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Evaluation of Antioxidant Activity of Synthesized Silver Nanoparticles from *Citrus Aurantium* Peels Extract by Using the Green Method

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Abstract: The substance which avoids oxidant of easily oxidizing substance even at low concentration is called antioxidant when antioxidant reacts with reactive oxygen species (ROS) or reactive nitrogen species (RNS) they often convert themself into antioxidant radical. Although such a radical has a reduced ability to react with imperative cellular targets, it can still cause damage. To decrease reactivity and reduction potential further down, the 'antioxidant radical' react with another antioxidant and this reaction continues in a stepwise manner until antioxidant radical' is no longer damage the cells like lipids, protein, DNA, and other important cellular molecules. In this research article, we had scanned our previously synthesized silver nanoparticles from *Citrus aurantium* peels extracts; Synthesis and characterization part of the research work is already in communication we are extending our research work on previously synthesized silver nanoparticles. In this research article, we had scanned the antioxidant activity of synthesized AgNPs was determined by H_2O_2 free radical scavenging activity, we obtained antioxidant activity of BHT with IC_{50} Value 65.52 µg/mL and antioxidant activity of synthesized AgNPs with IC_{50} Value 93.92 µg/mL. Antioxidant activity of synthesized AgNPs was also determined by DPPH free radical scavenging activity. We obtained antioxidant activity of BHT with IC_{50} Value 65.99 µg/mL and antioxidant activity of synthesized AgNPs with IC_{50} Value 99.68 µg/mL.

Keywords: Green method; Silver nanoparticle; Antioxidant activity; *Citrus aurantium*.

Introduction

Nobel metal nanoparticles especially silver nanoparticles have got great consideration in various fields of science and technology, due to its excellent utilization in various fields such as antimicrobial potential, environment remedy, chemical industry, photo-catalyst, drug delivery, and as biosensors (Wang et.al; 1991, Subbiah et.al; 2010). Additionally silver nanoparticles have several pharmacological applications like as antibacterial activity, anti-plasmodial activity, antifungal activity, anticancer, antiviral activity and antioxidant activity (Kuppusamy, et.al; 2016). In past few years antioxidant activity of silver nanoparticles is quite interesting to research topic for the scientific community due to their application in managing degenerative diseases.

Antioxidant agents which may be enzymatic or non-enzymatic materials regulate free radical,

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which is produced by various reactive oxygen species (ROS) or reactive nitrogen species (RNS).

The biological human body generates various free radicals inside our body to cure various diseases and antioxidant scavenged free radicals protect the damage of crucial bio-molecules. Antioxidants have a great advantage for the treatment of various unceasing diseases like diabetes, cancer, and brain dysfunction (Bhaumik et.al; 2015) sometimes antioxidants generated by our body may be insufficient to protect against various diseases so the human body needs the supply of antioxidants from outside. Alternatively silver nanoparticles show strong antioxidant potential than synthetic commercial antioxidants.

There are various methods which are reported for synthesis of silver nanoparticles (Khan et.at; 2011b , Chen et.al; 2007, Reicha et.al; 2012 , Abid et.al; 2012 , Khan et.al; 2011a , Alarcon, et.al; 2012), which include several physical and chemical methods but those methods are not safe for the environment due to hazardous and toxic side products obtained from those methods. So there is a great need for an eco-friendly method that does not harm the environment. Interestingly, the green methods have many advantages over other traditional methods (Krishnaraj et.al; 2010). There are various biological methods available for the synthesis of nanoparticle using bacteria, fungi, plant (Krishnaraj et.al; 2010). Several researchers have synthesized nanoparticle by using plant extract as reducing and capping agent (Sati et.al; 2020b).

