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Original Article

Evaluation of the Phytochemical, Antioxidant, Antibacterial and Anticancer Activity of *Prunus domestica* Fruit

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Abstract

Plants have been used long ago through man history of life for their use in food and medicinal drives. In modern life, natural products have been extracted and isolated from several kinds of plants for the development of new drugs. There are numerous interests in natural antioxidants extracted from medicinal plants, vegetables and fruits, which might help to prevent oxidative damage. One of such plants is plum *Prunus domestica* L., family Rosaceae. Samples from 'African Rose', and 'Santa Rosa' plum cultivars were collected from local market in Giza governorate, Egypt. The main phytochemicals of plums (fruit flesh and skin) were analyzed. Total polyphenols, flavonoids, tannins, anthocyanins, and reducing power were higher in 'African Rose' fruit. The ethanolic and ethyl acetate extracts of two plum cultivars were both high in the antioxidant effect with IC_{50} 13.923 and 18.416 µg/ml of ethanolic extract of 'African Rose', and 'Santa Rosa' respectively. The IC_{50} of 'African Rose' and 'Santa Rose' extract against Caco-2 was 4 and 8.5 µg/ml. GC-MS analysis was carried out, fourteen and twenty one compound were identified in 'Santa Rosa' and 'African Rose' respectively. The fruits had an antimicrobial action against gram positive and negative bacteria. There was anticancer activity against 3 cell lines: Liver cell line (HepG2), colorectal adenocarcinoma (Caco-2) cell line, and breast cell line (MCF-7).

Keywords: antibacterial; anticancer activity; antioxidant; DPPH; flavonoid; phenols; plum extract

Introduction

Medicinal plants have been widely used for therapeutic purposes since ancient times. The beneficial effects of fruits and vegetables are generally ascribed to the presence of phenolic compounds for example phenolic acids, flavonoids, tannins, and nitrogen compounds such as alkaloids and amines, as well as vitamins, terpenoids and other metabolites, which have a high antioxidant activity (Cai *et al*., 2004; Djeridane *et al*., 2006; Yang *et al*., 2009; Abdel-Rahim and El-Beltagi, 2010). This activity is because of the ability of these materials to reduce oxidative stress by neutralizing or scavenging of reactive species by hydrogen donation, before they attack cells and other biological components (Kobeasy *et al*., 2011; Erkan, 2012; Kesba and El-Beltagi, 2012). Reactive oxygen (ROS) and nitrogen (RNS) species are products of normal cellular metabolism. However, at high concentrations, these species may be significant mediators of damage to cellular structures, such as proteins, lipids and nucleic acids. The oxidation of any of these substrates, if uncontrolled, can contribute to the development of chronic diseases like cancer, hypertension, diabetes mellitus, cardiovascular and neurodegenerative diseases (Valko *et al*., 2007; Shallan *et al*., 2010). In this sense, there is great concern in finding natural antioxidants from plant materials, and various extracts and isolated compounds have been studied for their antioxidant activity, using different methods (El-Beltagi, 2011; Costa *et al*., 2012; El-Beltagi *et al*., 2018).

Plums are part of the Rosaceae family, Prunus genus. The fruits display a wide range of size, color, flavor, and texture (Dugalic *et al*., 2014). Consumers appreciate plum fruits for their flavour, color and aromatic features. High consumption of fruits and vegetables was linked to reduced incidence of degenerative diseases owing to their potential antioxidant capacity (Prior, 2003). It is considered as a respectable source of nutrients due to the content of ascorbic acid, monosaccharides, various minerals, dietary fiber, and phenolics, such as chlorogenic, benzoic, vanillic,

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and caffeic acid (Karabegovic *et al.,* 2014). These factors may supply important information to the consumer in terms of identifying a more nutritional fruit. The fruit of *P. domestica* is a potent antibacterial activity (Yaqeen *et al*., 2013). It is dried fruit contains large amounts of antioxidant constituents, such as neochlorogenic acid (3-O-caffeoylquinic acid), chlorogenic acid (5-O-caffeoylquinic acid), cryptochlorogenic acid (4-O-caffeoylquinic acid), (+)-abscisic acid, (+)-β-D-glucopyranosyl abscisate, (6S,9R)-roseoside, and two lignan glucosides [(+)-pinoresinol mono-β- D-glucopyranoside and 3-(β-D-glucopyranosyloxymethyl)- 2 -(4-hydroxy-3-methoxyphenyl)-5 -(3-hydroxypropyl)-7 -methoxy-(2R,3S) –dihydrobenzofuran] (Kikuzaki *et al*., 2004 and Kayano *et al*., 2002). In addition, the fruit contains flavonols (myricetin, quercetin, and kaempferol), carbohydrates (fructose, sucrose, glucose, sorbitol), organic acids (citric acid, malic acid), vitamins (α-tocopherol, γ-tocopherol, β-carotene), and minerals (sodium, potassium, magnesium, calcium, iron, zinc) (Lombardi-Boccia *et al*., 2004). The aim of this work was to evaluate of the phytochemical composition of *Prunus domestica* fruit cultivars ('Santa Rosa' and 'African Rose') and to study their effects as an antioxidant, antimicrobial and anticancer activity.

Materials and Methods

Plant material

Plums cultivars 'African Rose', and 'Santa Rosa' were collected from a local market in Egypt. *Prunus domestica* was botanically categorized by Dr. Samah Azooz - Botany Department, Faculty of Agriculture, Cairo University, Egypt.

