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Green Synthesis of Polyherbal Silver Nanoparticles from *Rosa Gallia officinalis, Citrus sinensis* and *Solanum tuberosum* Extract for antioxidant Potency

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ABSTRACT

Skin ageing is due to the combination of natural, largely genetically programmed and environmentally modulated changes which occur in the body system due to free radical damage. Silver Nanoparticle (AgNPs), were prepared by chemical reduction using green synthesis and they were evaluated for particle size in nanometer, zeta potential in millivolt, surface morphology by scanning electron microscopy (SEM) and percent entrapment efficiency. The polyphenols were quantified by chromatographic techniques and the antioxidant activity measured spectrophotometrically by DPPH (2,2 Diphenyl 1 picrylhydrazyl) assay. According to this study AgNPs showed a least particle size of 145.4±2.4nm, maximum zeta potential of -39.1±2.4 mV with desired polydispersity index of 0.358±0.02, the amount of polyphenols loaded in AgNPs was found to be 87.23±2.54%. Maximum phenolic content was found in F1 as 65.21 ± 3.721 mg equivalent GAE/g of extract. On comparing the IC_{50} values, F1 and F5 exhibited the lowest and highest values respectively. Therefore, F1 possesses higher DPPH radical scavenging potential.

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INTRODUCTION

Skin ageing process can be prematurely caused by various factors which include free radicals damaging the cells, exposure to the sun (photo-ageing) and pollution, environmental factors (smoking and drinking), diet and stress, and loss of subcutaneous support (Zhang and Duan, 2018; Tobin, 2017). It can be the result of a combination of natural, largely genetically programmed and environmentally modulated changes which occur in the body. Skin ageing is a predominantly natural change that cannot be completely reversed; however, it is possible to reduce the wrinkles and brown spots (Bau-

mann, 2007). Free radicals are unstable atoms or molecules having single and unshared electron. They are very reactive chemical radicals that can cause cellular damage (Park *et al.*, 2014). Damage occurs when free radicals try to stabilize by grasping additional electron from skin, proteins and cellular entities. Skin ageing will be rapid or speed up when the free radical over react with the cells which causes premature erosion. Today, this theory is widely accepted by most of the scientists to elucidate how free radicals could trigger skin ageing (Khan *et al.*, 2017). In order to prevent the reaction of free radical with cellular components antioxidants are majorly used the main role of the antioxidant to delay or prevent oxidation that build up at molecular level within tissues and cells (Silva-Beltran *et al.*, 2017). They neutralize the free reactive oxygen species and counteract their effects by forming a pair and stable radicals. Although complete reversal of ageing is nearly impossible, However antioxidants prevents from limited skin damage caused by free radicals and thereby they protect and improve its appearance (Wahab *et al.*, 2009; Nisa *et al.*, 2013).

Several medicinal plants have been investigated for therapeutic potential. Thus, they are widely used for development of new medicinal systems. Among all the novel drug delivery systems, nanosized systems are one of the great research interests. Nanoparticles are microscopic particles that exist on a nanoscale (1-100 nm). They are subcellular like structure thereby having unique material characteristics and more physiologically and biologically compatible. Nanoparticles can absorb and incorporate therapeutic substances, prevent chemical and enzymatic degradation, improve drug distribution and delivery properties (Song *et al.*, 2009).

In contrast to ancient studies, the current research suggests that the combination of antioxidants from different herbal sources could produce synergistic antioxidants effects to increasing their potency and efficacy against free radicals thorough anti-oxidant activity to treat anti-ageing.

MATERIALS AND METHODS

Materials used

The chemicals and reagents used are technical grade. Ethanol, Acetone, Toluene, Formic acid, Methanol, Glacial acetic acid, distilled water, Chloroform, Ethyl acetate, concentrated Sulfuric acid (H_2SO_4), 0.5N Alcoholic potassium hydroxide, Sodium hydroxide (NaOH), Lead acetate, Hydrochloric acid (HCl), Silver nitrate ($AgNO_3$),Ninhydrin reagent, Fehling's reagent 1&2, Folin-Ciocalteu reagent, Salkowski's reagent,1, 1-diphenyl-2-picylhydrazyl (DPPH), Ascorbic acid and all other non-specified reagents were procured from HiMedia Pvt Ltd, Mumbai, India.

