Research Article

In-vitro, Antiviral, Antimicrobial and Antioxidant Potential Activity of *Tomato pomace*

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ABSTRACT

This work aimed to evaluate the efficiency of different organic solvents such as, aqueous, ethanol and acetone for extraction of phenolic, flavonoid and tannin compounds (TPC, TFC and TTC, respectively) from the *Tomato pomace* powdered and their antiviral, antimicrobial and antioxidant activities. The aqueous extract showed the highest ($p \leq 0.05$) values for TPC. While, the acetone extract exhibited the highest values for TFC and TTC. Also, the results revealed that all extracts of the *Tomato pomace* exhibited variable antioxidant activity. Four methods were carried out to determine the antioxidant activity of *Tomato pomace*. *Tomato pomace* acetone extract exhibited greater $DPPH^{\cdot}$ scavenging activity than those extracted by other solvents. The same trend was achieved by using the $ABTS^{\cdot+}$, Fe^{2+}- chelating and reducing power assays. Some natural compounds were identified by HPLC in tomatoes pomace. Benzoic acid showed the major phenolic compounds presented of the *Tomato pomace* extracts. The aqueous *Tomato pomace* extract exhibited strong antimicrobial action in comparison with acetone and ethanol extracts. Increases in the concentration of extract resulted in an increase in its antimicrobial activities. As well, aqueous extract showed high antibacterial activity against gram-positive bacteria and yeast with MIC between 147 and 154 ppm. Aqueous extract had a dire effect on the biosynthesis of protein in cells of *Bacillus subtilis*, but the effect was slightly noted on the biosynthesis of nucleic acid. The results are of grand importance for improving the biological activity, functionality and health impact of *Tomato pomace* extract that could be applied in special food. The three tested *Tomato pomace* extracts were showed moderate anti avian influenza A H5N1 activity with 71% peak activity for acetone extract at 400µg/µl concentration. The obtained results showed that, the solvent play a vital role in the extraction of the plant constituents and *Tomato pomace* is a very promising source of bioactive compounds and can be used or its extracts as antiviral, antimicrobial and antioxidant agent.

Keywords: antiviral, antimicrobial, antioxidant, *Tomato pomace*.

INTRODUCTION

Tomato (*Solanum Lycopersicum* L.) is one of the world's major vegetable with a worldwide and Egypt production of 161.8 and 8.6 million tons, respectively [1]. Tomato is one of the most widely cultivated vegetable crops in Mediterranean countries. Tomatoes constitute the predominant source of lycopene and phenols in the Egyptian diet because of high utility in Egyptian culinary preparations and their cheap price. It is an excellent source of many nutrients and secondary metabolites that are important for human health; minerals, vitamin C and E, s-carotene, lycopene, flavonoids, organic acids, phenolics and chlorophyll [2, 3].Most of the food by-products usually represent an environmental problem for the industry, and many studies have been carried out about the potential utilization of several vegetable origin byproducts for their inclusion in the human diet, which could reduce industrial costs and justify new investments in equipment, providing a correct solution for the pollution problem connected with food processing [4, 5]. *Tomato pomace* is the mixture of tomato peels, crushed seeds and small amounts of pulp that remains after the processing of tomato for juice, paste and ketchup [6]. Dried *Tomato pomace* contains 22.6 - 24.7 % protein, 14.5 – 15.7% fat and 20.8 – 23.5% fiber and this by-product is a good source of vitamin B1, B2 and A [7]. In addition, *Tomato pomace* was reported to contain essential amino acids and the tomato seeds had higher amounts of minerals (Fe, Mn, Zn and Cu). Tomato peel contains high levels of lycopene compared to the pulp and seeds.[8] The majority of the flavonoids in tomatoes are present in the skin[9]. Several studies have revealed their antimicrobial, antithrombotic, antimutagenic, and anticarcinogenic activities [10, 11]. The antioxidants are isolated by solvent extraction and both extraction yield and antioxidant activity of the extracts are strongly dependent on the solvent, due to the variant antioxidant potential of compounds with different polarity. Solvents of higher polarity (ethanol or ethanol–water mixtures) additionally can extract flavonoid glycosides and higher molecular weight phenols, resulting higher yields of total extracted polyphenols. However, that can range in size from monomers to long-chain polymers such as tannins. The antimicrobial compounds from plants are of interest because antibiotic resistance is becoming a worldwide public health concern especially in terms of food-borne illness and nosocomial infections [12, 13, 14, 15]. Naturally occurring antimicrobials are being sought as replacements for synthetic preservatives such as parabens, butylatedhydroxytoluene (BHT) and butylatedhydroxanisole (BHA) that are under scrutiny as

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suspected cancer causing agents[16, 17]. Plants produce a massive amount of organic compounds that have antimicrobial activity. The compounds are found in various plant parts such as stems, roots, leaves, bark, flowers or fruits and seeds and include alliin/allicins, isothiocyanates, pigments, proteins, essential oils and phenolic compounds[18, 19].

Therefore, the objective of this study was to evaluate the efficiency of different organic solvents such as, aqueous ethanol, and acetone for extraction of (polyphenolic, flavonoids and tannins compounds (TFC, TPC and TTC respectively) from the *Tomato pomace* powdered and *in-vitro* evaluation their antiviral, antimicrobial and antioxidant activities.

