAB-MS) on MM 7070 E spectrometer (VG analytical). Some other compounds were subjected to electron spry ionization mass spectroscopic analysis (ESI – MS) a Varian

Mat112-ET Spectrometer. All measurements were carried out at Institute Fur Chemie, Humboldt Universität zu Berlin, Germany.

Methods

Extraction

Two kilograms of air dried leaves thoroughly crushed and exhaustively extracted under reflux over a water bath with 5 liters of a methanol / bi-distilled water (3: 1) mixture for 3 hours. The solvent was removed under reduced pressure at about 45 °C. The residual finally yielded 150 g of a sticky dark brown material.

For the primary detection of the phenolic content in methanolic extract, the following investigations were carried out

Two dimensional paper chromatography of the extract was applied on Whatman paper No 1, irrigated in the solvent system 6% (acetic acid – water) (HOAc – 6), followed by butanol: acetic: water (4:1:5) revealed the presence of mainly seventeen phenolic compounds, corresponding spots gave positive response towards $FeCl_3$ spray reagent, some of which appeared under UV light as dark purple spots which turned orange or lemon yellow or reddish orange when fumed with ammonia vapor or when sprayed with Naturstuff spray reagent[9].

A sample of 33 gm of the extract dissolved in 50 ml aqueous methanol (3:1) was fractionated over 300 gm polyamide 6S, Riedel-De Haen AG, Seelze – Hannover, Germany. Column (150 X 3.5 cm) and elution with methanol / bidistilled water mixtures of decreasing polarities for gradient elution led to the desorption of seven individual fractions (I-VII) which were dried, individually, in vacuum, and then subjected to rechromatography for several times to have the pure phenolic compounds.

Antioxidant assay

Antioxidant scavenger activities were spectrophotometrically determined at 517 nm according to method adopted by Brand-Williams[4], with some modification by Duarte-Almeida[6]. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:-

% Inhibition = [(AB –AA)/ AB] x 100

Where AB is the absorbance of blank sample and AA is the absorbance of tested extract.

Antimicrobial activities

The extract was dissolved in dimethylforamide (DMF) for antimicrobial investigation at the final concentration of (10 mg / 1 ml).

Antibacterial activity

Antibacterial activities of extract were tested using pour plate technique on nutrient agar medium. Culturing and incubated of different bacterial species were carried out at 27 °C for 24 hours. Extract was tested at two concentrations 0.1 ml and 0.3 ml (10 mg / 1 ml). After the elapse of incubation periods, the diameter of inhibition zones was measured (mm). Mean of 3 replicated was calculated. The inhibition zone formed by the extracts against the particular test bacterial strain determined as the antibacterial activities of the extract[17].

Antifungal activity

Czepak Dox media used for cultivation of fungal species. The medium was seeded with different fungal species. After solidification of media on plates, make pores in agar with cup porer (15 mm) diameter. Two concentrations 0.1 ml and 0.3 ml (10 mg / 1 ml) of the extract were transferred into the well. Dimethyl foramide (DMF) was used only as a control. The plates were incubated for 7 days at 30 °C. The inhibition zone (mm) formed by the extract against the particular test fungal strain determined as the antifungal activities of the extract.

Measurements of potential cytotoxicity by SRB assay

Potential cytotoxicity of the extract was tested using the method of Skehan[14]. Cells were plated in 96-multiwell plate (104 cells/well) for 24 hrs. Before treatment with the extract allow attachment of cell to the wall of the plate. Different concentrations of the extract under test (50, 100, 125, 250 and 500 µg /ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the extract for 48 hrs. at 37 °C and atmosphere of 5% CO_2. After 48 hrs. cells were fixed, washed and stained with Sulforhodamine B strain. Excess strain was washed with acetic acid and attached strain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and extract concentration is plotted to get the survival curve of each tumor cell line

Full Paper

after the specified compound.