Instruments and Equipment used

UV-visible spectrophotometer (Shimadzu, Japan), pH meter, Magnetic stirrer (5MLH Plus, REMI), Homogenizer, Nanoparticle size analyzer (Sz-100Z, HORIBA,Japan), Hot air oven, Analytical balance (MAB 220T Touch screen) and Fourier-transform infrared spectroscopy (FT-IR) [BRUKER, INDIA] were used.

Collection and drying of herbs

Rosa Gallia officinalis (Red rose petals), Family: *Rosacea* (1kg) was directly picked from the garden. A constant weight and moisture of 2-3% was ascertained after subsequent drying of the petals in shade and under hot air oven at 50ºC. *Citrus sinensis* (Orange peels), family: *Rutaceae* and *Solanum tuberosum* (Potato peels), family: *Solanaceae* (1kg each) were collected from the market respectively. The fresh peels were thoroughly cleaned under running tap water. The cleaned peels were further desiccated at 50^{o}C for 48 hours in hot air oven and allowed to shade dry for two days. Red rose petals, orange peels and potato peels were trodden into small pieces (1x1 cm approximately), powdered and mixed in 1:1:1 ratio. The obtained mixture was taken for extraction (Md *et al.*, 2010; Kovac-Besovic *et al.*, 2009).

Preparation of plant extract

The red rose petals, orange peels, and potato peels by taking 30gms each i.e. at the ratio 1:1:1 followed by the extraction is carried out by using Soxhlet apparatus as shown in Figure 1A. The process was repeated continuously for complete and exhaustive extraction of crude components (Peters, 2010; Philipson, 2007); The ethanolic extract as depicted in Figure 1B obtained was concentrated to dry residue under reduced pressure at room temperature. Concentrated residue was stored at 4^{o}C and used for further study (Kamijo *et al.*, 2008).

Preliminary phytochemical screening

Phytochemical investigation was performed on crude plant extract for detection of various bioactive compounds.

The various phytochemical tests performed were Dragendroff's test, Hager's test, Meyer's test, Wagner's test for alkaloids; test for phenols, test for flavonoids, test for glycosides, test for tannins, foam and froth tests for saponins and test for reducing sugar as shown in Figure 1C (Peters, 2010; Philipson, 2007).

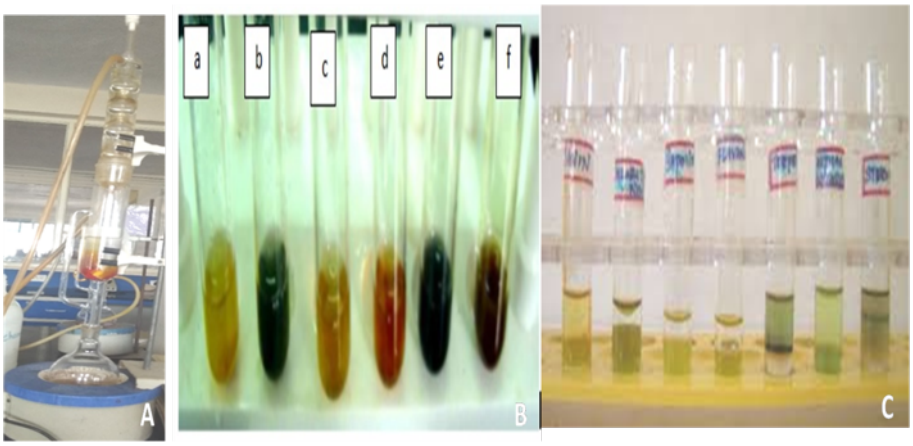

Figure 1: (A) Soxhlet apparatus; (B) Preliminary phytochemical screening tests of Flavonoids (a, c and d), Phenolic compounds (b), Polyphenols (e), Cardiacglycosides (f) (C) Preliminary phytochemical tests for Saponins, Alkaloids,Tannins