MATERIALS AND METHODS

Chemicals

$ABTS^+$ (2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), Folin–Ciocalteau reagents, Gallic acid, Quercetin, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, Ferrozine:

(3- (2 - pyridyl) - 5, 6- bis- (4-phenylsulfonic acid)-1, 2, 4-triazine, BHT: Butyl Hydroxy toluene and, potassium ferricyanide, were from (Sigma Chemical Co., St. Louis, MO, USA).

Preparation of plant extract

The *Tomato pomace* was extracted. Briefly, 10 g of the dried powder from pomace was soaked with 100 ml of water, 80% ethanol and 80% acetone and shaking at room temperature for 48 h. The extracts were filtered and the extraction was repeated twice. The resulting of different extracts was used for the determination of total phenolic, flavonoid, total tannins, antioxidant, antimicrobial and antiviral activities.

Total phenolic content

The total phenolic (TP) of *Tomato pomace* extracts were spectrophotometrically determined by Folin Ciocalteu reagent assay using gallic acid for the preparation of calibration curve (20 – 120 mg/L) [20].A suitable aliquot (1 ml) of each extract or standard solution was added to 25 ml volumetric flask, containing 9 ml of distilled water. One milliliter of Folin Ciocalteu`s phenol reagent was added to the mixture and shaken. After 5 min. 10 ml of 7 % Na_2CO_3 solution were added to the mixture. The solution was diluted to 25 ml with distilled water and mixed. After incubation for 90 min. at room temperature, the absorbance was determined at 750 nm with spectrophotometer (Unicum UV 300) against prepared reagent as blank. A total phenolic content in samples was expressed as mg gallic acid equivalents (GAE)/g dry weight. All samples were analyzed in triplicates.

Total flavonoid content

Total flavonoid (TF) of *Tomato pomace* extracts were spectrophotometrically determined by the aluminum chloride method using quercetin as a standard [21].One ml of extract or standard solution (quercetin, 20–120 mg/L) was added to 10 ml volumetric flask, containing 4 ml of distilled water. To the flask 0.3 ml 5 % $NaNO_2$ was added and after 5 min 0.3 ml 10 % $AlCl_3$ was added. At 6th min, 2 ml 1M NaOH were added and the total volume was made up to 10 ml with distilled water. The solutions were mixed well and the absorbance was measured against prepared reagent blank at 510 nm by using spectrophotometer (Unicum UV 300). Total flavonoids in sample were expressed as mg quercetin equivalents (QE)/ g fresh weight. Samples were analyzed in triplicates.

Total tannins content

Total tannins (TT) of *Tomato pomace* extracts were measured using the Folin-Ciocalteu reagent assay [22]. One ml of *Tomato pomace* extracts or standard solution of (tannic 20-120 mg/L) was added to 7.5 ml distilled water (dH_2O) then add 0.5 ml of Folin reagent and 1 ml of 35% sodium carbonate solution. The volume was made up for 10 ml with distilled water and absorbance was measured against prepared reagent blank at 775 nm by using spectrophotometer (Unicom UV 300). Total tannins in sample were expressed as mg tannic acid equivalent (TE)/g dry weight. All samples were analyzed in triplicates.

Antioxidant activity

DPPH· Free radical scavenging assay

Determination of DPPH· (2, 2-diphenyl-1-picryl hydrazyl) free radical scavenging activity was measured spectrophotometrically[23]. 0.1 mM of DPPH·in methyl alcohol was prepared and 0.5 ml of this solution was added to 1 ml of pomace extracts at different concentrations (25, 50, 75, 100 µg/ml). Methanol was used as blank. The mixture was shaken vigorously and allowed to stand at room temperature. Butyl Hydroxytoluene (BHT, Sigma) was used as positive control; and negative control contained the entire reaction reagent except the extracts. Then the absorbance was measured at 515 nm against blank. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capacity to scavenge the DPPH·radical was calculated using the following equation:

DPPH· scavenging effect (Inhibition %) =

$[(A_c – A_s / A_c) \times 100]$

Where:

A_c was the absorbance of the control reaction.

A_s the absorbance in the presence of the plant extracts.

Metal chelating activity

Metal chelating effects on ferrous ions was carried out colorimetrically[24]. One ml of pomace extracts, or EDTA solution as a positive control at different concentrations (25, 50, 75, 100 µg/ml) were spiked with 0.1 ml of 2 mM $FeCl_2$- $4H_2O$ and 0.2 ml of 5 mM ferrozine solution and 3.7 ml methanol were mixed in a test tube and reacted for 10

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min, at room temperature then the absorbance was measured at 562 nm. Mixture without extract was used as the control. A lower absorbance indicates a higher ferrous ion chelating capacity.

The percentage of ferrous ion chelating ability was calculated using the following equation:

Iron chelating activity (Inhibition %) =

$[(Ac - A_S / Ac) \times 100]$

Where Ac was the absorbance of the control reaction and as the absorbance in the presence of the plant extracts.

Determination of scavenging activities on $ABTS^{\bullet+}$ radicals

$ABTS^{\bullet+}$ assay was generated byoxidation of $ABTS^{\bullet+}$ with potassium persulphate[25]. $ABTS^{\bullet+}$ was dissolved in deionized water to 7.4 Mm concentration, and potassium persulphate added to a concentration of 2.6 Mm. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12-16 h at room temperature in the dark. The solution was then diluted by mixing 1mL $ABTS^{\bullet+}$ solution with 60 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using the spectrophotometer. Fresh $ABTS^{\bullet+}$ solution was prepared for each assay. *Tomato pomace* extracts (150 µl) at different concentration (25, 50, 75, 100 µg/ml) were allowed to react with 2850 µl of the $ABTS^{\bullet+}$ solution for 2 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. Results were expressed as in comparison with standard BHT. A bigger antioxidant capacity of the sample exhibited a smaller production of free radicals.

