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Chemical Constituents and Biological Activity of Successive Extracts and Silver Nanoparticles from Red Onion Peels

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Abstract

Egyptian onion is a famous economical plant rich with different primary and secondary metabolites which possess to have high biological actions such as antioxidant, antimicrobial, ant-inflammation and antiviral activities, The aim of the present research was to determine the antiradical and antioxidant effects of various individual organic and inorganic extracts obtained from unused red onion residues (red onion peels). In the present work, four extracts (Chloroform, ethyl acetate, ethanol and water) were assessed against different radical and oxidizing agents such as DPPH•, ABTS radicals' methods and potassium permanganate as a non-radical method compared with natural antioxidant standard (ascorbic acid) and silver nanoparticles of most promising extract (red onion water extract, Ag-NPs-W), in addition to analysis of active compounds of extract using Gas Chromatography–mass/mass spectrometry (GC-MS/MS). The obtained data revealed that: all antioxidant assays go parallel showing that the promising extract concentration and time of incubation are dependent and proportional to the concentration of phenolic compounds content. Water extract of red onion peels recorded the highest antioxidant activity when compared with the other three crude red onion peels extracts with 78.88 and 85.93 % against DPPH at 50 and 100 µg/ml respectively when compared with ascorbic acid as the natural standard (85.4 and 89.8 %) against DPPH at the same both concentrations, respectively. The data of antiradical activity of water extract showed the highest after 360 seconds (6 minutes) compared with antioxidant activity (30 min). From the obtained results it could be concluded that red onion peels water extract can be extensively used in providing of antioxidant, antiradical, and natural red pigments.

Keywords: *Allium cepa* L., Onion peel, Antioxidants Biological activities, Nanoparticles, TLC, GLC-mass.

1. Introduction

Allium species have been extensively used in different biological and chemical purposes especially in medical applications [1]. Recently, several published articles have verified the significant health roles of onion and its isolated compounds, these roles include radical scavenging activity [2], antimicrobial [3], anticancer [4] and antidiabetic [5], neuroprotective [6] and respiratory protective [7], digestive system protective [8]. Each of these roles are related to the high concentrations of bioactive chemicals such as organosulfur compounds, polyphenols and flavonoids, which are also present in other species but reduced to a great extent during processing [9,10]. Flavonoids are responsible for the color of onion skin.

The Egyptian *Allium* family includes the onion, which is a common vegetable. That species can be found in temperate climates. They're utilized to treat things such as to heat the intestines, stomach ulcers, hypertension, and malaria fever [11, 12].

Fredotovi´c et al. [13] retain a good radical scavenging activity which makes them one of the strongest antioxidants and free radicals' scavengers. Volatile sulfur compounds which present in onions are responsible as anticancer, antiviral, anti-inflammatory, and antimicrobial effects. The food industries waste a high amount of red onion peel this wastage is required searching for innovative method to use it Fredotovi´c et al. [10]. Given the existence of active ingredients in onion, which have medical uses, one way could be adapted to employ this by-product as a new and natural cause of products [14, 12]. Red onions are one of Egypt's most popular vegetable crops, and their peel is high in antioxidants that help people to lose weight and decrease their risk

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of diabetes. Because of its benefits to the health, Allium species and their extracts have been used since ancient times [12].

Additionally, Onion is rich in natural pigments such as red, yellow, purple and white, so, Fredotović, *et al.* [10] recognized the main anthocyanins and flavanols in peel extracts of two varieties of *A. cepa* and tested their antiproliferative, antimicrobial and antioxidant properties. quercetin 4`-monoglucoside, quercetin and Quercetin 3,4`-diglucolside are the most plentiful flavonols in all onion extracts detected by High-Performance Liquid Chromatography((HPLC) method.

2. Materials and Methods

3.1 Chemicals and reagents:

Pure chloroform, ethanol, ethyl acetate and methanol were purchased from E. Merck Co. (Darmstadt, Germany). Sulfarhodamine, 2, 2 diphenyl-1-picrylhydrazyl (DPPH), 2, 2'- azino-bis (ethylbenzthiazoline-6-sulfonic acid (ABTS.) were purchased from Sigma-Aldrich. Ascorbic acid and butylated hydroxyl toluene (BHT), were purchased from Sigma-Aldrich.

3.2 Extraction of active ingredients:

The collected red onion peels were air-dried and then ground to a fine powder. 20 g of the dried powder was subjected to extraction with successive selective solvents according to Marrelli *et al*. [15]. Chloroform, ethyl acetate, ethanol and water were used. The polarity was increased from non-polar to highly polar; the extraction process was repeated three times. The organic solvent extract was combined and concentrated under vacuum at 40 - 45 ºC to obtain a dry crude extract for each solvent used. In case of water extract, lyophilization was performed and the residue kept in dark bottle until used.

3.3 Determination of total lipids:

Lipids were extracted by a modified method described by *Xu et al*.[16]. The peels (5 g) were extracted twice with a mixture of chloroform and methanol (2:1; v/v) and sonicated for 10min. Then, sonicated cells were filtered through GF/C Whatman glass filter microfiber (47 mm). Chloroform (10 ml) and distilled water (10 ml) were added sequentially to the filtrate and sonicated again for 10 min. The resultant solution was filtered under vacuum through Whatman glass filter microfiber. The solvent was removed through evaporation at 40 ºC under reduced pressure. Then, the total lipids were weighted.

3.4 Determination of total soluble protein:

The determination of total soluble nitrogen was carried out according to Micro-Kjeldahel method. The crude protein was calculated by multiplying the total nitrogen percent by the factor of 6.25 [17].

3.5 Determination of total soluble carbohydrates:

Total soluble carbohydrates were spectrophotometrically determined using a 5% phenol /sulfuric acid reagent [18].

3.6 Analysis of anthocyanin content:

Anthocyanin content was extracted by using acidified methanol (1% HCl). The absorbance of the clear filtered pigment solution was measured spectrophotometrically at 535 nm using the molar absorption coefficient (29 500 M^{-1} cm^{-1} of cyaniding 3-glycoside) as described by *Stickland et al*. [19].