Citrus aurantium is an evergreen tree, leaves are generally oblong, or eclipsed, 9-15 cm long, incised, base cuneate (leaf shape; narrowly triangular, wider at the apex and tapering toward the base.), apex acute or blunt. Flowers are found in terminal or axillary clusters of 4-9, and generally bisexual pinkish-white Stamens 20-30. Fruits oblong, slightly notched, 8-10 cm across rind thick, generally oblate, not mamillate, usually orange-colored, Pulp sweet, yellow, and rarely red (Gaur 1999, Anonymous 2001, Kirtikar et.al; 1918). In this study we have evaluated antioxidant activity of synthesized AgNPs by H_2O_2 and DPPH free radical scavenging activity this is the novelty of this research work.

Fig.1: Fruits of *Citrus aurantium*

Synthesis and Characterization

We have synthesized silver nanoparticles from peels extract of *Citrus aurantium* on the basis of green chemistry principles [Bartwal et.al; 2020; Sati et.al; 2020a]. The brief synthesis protocol has illustrated in our previous article Sati et.al. 2020b. Characterization of synthesized AgNPs was done by several spectroscopic techniques like UV-Visible spectroscopy, X-ray diffraction method followed by SEM, EDAX, and TEM studies.

Evaluation of antioxidant activity

DPPH free radical Scavenging Activity of synthesized AgNPs

DPPH free radical Scavenging Activity of synthesized AgNPs was determined by the standard method (Cuendet et.al; 1997; Burits et.al., 2000). Five different concentrations of methanolic solution of the sample were added to 5 mL of a 100 µmol methanolic solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH in percent (I%) was calculated using the formula presented in the literature (Kharat et.al; 2006).Assays were carried out in triplicate. Synthetic antioxidant butylated hydroxytoluene (BHT) was used as a positive control.

Hydrogen Peroxide Radical Scavenging Activity

Hydrogen Peroxide Radical Scavenging Activity of synthesized nanoparticles was determined by standard method (Thampi et.al; 2015). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer saline (at pH 6.8). Different concentrations of nanoparticles and nanocomposite (20, 40, 60, 80, and 100 µg/mL) were prepared, from each concentration 4 mL of the test sample was mixed with 0.6 mL of previously prepared H_2O_2 solution. The absorbance of the solution was measured at 230 nm after 10 minutes against blank solution using a UV-Vis spectrophotometer. At 230 nm, the absorbance of hydrogen peroxide (control) was 2.698. Similarly free radical scavenging activity of aqueous extract of *Citrus aurantium* peels was done. Synthetic antioxidant butylated-hydroxytoluene (BHT) was used as a positive control. The percentage (%) scavenging of H_2O_2 by the test samples was calculated. Each experiment was carried out in triplicates and the results were recorded as a mean or % scavenging activity. The percentage of hydrogen peroxide scavenging by the test samples is calculated using the formula presented in the literature (Kharat et.al., 2016).

$$\% reduction = \frac{A_c - A_t}{A_c} \times 100$$

Where, $\mathbf{A_c}$ = Absorbance of the control; $\mathbf{A_t}$ = Absorbance of sample.

Results and discussion

Silver nanoparticles were fabricated by using green methods and *Citrus aurantium* peels extract was used as a stabilizing and capping agent. Synthesized AgNPs (silver nanoparticles) has been characterized by using different spectroscopic techniques like UV-Visible spectroscopy, X-RD, SEM, EDAX, and TEM. In UV-Visible absorption spectroscopy the peak was obtained at 465nm which is characteristics peak for silver nano particles. Resulted XRD patterns confirm the cubic crystalline structure of AgNPs. The average grain size 9.5 µm was observed by the SEM technique. The observed

EDAX spectrum indicated peaks, for the presence of Ag, C, O, K, Ca, and Cl. The observed Ag peak may be originated from AgNPs,

Antioxidant activity

DPPH free radical scavenging activity of AgNPs

The antioxidant activity of synthesized AgNPs was determined by DPPH free radical scavenging activity taking a different concentration of silver nanoparticle solution that is 20, 40, 60, 80, and 100 µg/mL. BHT solution was used as a positive control; the antioxidant activity of BHT and the antioxidant activity of synthesized AgNPs are shown in table 1. Obtained antioxidant activity of BHT with IC_{50} value 65.99 µg/mL [see Fig.2(a)] and antioxidant activity of synthesized AgNPs with IC_{50} value 99.68 µg/mL [Fig.2(b)].