Percent activity was calculated using the equation:

$\%\ \text{Inhibition} = [(A_0 - A_1) / A_0] \times 100\ \%$

Where: A_0 is the $ABTS^{\bullet+}$ absorbance of the control reaction

A_1 is the $ABTS^{\bullet+}$ absorbance in the presence of the sample

Reducing power

The reducing power was assayed spectrophotometrically[26]. Different concentrations (25, 50, 75, 100 µg/ml) of *Tomato pomace* extracts (1.0 ml) were mixed with 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 °C for 20 min. After, 2.5 ml of trichloroacetic acid (10 %) were added to the mixture, centrifuged at 3000 rpm for 10 min. Finally, 1.25 ml from the supernatant was mixed with 1.25 ml of distilled water and 0.25 ml $FeCl_3$ solution (0.1%, w/v). The absorbance was measured at 700 nm. The assays were carried out in triplicate and the results were expressed as mean values ± standard deviations. Increased absorbance values indicate a higher reducing power. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against the extract concentration. BHT was used as standard.

Antimicrobial activity

Different bacteria, including gram positive (*Bacillus subtilis* NRRL B-94, *Staphylococcus aureus* NRRL B-313), gram negative (*Escherichia coli* NRRL B-3703, *Pseudomonas aeruginosa* NRRL B-32), *Aspergillus niger* NRRL 599, *Aspergillis fluves*NRC, *Candida albicans* NRRL 477 and *Saccharomyces cerviciea* strains, were used, wherein the measurement of growth inhibition was carried out as previously described [27].

Procedure

Cells from cultures grown on nutrient slopes were inculated using a sterile loop into fresh nutrient broth and incubated overnight at 30 ºC. One mL of each culture were transferred to Petri dishes (120 mm in diameter) to which 50 mL molten nutrient agar (45 ºC) was added. Wells of 6 mm diameter were then mode in the solidified agar using proper sterile tubes. Plates were undisturbed for 30 min to allow diffusion of the sample (100, 200, 300 l) into the agar, then incubation inverted at 30 ºC for 48 h for bacteria and 72 h for fungi. Inhibition zones were measured, wherein the evaluation of antibacterial activity was carried out in triplicate with three replicates on each sample.

Determination of minimal inhibitory concentration (MIC)

The culture medium (25 ml) was poured into Petri dishes (9 cm in diameter) and maintained at 45°C until the samples were incorporated into the agar. The samples were added as 100, 150, 200 and 300 µg/mL. The different microbial strains were layered by using an automatic micropipette to place 30 µL over the surface of the solidified culture medium containing a sample. After the microorganisms were absorbed into the agar, the plates were incubated at 30°C for 24–48 h. MIC was determined as the lowest concentration of sample inhibiting the visible growth of each organism on the agar plate [28].

Mode of action

The effects of different concentrations of the aqueous extract on some biochemical activities were studied. Immediately after incubating flasks with *B. subtilis*, cells were harvested during the middle logarithmic growth phase, and aqueous extract was applied in concentrations of 1/4 and 1/2 MIC. Each test was repeated three times. Subsequently, the flasks were shaken using a rotary shaker of 150 rpm at 30 °C. Samples were withdrawn at onset of the experiment and after incubation periods of 24, 48, 72, 96, 120 and 144 min. The bacterial cells were subjected to the following determinations: total acid-soluble phosphorus compounds [29, 30], total lipids [31, 32], total soluble protein[33, 34], and total nucleic acids [35].

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Antiviral Bioassay

MTT cytotoxicity assay (TC_{50})

Extracts were diluted with Dulbecco's Modified Eagle's Medium (DMEM). Stock solutions of the test extracts were prepared in 10 % DMSO in ddH2O. The cytotoxic activity of the extracts were tested in Madin Darby Canine kidney (MDCK) cells by using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method with minor modification [36]. Briefly, the cells were seeded in 96 well-plates (100 µl/well at a density of 3×105 cells/ml) and incubated for 24 hrs at 37°C in 5% CO_2. After 24 hrs, cells were treated with various concentrations of the tested extracts in triplicates. After further 24 hrs, the supernatant was discarded and cell monolayers were washed with sterile phosphate buffer saline (PBS) 3 times and MTT solution (20 µl of 5 mg/ml stock solution) was added to each well and incubated at 37 °C for 4 hrs followed by medium aspiration. In each well, the formed formazan crystals were dissolved with 200 µl of acidified isopropanol (0.04 M HCl in absolute isopropanol = 0.073 ml HCL in 50 ml isopropanol). Absorbance of formazan solutions were measured at λmax540 nm with 620 nm as a reference wavelength using a multi-well plate reader. The percentage of cytotoxicity compared to the untreated cells was determined with the following equation.

$$\% \text{ Cytotoxicity} = [(A_0 - A_t) / A_0] \times 100$$

Where: A_0 is the absorbance of cell without treatment

A_t is the absorbance of cell with treatment

The plot of % cytotoxicity versus sample concentration was used to calculate the concentration which exhibited 50% cytotoxicity (LD50).