3.7 Phytochemical screening:

The extract was subjected to preliminary phytochemical screening include, testing for tannins, sterols, flavonoids, glycosides and reducing sugar as the following.

a. Test for sterol and terpenes:

Two milliliters from each extract were evaporated to dryness and the residue was dissolved in 2 ml chloroform and filtered. The filtrate was detected by libermann-Burchards test [20]. , Briefly in a test tube containing extract 1ml of acetic anhydride was added followed by a 3 ml of concentrated H_2SO_4 poured carefully down the side of the tube until the solution form two separate layers. The former red ring indicated the presence of sterol or terpene:

b. Test for flavonoids:

The test was carried out by adding concentrated hydrochloric acid (HCl) dropwise to 1 ml of a solution containing a fragment of magnesium ribbon [21], a positive result gave pinkish color.

c. Test for tannins:

2 ml of distilled water were added to 5 ml of extract, and filtrate. Ferric chloride solution (5%) was then added to the filtrate. The formations of yellowish- green color indicated the probable presence of tannins [22].

d. Test for glycosides and/or carbohydrates:

Extracts were tested for carbohydrates and reducing sugars in the usual manner using Molishs and Fehling reagents [23].

3.8 Thin Layer Chromatography (TLC):

The separation of active compounds from onion peels extracts were performed using Precoated silica gel plates (TLC F254) using petroleum ether: diethyl ether: acetic acid; petroleum ether: acetone; isopropanol: chloroform as mobile phases at the different ratio for comparison as the followings: (8.4:1.5:0.1 v/v); (7:3v/v); (0.15:9.85 v/v) respectively.

3.9 Bio-autography for Antioxidant Activity:

A rapid TLC screening method for antioxidant activity was done using the 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) as a spray reagent. TLC was performed for onion peels extracts as described earlier by Nair et al. [24]. The TLC plates were dried, and antioxidant activity was detected by spraying 0.2% from 2, 2-diphenyl-2-picrylhydrazyl (DPPH) dissolved in methanol. The development of yellow or

white spots against a purple background indicated the presence of antioxidant compound.

3.10 Radical scavenging activities:

a. DPPH radical assay:

The scavenging effects of successive extracts from onion peels and its nano-form were determined by the method of [25], where 1.0 ml of 0.16 mM DPPH solution (in methanol) was added to a test tube containing 1.0 mL aliquot of sample (extracts, fractions, and Ag-Nps-water) at 50 and 100 µg ml-1. The mixture was vortexed for1min and kept at room temperature for 30 min. in the dark. The absorbance of all the sample solutions, BHT synthetic standard and ascorbic acid as antioxidant natural standard were measured at 517 nm. The percentage (%) of scavenging activity was calculated according to the following equation (Eq. 1):

% Antioxidant activity = (Control-Sample x 100) / Control (Eq. 1)

Where: control in DPPH solution (0.16 mM).

b. ABTS radical cation assay:

This assay was based on the ability of different substances to scavenge (2, 2'- azino-bis ethylbenzthiazoline-6-sulfonic acid ($ABTS^{\cdot+}$) radical cation. The radical cation was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1/1, v/v) and leaving the mixture for 4-16 hrs. Until the reaction was completed, and the absorbance was stable. The ($ABTS^{\cdot+}$) solution was diluted with ethanol until it gives an optimum reading of 0.700 ± 0.05 at 734 nm in spectrophotometer according to Re et al. [26]. The photometric Assay was conducted on 0.9 mL of ($ABTS^{+}$ reagent), and 0.1 mL of tested samples at 50 and 100 μgml^{-1}, mixed for 45s , and the measurements were taken at 734 nm after 1min. The antioxidant activity of the tested samples was calculated by determining the decrease in absorbance at different concentrations using the following equation 2:

E= ((Ac-At)/Ac) x 100 (Eq. 2), where: At and Ac are the respective absorbance of tested samples and ABTS+ in comparison with BHT and ascorbic acid standard antioxidants.

c. Potassium permanganate assay:

The scavenging effects of the extract were according to Gaber et al. [27], where 1.0 ml of 0.02 M $KMnO_4$ solution (in methanol) was added to 1.0 ml of extract. The mixture was shaken for 1 min and reserved at room temperature for 30 min in the dark. The absorbances of all the samples and ascorbic acid as antioxidant standard were measured at 514 nm. The percentage (%) of scavenging activity was calculated as the following: %antioxidant activity = (control−sample) X 100 /control where control is $KMnO_4$ solution (0.02 M).

3.11 Determination of total phenolic content:

The total phenolic contents of successive extracts from onion peels were determined by the Folin-Ciocalteu method [28]. Briefly, 0.25 mL of each extract was mixed with 1.25 mL of 1 N Folin-Ciocalteu reagent. After 5 min, 1 mL of sodium carbonate aqueous solution (7.5 %, w/v) was added to the mixture and completed the reaction for 120 min at room temperature. The absorbance was measured at 765 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). The results were expressed in equivalent milligrams of gallic acid per gram of dry weight of plant extract (mg $GAE{\cdot}g^{-1}$ DW).

3.12 Measurement of antiradical activity of successive extracts

DPPH• radical in its radical form has characteristic absorbance at 517 nm respectively, which disappears after its reduction by an antiradical compound. The reduction of DPPH can thus be monitored by measuring the decrease in its absorbance at 517 nm when reacting with peel extracts or its fractions during the reaction time (30-300 sec) at 100 μgml^{-1}. All details to the relevant method are described by Shalaby and Shanab [29]. The antiradical activity (AU515) was calculated according to the equation3:

$AU515 = (A_0–A_1) – (A_0K–A_1K)$ (Eq3)

Where AU515 is the antiradical activity of the extract, A_0K is the absorbance of the control sample at the beginning of the reaction, and A_1K is the absorbance of the control sample after incubation times (30-300 sec) of the reaction. Because $A_0K–A_1K$ was always equal to 0, the above equation was simplified to: $AU515 = A_0–A_1$

3.13 Gas-Chromatographic-Mass-mass spectrum analysis (GC-MS/MS):

GC-MS/MS analysis was performed to identify and quantify active ingredients extracted from onion peels. The analysis was carried out using Trace GC1300-TSQ mass spectrometer from Thermo Scientific, Austin, TX, USA using helium at 1ml/min as carrier gas. A TG–5MS capillary column (30 m x 0.25 mm x 0.25 µm film thickness) was used. The oven temperature was programmed as follow: The initial temperature of 60°C was kept for 1 min. and the temperature was then increased, at a rate of 5.0 °C /min to 200 °C and maintained for 2 min at this temperature. The injector temperature was 260°C, and injections were made in the split mode with a split flow of 1:25. The mass spectrometer was

operating as follow: ion source temperature 250 °C, ionization energy 70 eV (electron impact ionization), m/z scanning range 50–650 Da, the acquisition of chromatographic data was performed by means of WILEY 09 and NIST 11 mass spectral database.