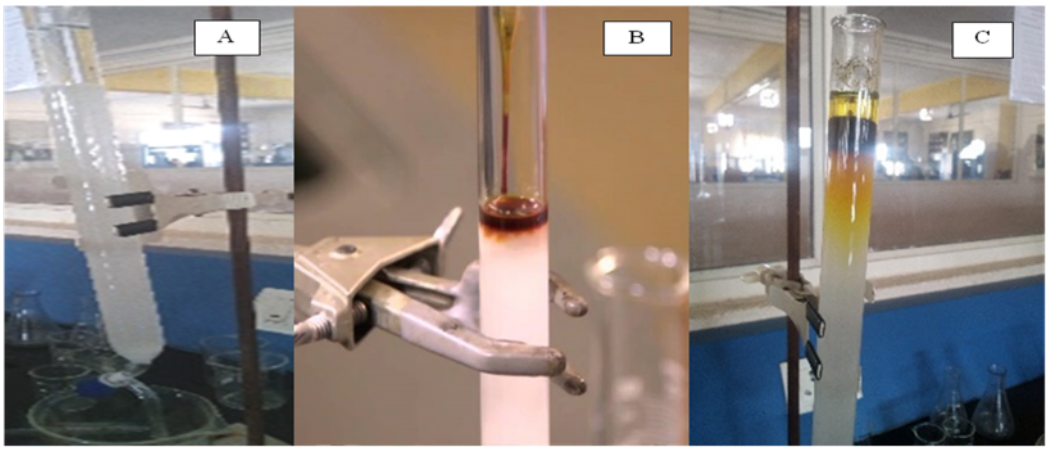

Figure 2: Column Chromatography- Development and separation of fractionates (a) Column bed prepared by wet packingmethod; (b) Loading of extract; (c) Elution of fractionate bands

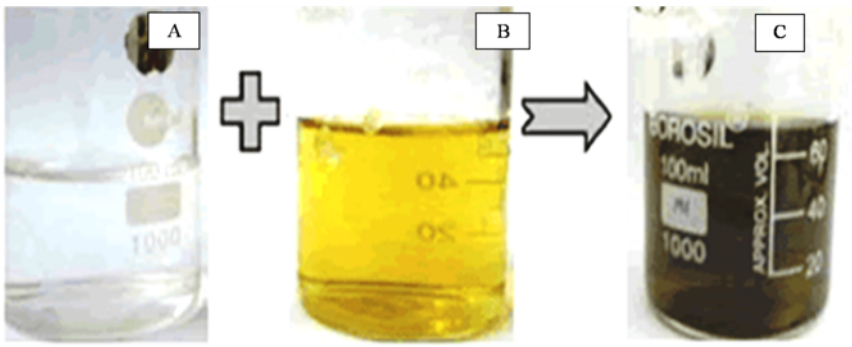

Figure 3: Preparation of silver nano particles (a) Silver nitrate solution; (b) Plant extract solution; (C) Silver Nano particles

Table 1: Preliminary phytochemical screening data of the mixture of crude extract of all the three herbs (red rose petals, orange peels, and potato peels).

Tests	Observations	Inferences
Test for Alkaloids 1.Dragendroff's test 2.Meyer's test 3.Wagner's test 4.Hager's test	Formation of reddish brown precipitate Reddish color precipitate was observed Yellow color precipitate was formed	Presence of alkaloids was confirmed
Test for Phenols	Formation of red color	Presence of phenols
Test for Flavonoids	Development of red color	Presence of flavonoids
Test for Cardiac glycosides	Development of yellow color	Presence of glycosides
Test for Tannins	Formation of greenish-black precipitate	Presence of tannins
Test for Saponins • i) • ii)	 No formation of 1 cm layer frothing No persistence of foam for 10 minutes	 Absence of saponins
Test for Reducing sugar	Orange red precipitate was formed	Presence of reducing sugar

Table 2: TLC of ethanolic extract and Rf values of the fractionates of the crude extract

S. No:	Mobile phase	Chemical constituents	Rf value
1	Toluene:Acetone (90:10)	Phenols	0.96
2	Chloroform: Ethylacetate (60:40)		0.97
3	Toluene: Ethyl acetate:Acetic acid (34:15:1)	Flavonoids	0.98
4	Methanol: Water (7:3)		0.97

Table 3: Phytochemical screening, TLC and UV spectra data of the fractionated compounds

S. N	Parameters	Specification	Fractionated compounds								
			F1	F2	F3	F4	F5	M1	M2	M3	M4
1	Phyto chemical screenings	Phenols	+	-	+	+	+	+	+	-	-
		Flavonoids	+	+	+	-	-	+	+	+	-
2	TLC technique	Rf values (phenols)	0.97	0.67	0.96	0.97	0.97	0.95	0.97	0.53	0.48
3	UV- Visible analysis	λmax in nm	270	300	273	270	271	274	273	245	260

