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Antioxidant Potential of the Biosynthesized Silver, Gold and Silver-Gold Alloy Nanoparticles using *Opuntia ficus-indica* extract

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Abstract

Nanobiotechnology has become a resourceful/crucial research area due to their copious applications in industrial, pharmacological and medical fields. In the current study, silver nanoparticles (OFI-AgNPs), gold nanoparticles (OFI -AuNPs) and bimetallic alloy nanoparticles (OFI-Ag-AuNPs) were mediated with *Opuntia ficus indica* (OFI) extract. Evaluation of antioxidant potential of the biosynthesized nanoparticles was done through total phenolic content (TPC), total flavonoid content (TFC) and Nitric oxide scavenging assay. The nitric oxide scavenging activity, total phenolic and total flavonoid contents of the synthesized nanoparticles increased in a dose dependent manner as compared to the standard. The OFI-AuNPs had highest total phenol of 258.28 µg GAE/g and OFI-extract gave the least value of 216.64 µg GAE/g at concentration of 100 µg/mL. The highest value of total flavonoid content (83.88 µg g^{-1} QE) was recorded at OFI-AgNPs, while the least value of 21.38 µg g^{-1} QE obtained in OFI-extract. OFI-Ag-AuNPs showed the highest nitric scavenging power of 50.8%, followed by OFI-AgNPs (42.2%), OFI-AuNPs (40.3%) and the least value of 29.7% were obtained in OFI-extract at concentration of 100 µg/mL. These results indicate *Opuntia ficus indica* as a suitable biomaterial for the synthesis of the nanoparticles which can be utilized as an antioxidant agent. In conclusion, these fascinating bioactivities exhibited by the synthesized nanoparticles established their usefulness in the production of antioxidants OFI-AgNPs, OFI-AuNPs and OFI-Ag-AuNPs for their biomedical applications

Keywords: *Opuntia ficus indica,* antioxidant activity, nanoparticles, nitric oxide, total phenolic (TPC), flavonoid content (TFC)

Introduction

Nanobiotechnology has become an emerging field of research interest to many scientists all over the world. This is a field of research which grows exponentially with a vast application in science and technology. It is an interdisciplinary area of well leading to the evolution of a novel approach to

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manipulate minutes particles resulting in the production of nanoparticles (NPs). Different methods and procedures for the preparation and synthesis of the nanoparticles like chemical and physical methods had been well established (Nath & Banerjee, 2013; Srinoi *et al*., 2018). However, due to the toxic and hazardous effect on the part of the chemically synthesized nanoparticles has rendered it unsuitable for the safety of human health and the environment (Mohan *et al*., 2014; John Leo & Oluwafemi, 2017).

Therefore, the call for the development of an eco-friendly procedure for the synthesis of nanoparticles beneficial to the human health and the environment has now received an extensive consideration. Green synthesis which involves the utilizations of various biological system such as yeast, fungi, bacteria and different plant parts and extracts are now in use for the synthesis of the nanoparticles (Unuofin *et al.,* 2020). Several plants parts and extracts had been reportedly used to synthesize nanoparticles. Some of those previously used include *Persea americana* fruit peel (Adebayo *et al.*, 2019 a), *Opuntia ficus indica* (Adebayo *et al.,* 2019b), cocoa pod (Lateef *et al.*, 2016), *Boerhaavia diffusa* (Ahmed *et al.,* 2020), *Cola nitida* (Lateef *et al.*, 2016), *Amaranthus caudatus* (Lateef *et al.,* 2017) and *Opuntia sticta* cladodes (Izuegbuna *et al.,* 2019). Biological method of nanoparticles synthesis has become preferable and safe method due to many factors such as low reduction cost, simplicity of the process and their resulting product which is compatible for pharmaceutical application and several biomedical use (Iravani *et al*., 2014; Pugazhendhi *et al*., 2019; Arif *et al.,* 2020; Verma *et al*., 2020; Jadoun *et al.,* 2020;).