3.14 Preparation of silver nanoparticles (Ag-NPs):

Ten mg of onion peels water extract (as a promising extract) was directly dissolved in 100 mL of 1mM $AgNO_3$ aqueous solution with stirring at room temperature. The pH of the obtained solutions was adjusted to 10 by KOH. After that, the reaction mixture was kept on a magnetic stirrer for 30 min under constant heating (70 °C). The reduction of Ag^+ ions to silver nanoparticles was monitored by visual inspection of the color change in solution and was apparent immediately after the beginning of the reaction. Particles synthesized with methanolic extract were designated Ag-NPs-Me. The nanoparticles were repeatedly centrifuged at 20,000xg for 30 min and washed with sterile bi-distilled water before further analysis [30].

3.15 Characterization of Ag-NPs-Me:

a. UV–vis spectrophotometric analysis:

The color change of the reaction medium was monitored initially by periodic sampling of the reaction solutions and then by measuring its UV–VIS absorption. The aliquots of reaction mixture were analyzed by UV-visible spectrophotometer in the range of 200–800 nm as described by Khattak et al. [30].

b. Fourier Transform Infrared (FTIR) spectroscopy:

Fourier Transform Infrared spectroscopy (FTIR) analysis was done for water extract of onion peels and silver NPs-Me with Shimadzu FTIR spectrometer at room temperature over the range of 4000−400 cm^{-1} at a resolution of 3 cm^{-1} in KBr pellets.

3.16 Statistical analysis:

Values are analyzed as means ± SE or SD. Statistical analysis was done utilizing "Costate" statistic computer program. Statistical analysis was established on One-way analysis of variance ANOVA followed by student-Newman Keuls test, and least significant difference (LSD) at $P< 0.05$.

3. Results and Discussion

Phytochemical screening:

A lot of medicinal plants are a biochemical factory as it contains different of secondary metabolites or active ingredients such as phenolic compounds, flavonoids, alkaloids, plant acids and glycosides.

The preliminary qualitative screening for phytochemicals of Egyptian red onion peels in successive extracts revealed that natural products such as flavonoids, phenols, terpenoids and tannins were detected in the examined four peels extracts (Table 1). However, only ethyl acetate extract contains most of the secondary metabolites tested (terpenoids, phenolic, flavonoids, carbohydrates, tannins and alkaloids).

Table 1: Phytochemical screening of successive extracts from red onion peels

Phytochemical	Peels successive extracts			
	Chloroform	Ethyl acetate	Ethanol	Water
Terpenoids	+	+	-	-
Phenolic	-	+	+	+
Carbohydrates	-	-	-	+
Tannins	+	+	+	-
Saponin	-	-	-	-
Alkaloids		+	+	-
Flavonoids	-	+	+	+

These results agreed with previous data obtained by [9] and [10] who reported that the biological activities of onion as antioxidant or radical scavenging activity are related to the high concentrations of different reducing compounds such as phenolic, flavonoids and organosulfur compounds.

Primary metabolites:

The collected data in Figure 1 reported that the onion peels have a higher amount of carbohydrates with 3.25% followed by lipids and proteins (1.4 and 0.029%) respectively. This data goes parallel with the result obtained by [31] who mentioned that the onion peel contains high content of carbohydrates and low protein.

Secondary metabolites:

a. Phenolic content:

Determination of phenolic compounds in the four extracts of red onion demonstrated that; water extract recorded the highest phenolic content at 4.5 mg/g followed by ethyl acetate at 1.071 mg/g then ethanol at 1.061 mg/g finally chloroform 0.248 mg/g as shown in Figure 2. These results may be due to the ability of water to dissolve the low molecular weight phenolic compounds.

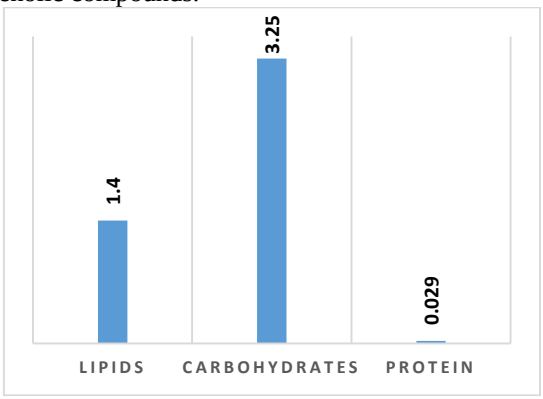


Figure 1. Lipids, carbohydrates, and protein contents (as %) of red onion peels.

These results matched well with the results obtained by Lee et al. [32] who reported that water extraction of onion peel yielded a higher number of phenolic compounds than ethanol extract [32].

b. Anthocyanin content:

The data in Figure 3 showed the total content of anthocyanin in red onion (*Allium cepa L.*) peels extracted using different solvents (chloroform, ethyl acetate, ethanol, water). The extraction process has a substantial impact on bioactive compounds and antioxidant activity.

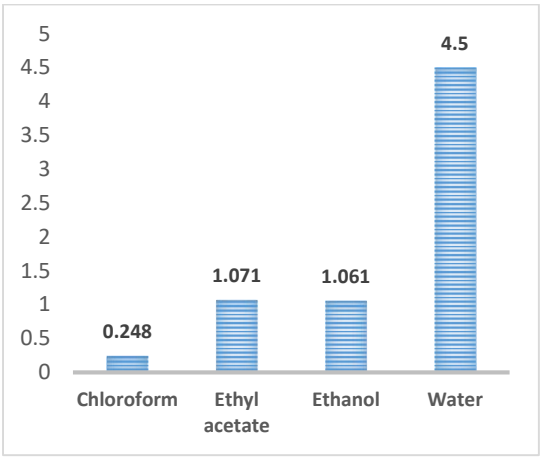


Figure 2: Phenolic compounds content (as mg GAE/g) of different red onion peels extracts.