Thin layer chromatography development

The crude extract was examined under Thin Layer Chromatography. It is extensively used for separation, finger print profiling and quantitative analysis of plant materials. About 3 μl of standard and test (filtered extract) solutions were loaded to a pre-coated plate using freshly prepared mobile phase (selected based on polarity) such as Toluene: Acetone (9:1); Ethyl acetate: Formic acid: Glacial Acetic Acid: Water (10:1.1:1.1:2.7); Chloroform: Ethyl acetate (6:4); Ethyl acetate: Toluene: Formic acid (7:3:1); Methanol: Water (7:3); Toluene: Ethyl acetate: Acetic acid (34:15:1). After development, the plates were dried under Hot air oven at 105ºC for about 10 minutes and visualization of spots was done under ultraviolet chamber after being sprayed with Folin-Ciocalteu's reagent for phenols, rutine for flavonoids, terpiniol for terpenoids. The retention factor (Rf values) was determined by the formula (Majors, 2003).

$$\text{Rf} = \frac{\text{Distance travelled by sample from origin line}}{\text{Distance travelled by solvent from origin line}}$$

Column Chromatography

The crude extract was further examined under column chromatography for isolation and separation of

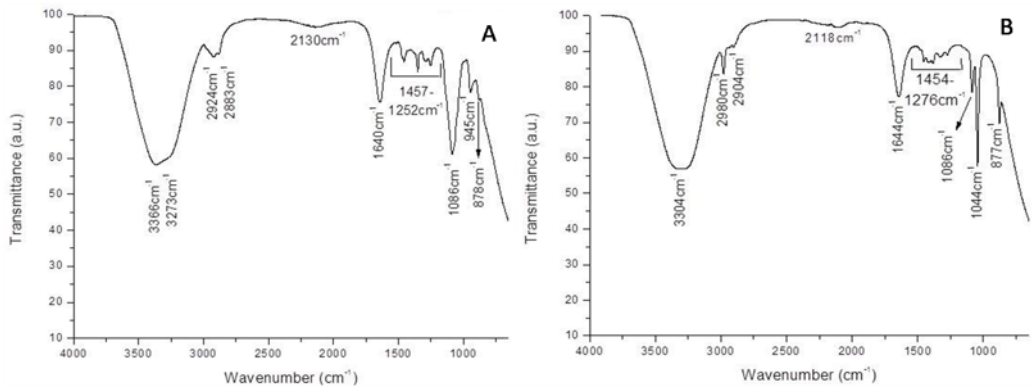

Figure 4: FT-IR spectral study (A) Sample (fractionates of the crude extract mixture); (B) Standard

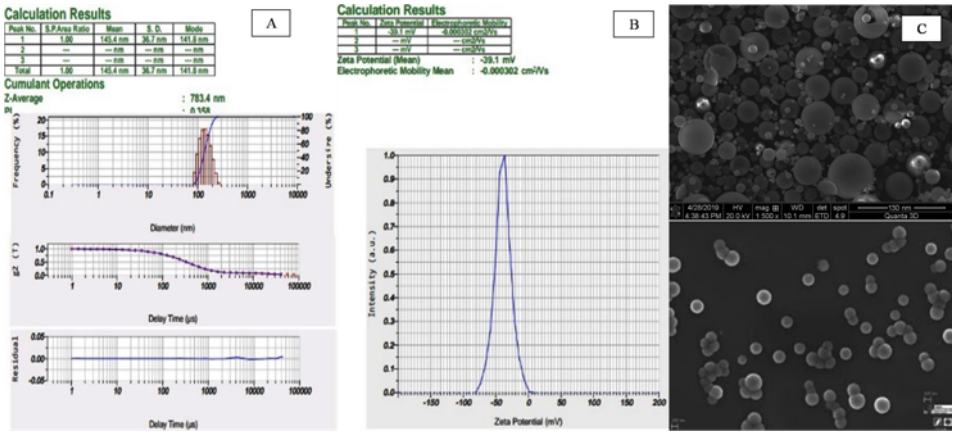

Figure 5: (A) Particle size and Poly dispersity index of F1; (B) Zeta potential data OF F1; (c) Scanning Electron Microscopy of F1 particle