Opuntia species is a genus that belongs to the family Cactaceae. The genus *Opuntia* spp. remains one of the largest family Cactaceae which has more than 1500 known species (Hegwood, 1990). *Opuntia* spp. like *Opuntia ficus-indica* have gained the interest of most researchers' sequel to their commercial value while other species were less documented. *Opuntia* spp. originated from Mexico but has now spread globally throughout the world (El-Mostafa *et al.,* 2014; Aparicio-Fernandez *et al*., 2017). The medicinal and therapeutic importance of *Opuntia ficus-indica* species had been known for a very long time in the traditional medicine (Knishinsky, 2014; González-Stuart & Rivera, 2019). It has been shown to be a satisfactory source of nutrients and antioxidants. It has health benefits such as protective effects of the cardiovascular system, hepatoprotector, chemopreventive, antiproliferative, anticancer and neuroprotective (Becerril & Valdivia, 2006; Livrea & Tesoriere, 2006; El-Mostafa *et al.,* 2014; Aparicio-Fernandez *et al*., 2017). *Opuntia* spp. have been used in the management of diseases that involves, diabetes, obesity, cancer and oxidative stress.

Normal biochemical reactions had been reported to elevate exposure to the environment and outrageous proportion of dietary xenobiotics effect in the productions of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Bagchi & Puri, 1998). ROS and RNS with free radicals are responsible for the oxidative stress in different pathophysiological conditions (Kim & Byzova, 2014). Recently, oxidative stress has gained the interest of most researchers. Oxidative stress is occasioned by the imbalance between reactive oxygen species (ROS) and endogenous antioxidants. Although, it is a normal physiological condition that is produced in order to sustain redox homeostasis but persistence in the imbalance leads to oxidative damage of proteins, fat, nucleic acids, and carbohydrates which eventually give rise to several diseases (Azab *et al.,* 2017). ROS had been reported as a chemical substance that act on some signaling pathways, modulating physiological responses (Valko *et al.,* 2007). The electron transport chain in the mitochondria and the cytochrome P450 usher into the production of ROS which majorly attack some macromolecules in the body, such as lipids, proteins and nucleic acid. However, modifications in some of the attacked macromolecules give rise to diverse diseases (Noori, 2012).

Several diseases have been traced to ROS most of which are chronic diseases such as atherosclerosis (Singh & Jialal, 2006), diabetes (Giacco & Brownlee, 2010), inflammatory diseases (Salzanoa *et al.,* 2014), cardiovascular diseases (Csanyi & Miller, 2014), cancer (Gupta *et al.*, 2014), cellular injury, hepatic disorders, neurodegenerative diseases, and kidney disease

(Losada-Barreiro & Bravo, 2017; Aryal *et al*., 2019). Most of these diseases had been reported to have a background inflammation, which is chronic in nature and involves the release of ROS. ROS is likewise involved in the expression of inflammatory markers (Onodera *et al.*, 2015), some of which take part in cell proliferation and metastasis (Sobolewski *et al.*, 2010) as well as mediate immunity (Lachininoto, 2013).

However, in order to prevent oxidative damage and other diseases caused by this oxidative stress, the neutralization of these oxidizing molecules occurs in the body through their interaction with a complex system of antioxidant processes mediated by endogenous antioxidative enzymes and substances (Kuratas, 2016). Nevertheless, the body endogenous antioxidants may be inadequate to combat serious or permanent oxidative stress, hence, an alternative process becomes very necessary.

Hence, involvement of an exogenous anti-oxidants are necessary to balance the rate of the ROS and to stop their unfavorable effects in human body. Over the years, antioxidant supplementation has been used for the elimination and management of ailments caused by oxidative stress (Arts and Hollman, 2005). Synthetic antioxidants such as butylatedhydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate (PG) and tert-butylhydroquinone (TBHQ) are very effective and frequently used in food formulations (Pokorny, 2007). Although, currently, pathological effects and carcinogenic potential of synthetic antioxidants have been carped (Jeetendra *et al.,* 2010). Thus, in recent years, there has been an increased interest in discovering natural alternatives especially those that originated from plants (Rahmat *et al.,* 2014). Supplementing the endogenous antioxidants defense with the natural exogeneous antioxidants against the reactive oxygen species will strengthen the body's endogenous and help them to reinstate the optimal balance by neutralizing the ROS (Albasha & Azab, 2014; Fetouh & Azab, 2014; Ivanov *et al.,* 2014; Rahmat *et al.,* 2014; Azab *et al*., 2017; Azab & Albasha, 2018).