Microbial strain

Table 1 showed the microorganisms which were used in this study and were acquired from the American Type Culture Collection (ATCC) in addition to the culture collection of the Microbiology Lab, Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University.

Extraction method

The fruits were cleaned and washed thoroughly under tap water, and then the fruits were freeze-dried and grinded into fine powder using an electric blender. The powder was dehydrated in an oven at 40 °C for 24 h. The fine powder sample (500mg) was extracted in 10 ml ethanol or ethyl acetate for 24 h using a shaker, then the extract was filtered and the samples were stored at 4 °C until use (Sumathy and Sumathy, 2011). All analysis was done in the labs of Cairo University. Research Park (CURP), Faculty of Agriculture, Cairo University, Cairo, Egypt.

Total polyphenolic content

The total phenols content was assessed by Folin Ciocalteu technique as described by Singleton and Rossi (1965). The absorbance was measured at 765 nm with spectrophotometer Thermo Scientific HERYIOS.

Total flavonoid content

The flavonoids content was determined by aluminium trichloride method as described by Zhishen *et al*. (1999). The absorbance was measured at 510 nm with a spectrophotometer.

Total tannin content

Tannin content in plums was determined using Folin-Denis reagent as mentioned by Saxena *et al*. (2013). The absorbance was read at 700 nm using spectrophotometer.

Total alkaloids content

Alkaloids were measured according to the method described by Harbone (1998). The absorbance was taken at 565 nm. The alkaloid concentration was calculated from the calibration curve of atropine used as standard and results expressed as g/100 g equivalent of atropine.

Total anthocyanin (TA) content

The total anthocyanin contents were determined by the pH differential method (Lee *et al*., 2005) using a spectrophotometer (Thermo Scientific HERYIOS). The absorbance of the fruit extract was measured at 515 and 700 nm in pH 1.0 and 4.5 buffers, respectively, using A = (A515 - A700) pH 1.0 - (A515 - A700) pH 4.5 with a molar extinction coefficient of 26,900. The results were expressed as mg of cyanidin-3-glucoside equivalent per 100 grams of fresh weight.

Determination of reducing power

The ability of the tested extracts to reduce Fe^{3+} was done by the method of Chou *et al.* (2009). The absorbance was measured at 700 nm. The results were expressed as µg of gallic acid equivalent per 100 g DW.

DPPH free Radical Scavenging Activity (RSA)

The antioxidant activity of the *Prunus domestica* extract was measured in terms of hydrogen donating or radical-scavenging capability using the stable DPPH method as modified by Park *et al*. (2006).

Table1. Microbial strains used to test the antimicrobial activities of 'Santa Rosa' and 'African rose' fruit extracts

Microbial group	Indicator strain	Positive control	Cultivation conditions
Gram positive bacteria	*Staphylococcus aureus* (ATCC 25923)	Kanamycin	Muller-Hinton broth, 37 °C/ 24 h
	Bacillus cereus (ATCC 33018)		Muller-Hinton broth, 30 °C / 24 h
Gram negative bacteria	*Escherichia coli* (ATCC 8739)	Polymyxin	Muller-Hinton broth, 37 °C / 24 h
	Salmonella typhimureum (ATCC 14028)		Muller-Hinton broth, 37 °C / 24 h
Fungus	*Aspergillus niger* (nrrl 326)	Nystatin	Sabouraud dextrose broth, 25 °C / 3days
	Candida albicans ATCC 10231		Sabouraud dextrose broth, 25 °C / 24 h

The reaction mixture containing 1 ml of the extract at diverse concentrations (40, 80, 120, 150 µg/ml) + 1 ml of DPPH (0.2 mM) was vigorously shaken and incubated in darkness at room temperature for 30 minutes. The absorbance was read at 517 nm using UV-visible spectrophotometer. Radical scavenging activity was stated as percent of inhibition and was calculated using the following formula:

%DPPH = [Absorbance of Control – Absorbance of Sample / Absorbance of Control] × 100.

Extraction of phenolic and flavonoid compounds

0.2 g dry sample extracted with 20 ml ethanol 80%, soak in brown bottle for 24 hr at room temperature, then centrifuged for 5 min, volume adjusted to 25 ml by ethanol 80%, and filtered through Whatman filter paper, 10 ml of the solution evaporated to dryness then dissolved in 5 ml HPLC grade methanol 50%, and filtered through PTFE filter with pore size 0.2 µm.

Instrument condition for phenolic compounds

Agilent 1260 infinity HPLC Series (Ag-ilent, USA), equipped with Quaternary pump, a Zorbax Eclipse plusC18 column 100 mm × 4.6 mm i.d. (Agilent Technologies, USA), operated at 30 °C. The separation is completed using a ternary linear elution gradient with (A) HPLC grade water 0.2 % H_3PO_4 (v/v), (B) methanol and (C) acetonitrile. The injected volume was 20 µl. Detection: VWD detector was set at 284 nm.

Instrument condition for flavonoids

HPLC, Smart line, Knauer, Germany., equipped with binary pump, a Zorbax Eclipse plus C18 column 150 mm x 4.6 mm i.d. (Agilent Technologies, USA), operated at 35 °C. Eluent: methanol: H_2O with 0.5% H_3PO_4, 50:50 with flow rate 0.7 ml/min, and the injected volume was 20 µl. Detection: UV detector set at 273 nm and data integration by clari-tychrom@ software. This method was the modified of methods Goupy *et al.* (1999) and Mattila *et al.* (2000) for fractionate the polyphenols and flavonoids, respectively.