individual bioactive compounds. The column bed (Figure 2A) was prepared by flowing a mixture of petroleum ether and silica gel G (50g, 60-120#) through a cleaned and dried column (stationary phase), allowing silica to be adequately retained with no pores. Crude extract (2gms) (Figure 2B) was added onto the prepacked column via wet loading. Different solvent systems (mobile phase) were continuously used for elution of different components (Figure 2C). Fractionates were collected (Figure 2C), pooled together based on similar results and further analyzed for the presence of phenols (LaCourse, 2002; Rasool *et al.*, 2011). As suggested by thin layer chromatography (TLC), Toluene: Acetone (9:1) was ideal solvent system for separation and elution of phenolic components. The development of column and separation of fractionates are shown in (Figure 2) (Anagnostopoulou *et al.*, 2006).

TLC, Ultraviolet spectroscopy and Fourier-transform infrared spectroscopy (FT- IR) spectroscopic analysis of fractionates

The phenolic components in fractionates were investigated by TLC, UV spectrophotometer and FT-IR spectroscopy for detection, characterization and structural elucidation.

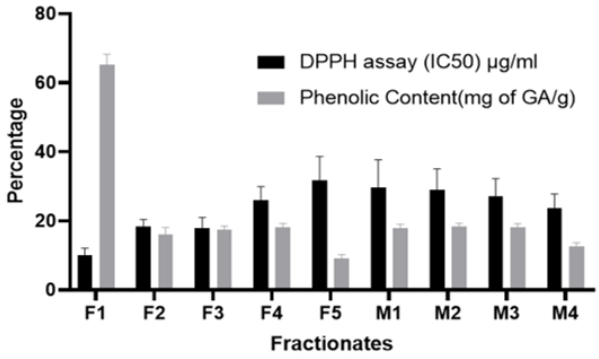

Figure 6: DPPH scavenging activity and total phenolic content of fractionates

Phytochemical screening

The phytochemical analysis of the fractionates was conducted by subjecting the fractionates to different confirmatory tests. The aim is to determine the presence of certain phytochemical classes mainly focusing on phenolic groups. The tests for Phenols and Flavonoids were performed (Peters, 2010; Philipson, 2007).

Thin layer chromatography (TLC)

The TLC analysis was performed using Toluene:

Acetone (9:1) solvent system. The mobile phase was prepared and kept in TLC chamber while the fractionates were loaded on the solvent front line (1 cm height and 1 cm apart). The prepared stationary phase was kept in the TLC chamber for 15 minutes. As the mobile phase travels from the front line, there is elution of phenolic compounds. Yellowish spots were seen after spraying phenol reagent and drying in oven at 105ºC for 10 minutes (Rolim *et al.*, 2019). The retardation factor (Rf) values were calculated using the formula:

$$Rf = \frac{\text{Distance travelled by sample from origin line}}{\text{Distance travelled by solvent from origin line}}$$

UV visible spectroscopy

The fractionates were filtered and analyzed in UV visible spectroscopy using water and acetone as blank region with a resolution of 1nm, from 200-500 nm to determine their absorbance.

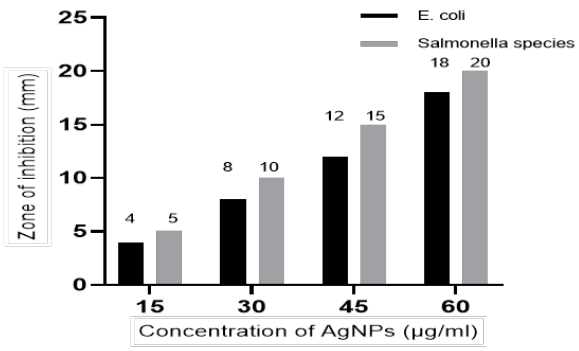

Figure 7: Antimicrobial activity of best AgNPs (F1) formulation

FT-IR spectroscopy

FT-IR investigation was done on each filtered fractionate in the range approximately 4000-400 cm^{-1}. The analysis was performed by placing a tiny sample as small as 10 microns in Alpha-Bruker FT-IR spectrophotometer. The amount of IR beam refracted and the sample absorbance frequencies were recorded. A reference database was used for identification of samples.