In this context, plants containing high concentration of numerous redox-active secondary metabolites or antioxidants, such as ascorbic acid, phenolic, carotenoids, flavonoid, glutathione, tocopherols, polyphenols and other non-nutrient substances, received a great attention due to their capacity to eliminate the deleterious effect of oxidative molecules (Uddin *et al.,* 2008; Ivanov *et al*., 2014). The natural antioxidants are credited to have relatively insignificant side effects in comparison to the synthetic antioxidants, which tend to be unstable with different significant side effect (Chandra *et al.,* 2014)

Sequel to these findings, this study aimed at evaluating the *in vitro* antioxidant activity of silver, gold and silver-gold alloy nanoparticles mediated by *Opuntia ficus–indica* extract using total phenolic and flavonoid content and nitric oxide (NO) scavenging activity. To the best of our knowledge, this study will be the first time where the evaluation of nitric oxide (NO) scavenging activity, total phenolic and flavonoid contents of the green synthesis of the nanoparticles NPs using *Opuntia ficus-indica* fruit peel extract would be reported.

Materials and Method

Reagents

All reagents were of high analytical grade obtained from Sigma Aldrich Chemical and used without any further purification.

Preparation of the Plant Extract

Opuntia ficus-indica was procured from Station Road Area, Offa, Kwara State, Nigeria (8.1491^0N, 4.7207^0E). Its identity was confirmed and authenticated at Department of Pure and Applied Biology, LAUTECH, Ogbomoso. The spines were separated, clean and dried at 40 ^{o}C inside oven. It was pulverized using electrical blender (Euro premium, 750 watt) for 10 s. Ten grams (10 g) of the powdery *O. ficus-indica* was obtained and suspended into 150 mL of distilled water inside the tight closed container for 24 h in the dark cupboard. The mixture was filtered, centrifuged at 4000 rpm for 20 min. The supernatant was collected and stored for use (Adebayo *et al*., 2019a).

Biosynthesis and Characterization of the nanoparticles

Silver nanoparticles, gold nanoparticles and silver-gold alloy nanoparticles were mediated by *Opuntia ficus-indica* extracts and characterized as reported by Adebayo *et al.* (2019a). Briefly, 1 mL

of the extract was added to 24 mL of 1 mM silver nitrate ($AgNO_3$) and Chloroauric acid (HAuCl4) solution. Alloy was prepared by reacting together 1 mM Silver (AgNO3) and 1 mM Chloroauric acid (HAuCl4) in the ratio 4:1 (v/v) respectively. 1 mL of the sample *Opuntia ficus-indica* extract was added to a reaction vessel containing 24 mL of alloy solutions for the biosynthesis of alloy nanoparticles. The reaction was completed under static conditions at room temperature (30 ± 2 ºC) for 2 h. Materials used were remain under ambient temperature (30 ± 2 ºC) for change in colour as a result of nanoparticles formation.

The changes in colour were monitored while the quantitatively surveilled were done by measuring the absorbance spectra of the reaction mixtures, using UV-Spectrophotometer, Fourier Transform Infrared (FTIR) spectroscopy, Transmission Electron Microscopy (TEM) Micrograph, Energy Dispersive X-ray (EDX) Spectroscopy, X-ray Diffraction (XRD) and Selected Area Electron Diffraction (SAED).

Antioxidant Activity

Determination of Total phenolic content

The total phenolic content of the OFI-extract and the synthesized OFI-AgNPs, OFI-AuNPS and OFI-Ag-AuNPs were measured spectrophotometer by using Folin-Ciocaltaeu method as suggested by Zhou (2006). The samples (150 µL) and 2400 µL of ultrapure water and 150 µL of 0.25 N Folin-ciocalteu reagent were added together, mixed well and was allowed to react for 3 min. 300 µL of 1 N sodium carbonate solution was added to the mixture and mixed well for uniform concentration. The solution was then incubated at room temperature in the dark for 2 h. The absorbance of the solution was measured at 516 nm by using a spectrophotometer and the results are expressed in µg of GA equivalents per gram of the extract fraction.