Fruits extraction for GC/MS analysis

The fruits were washed, shade dried and ground to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to stoppard flask and treated with ethanol until the powder is fully dipped. The flask was shaken every hour for the first 6 hours and then it was kept away and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness using a vacuum distillation unit. The final residue gained was then subjected to GC-MS analysis.

GC-MS analysis

GC-MS analysis of these extracts was performed using an Agilent 7000 Series Triple, Quad Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS/) equipped With a Elite-5MS (5% diphenyl / 95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection an electron Ionization system with ionizing energy of 70ev was used. Helium gas (99.999%) was used as carrier gas at a constant flow rate 1 ml/min and injection volume of 2 µl was employed (split ratio of 10:1); the injector temperature 250 °C; ion-source temperature 200 °C. The oven temperature programmed from 110 °C (iso thermal for 2 min) with a rise of 10 °C/min to 200 °C, then 5 °C/min to 280 °C, ending with a 9 min iso thermal at 280 °C, mass spectra were taken at 70ev: a scan interval of 0.5 second and fragments from 45 to 450Da, total GC Running time was 36 minutes. The relative % quantity of each component was calculated by relating its average Peak area to the total areas. Software approved to handle mass spectra and chromatograms was Turbomass.

Clarification of mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components kept in the NIST library. The name, structure and molecular weight of the components of the test materials were determined (Bagavathi and Ramasamy, 2012).

Antibacterial activity

Agar disc diffusion method was done to evaluate antibacterial activity of plum as describe by Bauer *et al.* (1966). The strains were grown on Mueller-Hinton agar slants at 37 °C for 24 h and checkered for purity. After the incubation, the cells were washed off the surface of agar and suspended in sterile physiological solution. The amount of cells in 1 ml of suspension for inoculation measured by McFarland nefelometer was 5 × 107 CFU/ml. 1 ml of these suspensions was homogenized with 9 ml of melted (45 °C) Mueller-Hinton agar and dispensed into Petri dishes. On the agar surface, 5 mm diameter paper discs (HiMedia®, Mumbai, India) were applied and impregnated with 15 µl of samples. The plates were incubated at the optimal temperature for each indicator strain (Table 1) and tested after 24, 48 and 72 h. Inhibition of growth was scored positive in the presence of a detectable clear zone (ZI) around the disc and expressed in mm. Experiments were car-out in triplicates and the inhibition zone was recorded as the average of the replicates± SD.

In vitro cytotoxicity assay

Human lung cancer (A549), colorectal adenocarcinoma Caco-2, and MCF-7 human breast cancer were purchased from CURP, faculty of agriculture at Cairo University (Egypt). Cells were kept in (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 µg/ml streptomycin and 100 unit/ml penicillin g potassium, in a humidified 90% and 5% (V/V) CO_2 atmosphere at 37 °C. The cytotoxicity of ethanolic extracts was tested by the neutral red (NR) assay as before described (Repetto *et al.*, 2008). Exponentially growing cells were collected using 0.25% Trypsin-EDTA and seeded in 96- well plates at 20000 cells/well. After incubation (overnight), extracts were added in many concentrations (10, 50, 100, 200, 400, and 800 µg/ml); 4 wells for each concentration. After treatment with extracts for 24h., media were removed and cells were exposed to neutral red solution for 4 hours at 37 °C. Destin solution was used to dissolve the NR stained cells and color was measured at 540 nm microplate reader (Biotek, ELX808).

Statistical analysis

All results were expressed as mean values ± standard deviation. Comparisons were performed by analysis of variance (ANOVA). Statistical analyses were run using SAS software.

Results and Discussion

Phytochemical constituents of plums

As illustrated in Table 2, the chemical constituents of ethanolic and ethyl acetate extracts of two species of plum fruit extracts 'Santa Rosa' and 'African Rose'. First, for 'Santa Rosa' ethanolic extract contain total phenolic (122.17 ± 2.05 mg /g DW) while ethyl acetate (64.94 ± 0.16 mg /g DW) and for 'African Rose' ethanolic and ethyl acetate extract 486.08 ± 4.07 and 116.849 ± 1.15 mg /g DW respectively. Total phenolic content is frequently used to measure the antioxidant properties of plant extracts since it constitutes an effective, fast and inexpensive assay (Dai and Mumper, 2010). A direct relationship has been detected between the total polyphenolic contents and antioxidant activity of several natural product extracts (Vinson *et al*., 2001). Several studies have examined the polyphenolic content of different species of the genus *Prunus*, Demir *et al.* (2017) found that Total polyphenolic content was 33.7 ± 0.13 mg GAE per g sample of *Prunus laurocerasus*. The total polyphenolic content values of many extracts of *L. officinalis* fruits from different regions are reported to range between 23.6 and 64.6 mg GAE per g extract (Orhan and Akkol, 2011; Celep *et al*., 2012). Another study showed that ethyl acetate extract got 33.88 ± 0.18 mg/g of total poly phenolic content (Dhingra *et al*., 2014). According to one article, methanolic extract has 4.42 mg /g dry weight (Eivind and Rune, 2006); However, Gil *et al*. (2002) found that, phenolics content varied from 0.42 to 1.09 mg/ g fresh weight in five plum cultivars, while, Kim *et al*. (2003) found a range from 1.74 to 3.75 mg/ g fresh weight in six plum cultivars. Vlaic *et al*. (2017) found that the phenolics measured from the plum samples varied between 60.31 and 699.92 mg GAE 100 g^{-1}.