Preparation of silver nanoparticles

Preparation of plant extract

The extract sample was prepared by dissolving different weights (0.5g; 1g and 2g) of powdered crude extract up to 100 ml of distilled water, with continuous stirring and heating at about 60ºC followed by filtration using Whatmann No. 1 paper.

Preparation of Silver nitrate solution

Different strengths (1, 2.5 and 5 millimolar) of silver nitrate solutions were prepared by dissolving 0.0169, 0.0425 and 0.085g in 100 ml of distilled water each respectively.

Preparation of silver nanoparticles by Green synthesis

Silver nanoparticles were prepared by adding dropwise 25 ml of $AgNO_3$ solution to 25 ml of extract solution with continuous shaking at room temperature until the solution turns to grey black as shown in Figure 3 A, B, C. The polyphenols act as reducing, capping and stabilizing agent. The pH of the final suspension was brought to 10.5 by dropping sodium hydroxide (NaOH) (1 ml/L) to the suspension. Furthermore, the suspension was centrifuged at 5000 rotation per minute (rpm) for 10 min and the precipitated AgNPs were collected, washed twice and freeze dried. The optimization design used was the 2^3 factorial design and its levels are shown in Table 4. Chemical reduction by green synthesis was the method used for preparation of silver nanoparticles. Green synthesis is an environmentally friendly method for the synthesis of the nanoparticles since the toxic chemical which is produced during the biosynthesis can be degraded with help of the enzyme which is present in the microbes (Suber *et al.*, 2005; Bogle *et al.*, 2006).

Experimental design for formulation of silver nanoparticles

2^3 experimental design was used to optimize the formulation technique. The aim of this design to decide the best combination possible and to establish a relationship between factors/inputs (independent variables)which includes Extract concentration (A in mg), Silver nitrate concentration (B in mM) and Stirring speed (C in rpm), and outputs (dependent variables) Particle size (Y1), Zeta potential (Y2) and Polydispersity index (Y3). According to this design, high and low levels of factors can be combined in 8 different ways as depicted in Table 4 (Yoosaf *et al.*, 2007; He *et al.*, 2004) .

Evaluation parameters of nanoparticles

Particle size

Horiba Nanoparticle size analyzer equipped with the Horiba software was used for nanoparticles size characterization. Measuring particle size and size distribution reveals the in-vivo distribution, biological fat, toxicity and targeting ability of the nanosystem (Li and Zhang, 2010). Additionally, it also relates drug loading, drug release and stability of nanoparticles. Photon-correlation spectroscopy or dynamic light scattering is the most widely used technique for determining particle size. The obtained result is double checked by Scanning Electron Microscopy (SEM) (Choi *et al.*, 2007; Sun *et al.*, 2000).

Table 4: Particlesize, Zeta potential and Polydispersity index data

Parameters	Independent Variables with level code			Dependent Variables		
Formulation	Factor A Extract conc. (g)	Factor B AgNO3 conc.(mm)	Factor C Stirring speed(rpm)	Response X1 Particle size (nm)	Response X2 Zeta potential (mV)	Response X3 Polydispersity index
F1	2 .0(+1)	5 (+1)	5000 (+1)	145.4±2.4	-39.1±2.4	0.358 ± 0.02
F2	2.0 (+1)	5 (+1)	2000 (-1)	18.9 ± 1.6	-5.4 ± 0.6	3.61 ± 1.2
F3	0.5 (-1)	5 (+1)	2000 (-1)	531.4±2.4	-3.1 ± 0.6	1.147 ± 1.4
F4	2.0 (+1)	1 (-1)	5000 (+1)	1.3 ± 0.4	-3.8 ± 0.8	0.60 ± 0.02
F5	0.5 (-1)	1 (-1)	5000 (+1)	478.5±2.8	0.0 ± 0	1.723 ± 0.24
F6	2.0 (+1)	1 (-1)	2000 (-1)	0.6±0.13	-1.2 ± 0.9	7.40 ± 1.4
F7	0.5 (-1)	1 (-1)	2000 (-1)	0.5±0.02	-8.0 ± 0.4	0.264 ± 0.06
F8	0.5 (-1)	5 (+1)	5000 (+1)	511.1±2.5	-6.0 ± 0.4	1.658 ± 0.4

Zeta potential

Zeta potential (ZP) is a measure of the surface property. It predicts the electrical potential of particles and is dependent on the composition of the nanoformulation and the pH of the medium (Suber *et al.*, 2005; Bogle *et al.*, 2006). Nanosuspension with zeta potential above +30mV or less than -30mV are said to be stable, as the surface charge prevents particles aggregation.