Plaque reduction assay

Assay was carried out according to the method of [37]in a six well plate where MDCK cells (10^5 cells / ml) were cultivated for 24 hrs at 37°C. A/CHICKEN/M7217B/1/2013 (H5N1) virus was diluted to give 10^4 PFU/ well and mixed with the safe concentration of the tested extracts, and incubated for 1 hour at 37°C before being added to the cells. Growth medium was removed from the cell culture plates and the cells were inoculated with (100 µl / well) virus, After 1 hour contact time for virus adsorption, 3 ml of DMEM supplemented with 2% agarose and the tested compounds was added onto the cell monolayer, plates were left to solidify and incubated at 37°C till formation of viral plaques (3 to 4 days). Formalin (10%) was added for two hours then plates were stained with 0.1 %crystal violet in distilled water. Control wells were included where untreated virus was incubated with MDCK cells and finally plaques were counted and percentage reduction in plaques formation in comparison to control wells was recorded as following

% inhibition = viral count (untreated)-viral count (treated)/viral count (untreated) x100

HPLC Identification

Identification of phenolic compounds of *Tomato pomace* extracts was performed using HPLC system (Agilent 1100 series) coupled with UV-Vis detector (G1315B) and (G1322A) DEGASSER. Sample injections of 5 µl were made from an Agilent 1100 series auto-sampler; the chromatographic separations were performed on ZORBAX-EclipseXDB-C18 column (4.6×250 mm, particle size 5 µm). A constant flow rate of 1 ml /min was used with two mobile phases: (A) 0.5% acetic acid in distilled water at pH 2.65; and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear starting with (A) and ending with (B) over 50 min, using an UV detector set at wavelength 280 nm [38]. Phenolic compounds of each extracts was identified by comparing their relative retention times with those of the standard mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements, and then converted to µg phenolic / g dry weight. All chemicals and solvents used were HPLC spectral grade, and obtained from Sigma (St. louis, USA) from Merck – Shcuchrdt (Munich, Germany).

Statistical analysis

Data were statistically analyzed using Costat statistical package [39].

RESULTS AND DISCUSSION

Phenolics, Flavonoids and Tannins contents

Data of polyphenolic contents of *Tomato pomace* samples are presented in Table 1. Results reveal that the total phenols in 80% acetone extracts were (2.50 mg GA/g) lower than in aqueous (6.47mg GA/g) and ethanol 80% (5.49 mg GA/g). The highest concentration of total phenolic was found in aqueous extract. The extractive capacity of phenolic components from pomace material is considerably depend on the type of solvents.

The best extraction efficiency was achieved by aqueous and 80 % ethanol whereas acetone resulted in poor phenolic contents. The technique of phenolic isolation from a plant material, including the methods and type of extracting solvent, depends generally on the type of phenolic compound and the solvents [40].

The yields of flavonoids and tannins obtained by using various extractants (solvents) are shown in Table 1. The highest content of flavonoids was obtained with acetone 80% (5.22 mg Q/g) and the lowest one with aqueous (2.76 mgQ/g). The same trend was observed with tannins. The highest value was gained with 80% acetone (3.38 mg/g) and the lowest value (2.95 mg/g) was observed with aqueous extract.

This study showed that extracts obtained using different solvents, had different total phenolic ,total flavonoids and total tannins contents . The differences observed could be related to the polarity of particular solvent used in the extraction. From these results, the content of phenolic

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followed the following order: aqueous > ethanol > acetone 80%. This may be attributed to higher polarity of aqueous extract for phenolic components extraction [41]. This indicates that phenolic compounds of *Tomato pomace* are better extracted with aqueous than all the other solvents tested.

The total phenolics content of *Tomato pomace* (2.50: 6.47 mg/g) was less than (182.78 mg/g) which obtained by [42]. The content of total phenolic compounds of *Tomato pomace* was 6.47 mg GAE/g showing a higher content than those reported in other fruits; for example, orange (*C. aurantium cv. Canoneta*) (0.51 mg GAE/100 g) [43], orange (*C. sinensis cv. Valencia*) (0.16 mg GAE/100 g), and lime (*C. aurantifolia cv. Persa*) (0.35 mg GAE/100 g).[44]

The differences between our results of Total Phenolics, Flavonoids and Tannins contents and other investigators may be attributed to plant species, environmental condition and sample preparation. The differences in phenolic content could be related to the part of fruit used for making the extract and type of solvent. Phenolics might tend to accumulate in the dermal tissues of plant body due to their potential role in protection against UV radiations, acting as attractants in fruit dispersal, and as defense chemicals against pathogens and predators [45].

In-vitro Antioxidant activity

DPPH· scavenging activity of Tomato pomace

DPPH· free radical test is based on the exchange of a proton between the antioxidant and the stable DPPH· free radical and shows absorption at 515 nm. In principle, the reaction brings about the reduction of DPPH· radicals to the corresponding hydrazine, which can be observed by a rapid color change from purple to yellow and can be monitored spectrophotometrically[46]. The decrease in absorbance of DPPH· radical is caused by antioxidant through the reaction between antioxidant molecule and radical results in the scavenging of the radical by hydrogen donation [47].The results for 80 % acetone, 80% ethanol, and aqueous extracts are shown in (Table 2).