Results stated by Díaz Mula *et al*. (2009), admiring that the variety has a very significant role in the biosynthesis of phenolic compound. Another study showed that the total phenolic content of dried plum was found to be 1.05 mg GAE/100 mg extract (Mehta *et al*., 2014). In the same concern, Sójka *et al*. (2015) found that polyphenol content was found in plums pomaces was 50 g/100 g.

As flavonoids are well recognized antioxidants, it is thought that the plants which are high in flavonoids have strong antioxidant activity. The highest value of total flavonoid was for ethanolic extract of 'African Rose' (45.991 ± 1.26 mg /g DW), then its ethyl acetate extract (35.61 ± 1.07 mg /g DW) then 'Santa Rosa' ethanolic extract with value (30.02 ± 0.15 mg /g DW) and the lowest value was for 'Santa Rosa' ethyl acetate extract (15.79 ± 0.37 mg /g DW). Dhingra *et al*. (2014) reported that a proper amount of total flavonoid content (47.96 ± 0.36 mg /g) was found to be existing in ethyl acetate fraction of *P. domestica*, while minimum concentration was existing in the hexane fraction (2.06 ± 0.3 mg /g). Result of an earlier report was 1.03 mg /g fresh weight of flavonoids in the pulp of *P. domestica* pulp (Sandra *et al*., 2009). Another study submitted that the total flavonoid content of dried plum was 0.583 mg CE/100 mg extract (Mehta *et al*., 2014). So our process seems to provide a much better yield in ethanol and ethyl acetate fractions.

Total tannins were high in ethanolic extract of 'African Rose' and 'Santa Rosa' to give 9.355 ± 0.21 and 8.71 ± 0.14 mg/g DW respectively. Contrarily, the 'Santa Rosa' is higher in total alkaloids with values 4.84 ± 0.08 and 3.74 ± 0.11 mg/g DW for ethanolic and ethyl acetate fruit extracts respectively. Then the 'African Rose' got lower values 2.425 ± 0.31 and 1.316 ± 0.11 mg/g DW for ethanolic and ethyl acetate fruit extracts respectively.

Total anthocyanin was the highest in 'African Rose' ethanolic extract to give 326.83 ± 1.68 mg/g DW followed by its ethyl acetate extract 206.48 ± 2.08, then 'Santa Rosa' ethanolic extract 164.13 1.78, then the ethyl acetate extract 97.51 ± 0.46 mg/g DW. Vlaic *et al*. (2017) found that the amount of anthocyanins during maturity was between 1.65 to 1.76 mg CE 100 g^{-1}.

Antioxidant activity of plums

The effect of antioxidants on DPPH radical scavenging was assumed to result from their hydrogen donating capability. DPPH is a stable free radical and accepts an electron or hydrogen radical to develop a stable molecule. DPPH is a stable free radical compounds and has an absorbance in its oxidized form around 515-520 nm. It is visually clear as a discoloration from purple to yellow. The scavenging of DPPH radicals increased with increasing extract concentration from 40, 80, 120 and 150 µg/ml (Table 3). The best IC_{50} was the value of ethanolic extract of 'African Rose' (13.923 µg/ml) followed by the ethanolic

Table 2. Quantitative phytochemical analysis of 'Santa Rosa' and 'African Rose' fruit extracts

Constituents	'Santa Rosa' extracts		'African Rose' extracts	
	Ethanolic	Ethyl acetate	Ethanolic	Ethyl acetate
Total phenolic (mg Gallic acid /g DW)	122.17±2.05[a]	64.94±0.16[b]	486.08±4.07[a]	116.849±1.15[b]
Total flavonoid (mg Quercetin /g DW)	30.02±0.15[a]	15.79±0.37[b]	45.991±1.26[a]	35.61±1.07[b]
Total tannin (mg Tannic acid /g DW)	8.71±0.14[a]	5.33±0.25[b]	9.355±0.21[a]	5.823±0.34[b]
Total alkaloid (g/100g DW)	4.84±0.08[a]	3.74±0.11[b]	2.425±0.31[a]	1.316±0.11[b]
Total anthocyanin (mg cy-3-glu /100g FW)	164.13±1.78[a]	97.51±0.46[b]	326.83±1.68[a]	206.48±2.08[b]

Values are mean ± SD of three replicate analyses

extract of 'Santa Rosa' 18.416 then the ethyl acetate extract of 'African rose' 28.532 and 'Santa Rosa' 31.522 was the lowest. IC_{50} value indicates the concentration of the test sample required to inhibit 50% of free radicals. The IC_{50} value is widely a factor used to measure the free radical scavenging activity (Cuvelier *et al*., 1992); a lesser IC_{50} value corresponds to a higher antioxidant activity. The four extracts of plum showed a significant dose-dependent inhibition of DPPH activity. Enzymatic and nonenzymatic antioxidants are molecules that have the capability to scavenge free radicals before they damage cells. Antioxidants can be endogenous or obtained exogenously, either from diet or from dietary supplements (Li *et al*., 2014). Scientific evidence recommends that antioxidant compounds in food play a significant role as a health protecting factor.