Determination of Total flavonoid content

The total flavonoid of the synthesized nanoparticles was estimated spectrophotometrically by using method describe by Edewor *et al.* (2015). The solution was made up of 10 mL of 30% (v/v) ethanol, mixed with 0.7 mL of 5% (w/w) sodium nitrite and a dilute solution (1mL) of the samples of each concentration (20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL). The mixture was stirred for 5 minutes and 0.7 mL of 10% aluminum chloride (w/w) was added. The mixture was stirred again, and then 5 mL of 1 mol/l sodium hydroxide was added. This was again diluted with 5 mL of 30% (v/v) of ethanol and left standing for 10 minutes. The absorbance was measured at a wavelength of 500 nm. Quercetin was used as the standard and different concentrations of it were prepared and the absorbance readings obtained at 500 nm. This was used to obtain a graph and the total flavonoid content were determined from the graph

Nitric oxide scavenging (NOS) activity

Nitric oxide scavenging potential of the OFI-extract and the synthesized silver, gold and alloy nanoparticles was determined according to Green *et al*. (1982) with some modifications. Nitric oxide generated from sodium nitroprusside interacts with oxygen to produce nitrite ions, assayed by Griess reaction. Sodium nitroprusside (5 mM in Phosphate Buffer Saline) were incubating with 1mL of various concentrations (20µg/mL-100 µg/mL) of the OFI-extract and the synthesized OFI-AgNPs, OFI-AuNPS and OFI-Ag-AuNPs at 25 °C. After 2 h, 0.5 mL of Griess reagent was added and the absorbance was measured at 550 nm. The percentage inhibition of NO was calculated by using the formula: $=\left(1-\frac{As}{Ac}\right)x100$

Where; As: absorbance of the sample, Ac: absorbance of control

Results

Biosynthesis and Characterization of Silver, Gold and Alloy nanoparticles

The UV-vis spectroscopy showed silver, gold and silver-gold alloys nanoparticles with surface plasmon resonance at 462, 545 and 539 nm, respectively. FTIR peaks at 3300 and 1635 cm^{-1}, 3288 and 1635 cm^{-1} and 3307 and 1637 cm^{-1} for the biosynthesized OFI-AgNPs, OFI-AuNPs and OFI-Ag- AuNPs respectively. Generally, the particles were spherical with size range of 27-38 nm, 11-28 nm and 15-54 nm for the biosynthesized

OFI-AgNPs, OFI-AuNPs and OFI-Ag-AuNPs nanoparticles respectively. Furthermore, the TEM images of the biosynthesized OFI-AgNPs, OFI-AuNPs and OFI-Ag- AuNPs showed that the nanoparticles formed were anisotropic, mostly spherical in shape with some occasional aggregation to form rod-like structure (Adebayo *et al.*, 2019b).

Total Phenolic Contents

Total phenolic content of the OFI-extract and the biosynthesized OFI-AgNPs, OFI-AuNPs and OFI-Ag- AuNPs were recorded in the table 1. The total phenolic content of the samples was derived from the utilization of the calibration curve with the equation $y = 0.0026x + 0.2695$, $R^2 = 0.9742$ of gallic acid (standard). The concentration used ranged from 20–100 µg/mL and expressed in gallic acid equivalents (GAE) per g of sample (Table 1). The concentration of the samples increases with the increase in the concentration of the phenolic contents as recorded in the table 1. Total phenolic content of the biosynthesized nanoparticles ranges from 104.81-236.74 µg GAE/g (OFI-AgNPs), 157.12-258.28 µg GAE/g (OFI-AuNPs), 150.58-248.66 µg GAE/g (OFI-Ag-AuNPs) and 126.23-216.64 µg GAE/g (OFI-extract). The highest total phenolic content of 258.28 µg GAE/g was obtained from OFI-AuNPs, followed by 248.66 µg GAE/g from OFI-AgNPs, 114.82 µg GAE/g from OFI-AgNPs while the lowest value of 216.64 µg GAE/g was obtained from OFI-extract at 100 µg/mL.