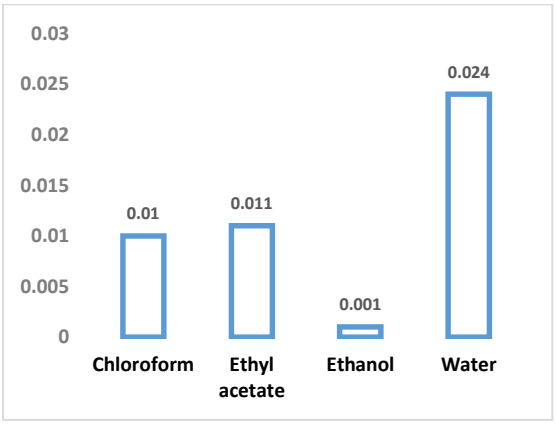


Figure 3. Anthocyanin content (as mg/100g) of different extracts of red Onion peels.

In general, the water extract showed the highest extraction content of total anthocyanins by 0.024mg/100g, compared to those in ethyl acetate, chloroform, and ethanol extracts (0.011, 0.01 and 0.001mg/100g) respectively. These results may be due to the polarity of solvent used for extraction of anthocyanin from onion peels. The yield of anthocyanin extraction was shown to be affected by extract nature, higher temperatures and longer extraction duration [33, 34].

Antioxidant activity:

a. DPPH radical assay:

The antioxidant activity of successive extracts from red onion (*Allium cepa L.*) peels was evaluated using DPPH radical scavenging method. The obtained results in Table 2 revealed that; the method is dependent on the concentration of extract and incubation time.

Table 2: The antioxidant activity (%) of different extracts of red onion against DPPH at different concentrations

Extract	Concentration (µg/ml)	
	50	**100**
Chloroform	10.10	22.93
Ethyl acetate	11.81	31.56
Ethanol	50.21	61.06
Water	78.88	85.93
Ascorbic acid	84.7	89.43
BHT	83.2	89.07

The obtained data demonstrate that water extract recorded remarkably the highest antioxidant activity against DPPH radical by 78.88% at 50 µg mL^{-1} in addition to 85.93% at 100 µg mL^{-1} followed in descending order by ethanol extract by 50.21% at 50 µg mL-1 in addition to 61.06% at 100 µg mL^{-1} followed by ethyl acetate extract which recorded 11.81% at 50 µg mL^{-1} and 31.56% at 100 µg mL^{-1} followed by chloroform with 10.10% at 50 µg mL^{-1} and 22.93% at 100 µg mL^{-1}.

These results may be due to the polarity index of water solvent which allows the phenolic compounds or compounds with antioxidant activity to be extracted in higher amounts than other solvents. This result was in agreement with the result obtained by [35, 36] they mentioned that there is high antioxidant activity of 70% ethanol and water extracts from red onion cultivars against DPPH radical assay.

b. ABTS radical assay:

The antioxidant activity of successive extracts from red onion (*Allium cepa L.*) peels was investigated using ABTS radical scavenging method. The observed results go parallel with the results obtained with DPPH radical assay as shown in Table (2). The gathered results that are reported in Table 3, revealed that; the water extract possessed the highest antioxidant activity against ABTS radical scavenging method by 46.43%, followed in descending order by ethyl acetate, chloroform, and ethanol extracts (32.14, 5.63 and 1.79%) respectively.

Table 3: The antioxidant activity (%) of different extracts of red onion against ABTS at different concentrations

Extract	Concentration (µg/ml)	
	50	**100**
Chloroform	8.43	19.40
Ethyl acetate	15.61	29.92
Ethanol	46.70	63.06
Water	74.76	83.21
Ascorbic acid	82.01	87.45
BHT	79.29	88.87

This could be due to the high levels of total phenolic compounds and total flavonoid compounds; this goes parallel with the data investigated by Masood et al. [36] who reported that there are great correlation between antioxidant activity and the concentration of phenolic and flavonoid compounds.

These findings showed that the peels could be used as a natural antioxidant in the same way as tea which suggested by [37].

It has been stated that incidence of multiple hydroxyl groups in flavonoids rises their antioxidant activity against peroxyl radicals [38].

The bigger antioxidant activity of onion peel's ethanolic extract shows its probable role in the inhibition of oxidative degradation of lipid bilayer of cell membranes which is quite related in terms of avoidance of chronic diseases development [36].

c. $KMnO_4$ assay:

The reaction with potassium permanganate depends on the oxidation and reduction reactions: in details: the reaction happened by oxidation of unsaturated bonds in antioxidant agents and convert it diol compounds, the obtained data of red onion peels extract with potassium permanganate reported that all red onion peel extract exhibited reducing activity lower than the reaction with anther radical reaction assay (ABTS and DPPH) as shown in Table 4. Moreover, the polar extracts (water and ethanol) exhibited high antioxidant activity than non-polar extracts and these findings may be due to their contents from antioxidant compounds such as flavonoids, phenolic compounds and mono sugars. These data were in agreement with the results obtained by [27] who reported that the obtained data of radical scavenging activity of fruit peels (Gauva and pomegrnant) revealed that the highest antioxidant activities were recorded with ABTS and DPPH method more than potassium permanganate, this may be due to that the radical assay such as DPPH and ABTS are unstable agents and react faster with different antioxidant compounds when compared with non-radical methods.

Table 4: The antioxidant activity (%) of different extracts of red onion against $KMnO_4$ at different concentrations

Extract	Concentration (µg/ml)	
	50	**100**
Chloroform	5.43	13.90
Ethyl acetate	10.45	17.65
Ethanol	11.09	34.67
Water	53.23	60.89
Ascorbic acid	75.90	78.56
BHT	73.23	76.09

Antiradical activity

Antiradical activity of successive extracts of red onion peels was determined using DPPH radical scavenging methods at 100 μgml^{-1} during the incubation times (0-360 sec).

The obtained results recorded in Figure (4), revealed that, the antiradical activity was shown to be incubation time-dependent. Ethyl acetate extract showed the highest antiradical activity represented as AU or antiradical unit against DPPH (0.122 and 0.105) when compared to the other extracts.