RESULTS AND DISCUSSION

Results of phenolic constituents in methanolic extract of *strawberry* leaves

Investigation of the phenolic compounds was done by fractionation of the extract, over polyamide column and elution with methanol/bi-distilled water mixtures of decreasing polarities for gradient elution led to the desorption of seven individual fractions (I-VII) which were dried, individually, in vacuum, and then subjected to rechromatography for several times led to the separation of seventeen pure phenolic compounds. Fraction I; was found to contain three compounds 1) Gallic, 2) Vanillic and 3-) ρ- hydroxy benzoic, fraction II; contain 4) Quercetin-3-O-rutinoside, 5) Quercetin -3-O-glucopyranuronide and 6) Kaempferol-3-O-glucopyranuronide, fraction III; contain 7) Quercetin-3-O- β-glucopyranoside and 8) Quercetin 3-O-α-$^{1}C_{4}$-rhamnopyranoside, fraction IV; contain 9) 1-O-galloyl-2,3-O-hexahydroxydiphenoyl-$^{4}C_{1}$-β-glucopyranose and 10) 2,3-O-hexahydroxy- diphenoyl - (α/β)-glucose, fraction V; contain 11) Catechin, fraction VI; contain 12) Caffeic acid, 13) Ellagic acid and 14) ρ-coumaric acid, fraction VII; contain 15) Quercetin, 16) Myricetin and 17) Kaempferol. The structure of these compounds were confirmed by chromatographic, UV, MS, H- NMR spectral data and the results were listed in TABLE 1.

Biological activates of *strawberry* leave extracts

Assay for antioxidant capacity

The methanolic extract of *strawberry* completely inhibited DPPH absorbance at three different concentrations used (19, 38 and 77µl), (Figure 1) and (TABLE 2). The percentages obtained can be considered as a full absorbance inhibition of DPPH, because after completing the reaction, the final solution always possesses some yellowish color and therefore its absorbance inhibition compared to colorless methanol solution can not reach 100%. Permanent residual absorbance results in up to 7% of the total absorbance inhibition.

Results of antimicrobial activity

Antibacterial activity

Data in Figure 2 evaluate that *Staphyllococcus aureus* is the highest resistance species to the extract at 3 mg concentration under investigation. Moreover both concentrations were resulted dramatic inhibitory effects to *Kelbsiella sp.*, *Salmonella typhi*, *Escherichia coli* species less than the higher concentration one 0, 3 ml (10 mg / 1 ml). The maximum inhibitory responses are indicated after the treatment of *Pseudomonas aeroginosa*, *Kelbseilla sp.*, *Salmonella typhi* with highest concentration of methanolic extract. These results indicated that these three species are highly sensitive against extract when compared with the two other species *Staphyllococcus aureus* and *Escherichia-coli*. These results agreement with that obtained by Ayoola[3],

TABLE 1 : Chromatographic and spectral analysis of the phenolic compounds from methanolic extract of strawberry leave (*Fragaria ananassa*).

Compd. No.	Rf values (x 100)	UV λ_{max} (nm) MeOH	MS	^{1}H- NMR Spectral Data (DMSO-d_6)δ (ppm)
1	44 (H2O),55 (HOAC) ,72 (BAW)	272	170	6.98 (*s*, H-2 and H-6)
2	0 (H2O),77 (HOAC), 90 (BAW)	276	168	7.32 (s, H-2), 6.64(d,J=8 Hz,H-5), 7.24(d, J=8 Hz, H6), 3.69(O-Me at position 3
3	0 (H2O),36 (HOAC), 84 (BAW)	253.	138	7.8(d, J=9 Hz), 6.8(d, J=9 Hz).
4	0 (H2O),48 (HOAC), 42 (BAW)	359, 299*, 266*, 259	610	6.18 (d, J=2.5Hz,H-6),6.37 (d, J-2.5 Hz,H-8),7.55 (d, J=2.5 Hz,H-2'), 6.85 (d, J=8 Hz,H-5'),7.56 (dd, J=2.5 & 8 Hz,H-6'),5.32 (d, J=8 Hz,H-1"), 3-3.75 (m, Hz,H-2"-6"),4.35(broad s, $\Delta\nu_{1/2}$=4),3-3.75 (m,H-1''',2''',5'''),0.97 (d, J=6 Hz, CH_3-rhamnose)
5	67 (H2O),41(HOAC), 38 (BAW)	255, 268*, 357	478	7.6 (dd, J=2.5 ,8Hz, H-6'), 7.55 (d, J=2.5 Hz, H-2'), 6.86 (d, J= 8Hz, H-5'), 6.45 (d, J=2.5Hz, H-8), 6.2 (d, J=2.5 Hz,H-6), 5.5 (d, J=9Hz,H-1''),3.2, 3.9 (m, glucuronic acid protons hidden by H2O protons signal).
6	72 (H2O),45 (HOAC), 42(BAW)	366, 350.	462	8 (d, J= 8Hz, H-2' and H-6'), 6.84 (d, J= 8Hz, H-3' and H-5'),6.4 (d, J=2.5 Hz, H-8), 6.2 (d, J=2.5 Hz, H-6), 5.48 (d, J=9Hz,H-1''), 3.2, 3.8 (m, sugar protons overlaping with OH protons).