The ethanolic extract of 'African rose' and 'Santa Rosa' has got a higher reducing power 30.24 ± 0.45 and 25.34 ± 0.53 respectively, then the ethyl acetate extract of them 21.08 ± 0.18 and 16.21 ± 0.25 mg/g DW respectively.

According to Phongpaichit *et al.* (2007), extracts which have IC_{50} values ranging from 50 to 100 mg / ml is considered to display intermediate antioxidant activity. For now, extracts with IC_{50} values ranging between 10 to 50 mg / ml is considered to have strong antioxidant activity. In this case, the four extracts of 'African Rose' and 'Santa Rosa' possessed strong antioxidant activity. While the ethanolic extracts are the best of them. The antioxidant capacity found in plum fruit pulp values was set between 44.18 and 49.23% (Vlaic *et al*. 2017). They assumed that Differences depending on the variety antioxidant capacity registered during maturation. Also Sójka *et al*. (2015) found that antioxidant activity, 1.036 mM TE/g.

A Pearson product-moment correlation coefficient was computed to evaluate the relationship between total phenolics and IC_{50} (µg/ml) of different extracts. The results suggest that all the correlation was statistically significant. Statistical analysis indicated A strong negative correlation were found between both Total phenolics and IC_{50} ($r2 = 0.635$, $p \leq 0.05$) (Fig. 1). Results identified by Díaz Mula *et al*. (2009), approving that the phenolic compounds, are in correlation with the antioxidant capacity.

HPLC analysis of phenolic and flavonoid compounds

Data in Table 4 showed that the concentration of several phenolic compounds and flavonoids was identified. Myricetin was dominating (97.62 µg/mg DW) in 'Santa Rosa' fruit followed by ellagic acid and benzoic acid. While in 'African rose' fruit extract Ellagic acid was dominating (122.30 µg/mg DW) followed by myricetin then vanillin. chlorogenic acid was dominant in three plum species *P. cerasifera Ehrh*., *P. domestica L*., and *P. spinosa L*. 11.95, 11.565, and 12.985 mg kg^{-1} fw respectively (Celik *et al*., 2017). Additionally, Sójka *et al*. (2015) found that the dominant compound in fresh plum pomace was

Table 3. Antioxidant activity of 'Santa Rosa' and 'African Rose' fruit extracts against DPPH method

Conc. (µg/ml)	'Santa Rosa'		African Rose'	
	DPPH % in ethanolic extract	DPPH % in ethyl acetate extract	DPPH % in ethanolic extract	DPPH % in ethyl acetate extract
40	43.897	43.447	58.957	56.287
80	48.933	46.862	66.667	61.252
120	58.463	54.589	73.156	62.008
150	70.032	63.677	81.143	62.763
IC_{50} (µg/ml)	18.416	31.522	13.923	28.532
Reducing power activity (µg Gallic acid /100g DW	25.34±0.53	16.21±0.25	30.24±0.45	21.08±0.18

Table 4. HPLC analysis of phenolic and flavonoid compounds 'Santa Rosa' and 'African Rose' fruit extracts

No.	Compounds	Conc. µg/mg DW	
		'Santa Rosa'	'African Rose'
1	Pyrogallol	4.5	6.38
2	Quinol	1.27	-
3	Gallic acid	2.40	2.45
4	Catechol	1.55	5.04
5	*p*-Hydroxy benzoic acid	1.45	5.92
6	Caffeine	2.52	5.10
7	Chlorgenic acid	1.57	1.24
8	Vanillic acid	1.81	3.79
9	Caffeic acid	1.08	3.53
10	Syringic acid	1.81	4.52
11	Vanillin	5.35	14.17
12	*p*-Coumaric acid	1.83	2.60
13	Ferulic acid	1.41	1.06
14	Benzoic acid	17.37	4.18
15	Rutin	4.9	12.88
16	Ellagic acid	37.21	122.30
17	o- Coumaric acid	4.52	9.79
18	Salicylic acid	1.17	5.43
19	Myricetin	97.62	19.24
20	Cinnamic acid	9.32	1.07
21	Quercitin	1.50	6.93
22	Rosmarinic acid	8.14	1.02
23	Neringenin	4.30	2.44
24	Kaempferol	8.33	3.20

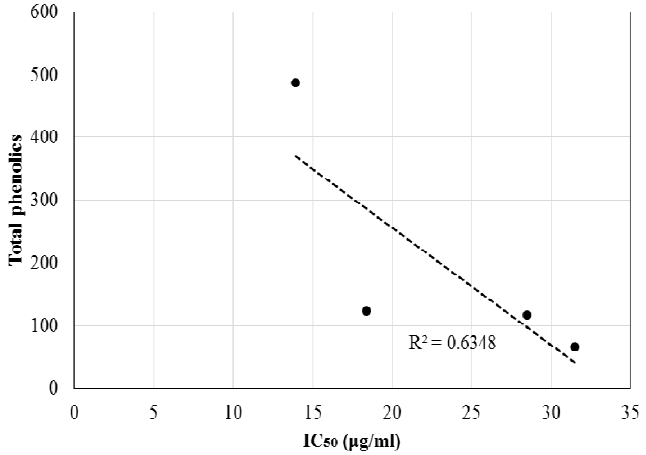

Fig. 1. Correlation between total phenolics and IC_{50} (µg/ml)

neochlorogenic acid (6.42 mg/100 g fresh weight).