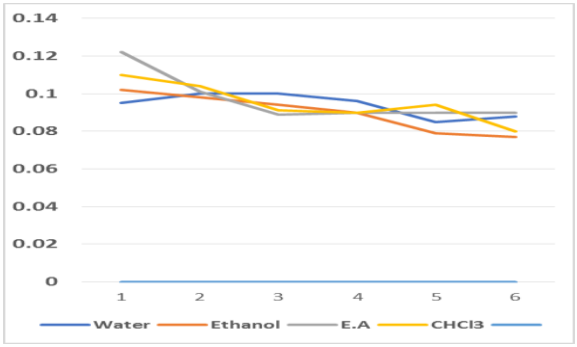


Figure 4. Antiradical Unit (AU) against DPPH of different extracts from red onion peels at 100 µg/ml.

The obtained data agreed with those reported by [39, 29] and in the same context [40]. Studied the radical scavenging and antiradical activities of aqueous methanolic extract of fenugreek seeds using different radical assays such as DPPH and ABTS and they revealed that the activities could be correlated with the content of phenolic compound concentration. The same data were mentioned by [41] who found that there was a very significant correlation between antioxidant, antiradical activities and total content of phenolic compounds in the methanol extracts of Rumex species.

GC-MS/MS analysis of water extract:

The GC-MS/MS analysis of water extract of red onion peels showed the presence of various phytocomponents. The phytocomponents of extract is presented separately in Table 5 and the GC-MS chromatogram with the peak area of each extract is

also shown in Figure 5. Totally seven constituents were identified in red onion peels water extract. The major compounds are β-D-glucopyranose (31.51%) followed by Xylose and Pseudojervine (8.46 and 6.45% respectively) no previous published data for phytochemical identified from red onion peels.

Bio-autography for Antioxidant Activity:

Antioxidant potential compounds separated on thin-layer chromatography plates were identified using ABTS radical reagent a shown in Figure 6.

The separated spots from the promising extract producing white or yellowish color on the green background were considered a strong radical scavenging activity. The obtained data in Figure 6 revealed that the nearest fractions from the start line showed white bands being formed at the region at retardation factors 0.15, 0.25 and 0.30 on exposure to ABTS. ABTS reagent measures the electron-donating activity of various active compounds and asses' antioxidant activity [42].

Synthesis of silver nanoparticles (Ag-NPs-W); UV-visible of NPs:

The water extract of red onion peels was added to the silver nitrate solution. The reaction colour was generated right away, and at the end of the reaction, it went from colourless to brown. Within one hour, the brown colour intensity grew significantly (during 5 min.) and remained consistent for around 1 hour. Because of their characteristic stimulation of surface plasmons in the 400–490 nm region, silver nanoparticles has a brown colour [43]. As a result, a change in the colour of the solution from colourless to brown indicates the formation of silver nanoparticles [44].

Table 5. List of phytochemical constituents (as Relative percentage) in water extracts of onion peels by GC-MS/MS.

	RT	Chemical name	Relative percentage	Biological activities	References
1	8.99	4-ethylbenzoic acid	4.3	Antimicrobial	(Cho *et al* 1998)
2	14.92	Aralionine	0.95	No activity reported	
3	32.88	Pseudojervine	6.45	Antimicrobial	(Han & Woo, 1973)
4	41.51	Xylose	8.46	Antibacterial	(Dreger *et al*., 2021; Singh, 2014)
5	45.27	β-D-glucopyranose	31.51	Antioxidant and Anti-Inflammatory Activities	(Legault *et al*., 2011)
6	50.52	Heptadecenoic acid	0.60	Antioxidant activity	(Al-Douri & Shakya, 2019; Elagbar *et al*., 2016)
7	57.27	Corlumine	0.47	Spasmolytic activity	(Bhakuni, 1984)

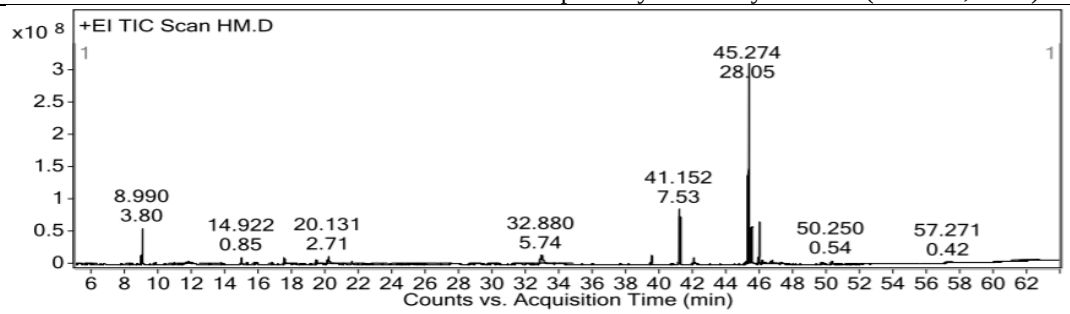
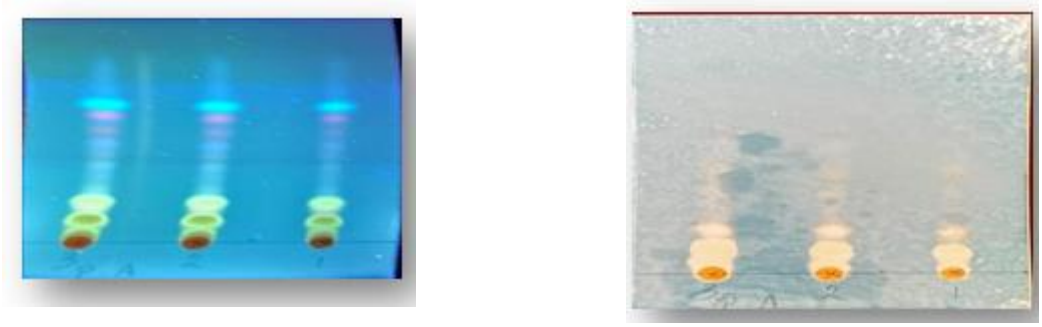


Figure 5. GC/MS chromatogram of red onion peels water extract.



Petroleum ether: Acetone 7: 3 **Petroleum ether: Acetone 7: 3 (After sprayed with DPPH)**

Figure 6. TLC chromatogram of red onion peels water extracts (under short UV lamp at 254 nm) and TLC-bioautography of water extracts (Sprayed with and without DPPH reagent).