Table 1: Total Phenolics, Flavonoids and Tannins contents of tomato pomace extracts

Extracts	Phenolics (mg/g)	Flavonoids (mg/g)	Tannins (mg/g)
Aqueous	6.47 ^{c} ± 0.09	2.76^{a} ± 0.06	2.95 ^{a} ± 0.04
Ethanol 80%	5.69 ^{b} ± 0.07	4.43 ^{b} ± 0.14	3.16^{b} ± 0.09
Acetone 80%	2.50 ^{a} ± 0.14	5.22 ^{c} ± 0.10	3.38 ^{c} ± 0.06
LSD at 0.05	0.29	0.26	0.15

All values with the same letters are not significantly different at p≤ 0.05

Table 2: IC_{50} of Tomato pomace with different solvent extracts

Extracts	Inhibition %				IC_{50} µg/ml
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	
Aqueous	6.21 ^{a} ± 0.60	19.37 ^{a} ± 0.90	30.79 ^{a} ± 0.61	38.81 ^{a} ± 0.31	106.42 ^{d} ± 0.59
Ethanol 80%	12.76 ^{b} ± 0.61	23.91 ^{b} ± 0.83	38.81 ^{b} ± 0.50	51.17 ^{b} ± 0.93	95.20 ^{c} ± 0.82
Acetone 80%	18.34 ^{c} ± 0.65	31.26 ^{c} ± 0.92	47.90 ^{c} ± 0.80	57.11 ^{c} ± 0.60	54.85 ^{b} ± 1.46
BHT standard	57.62 ^{d} ± 0.37	73.04 ^{d} ± 0.40	86.38 ^{d} ± 0.49	96.12 ^{d} ± 0.50	7.61^{a} ± 0.33
LSD at 0.05	1.23	1.79	1.24	1.44	1.83

All values with the same letters are not significantly different at p≤ 0.05

The scavenging ability of all extracts on DPPH· radicals exhibited concentration dependence. Their scavenging capacity increased with increase in concentration. Eighteen percent of acetone extract exhibited the highest DPPH· scavenging activity, followed by 80% ethanol, and aqueous.

The values obtained were 57.11, 51.17 and 38.31% antioxidant radical scavenging activities respectively at a concentration of 100µg/ml. In addition, the acetone extract had the highest scavenging activity at all concentrations. Statistical analysis revealed that the difference between the 80% acetone extract and the other samples was significant ($p \leq 0.05$). Similar results were also found with respect to IC_{50}values as indicated in (Table 2).

Acetone had the least value of IC_{50} (54.85 µg/mL), followed by ethanol (95.20 µg/mL), aqueous (106.42 µg/mL). A lower IC_{50}value means better efficiency of antioxidant activity of the sample. From these results acetone extract was the most potent solvent. The same observation for its ability to scavenge $ABTS^{\cdot+}$(Table 4) was observed than of the other solvent extracts. It could be attributed that the phenolic compounds in different extracts had different mechanism against diverse free radicals scavenging activity. These results also demonstrated that 80% acetone might be a more suitable solvent for extracting antioxidants from *Tomato pomace* powder.

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In-vitro Fe^{2+}-chelating activity of Tomato pomace

Iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. In this direction, the ability of substances or plant extracts to chelate iron can be important mechanism for antioxidant property [48]. Therefore assessed the ferrous ion chelating capacity of extracts by measuring Fe^{+2}-ferrozine test system. The results are expressed as EDTA an equivalents, which is known as an excellent chelator and summarized in (Table 3).

It is clear that chelating powers of ethanol and aqueous extracts were higher as compared to acetone extracts.

It is clear that chelating powers of ethanol and aqueous extracts were higher as compared to acetone extracts. Ethanol extract of *Tomato pomace* at 100 µg/ml had the strongest metal chelating activity (26.88 %) followed by aqueous extract (23.19 %) while acetone extract of *Tomato pomace* at the same concentration showed lowest activity (19.54 %) among the extracts tested. In this case, this may indicate the presence of non-phenolic antioxidants, such as ascorbic acid and citric acid responsible for metal chelation.[49]

In-vitro $ABTS^{\cdot+}$ radical scavenging activity of Tomato pomace

$ABTS^{\cdot+}$ scavenging activities of *Tomato pomace* extracts are presented in (Table 4). The results indicated that *Tomato pomace* extracted by three different solvent have ABTS radical scavenging activity at the concentrations of 25 - 100 µg/mL. There was a significant ($p \leq 0.05$) in the concentration of $ABTS^{\cdot+}$ due to the scavenging capacity of each sample: acetone, aqueous and ethanol extract. The scavenging effect on ABTS radical was in the following order: 80% acetone > aqueous > 80% ethanol and the values were 53.32, 46.03 and 42.64 %, respectively at a concentration of 100 µg/mL. Similar to $DPPH^{\cdot}$ results, the highest $ABTS^{\cdot+}$ scavenging activity was in the acetone extracts. Similar results were also found with respect to IC_{50} values (Table 2).

Table 3: Fe^{2+}- chelating activity of tomato pomace extracts

Extracts	Inhibition %			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Aqueous	11.41 ^{b} ± 0.29	15.38 ^{b} ± 0.35	20.90 ^{b} ± 0.37	23.19 ^{b} ± 0.21
Ethanol 80%	13.79 ^{c} ± 0.35	18.09 ^{c} ± 0.42	23.70 ^{c} ± 0.37	26.88 ^{c} ± 0.21
Acetone 80%	4.21 ^{a} ± 0.28	13.65 ^{a} ± 0.35	17.11 ^{a} ± 0.42	19.54 ^{a} ± 0.35
EDTA standard	73.29 ^{d} ± 0.29	81.62 ^{d} ± 0.30	85.52 ^{d} ± 0.47	90.78 ^{d} ± 0.34
LSD at 0.05	0.70	0.91	0.89	0.62