GC-MS analysis of biomolecules on the plum fruit extract

The GC-MS analysis of 'Santa Rosa' fruit revealed the presence of fourteen compounds while in 'African rose' there was twenty one compounds that could contribute the therapeutic quality of the plant (Figs. 2 and 3). The identification of the phytochemical compounds was based on the peak area, retention time and molecular formula. The active principles with their retention time (RT), molecular weight (MW), molecular formula and peak area in percentage of 'Santa Rosa' are presented in Fig. 1. The main compounds present in the fruits were Cyclohexanol, 5-methyl-2-(1-methylethyl)- 3-p-Menthanol (35.28%), Thymol (14.15%) etc. other minor compounds were also present (Table 5). Where GC-MS profiles of 'African rose' fruit is shown in Fig. 2. The main compounds present in the fruits were 7-Methyl-7-(1-methylethenyl)-2-phenylbicyclo [4.2.0]oct-1-ene (35.33%), glycine, N-methyl-N-methoxy-carbonyl-, nonyl ester (21.07 %) etc. other major and minor compounds were also present (Table 6).

Thymol is one among the 14 compounds from the fruit of 'Santa Rosa'. Thymol is a natural mono-terpenoid phenol which has strong antiseptic properties. The terpenoids, mainly the mono-terpenols, donate fruity characteristics to the aroma of fruits, most of which are defined as very pleasant (Coelho *et al*., 2006). Likewise, Chai *et al*. (2012) described that the terpenoid content of *P. domestica* was relatively low as the main content was only 2.52 µg kg-1 FW. In *P. salicina* hybrids, the terpenoid content was relatively high and represented 10.0% of the total volatiles.

Antimicrobial activity of plums

The agar diffusion method used to evaluate the antibacterial and antifungal activity of ethanolic and ethyl acetate extracts of 'Santa Rosa' and 'African rose' plums by using selected gram-positive, gram-negative bacteria and fungus. The diameter of the inhibition zone (ZI) is shown in Table 7. The data show that the extract exhibited the activity against the examined food pathogens. Gram positive bacteria *Staphylococcus aureus* and *Bacillus cereus* demonstrated almost the same susceptibility of the Gram-negative *Escherichia coli* and *Salmonella typhimureum*. The 'African rose' ethanolic extract exhibited antibacterial activity against *Staphylococcus aureus* (ZI = 18.51 mm), one of the most common gram-positive bacterium causing food poisoning. On the other hand, a weak antimicrobial activity of 'Santa Rosa' ethyl acetate extract was detected against *B. cereus* (ZI = 9.18 mm). The effect was equivalent to that of kanamycin, and polymyxin used as positive control. The extracts exposed no effect on the fungus used (*Aspergillus niger* and *Candida albicans*). In terms of antimicrobial potential, gram-positive and gram-negative bacteria have been found susceptible to the *P. domestica* extract for example Gram-positive strain of *S. aureus* (19.7 ± 0.4 mm) and Gram negative strains of *E. coli* (14.4 ± 0.7 mm) (Islam *et al*., 2017). Moreover, Mehta *et al*. (2014) found that *S. aureus* (ZI = 16 mm) which is near to our results. Sójka *et al*. (2015) results haven't shown antimicrobial effects of plum pomaces against e gram-negative bacteria *E. coli*.

Anticancer activity of plums

Results showed that 'Santa Rosa' and 'African rose' extracts had cytotoxic effects on Liver cell line, colorectal adenocarcinoma, and breast cancer cell line and this effect were concentration dependent (Table 8). We used five different concentration of plant extract, it was found that as plant concentration was increased, the cell viability decreased and anticancer activity increased. The IC_{50} of 'African rose' and 'Santa Rosa' against Colorectal adenocarcinoma (Caco-2) was 4 and 8.5 µg/ml respectively. The value of IC_{50} was found to be 99.97µg/ml against MCF-7 (breast cancer) cell line (Poongodi *et al*., 2015). While our results showed that the cytotoxicity assay, which was carried out in MCF-7 (breast cancer) cell line had an IC_{50} of 'African rose' and 'Santa Rosa' 38.8 and 43.5 µg/ml respectively. Also Demir *et al*. (2017) found that IC_{50} of their extract of *Prunus laurocerasus* against MCF-7 was > 500 µg/ml. Their results also showed IC_{50} against HepG2 was 357.1 µg/ml. The IC_{50} of *Prunus domestica* fruit peel against HepG2 was 23.2 µg/ml (Ateya *et al.,* 2014). While our results showed that IC_{50} of *Prunus domestica* fruit against HepG2 was 25.5 and 23.1 µg/ml of 'Santa Rosa' and 'African rose' respectively.