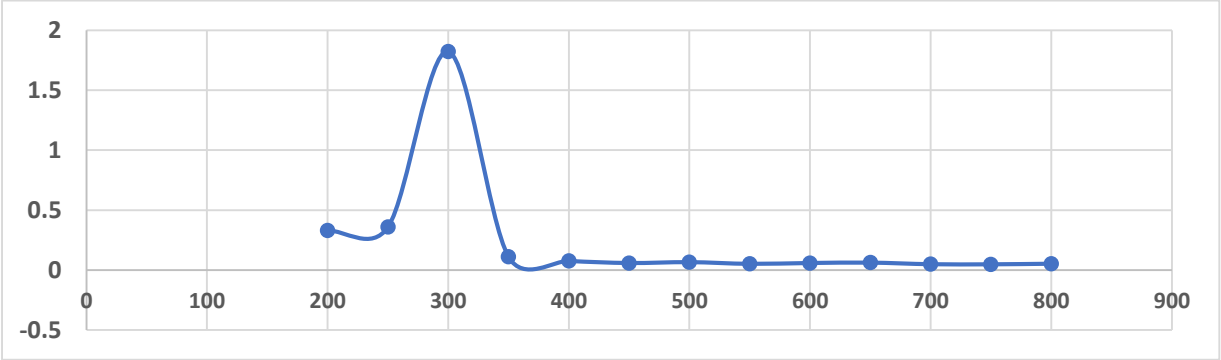


Figure 7. UV–VIS spectra of Onion peels water extract with (Ag-NPs-W)

The aqueous extract of red onion peels had a significant reduction potential for reduced silver ions and the synthesis of Ag-NPs, according to this study. As shown in Fig. 7, the UV–VIS spectra of produced silver nanoparticles had a maximum peak at 300 nm, which was similar with the spectra of spherical silver nanoparticles in the 380–450 nm wavelength range. [45, 46, 47] all have shown similar surface plasmon resonance peaks in green production of silver nanoparticles (2021).

FTIR of nanoparticles

FTIR measurements were carried out to identify the promising biomolecules in the red onion peels water extract accountable for the silver ion reduction and the capping agent liable for the reduced AgNPs stability.

The FTIR spectra of aqueous extract of red onion peels and its silver nanoparticles, as illustrated in Figures 8 a and b and Table 6, were recorded in the frequency range between 4400 and 400 cm 1. The obtained results revealed that the FTIR spectra peaks of red onion peels extract (3448 and 1635 cm $^{-1}$) and manufactured AgNPs (3455 and 1636 cm $^{-1}$) were slightly shifted. This could be because some active groups are involved in the reduction reaction that converts silver ions to metal. According to the FT-IR analysis, the reasonable mechanism for the creation of silver nanoparticles could be due to the reduction of silver ions that occurs in conjunction with the oxidation of flavonoids and phenolic components of polyols or other compounds. [48, 49].

Antioxidant activity of Ag-NPs-water

The results obtained in Table 7 indicates the antioxidant activity of water extract and Ag-NPs of red onion by $KMnO_4$ radical scavenging method, which revealed the antioxidant activity increased in case of silver nanoparticle water extract by 74.29% when compared with native water extract (60.89%). This activity may be due to the increase of particle size area in case of nano form (at the same concentration of native extract) which increase the capping of active groups such as hydroxyl, carbonyl, carboxyl and amine from water extract of onion peels. These chemical groups increase the radical scavenging activity of Ag-NPs when compared with native extract of onion peels.

Table 6. Wavenumbers range of characteristic bands and corresponding assignments for red onion peels and Ag-NPs-W.

Wave-number range (cm $^{-1}$)	Function groups assigned	Sample water extract	Sample Ag-NPs-W
3300-4000	Polymeric hydroxyl compound O-H stretching	3448	3455
1700-1630	C=O stretching vibration, C-N stretching, Lipids, Ester carbonyl – COOR and carboxylate ion stretching (-COO-)-	1635	1636

Table 7. The antioxidant activity (%) of Ag-NPs and water extract of red onion against $KMnO_4$.

Extracts	Antioxidant activity %
Ag-NPs	74.29%
Water	60.89%

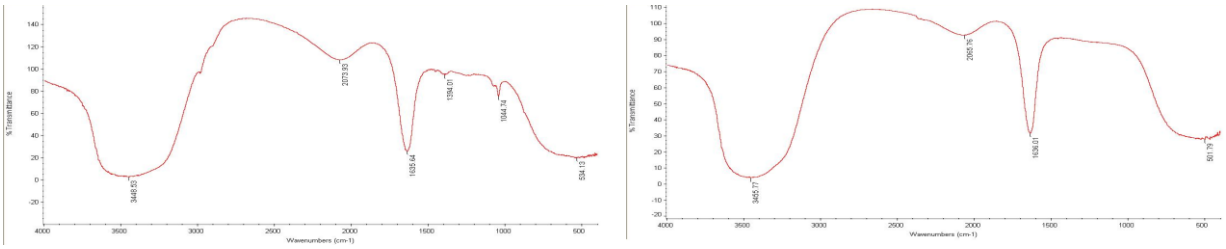


Figure 8: FTIR spectra of Onion peels water extract (a, above picture) and silver nanoparticles synthesized solution (b).

Conclusions

The current study used the peels from Egyptian red onion for the first time as source of biological compounds as antioxidants. From the obtained data in the current work, we can be concluded that the crude polar and non-polar extracts of red onion peels contain a wide variety of natural products such as phenolic and flavonoids compounds that could serve as antioxidant, anticancer, antiradical and reducing or capping agents in the synthesis of nanoparticles. The biological activities of red onion peels extracts were dependent on time and concentration of extract.

Moreover, the obtained results revealed that water extract of red onion peels exhibited the highest antioxidant and antiradical activity when compared with other three crude extracts and natural antioxidant standard (ascorbic acid). As, the antiradical activity being defined as the ability of a compound to react with free radicals in a single free radical reaction, the calculated antiradical activity units showed the highest values after 360 seconds (6 minutes) compared with antioxidant activity (30 min). Also, there are great correlation the phenolic compounds concentration in peels extract and the antioxidant and antiradical activity.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data used and analyzed in this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

References

[1] Cascajosa-Lira, A., Puerto, M., Prieto, A. I., Pichardo, S., Díez-Quijada Jiménez, L., Baños, A., Guillamón, E., Moyano, R., Molina-Hernández, V., Jos, Á., Cameán, AM. Cameán, A. M. Genotoxicity Evaluation of Propyl-Propane-Thiosulfinate (PTS) from Allium genus Essential Oils by a Combination of Micronucleus and Comet Assays in Rats. Foods, 10(5), 989. (2021).