All values with the same letters are not significantly different at p 0.05

Table 4: $ABTS^{\cdot+}$ scavenging capacity of tomato pomace extracts

Extracts	Inhibition %			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Aqueous	21.07 ^{b} ± 0.70	30.30 ^{b} ± 0.43	38.38 ^{b} ± 0.33	46.03 ^{b} ± 0.82
Ethanol 80%	18.18 ^{a} ± 1.52	25.76 ^{a} ± 0.57	33.98 ^{a} ± 0.57	42.64 ^{a} ± 0.43
Acetone 80%	25.47 ^{c} ± 0.54	35.14 ^{c} ± 0.54	45.02 ^{c} ± 0.22	53.32 ^{c} ± 0.90
Trolox	76. 98 ^{d} ± 0.38	84.39 ^{d} ± 0.18	87.37 ^{d} ± 0.41	95.14 ^{d} ± 0.41
LSD at 0.05	1.44	0.92	0.85	1.50

All values with the same letters are not significantly different at p≤ 0.05

In-vitro Reducing power of Tomato pomace

The presence of antioxidants caused the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form and the yellow color of the test solution changed to various shades of green and blue depending on the reducing power of each compound. Figure 1 shows the reducing power of different solvent extracts at different concentrations from the *Tomato pomace*.

Different solvent extracts showed significant ($p \leq 0.05$) differences in their reducing power, indicating that *Tomato pomace* reducing power can also be influenced by the type of extraction solvent. The reducing power of different solvent extracts increased with increase in amount of sample concentration (25, 50, 75 and 100 µg/mL). The reducing power of the different extracts was in the following order: 80% acetone > 80% ethanol and aqueous.

This data indicates that *Tomato pomace* extracts are capable of donating electrons, which can react with free radicals to convert them to stable products and strongly inhibit radical chain reaction. These results were previously recorded [50, 51] indicating that the reducing power of bioactive compounds is associated with

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antioxidant activity. Thus, it is necessary to determine the reducing power of phenolic constituents to elucidate the relationship between their antioxidant effects and the reducing power[51].

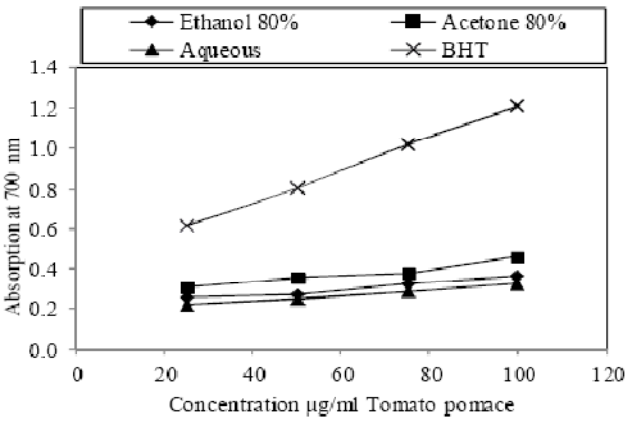

Figure 1: EC_{50} of *Tomato pomace* extracts

In vitro Antimicrobial activity

Phenolic compounds disturb cell membranes, denature proteins, and inactivate enzymes. They are used as surface disinfectants and to demolish discarded cultures because organic materials do not reduce their antimicrobial actions [52]. It has been demonstrated that phenolic compounds present in pomace extracts, such as Ellagic, Gallic, Pyrogallol and Benzoic, have ability to inhibit the growth of a range of bacteria and fungi [53, 54]. The *in-vitro* antimicrobial activity of aqueous, ethanol and acetone extracts of pomace were evaluated by agar diffusion method using different microbial species, including gram-negative bacteria, gram-positive bacteria, yeasts, and fungi. The examination of antimicrobial activity of the aqueous, ethanol and acetone extracts inhibited the growth of all microorganisms tested (Table 5).

The aqueous extract was more active against bacteria, fungi and yeasts than the ethanol and acetone extracts. The maximum inhibition zone obtained with 600 ppm was that against *St. aureus* (20.23 mm). On the other hand, the ethanol and acetone extracts led to the highest inhibition of gram-positive bacteria, wherein the inhibition zones were between 18.61 mm and 18.20 mm at the concentration of 600 ppm. The minimum inhibitory concentrations (MIC) are shown in Table (6).

Table 5: Antimicrobial activity of tomato pomace extracts

Extracts	Concentration µg/ml (ppm)	Diameter of Inhibition (mm)							
		Bacteria				Fungus		Yeast	
		B. subtilis	*St. aureus*	*E. coli*	*P. aueginosa*	*A. niger*	*A. fluves*	*S. cervisiae*	*C. albicans*
Aqueous	200	08.22	09.43	00.00	00.00	00.00	00.00	08.00	09.44
	400	13.80	15.00	13.17	12.00	11.43	10.65	13.88	14.68
	600	19.46	20.23	18.56	16.66	15.33	14.15	17.88	17.66
Ethanol	200	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
	400	12.83	12.50	00.00	00.00	11.66	11.37	10.55	10.67
	600	17.64	17.46	00.00	00.00	15.88	14.87	15.36	14.70
Acetone	200	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
	400	12.62	13.31	10.85	09.64	12.70	12.34	11.20	11.18
	600	18.61	18.20	15.66	14.55	16.75	17.52	16.10	15.65