Table 1: DPPH free radical scavenging activity of Standard (BHT) and AgNPs

S.No.	Concentration (µg/mL)	Absorbance		% Reduction		IC_{50} Value (µg/mL)
		Standard	AgNPs	Standard	AgNPs	
1	20	0.362	0.419	19.55	6.84	**Standard**
2	40	0.336	0.397	25.33	11.77	**65.99**
3	60	0.252	0.341	44	22.81	
4	80	0.172	0.270	62.00	40.00	**AgNPs**
5	100	0.101	0.200	76.30	51.11	**99.68**

It is observed that there is a significant change in the DPPH radical scavenging activity for studied samples. The scavenging capacity increased in a dose-dependent manner. The recorded % reduction was found to be minimum at the lowest concentration that is at 20 µg/mL, BHT reduced to 19.55% while when the concentration reaches 100 µg/mL BHT reduced upto 76.30%. While for the sample (AgNPs) the scavenging capacity increased in a dose-dependent manner. The recorded % reduction was found to be minimum at the lowest concentration that is at 20 µg/mL sample reduced to 6.84 % while when the concentration reaches 100 µg/mL sample reduced upto 51.11%.

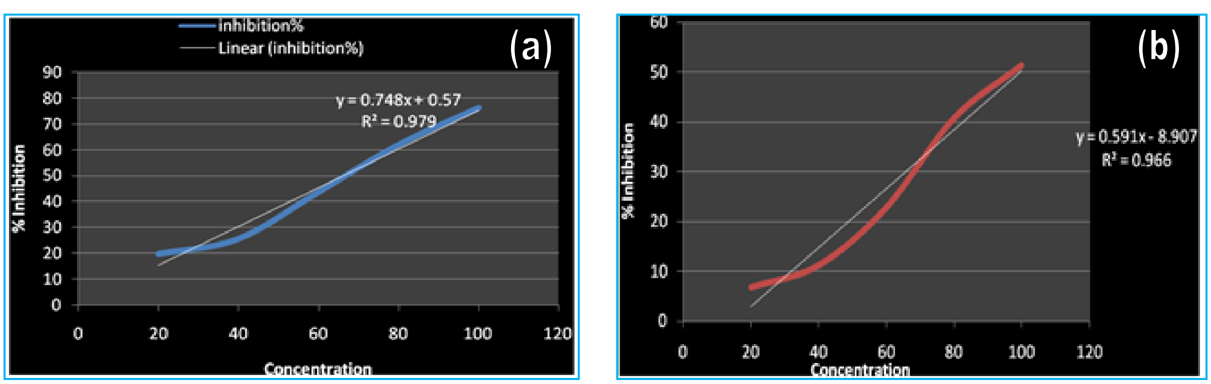

Fig.2: DPPH free radical scavenging activity of **(a)** BHT; **(b)** AgNPs

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Literature survey reveals that there is various research study carried by the researcher in the field of nanoscience especially on the medicinal plant but no report is available on antioxidant activity of synthesized silver nanoparticles from *Citrus aurantium* peels extract. But there is several research data available on antioxidant activity of orange varieties (*Citrus sinensis* L. and *Citrus aurantium* L.) cultivated in Algeria: peels and leaves (Lagha et.al., 2013).

H_2O_2 free radical scavenging activity of AgNPs from *Citrus aurantium* peels extract

The antioxidant activity of synthesized AgNPs was determined by H_2O_2 free radical scavenging activity taking a different concentration of silver nanoparticle solution that is 20, 40, 60, 80, and 100 µg/mL. BHT solution was used as a positive control. Antioxidant activity of BHT and synthesized AgNPs are shown in table 2.