[2] Ouyang H, Hou K, Peng WX, Liu ZL, Deng HP. Antioxidant and xanthine oxidase inhibitory activities of total polyphenols from onion. Saudi J Biol Sci. 25:1509–13. (2018) doi: 10.1016/j.sjbs.2017.08.005

[3] Loredana L, Giuseppina A, Filomena N, Florinda F, Marisa D, Donatella A. Biochemical, antioxidant properties and antimicrobial activity of different onion varieties in the Mediterranean area. J Food Meas Charact 13:1232–41. (2019) doi: 10.1007/s11694-019-00038-2.

[4] Jakaria M, Azam S, Cho DY, Haque ME, Kim IS, Choi DK. The methanol extract of Allium cepa L. protects inflammatory markers in lps-induced bv-2 microglial cells and upregulates the antiapoptotic gene and antioxidant enzymes in N27-A cells. Antioxidants. 8:348. (2019) doi: 10.3390/antiox8090348.

[5] Jini D, Sharmila S. Green synthesis of silver nanoparticles from Allium cepa and its in vitro antidiabetic activity. Mater Today Proc. 22:432–8. . (2020). doi: 10.1016/j.matpr.2019.07.672.

[6] Salami M, Tamtaji O, Mohammadifar M, Talaei S, Tameh A, Abed A, Shirkhoda, S., Dadgostar, E. and Taghizadeh, M. Neuroprotective effects of onion (Allium cepa) ethanolic extract on animal model of parkinson's disease induced by 6-hydroxydopamine: a behavioral, biochemical, and histological study. Gazi Med J. 31:25– 9. (2020) doi: 10.12996/gmj.2020.07.

[7] El-Hashim AZ, Khajah MA, Orabi KY, Balakrishnan S, Sary HG, Abdelali AA. Onion bulb extract downregulates EGFR/ERK1/2/AKT signaling pathway and synergizes with steroids to inhibit allergic inflammation. Front Pharmacol. 11:551683. (2020). doi: 10.3389/fphar.2020. 551683.

[8] Khajah MA, El-Hashim AZ, Orabi KY, Hawai S, Sary HG. Onion bulb extract can both reverse

and prevent colitis in mice via inhibition of pro-inflammatory signaling molecules and neutrophil activity. PloS ONE. (2020) 15:e0233938. doi: 10.1371/journal.pone.02 33938.

[9] Putnik, P., Gabrić, D., Roohinejad, S., Barba, F. J., Granato, D., Mallikarjunan, K., Lorenzo, J. M., & Kovačević, D. B. An overview of organosulfur compounds from Allium spp.: From processing and preservation to evaluation of their bioavailability, antimicrobial, and anti-inflammatory properties. Food Chemistry, 276, 680–691. (2019).

[10] Fredotović, Ž., Soldo, B., Šprung, M., Marijanović, Z., Jerković, I., & Puizina, J. Comparison of organosulfur and amino acid composition between triploid onion Allium cornutum Clementi ex Visiani, 1842, and Common Onion Allium cepa L., and evidences for antiproliferative activity of their extracts. Plants, 9(1), 98. (2020).

[11] Akaranta, O., & Akaho, A. A. Synergic effect of Citric Acid and Red Onion skin extract on the Oxidative stability of Vegetable Oil. Journal of Applied Sciences and Environmental Management, 16(4). (2012).

[12] Hammouda, A. Z. Efficiency of Red Onion Peel Extract Capsules on Obesity and Blood Sugar. Pakistan Journal of Biological Sciences: PJBS, 24(1), 99–111. (2021).

[13] Fredotovi´c, Ž.; Šprung, M.; Soldo, B.; Ljubenkov, I.; Budic´-Leto, I.; Bilušic´, T.; C˘ ikeš-C˘ ulic´, V.; Puizina, J.Chemical Composition and Biological Activity of Allium cepa L. and Allium cornutum (Clementi ex Visiani1842) Methanolic Extracts. Molecules 2017, 22, 448.

[14] El-fayoumy EA, Shanab SM, Gaballa HS, Tantawy MA, Shalaby EA. Evaluation of the antioxidant and anticancer activity of crude extracts and different fractions of Chlorella vulgaris axenic culture grown under various concentrations of copper ions. BMC Complementary Medicine and Therapies, 21:51, 1-16. (2021).

[15] Marrelli, M., Amodeo, V., Statti, G., & Conforti, F. (2019). Biological properties and bioactive components of Allium cepa L.: Focus on potential benefits in the treatment of obesity and related comorbidities. Molecules, 24(1), 119.

[16] Xu, X.; J. and Hallam, D. N. Modification of fatty acid composition in halophilic antractic microalgae. Phytochemistry, 49: 1249-1252. (1998).

[17] AOAC. Official Methods of analysis of official analytical chemistry. Pub. By the Association of Analytical Chemistry, Inc., Arlington, West Virginia, USA. (1990).

[18] Dubios, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A. and Smith, F. Anal. Chem., 28: 350. (1956).

[19] Stickland GG, Sunderland N. Production of anthocyanin, flavonols and chlorogenic acid by cultured callus tissues of Haplopappus gracilis. Ann. Bot. 36: 443-457. (1972)

[20] Berieskern, C.H. and Klinger-Handpolonius, W. Triterpene and sterol in leaves of Saliva trilopa and Pyrus malus. Arch. Pharm., 294: 380-391. (1961).

[21] Wall, M. E.; Kreider, M. M.; Kremson, C. F.; Eddy, C. R.; Williaman, J. J.; Coree, D. S. and Gentry, H. S. Steroidal saponins: Survey of plants for steroidal of sapogenins and other constituents. J. Pharm. Soc., 43: 1-3. (1954)

[22] Claus, E. R. Pharmacognosy 5th Ed. Herny Kimpton, Co. Inc., London. (1967).

[23] Harper, H. A. Review of physiological chemistry 15th Ed. Long Medicinal (1975).

[24] Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turk J Biol; 29:41- 47. (2005).

[25] Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenecity. Journal of Agriculture Food Chemistry. 43, 27–37. (1995).