Total Flavonoid Contents (TFC)

The total flavonoid of the biosynthesized nanoparticles was shown in the table 2. The equation; $y = 0.0005x + 0.0051$, $R^2 = 0.9919$ of quercetin in the calibration curve was used to obtained TFC result. The used concentration ranged from 20–100 µg/mL and expressed in quercetin (standard) equivalents (QE) per g of sample. Total flavonoid contents increase with increasing concentration of the samples as observed from the table 2. The value of the flavonoids content ranged from 25.43-129.73 µg g^{-1} QE, 15.73-118.07 µg g^{-1} QE, 34.13-82.10 µg g^{-1} QE and 9.55-21.38 µg g^{-1} QE for OFI-AgNPs, OFI-AuNPs, OFI-Ag-AuNPs and OFI-extract respectively. OFI-AgNPs gave the highest flavonoid content of 83.88 µg g^{-1} QE which is very significantly higher compare to OFI-AuNPs (37.58 µg g^{-1} QE), OFI-Ag-AuNPs (29.68 µg g^{-1} QE) and the least valve of 21.38 µg g^{-1} QE from OFI-extract at 100 µg/mL.

Nitric Oxide Scavenging Activity

Nitric oxide scavenging activity of the biosynthesized nanoparticles were showed in the Table 3. The values showed the percentage scavenging activity of the samples with the concentration ranges from 20-100 µg/mL and Ascorbic acid used as a standard. It was observed that all the three biosynthesized nanoparticles (OFI-AgNPs, OFI-AuNPs and OFI-Ag-AuNPs) showed a robust potential to scavenge nitric oxide which is higher than that of the OFI-extract but lower than the standard ascorbic acid. The percentage scavenging power ranges from 10.7 to 42.2%, 8.7 to 40.3%, 10.8 to 50.8% and 8.6-29.7% for OFI-Ag-AuNPs, OFI-Ag-AuNPs, OFI-Ag-AuNPs and OFI-extract respectively while that of the standard ascorbic acid range from 72.3 to 80%. OFI-Ag-AuNPs gave the highest percentage of nitric oxide scavenging activity (50.8%), followed by OFI-AgNPs (42.2%), OFI-AuNPs (40.3%) and the least value was recorded by OFI-extract (29.7%).

Table 1: Total Phenolic Content (TPC) (µg GAE/g**) of the Biosynthesized OFI-AgNPs, OFI-AuNPs and OFI-Ag-AuNPs**

Samples	Concentrations µg/mL				
	20	40	60	80	100
OFI-AgNPs	104.81±0.00^{d}	200.58±0.00^{a}	211.35±0.00^{b}	215.97±0.00^{b}	236.74±0.00^{c}
OFI-AuNPs	157.12±0.00^{a}	170.97±0.00^{b}	187.55±0.00^{c}	204.81±0.00^{c}	258.28±0.01^{a}
OFI-Ag-AuNPs	150.58±0.00^{ab}	200.58±0.00^{a}	238.27±0.01^{a}	242.56±0.01^{a}	248.66±0.00^{b}
OFI-Extract	116.23±0.00^{c}	150.43±0.00^{c}	177.44±0.01^{d}	194.51±0.00^{d}	216.64±0.01^{d}

Values are expressed as the mean ± standard deviation. All superscripts indicated a significant difference ($P < 0.05$) between the means ($n = 3$). Values on the same column with different superscripts are significantly different.