Full Paper

Compd. No.	Rf values (x 100)	UV λ_{max} (nm) MeOH	MS	^{1}H- NMR Spectral Data (DMSO-d_6)δ (ppm)
7	17 (H2O),34 (HOAC), 45 (BAW)	256, 356	463	6.21 (d, J=2.5 Hz, H-6), 6.43 (d, J=2.5Hz, H-8), 7.56 (d, J=2.5 Hz, H-2'), 6.89 (d, J=8 Hz, H-5'), 7.56 (dd, J=2.5 & 8 Hz, H-6'), 5.5 (d, J=8 Hz, H-1''), 3.2-3.8 (m, H-2''-H-6'')
8	22 (H2O), 48 (HOAC), 68 (BAW)	259, 297 sh., 348	448	Quercetin moiety: 6.17 (*d*, J=2.5 Hz, H-6), 6.36 (*d*, J=2.5 Hz, H-8), 7.256 (*d*, J=2.5, H-2'), 6.82 (*d*, J=8 Hz, H-5'), 7.251 (dd, J=2.5 and 8 Hz, H-6') Rhamnose moiety: 5.20 ($\Delta\nu_{1/2}$ = 4 Hz, H-1''), 3.1 – 3.9 (*m*, overlapped with water proton resonances, H-2''-H-6'').
9	66 (H2O), 78 (HOAC), 22 (BAW)	255, 362	-	Glucose moiety: 6.12(d, J=8, H-1), 4.87(t, J=8, H-2), 5.15(t, J=8, H-3), 3.2–3.8 (m, H-4), 3.2-3.8(m, H-5) 3.3-3.8(m, H-6) Galloyl moiety: 7.02 (s, H-2 and H-6). Hexahydroxydiphenoyl: 6.48 (s, H-3), 6.15 (s, H-3').
10	66 (H2O), 78 (HOAC), 22 (BAW)	259 (inflection)	-	Glucosemoiety:.25(*d*, J=3.5, α-H-1'), 4.64 (*d*, J=8 Hz, β- H-1'), 4.44(*dd*, J=8, 7.5, β-H-2'), 4.68 (*dd*, J=3.5 and 8 Hz, α-H-2'), 4.83 (*t*, J=7.5 Hz, β-H-3'), 5.08 (*t*, J=8 Hz, α-H-3') , 3.3 -3.8 (*m*, H-4'-H-6') , Hexahydroxydiphenoyl: 6.32, 6.323, 6.45 and 6.46 (*s*, H-3 and H-3')
11	33 (H2O), 54 (HOAC), 60 (BAW)	278	390	4.51 (*d*, J= 7.3, H-2), 3.84 (*m*, H-3), 2.38 (ax, *dd* J=16.0, 7.9, H-4) 2.68 (eq, *dd*, J= 16.0, 5.3, H-4) 5.90 (*d*, J= 2.2, H-6), 5.72 (*d*, J=2.2, H-8), 6.74 (*d*, J= 1.9, H-2'), 6.7 (*d*, J= 8, H-5'), 6.61(*dd*, J= 8, 1.9 , H-6')
12	25 (H2O), 45 (HOAC), 81 (BAW)	218, 245, 298, 325.	180	6.2 (*d*, J= 16Hz, β-H), 6.76(J=7.5 Hz, 5-H), 6.88 (dd, J=7.5 Hz and J=2.5 Hz, 6-H), 6.98(*d*, J= 2.5Hz, 2-H), 7.48 (*d*, J= 16Hz, α-H).
13	43 (H2O), 45 (HOAC) , 90 (BAW)	266, 310.	302	6.2 (*d*, J= 15Hz, α-H), 6.72(J=8 Hz, 3-H and 5-H), 7.32 (d, J=8 Hz, H-2and, 6-H), 7.52 (*d*, J=15Hz, β-H).
14	0 (H2O), 9 (HOAC), 48 (BAW)	255, 362.	164	7.48 (*s*, H- 5 and H-5')
15	0 (H2O), 7 (HOAC), 75 (BAW)	255, 268, 370	302	6.19 (*d*, *J* =2.5, H-6), 6.4 (*d*, *J* =2.5, H-8), 7.64 (*d*, *J* =2.5, H-2'), 6.88 (*d*, *J* =8.5, H-5'), 7.53 (*dd*, *J* =2.5 &8.5, H-6')
16	0 (H2O), 9 (HOAC), 48 (BAW)	265, 376	318	6.19 (*d*, *J* =2.5, H-6), 6.4 (*d*, *J* =2.5, H-8), 7.64 (*d*, *J* =2.5, H-2'), 6.88 (*d*, *J* =8.5, H-5'), 7.53 (*dd*, *J* =2.5 &8.5, H-6')
17	0 (H2O), 10 (HOAC), 85 (BAW)	268, 369	286	6.4 (*d*, *J*=2.5, H-8), 6.18 (*d*, *J*=2.5, H-6) 8.14 (*d.*, *J*=8, H-2' and H-6'), 6.89 (*d*, *J*=8, H-3' and H-5')