Table 2: H_2O_2 free radical scavenging activity of Standard (BHT) and AgNPs

S.No.	Concentration (µg/mL)	Absorbance		% Reduction		IC_{50} Value (µg/mL)
		Standard	AgNPs	Standard	AgNPs	
1	20	2.224	2.390	17.62	11.48	**Standard 65.52**
2	40	1.966	2.247	27.18	16.77	
3	60	1.483	1.963	45.07	17.29	
4	80	1.022	1.456	62.14	46.07	**AgNPs 93.92**
5	100	0.620	1.163	77.03	56.92	

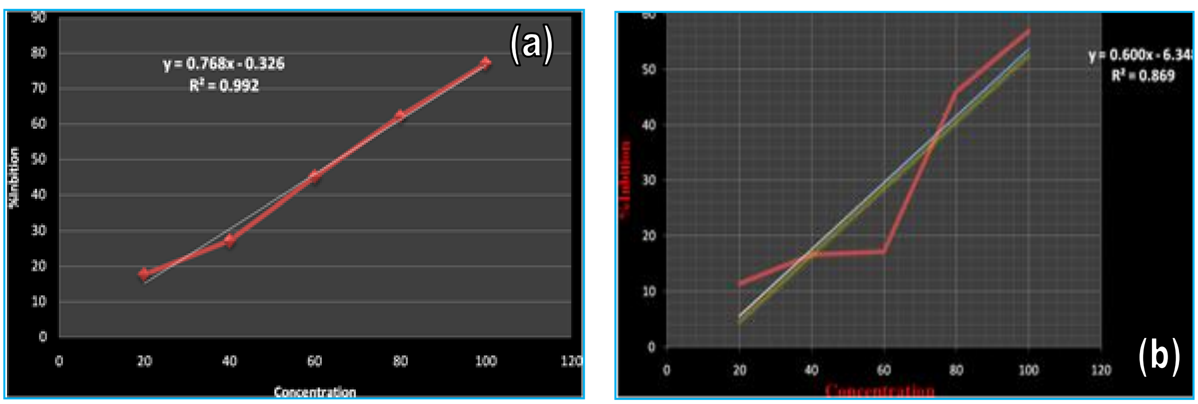

Fig.3: H_2O_2 free radical scavenging activity of **(a)** BHT; **(b)** AgNPs

We obtained antioxidant activity of BHT with IC_{50} Value 65.52 µg/mL [Fig.3(a)] and antioxidant activity of synthesized AgNPs with IC_{50} Value 93.92 µg/mL [see Fig. 3(b)].

Conclusion

AgNPs has been synthesized by using peels extract of *Citrus aurantium* as a stabilizing and capping agent. Synthesized silver nanoparticles have been characterized by UV-Visible spectroscopy, X-RD, SEM, E-DAX, and TEM. In UV-Visible absorption spectroscopy the peak was obtained at 465 nm which is characteristics peak for silver nano particles. XRD result shows that the synthesized AgNPs have cubic crystalline structure. The average grain size of AgNPs was 9.5 µm, which was observed by the SEM technique. The EDAX spectrum shows the peaks, for the presence of Ag, C, O, K, Ca, and Cl. Synthesized AgNPs from *Citrus aurantium* peels extracts has significant potential for an antioxidant activity like H_2O_2 free radical scavenging activity, we obtained antioxidant activity of BHT with IC_{50} Value 65.52 µg/mL and antioxidant activity of synthesized AgNPs with IC_{50} Value 93.92 µg/ml. Antioxidant activity of synthesized AgNPs was also determined by DPPH free radical scavenging activity we obtained antioxidant activity of BHT with IC_{50} Value 65.99 µg/mL and antioxidant activity of synthesized AgNPs with IC_{50} Value 99.68 µg/mL.

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