Table 2: Total flavonoid contents (µg g^{-1} QE) of the biosynthesized OFI-AgNPs, OFI-AuNPs and OFI-Ag-AuNPs

Samples	Concentration µg/mL				
	20	40	60	80	100
OFI-AgNPs	11.75 ± 0.12^{ab}	19.88 ± 0.06^{a}	39.65 ± 0.12^{a}	67.81 ± 0.09^{a}	83.88 ± 0.06^{a}
OFI-AuNPs	9.75 ± 0.09^{abc}	13.78 ± 0.06^{c}	27.88 ± 0.06^{b}	31.85 ± 0.09^{b}	37.58 ± 0.27^{b}
OFI-Ag-AuNPs	15.58 ± 0.12^{a}	17.85 ± 0.09^{ab}	19.65 ± 0.09^{c}	21.89 ± 0.06^{c}	29.68 ± 0.12^{c}
OFI-Extract	9.55 ± 0.04^{abc}	10.28 ± 0.06^{d}	13.95 ± 0.02^{d}	17.19 ± 0.06^{d}	21.38 ± 0.09^{d}

Values are expressed as the mean ± standard deviation. All superscripts indicated a significant difference ($P < 0.05$) between the means ($n = 3$). Values in the same column with different superscripts are significantly different.

Table 3: Nitric acid scavenging activity (%) of biosynthesized OFI-AgNPs, OFI-AuNPs and OFI-Ag-AuNPs

Samples	Concentrations µg/mL				
	20	40	60	80	100
OFI-AgNPs	10.70	23.70	23.90	35.40	42.20
OFI-AuNPs	8.70	18.10	19.30	23.50	40.30
OFI-Ag-AuNPs	10.80	17.30	29.40	35.40	50.80
OFI-Extract	8.60	10.40	13.20	17.30	29.70
Ascorbic acid	72.30	74.30	77.00	78.50	80.00

Discussion

The biosynthesized nanoparticles (OFI-AgNPs, OFI-AuNPs, and OFI-Ag-AuNPs) reported to show absorbance (UV-Vis spectrum) values of 455.5, 538 and 540.5 nm respectively, with the existence of $-NH_2$ and -OH functional groups shown by FTIR absorption spectra. The indication is that biomolecules are rich in amine (N-H) and hydroxyl (O-H) groups which accounted for the reduction of the metal ions (Ag^{+} and Au^{3+}) and capping of OFI-AgNPs, OFI-AuNPs, and OFI-Ag-AuNPs (Adebayo *et al.,* 2019 b). It is therefore evident that proteins present in the *Opuntia ficus indica* extract accounted for the capping and stabilization of the OFI-AgNPs, OFI-AuNPs, and OFI-Ag-AuNPs. Furthermore, the TEM images of the biosynthesized OFI-AgNPs, OFI-AuNPs, and OFI-Ag-AuNPs showed that the nanoparticles formed were anisotropic, mostly spherical in shape with some occasional aggregation to form rod-like structure (Adebayo *et al.,* 2019 b).

Plants remain one of the major potential origins of natural antioxidants. Natural substance like plant-based phytochemicals play a significant role in plant as a defense system and also serve several medical and therapeutic purposes (Hussain *et al.,* 2017). Flavonoid, terpenoids, alkanoids, carotenoids and phenolic which are known as polyphenolic compounds are all group of phytochemicals. They are generated to annul the stress condition such as oxidative stress and as well possessed the ability to donate an electron, act as metal chelators, act as singlet and triplet oxygen quenchers (Bakhtiar *et al*., 2015; Unuofin *et al.,*

2018; Unuofin *et al*., 2020). Based on their activity they belong to the non-enzymatic type of antioxidant which act by interrupting the free radical chain reaction (Nimse *et al.,* 2015).

The antioxidant activity of the phenolic has been documented as their main function. Phenolic compounds have been reported for their different medical application such as antidiabetics, anticancer, anti-inflammatory, antibacterial, antifungal, antiviral, cholesterol-lowering and other health effect, however they function primarily as antioxidant (Ganesan, 2017; 2018). Phenolic has been known as a good source of antioxidant owning to diverse mechanism such as free radical-scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelating and acting as a substrate for radicals such as superoxide and hydroxyl (Adwas *et al.,* 2019).