TABLE 2 : Antioxidant activity curve of methanolic and aqueous methanolic extracts *strawberry leaves*.

Methanolic solution (µl) of the substance added to 3ml DPPH	%Inhibition		
	Ascorbic acid	Aqoeous methanolic of *strawberry*	Methanolic of *strawberry*
0	0	0	0
10	92.15	23	49.92
19	98.8	26.16	56.69
38	98.27	44.96	91.42
77	98.35	93.53	94.58
100	98.37	81.5	94.73

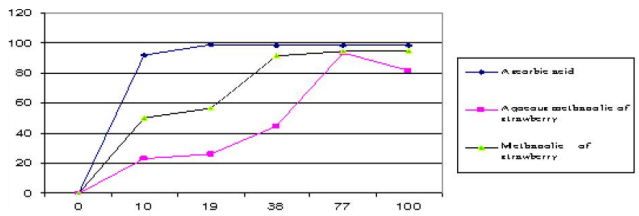

Figure 1 : Antioxidant activity curve of the extracts of *strawberry*.

and ayad[1]. A yoola; reported that, the alcoholic extracts of clove, ginger, peppermint spearmint and thyme were the most effective than aqueous extracts against *E.coli* isolated.

TABLE 3 : Antibacterial activity of methanolic and aqueous methanolic extracts of strawberry leaves.

bacterial strains	Inhibition zone diameter(mm)			
	Methanolic		Aqueous methanolic	
	0.1 ml	0.3ml	0.1 ml	0.3 ml
Pseudomonas aeroginosa	_	10	_	6
Kelbsiella sp.	5	10	2	18
Salmonella typhi	3	10	10	21
Staphyllococcus aureus	_	2	5	15
Escherichia coli	3	8	_	9

Antifungal activity

The two extracts had different antifungal activities *against* the tested fungal strains. Methanolic and aque-

Full Paper

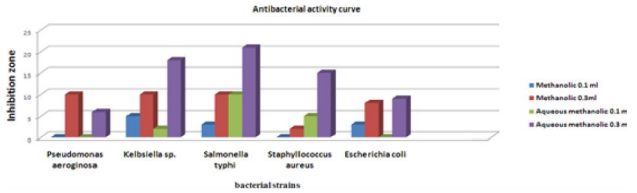

Figure 2 : Antibacterial activity of the methanolic and aqueous methanolic extract of *strawberry*

ous methanolic extracts of *strawberry* leaves showed no inhibitory activity against all species at 0.1 ml concentration, but at 0.3 ml concentration showed inhibitory activity against all species (Figure 3) and (TABLE 4).

TABLE 4 : Antifungal activity of methanolic and aqueous methanolic extracts of *strawberry* leaves.

fungal strains	Inhibition zone diameter (mm)			
	Methanolic		Aqueous methanolic	
	0.1 ml	0.3 ml	0.1 ml	0.3 ml
Fusarium oxysporum	_	5	_	9
Aspergillus niger	_	10	_	10
penicillium sp.	_	8	_	5
Cladosporium sp.	_	5	_	4.5
Aspergillus flavus	_	2	_	3

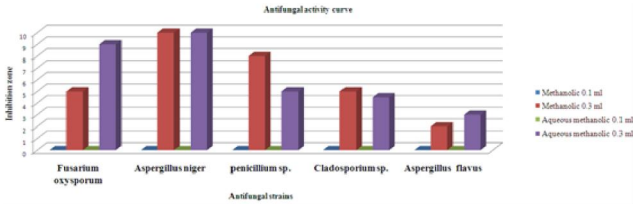

Figure 3 : Antifungal activity curve of the methanolic and aqueous methanolic extract of *strawberry* leaves.