Table 6: Minimum inhibition concentration (MIC) of tomato pomace extracts

Extracts	MIC (ppm)							
	Bacteria				Fungus		Yeast	
	B. subtilis	*St. aureus*	*E. coli*	*P. aueginosa*	*A. niger*	*A. fluves*	*S. cervisiae*	*C. albicans*
Aqueous	150	165	220	237	286	290	147	154
Acetone	325	320	345	315	350	340	335	340

The data indicate that the aqueous extract exhibited the activity against the investigated food pathogens. Gram-positive bacteria *St. aureus* and *B. cereus* demonstrated higher vulnerability than gram-negative bacteria *E. coli* and *P. aeruginosa*. An important information is that the aqueous extract showed antibacterial activity against *St. aureus* (MIC = 165 ppm) and *B. cereus* (MIC = 150 ppm).On the other hand, a weak antimicrobial activity was found against *E. coli* (MIC = 220 ppm) and *P. aeruginosa* (MIC = 237 ppm). Phenolic compounds e.g. carvacrol, oxygenated derivatives and its precursor's *p*-cymene and *γ*-terpinene possess high levels of antimicrobial activity [55, 56]. It seemed that phenolic

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compounds were more active against gram-positive bacteria than gram-negative bacteria [57].

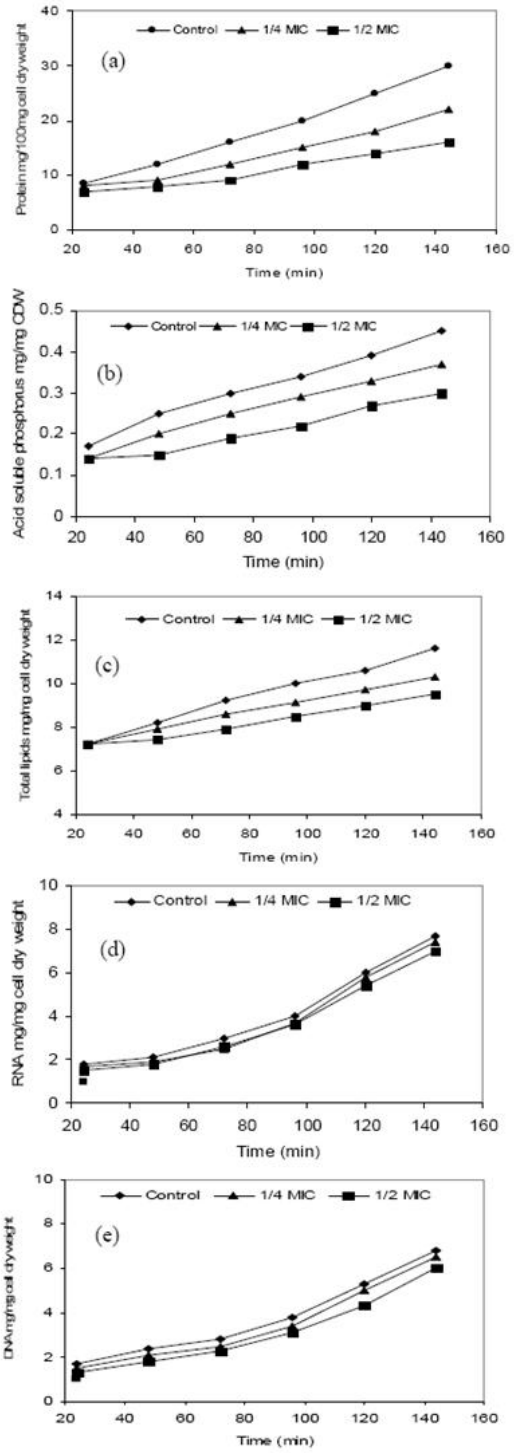

Figure 2: Effect of aqueous extract on total protein (a), acid soluble phosphorus (b), total lipid (c), RNA weight (d) and DNA weight (e) of *B. subtilis* NRRL B-94

The antimicrobial activities of phenolic compounds from different plant sources have been studied [58]. These compounds play an important role in fruits protection against pathogenic microorganisms [59, 60]. The mixture of phytochemical constituents in plant extracts can be an advantage due to the synergistic effect that the constituents may have[61]. The impact of different concentrations of pomace extract on the biosynthesis of total acid-soluble phosphorus compounds, total lipids, total protein and total nucleic acids (DNA and RNA) in the cells of *B. subtilis* was studied, and the data are presented in Figure 2 (a, b, c, d and e)**.** It was found that pomace extract had a strong effect on the biosynthesis of protein and total lipids in cells of *B. subtilis* Figure 2 (a and b)**.** This effect increased with increasing the concentration and incubation period. On the other hand, pomace extract had an insignificant impact on the biosynthesis of acid-soluble phosphorus and nucleic acids (DNA and RNA) in the cells of *B. subtilis* Figure 2 (c, d & e)**.** These results indicated that pomace extract greatly affect the biosynthesis of proteins by inhibiting some steps in the complex process of translation. On the other hand, some drugs affect the synthesis of DNA and/or RNA, so that their messages cannot be read. The majorities of these drugs are undiscerning and affect animal and bacterial cells alike; thus, these drugs have no therapeutic applications [62]. Most of the studies on the mechanism of phenolic compounds focused on their effects on cellular membranes, altering their function and in some instances their structure, causing swelling and increasing their permeability. The increases in cytoplasmic membrane permeability appear to be a consequence of the loss of the cellular pH gradient, decreased ATP levels, and the loss of the proton motive force, which lead to cell death[63].

Antiviral activity

The three tested extracts were highly safe to start antiviral bioassay (Figure 3 and Table 7). These extracts were tested against AIV H5N1 (A / chicken / Egypt / M7217B / 2013(H5N1)).