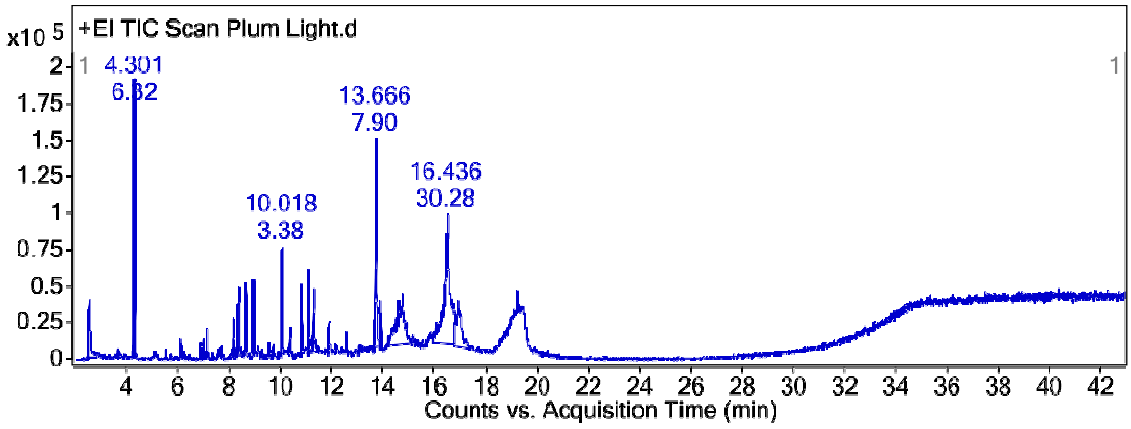

Fig 2. GC-MS profiles of 'Santa Rosa' fruit

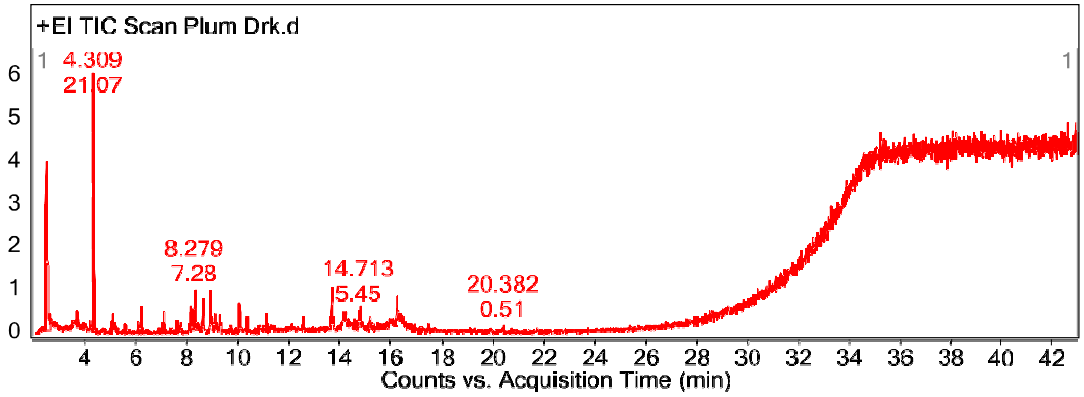

Fig. 3. GC-MS profiles of 'African Rose' fruit

Table 5. Compounds present in the 'Santa Rosa' fruit using GC-MS analysis

No.	Peak name	Retention time	Peak area	% Peak area
1	Ambrosiol (8,9-dihydroxy-6,9a-dimethyl-3-methylidene-decahydro-azuleno[4,5-b]furan-2(3h)-one) Formula: $C_{15}H_{22}O_4$ MW: 266.337	2.521	251220.64	5.25
2	2-Imidazolidinethione Formula: $C_8H_{16}N_4O_4$ MW:102.16	3.649	65602.97	2.37
3	4-amino-3,5-bis(dimethoxymethyl)-4H-1,2,4-triazole Formula: $C_{15}H_{22}O_4$ MW:232.237	4.301	302154.31	6.32
4	4-methylhex-4-en-1-ol Formula: $C_7H_{14}O$ MW:114.188	8.335	171532.11	3.59
5	2-Octanone Formula: $C_8H_{16}O$ MW: 128.215	8.625	134606.55	3.82
6	2,2-Dimethylcyclohexanone Formula: $C_8H_{14}O$ MW: 126.2	8.931	144701.63	4.03
7	Ethyl 4-oxo-8-methyldecanoate Formula: $C_{13}H_{24}O_3$ MW: 228.332	10.018	161382.5	4.38
8	2-heptanoylfuran Formula:$C_{11}H_{16}O_2$ MW: 180.247	10.783	122799.66	2.63
9	β.-methylstyrene Formula: C_9H_{10} MW: 118.179	11.089	123993.72	3.99
10	Phosphonic acid, methyl-, bis(trimethylsilyl) ester Formula:$C_7H_{21}O_3PSi_2$ MW: 240.38	11.838	57248.4	2.2
11	2,4-Dicyanoaniline Formula: $C_8H_5N_3$ MW: 143.149	13.666	377501.08	9.9
12	2,2-Bis(4-hydroxyphenyl) propane Formula: $C_{15}H_{16}O_2$ MW: 228.29	13.876	52031.07	2.09
13	Cyclohexanol, 5-methyl-2-(1-methylethyl)- 3-p-Menthanol Formula: $C_{10}H_{20}O$ MW: 156.2652	16.436	1447840.82	35.28
14	Thymol Formula: $C_{10}H_{14}O$ MW: 150.221	16.887	437589.04	14.15