Results of anti tumor activity

The methanolic extract of *strawberry* leaves were tested against three human cell lines [HEPG2 (liver carcinoma cell line), MCF7 (breast carcinoma cell line) and HCT 116 (colon carcinoma cell line)]. The results showed that the extract has activity against all cell lines tested (TABLE 5) and (Figure 4). IC50 of HEPG2= 120 µg / ml, MCF7=170 µg / ml and HCT= 165 µg / ml.

TABLE (5) Cytotoxic activity of the methanolic extract of *strawberry* leaves against colon, breast and liver carcinoma cell lines.

Conc. µg/ml	HCT	MCF7	HEPG2
0.0	1.000000	1.000000	1.000000
50.0	0.882024	0.927359	0.694252
125.0	0.539317	0.591482	0.493754
250.0	0.426975	0.325955	0.211873
500.0	0.411150	0.248703	0.194443

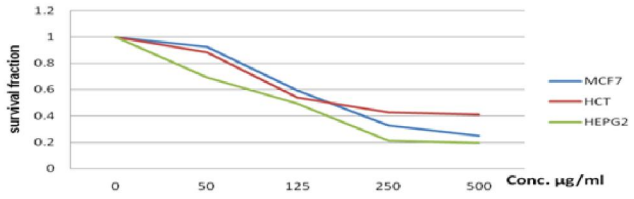

Figure 4 : % of survival fraction of colon, breast and liver carcinoma cell lines against concentration (µg/ml) of methanolic extract of *strawberry* leaves.

CONCLUSION

The results of this study suggest the possibility of using these extracts as natural food preservatives and potential sources of antibacterial, antifungal and antioxidant ingredients for the food and pharmaceutical industry.

REFERENCES

[1] A.Ayad; Pathogens, Medical Science, Faculty of Science, Benha Univerisity, Botany Department, **(2009)**.
[2] F.A.Ayaz, M.Kucukislamoglu, M.Reunanen; Journal of Food Composition and Analysis, **13**, 171 **(2000)**.
[3] G.A.Ayoola, F.M.Lqwore, T.Adelowotan, E.Adenipekun, T.O.Odugbemi; Africa Journal of Microbiology Research, **2**, 162 **(2008)**.
[4] W.Brand-Williams, M.E.Cuvelier, C.Berest; Lebensmittel-Wissenschaft and Technologie, **28**, 25 **(1995)**.
[5] B.R.Cordenunsi, J.R.O.Nascimento, M.I.Genovese, F.M.Lajolo; Journal of Agricultural and Food Chemistry, **50**, 2581 **(2002)**.
[6] J.M.Duarte-Almeida, R.J.Santos, M.I.Genovese, F.M.Lajolo; Ciência e Tecnologia de Alimentos, **26**, 446 **(2006)**.
[7] S.Hakkinen, M.Heinonen, S.Karenlampi, H.Mykkanen, J.Ruuskanen, R.Torronen; Food Research International, **32**, 345 **(1999)**.
[8] J.B.Harborne, C.A.William; Chapman and Hall, London, **(1975)**.
[9] J.B.Harborne; Chapman and Hall, London, **(1982)**.
[10] J.J.Machiex, A.Fleuriet, J.Billot; Boca Raton, FL: CRC, **(1990)**.
[11] J.A.Maga; Food Science and Nutrition, **10**, 323 **(1978)**.

[12] T.J Mabry, K.R.Markham, M.B.Thomas; Springer, New York, **(1969)**.
[13] N.P.Seeram, R.Lee, H.S.Scheuller, D.Heber; Food Chemistry, **97**, 1 **(2006)**.
[14] P.Skehan, R.Storeny; Journal of the National Cancer Institute, **82**, 1107 **(1990)**.
[15] L.A.Terry, D.C.Joyce, N.K.B.Adikaram, B.P.S.Khambay; Postharvest Biology and Technology, **31**, 201 **(2004)**.
[16] S.Tulipani, J.M.Alvarez-Suarez, F.Busco, S.Bompadre, J.L.Quiles, B.Mezzetti, M.Battino; Food Chemistry, **128**, 180 **(2011)**.
[17] Y.K.Vaghasiya, R.Nair, M.Soni, S.Balujs, S.Chanda; Journal Serbian Chemical Socity, **69**, 991 **(2004)**.
[18] H.Wang, G.Cao, R.L Prior; Journal of Agricultural and Food Chemistry, **44**, 701 **(1996)**.