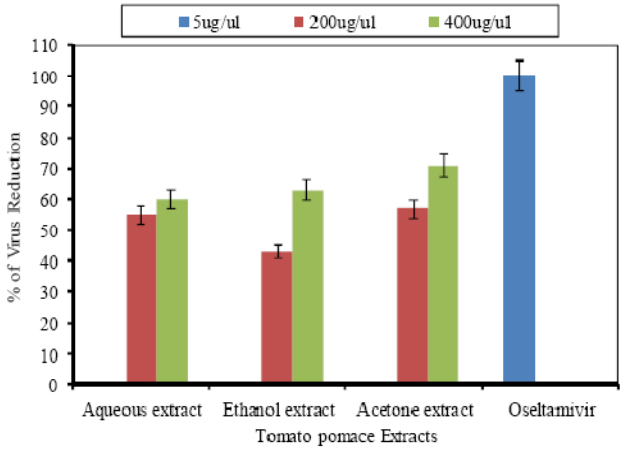

Figure 3: Cytotoxicity and virus reduction percentage of the tested Extracts.

The obtained results were not categorized the extracts as promising materials for antiviral bioactivity where the maximum reduction percentage was 71 at concentration of 400 µg/µl of the acetone extract of *Tomato pomace*. Further fractionation of the extract may give good results in one or more fractions.

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Table 7: Cytotoxicity and virus reduction percentage of the tomato pomace extracts

Extracts	Cytotoxicity TC_{50}	Conc. µg/µl	Initial viral count	Viral count (PFU/ml)	Inhibition %
Aqueous	725 µg/µl	200	$10 X 10^6$	$4.5 X 10^6$	55
		400		$4 X 10^6$	60
Ethanol	579µg/µl	200	$7 X 10^6$	$4 X 10^6$	43
		400		$2.6 X 10^6$	63
Acetone	822µg/µl	200	$7 X 10^6$	$3 X 10^6$	57
		400		$2 X 10^6$	71
Oseltamivir		5	$10 X 10^6$	0	100

Identification of phenolic compounds of *Tomato pomace*

It is obvious that the total phenolic content measured by the Folin-Ciocalteu procedure does not give a full picture of the quality or quantity of the phenolic constituents in the extracts as reported in literature [64, 65]. Various factors affect HPLC analysis of phenolics, including sample purification, mobile phase, column types and detectors [66].The HPLC analysis of the phenolic compounds in different extracts (Aqueous, 80% and 80 % acetone were employed using the previous condition and were compiled in Table 8. Several phenolic compounds, which are representative of the diverse structural types, were identified. Table 8 reveals that the aqueous extract from red tomatoes pomace had the highest amounts of benzoic, pyrogallol and ellagic acids (126.215, 19.855 and 18,436mg/100g respectively). The 80 % ethanol pomace extract had the highest amount of benzoic, ellagic, gallic and chlorogenic acid (142.516, 23.407, 12.907 and 10.283 mg/100g respectively). For other analyzed phenols, such as ellagic, gallic and pyrogallol higher contents (83.99, 17.07and 22.397 mg/100g) were gained with acetone 80% than with ethanol 80% (23.047, 12.097 mg/100g) and (18.436, 9.257 and 19.855mg/100g).

Table 8: Phenolic compounds of *Tomato pomace* extracts (mg/100 g DW)

Phenolic compounds (mg/100g DW)	Aqueous	Ethanol 80%	Acetone 80%
Ellagic	18.436	23.407	83.993
Tannic acid	1.321	4.329	3.449
Gallic	9.257	12.907	17.072
Chlorogenic	1.803	10.283	6.739
Pyrogallol	19.855	-	22.397
Vanillic	-	1.940	-
Genistein	-	-	1.079
Benzoic	126.215	142.516	140.576
Acacetin	0.113	1.096	1.252

It was observed that vanillic and genistein not detected with aqueous extract, whereas, genistein not detected in aqueous and 80% ethanol extract. The individual phenolics extraction from *Tomato pomace* depended on the use of solvent[67]. The extraction yield of phenolic compounds is greatly depending on the solvent polarity[68,69]. It has been reported that aqueous solutions of ethanol, methanol was better than a pure compound solvent system for the extraction of phenolics compound from Muscadine seed[70].

Also, it have been reported that the individual phenolics and flavonoids content are strongly depend on the type of the solvent as well as on the different concentrations used[69]. It has been showed that70% solvent-water extract was more efficient and contained more complex mixtures of phenolic compounds than the pure solvent extract [68].Many authors established that the extraction yield of phenols is greatly depending on the solvent polarity [67, 69, 71].In contrast it has been found that, ethanol and methanol were more effective than water for phenols from peanut skin[71]. Meanwhile, the methanol was the best for pine sawdust, while in almond hulls ethanol was the best extraction solvent [72]. The ethanol extracts contained higher amounts of total phenolics and flavonoids than water and methanol extracts from wild ginseng leaves[73].

CONCLUSION

Utilization of *Tomato pomace* for extraction of beneficial antioxidants compounds not only provides health benefits, but also adds value to the waste generated by the tomato processing industries. In our study aqueous extract proved to be effective on the extraction of phenolics, flavonoids and tannins. Therefore, it can be concluded that, the solvent play a vital role in the extraction of the plant constituents and the efficiency of the active compounds (phenolic, flavonoids and tannins) as anti-viral, antimicrobial and antioxidant agents.

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