Table 6. Compounds present in the 'African Rose' fruit using GC-MS analysis

No.	Peak name	Retention time	Peak area	% Peak area
1	7-Methyl-7-(1-methylethenyl)-2-phenylbicyclo[4.2.0]oct-1-ene Formula: $C_{18}H_{22}$ MW: 238.374	2.473	177523.2	35.33
2	Glycine, N-methyl-N-methoxycarbonyl-, nonyl ester Formula: $C_{14}H_{27}NO_4$ MW: 273.3685	4.309	105757.53	21.07
3	4-Methyl-2,4-bis(4'-trimethylsilyloxyphenyl)pentene-1 Formula: C24H36O2Si2 MW: 412.72	4.72	3480.78	0.69
4	Tri chloro mono fluoro methane Formula: CFCl3 MW: 137.359	5.082	10950.26	3.72
5	Glycine, n-methyl-n-ethoxycarbonyl-, ethyl ester Formula: $C_8H_{15}NO_4$ MW: 189.21	5.541	3440.43	0.69
6	3-Phenyl-1,3-pentanediol Formula: $C_{11}H_{16}O_2$ MW: 180.247	6.065	3821.97	0.76
7	Bromoacetylene	7.039	4288.74	0.85
8	Benzene, 1-fluoro-4-methyl-Formula:C_7H_7F MW: 110.1289	7.095	8966.41	1.79
9	Ethyl 2-(ethoxycarbonyloxy) ethylcarbamate Formula:$C_8H_{15}NO_5$ MW: 205.2084	7.506	3834.27	0.76
10	2-methyl-5-(methylthio)- Furan Formula:C_6H_8OS MW: 128.189	7.578	4172.29	0.83
11	1-Phenethyl-4-piperidone Formula:$C_{13}H_{17}NO$ MW: 203.28	8.134	19394.37	3.86
12	Pentanamide, 2-(dimethylamino)-N-[7-(hydroxyphenylmethyl)-3-(1-methylethyl)-5,8-dioxo-2-oxa-6,9-diazabicyclo hexadeca-12,14,15-trien-4-yl]-3-methyl Formula: $C_{31}H_{44}N_4O_5$ MW: 552.716	8.279	36551.51	7.28
13	D-proline, n-methoxycarbonyl-, octyl ester Formula: $C_{15}H_{27}NO_4$ MW: 285.38	8.577	18150.9	3.62
14	Ropivacaine Formula:$C_{17}H_{26}N_2O$ MW: 274.4054	8.883	28321.83	5.64
15	Acetoxyacetic acid, 2-naphthyl ester Formula: $C_{14}H_{12}O_4$ MW: 244.24	10.01	17026.87	3.39
16	Phenol, 4-ethyl- Formula: $C_8H_{10}O$ MW: 122.1644	10.324	5025.6	1
17	1,4-dihydro-1-methyl-3-Pyridinecarbonitrile Formula: $C_7H_6N_2$ MW: 118.1	11.089	6114.98	1.22
18	3-Cyano-4,6-dimethyl-2-hydroxypyridine Formula: $C_8H_8N_2O$ MW: 148.16	12.531	4907.41	0.98
19	N-(1-Naphthyl) cyclohexanecarboxamide Formula: $C_{17}H_{19}NO$ MW: 253.339	14.713	27357.26	5.45
20	Isophthalic acid, 2-formylphenyl propyl ester Formula: $C_{18}H_{15}O_5$ MW: 312.3166	15.099	2812.5	0.56
21	Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2yl)eth] pentyl ester Formula: $C_{19}H_{25}NO_5$ MW: 347.411	20.382	2552.25	0.51

Table 7. Antibacterial activities of 'Santa Rosa' and 'African Rose' fruit extracts against selected bacterial strains and fungus

Samples	Inhibition zone (mm)*					
	Gram positive bacteria		Gram negative bacteria		Fungus	
	S. aureus	*B. cereus*	*E. coli*	*S. typhimureum*	*A. niger*	*C. albicans*
'Santa Rosa' ethanolic extract	15.24±0.15	11.34±0.22	13.17±0.36	16.22±0.44	-	-
'Santa Rosa' ethyl acetat extract	12.14±0.31	9.18±0.43	10.55±0.28	12.19±0.35	-	-
'African Rose' ethanolic extract	18.51±0.17	14.10±0.31	15.41±0.30	18.36±0.21	-	-
'African Rose' ethyl acetat extract	13.62±0.27	12.58±0.27	12.19±0.08	14.63±0.48	-	-

Values are mean ± SD of three replicate analyses

Table 8. Anticancer activities of 'Santa Rosa' and 'African Rose' ethanolic extracts

Concentrations (µg/ml)	Liver cell line (HepG2) Viability %		Colorectal adenocarcinoma (Caco-2) cell line Viability %		Breast cell line (MCF-7) Viability %	
	'Santa Rosa'	'African Rose'	'Santa Rosa'	'African Rose'	'Santa Rosa'	'African Rose'
500	100	100	100	95	100	100
1000	89.5	87	99	93.6	88.8	86.7
1500	80.3	77	87.6	82.6	83	82.6
2000	72	70	79	71	78	79
3000	65	61	57	51	67	64
IC_{50} (µg/ml)	25.5	23.1	8.5	4	43.5	38.8

Conclusions

The multitarget therapeutic potential of the *P. domestica* was explored in different *in vitro* testing examples. Both the 'African rose' and 'Santa Rosa' possessed selective effects in inhibition of cancer cells, pathogenic bacteria, oxidation, thereby representing its use as a value-added component for functional food.

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