[26] Re R, Pellegrini RN, Proteggente A, Pannala A, Yang M, Rice- Evans C. Antioxidant activity applying improved ABTS radical cationdecolorization assay. Free Radical Biology and Medicinal, 26: 1231-1237. (1999).

[27] Gaber, N.B., El-Dahy, S.I. & Shalaby, E.A. Comparison of ABTS, DPPH, permanganate, and methylene blue assays for determining antioxidant potential of successive extracts from pomegranate and guava residues. Biomass Conv. Bioref. (2021). https://doi.org/10.1007/s13399-021-01386-0.

[28] Wen XB, Miao F, Zhou L, Zhang M, He QL. In vitro antioxidant activity of Parnassia wightiana

W. extracts, Chinese Journal of Natural Medicines, 10(3): 190-195. (2012).

[29] Shalaby EA, Shanab SMM. Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of Spirulina platensis. Indian Journal of Geo-Marine Sciences 42(5):556-564. (2013).

[30] Khattak U, Ullah R, Khan S, Afriq S, Rauf A, Hassanien M. Synthesis, characteristics, and biological activities of silver nanoparticles from Euphorbia dracunculiases, EurAsia J. BioSci. 13(2): 2249–2260. (2019).

[31] Ifesan, B. O. T. Chemical composition of onion peel (Allium cepa) and its ability to serve as a preservative in cooked beef. Int. J. Sci. Res. Methodol, 7(4), 1–10. . (2017).

[32] Lee, K. A., Kim, K.-T., Kim, H. J., Chung, M.-S., Chang, P.-S., Park, H., & Pai, H.-D. Antioxidant activities of onion (Allium cepa L.) peel extracts produced by ethanol, hot water, and subcritical water extraction. Food Science and Biotechnology, 23(2), 615–621. (2014).

[33] Moldovan, B., David, L., Chişbora, C., & Cimpoiu, C. Degradation kinetics of anthocyanins from European cranberrybush (Viburnum opulus L.) fruit extracts. Effects of temperature, pH and storage solvent. Molecules, 17(10), 11655–11666. (2012).

[34] Ali, O.-H., Al-sayed, H., Yasin, N., & Afifi, E. Effect of different extraction methods on stablity of anthocyanins extracted from red onion peels (Allium cepa) and its uses as food colorants. Bulletin of the National Nutrition Institute of the Arab Republic of Egypt, 47(2), 1–24. (2016).

[35] Yang, S. J., Paudel, P., Shrestha, S., Seong, S. H., Jung, H. A., & Choi, J. S. In vitro protein tyrosine phosphatase 1B inhibition and antioxidant property of different onion peel cultivars: A comparative study. Food Science & Nutrition, 7(1), 205–215. (2019).

[36] Masood, S., Ihsan, M. A., Shahzad, K., Sabir, M., Alam, S., Ahmed, W. & Chung, G. Antioxidant potential and α-glucosidase inhibitory activity of onion (Allium cepa L.) peel and bulb extracts. Brazilian Journal of Biology, 83. (2021).

[37] Suzuki, T., Pervin, M., Goto, S., Isemura, M., & Nakamura, Y. Beneficial effects of tea and the green tea catechin epigallocatechin-3-gallate on obesity. Molecules, 21(10), 1305. (2016).

[38] Vu, N. K., Kim, C. S., Ha, M. T., Ngo, Q. M. T., Park, S. E., Kwon, H., ... & Min, B. S. Antioxidant and antidiabetic activities of flavonoid derivatives from the outer skins of Allium cepa L. Journal of agricultural and food chemistry, 68(33), 8797-8811. (2020).

[39] Nivas D, Gaikwad DK, Havan PD. Antiradical activity of radically important Morinda pubescens fruits. International J. Pharma and BioScience 1 (3): 1-4. (2010).

[40] Kaviarasan S, Naik GH, Gangabhagirathi R, Anuradha CV, Priyadarsini KI In vitro studies on antiradical and antioxidant activities of Fenugreek (Trigonella foeveem) seeds. Food Chemistry, 103: 31-37. (2007).

[41] Shafiq N, Rafiq N, Saleem M and Rafiq S: Chemical and biological analysis of the extract from the plant Rumex hastatus for its secondary metabolites Bioinf Pharm Chem Sci; 3: 40-4. (2017).

[42] Prema, R., Sekar, D. S., Sekhar, K. B., & Jeevanandham, S. In vitro cytotoxicity study on combined plants extracts (Cissus quadrangularis and Aegle marmelos). Euro J Exp Bio, 2, 882–888. (2012).

[43] Gilaki M. Biosynthesis of Silver Nanoparticles using Plant Extracts. Journal of Biological Sciences 10(5):465-467. (2010).

[44] Vanaja M, Shanmugam R, Paulkumar K, Gnanajobitha G. Kinetic study on green synthesis of silver nanoparticles using Coleus aromaticus leaf extract. Advances in Applied Science Research 4(3):50-55. (2013).

[45] Yugaya YA, Usoltsevab RV, Silantevc VE, Egorovaad AE, Karabtsove AA, Kumeikodf VV, Ermakovab SP, Bulgakova VP, Shkryl YN. Synthesis of bioactive silver nanoparticles using alginate, fucoidan and laminaran from brown algae as a reducing and stabilizing agent. Carbohydrate polymers, 245: 116547. (2020).

[46] Ndikau M, Noah NM, Andala DM, Masika E Green Synthesis and Characterization of Silver Nanoparticles Using Citrullus lanatus Fruit Rind Extract. International Journal of Analytical Chemistry. (2017). ID 8108504 | https://doi.org/10.1155/2017/8108504.

[47] Desai, R.; Mankad, V.; Gupta2, S.K.; and Jha, P. K. Size Distribution of Silver Nanoparticles: UV-Visible Spectroscopic Assessment, Nanoscience and Nanotechnology Letters Vol. 4, 30–34. (2012).

[48] Gandhi, MSA, Kumar VS, Li Q. Synthesis of

Silver Nanoparticles using Rumex Crispus Extract and Evaluation of their Antibacterial Activities. Asia Communications and Photonics Conference 2020. (2020).

[49] Younes, K. M., Romeilah, R. M., El-Beltagi, H. S., Hani, E. L., Rajendrasozhan, S., El-Shemy, H. A., & Shalaby, E. A. In-vitro evaluation of antioxidant and antiradical potential of successive extracts, semi-purified fractions and biosynthesized silver nanoparticles(2021).