In this study, the total phenolic contents increase with the increasing concentration of the samples. The biosynthesized OFI-AuNPs gave the highest phenolic contents (258.28 µg GAE/g) compare to OFI-AgNPs (248.66 µg GAE/g), OFI-AgNPs (114.82 µg GAE/g) and OFI-Extract (216.64 µg GAE/g) at a concentration 100 µg/mL. Several factors had been established that could be accounted for the observed phenolic content values obtained in this current study which differ slightly compared to those in the literatures. These factors include differences in the methodology used, quantity of sugars, carotenoids, ascorbic acid and solvents used. Other factors are plant age, extraction time, differences in geographical location and methods of extraction used which may change the quantity of phenolics (Upadhya *et al.,* 2015; Burri *et al*., 2017; Oke *et al.,* 2021). Furthermore, the nucleophilic nature of the aforementioned phytochemicals, which help in the reduction and chelation of transitional metals also assist in stabilizing them (Sivaraman *et al*., 2009; Raghunandan *et al.,* 2010).

Many studies have focused on the biological activities of phenolics which are potent antioxidants and free radical scavengers. The antioxidant activity of phenolics is mainly due to their redox properties, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. Phenolic compounds are also known to play an important role in stabilizing lipids against peroxidation and inhibiting various types of oxidizing enzymes. This is because phenolic compounds are free radical terminators that contribute directly to antioxidant activity (Shahidi *et al.,* 2009).

Flavonoid is needed by the human body to maintain good health (Shi *et al.,* 2019). In this study the concentration of the flavonoid compounds increased with increasing concentration of the sample in a dose dependent. OFI-AgNPs displayed the greatest flavonoids contents (129.73 µg g^{-1} QE) compare to OFI-AuNPs (118.07 µg g^{-1} QE), OFI-Ag-AuNPs (82.10 µg g^{-1} QE) and OFI-extract (21.38 µg g^{-1} QE) at 100 µg/mL. The biosynthesized nanoparticles showed a robust flavonoid content better than that of the OFI-extract alone. Flavonoids are said to be a secondary metabolite with antioxidant activity. The antioxidant potency of the flavonoid relies on the amount and position of free OH groups (Panche *et al.,* 2016). At a concentration 100 µg/mL, the highest flavonoid content was obtained in biosynthesized OFI-AgNPs. Hence the flavonoid content activity could be rated in this order: OFI-AgNPs > OFI-AuNPs > OFI-Ag-ANPs > OFI-extract. Some factors like genetic diversity, environmental, biological, seasonal and year-to-year variations could significantly affect the flavonoid content (Kumar *et al.,* 2018). The high phenolic and flavonoid contents of the *Opuntia ficus indica* has been reported by Saravanakuma *et al.* (2015) & Oke *et al*. (2021).

The results of this study showed that TPC and TFC was higher in the synthesized nanoparticles compared to the aqueous *Opuntia ficus-indica* (OFI) extract alone. Compounds such as phenolics, flavanoids, terpenoids, and soluble proteins have been documented to act as capping agents (Ramamurthy *et al.,* 2013). In line with these results, Abdel-Aziz *et al.,* (2014) and Sultana *et al.,* (2015) documented higher total phenol and flavonoid content in the synthesized AgNPs compared to the *Chenopodium murale* and *Houttuynia cordata* leaf extract respectively.

This study showed that *Opuntia ficus-indica* (OFI) is a good source of phenolic and flavonoids compounds. Phenolic and flavonoids have been

documented to be the most important phytochemicals responsible for the antioxidant capacity (Saumya & Basha, 2011). In this study the nanoparticles synthesized using fruit extract of *Opuntia ficus-indica* showed antioxidant activity due to capped phenolic compounds. Philip, (2011) reported that phenolic group facilitates the conversion of silver nitrate to AgNps due to its electron donating ability.

Nitric Oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons. The excessive production of nitric oxide had been reported to be associated with several unhealthy conditions such as shock, dermatology, neurology and oncology (Wong & Lerner, 2015; Dzoyem & Eloff, 2015). NO is generated in the biological tissues by the actions of an enzyme called nitric oxide synthase (NOS) and downstream mediation process in human metabolic processes, which metabolizes arginine to citrulline with the formation of NO via a five-electron oxidative reaction (Wong & Lerner, 2015). These compounds are responsible for altering the structural and functional behavior of many cellular components (Wong & Lerner, 2015). Hence, the mechanism of action of the biosynthesized nanoparticles may be through the inhibition of the activity of the enzyme nitric oxide synthase and downstream mediator (Wong & Lerner, 2015).

In the present study all the biosynthesized nanoparticles showed a good nitric oxide scavenging activity higher than that of the OFI-extract alone but lesser when compared with the standard. However, the highest nitric acid scavenging percentage of 50.8% was obtained by OFI-Ag-AuNPs compared to OFI-AgNPs, OFI-AuNPs and OFI-extract. Hence, the biosynthesized nanoparticles revealed a robust capacity of scavenging the free radicals in a dose dependents manner with the bimetallic OFI-Ag-AuNPs having the highest antioxidant potential compared to OFI-AgNPs, OFI-AuNPs and OFI-extract. The different functional group attached to them may be the reason for the differences in their activity. Likewise, the combinations of two different properties of nanoparticles via silver and gold may be accountable for the best scavenging activity observed in the bimetallic alloy nanoparticles. Ability to donate hydrogen molecules and other biomolecules with the interference of several functional groups present in the extract could be linked with their robust scavenging power (Netala *et al.,* 2016) which could as well be responsible for the synthesized nanoparticles as capping and stabilizing agents (Xia *et al.,* 2013; Reddy *et al.,* 2014; Kumari *et al.,* 2015; Gudikandula *et al.,* 2017).

Furthermore, the radical scavenging activity of the synthesized nanoparticles (OFI-AgNPs, OFI-AuNPs and OFI-Ag-AuNPs) have been established to be as a result of integration or absorption of more bioreductant molecules or bioactive compounds of plant (*Opuntia ficus-indica*) extract on the surface of the OFI-AgNPs, OFI-AuNPs and OFI-Ag-AuNPs which enlarge the surface area for antioxidant activity (Mabrouki *et al*., 2015; He *et al.,* 2017; Chandrasekhar & Vinay, 2017; Bhutto *et al*., 2018; Ogochukwu *et al*., 2019). Hence, the reducing action of the synthesized OFI-AgNPs, OFI-AuNPs and OFI-Ag-AuNPs might be credited to the existence of phenolic functional groups on the surface

This work agreed with the previous result reported by Adebayo *et al.* (2019b) where antioxidant activity of the biosynthesized silver, gold and the bimetallic silver-gold nanoparticles from *Opuntia ficus-indica* were determined by using DPPH and ABTS assay. Das *et al.,* (2019) had reported similar result where the antioxidant activity of the silver nanoparticles synthesized using the outer peel extract of Ananas comosus (L.) were evaluated using DPPH, ABTS and Nitric Oxide activity. This work also was confirmed by the work reported by Johnson *et al.,* (2018) & Zhao *et al.,* (2018). This study confirms the use of OFI-AgNPs, OFI-AuNPs and OFI-Ag-AuNPs as potential agent of antioxidant formulations in biomedical/ pharmaceutical areas.

The present study shows a positive indication of the biosynthesized nanoparticles of their utility in preventing body from the oxidative stress and the associated disease. The study suggested the use of biosynthesized OFI-AgNPs, OFI-AuNPs and OFI-Ag-AuNPs as natural antioxidants. Hence, these nanoparticles can be used as a natural antioxidant in the pro-oxidants and antioxidants, to balance reactive oxygen species (ROS) levels. Moreover,

since antioxidants play a significant role in the curing of some free radical associated diseases like cancers, *Opuntia ficus-indica* with its rich level of phytochemicals can be used as either dietary or complementary agents.

In conclusion, this study has established the antioxidant activity of the biosynthesized OFI-AgNPs, OFI-AuNPs and OFI-Ag-AuNPs which may be due to the high presence of phenolics and flavonoids content present in the used extract. These compounds have been shown as a strong antioxidant with high reducing capacity and free radical scavenging capability. The ability of the *Opuntia ficus-indica* extract to reduce silver/gold ions or for the formation of nanoparticles by reducing silver and gold ions might be due to the presence of phenolic and flavonoid compounds which are electron donors. Therefore, *Opuntia ficus-indica* can serve as a source of natural reducing agents for green synthesis.

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