Surface morphology

Surface morphology of the prepared silver nanoparticles was analyzed using Horiba nanoparticle analyzer. About 30 microns of silver nanoparticles solution was initially dried, then mounted on sample holder. This was followed by coating with gold using a sputter coater (Yoosaf *et al.*, 2007; He *et al.*, 2004) and further scanned for nanoparticles size and shape characterization.

Entrapment efficiency

The entrapment efficiency (EE) was expressed as the ratio of the amount of drug released from lysed nanoparticles to the amount of drug initially taken to prepare the nanoparticles (Suber *et al.*, 2005; Bogle *et al.*, 2006). Unentrapped drug molecules were separated by ultracentrifugation and subsequent decantation of the resulting supernatant into phosphate buffer p^H 7.4 (Yoosaf *et al.*, 2007; He *et al.*, 2004).

$$\mathrm{EE}[\%] = \frac{\text{Amount of drug released from thelysed nanoparticle}}{\text{Amount of drug initially taken to prepare the nanoparticle}} \times 100$$

Polydispersity index

Polydispersity index (PI) is a measure of non-uniformity of size distribution of particles. It is dimensionless and it indicates the degree of homogeneity of the medium. Nanoparticles with PIs less than 0.5 are considered to be monodisperse and exhibit less particles aggregation (Yoosaf *et al.*, 2007; He *et al.*, 2004).

Antioxidant activity assays DPPH (2, 2-diphenyl-1-picryl-hydrazyl) scavenging activity

The antioxidant potency of prepared silver nanoparticles was determined by DPPH assay method. The DPPH radicals inhibiting capacity of each formulation was compared to Ascorbic acid to establish efficient antioxidant action (Czyzowska *et al.*, 2015; Kumar *et al.*, 2013). Assay samples were prepared by adding 0.8 ml of 0.1 mM DPPH methanol solution to 2.4 ml of test solutions or ascorbic acid as control. The mixtures were incubated for 10 minutes at room temperature and their absorbance was read under UV spectrophotometer at 517 nm (Iqbal *et al.*, 2017). The antioxidant capacities were expressed as IC_{50} values (μg/ml) and calculated according to the formula (Kähkönen *et al.*, 1999; Singh and Rajini, 2004).

$$IC_{50} = [(\text{A control} - \text{A sample})\,/\text{A control}] \times 100$$

Determination of total phenolic content

The total phenolic count was expressed as mg of Gallic acid equivalents (GAE) per gram of extract and determined by Folin-Ciocalteu assay (Baydar and Baydar, 2013).

0.1 ml of crude extract was subsequently mixed with 1.5 ml of Folin-Ciocalteu reagent and 1.4 ml sodium carbonate.

The mixtures were incubated for 30 minutes and their absorbance detected at 765 nm (Ng *et al.*, 2004; Bocco *et al.*, 1998).

Antimicrobial activity

Media preparation and inoculation of microorganisms

The antimicrobial activity of prepared silver nanoparticles against *E. coli* and *Salmonella* species was investigated using standard well diffusion method. Assay medium was prepared by dissolving 3.5 g of nutrient agar in 1000 ml of distilled water, and then equally poured on petri plates. The bacteria were inoculated in the media with a sterile pipette and the mixtures were left out to dry for 15 min (Khan *et al.*, 2017).

Silver nanoparticles diffusion

Different concentrations (15μg/ml, 30μg/ml, 45μg/ml and 60μg/ml) of silver nanoparticles were added into the culture media and the plates were further incubated at 37ºC for 24 hours.

Measurement of antimicrobial capacity

The approximate antimicrobial capacity of silver nanoparticles against *E. coli* and *Salmonella* species was estimated by measuring the diameter of the inhibition zones (Nisa *et al.*, 2013).

RESULTS AND DISCUSSION

Extraction processes

Solvent extraction is of choice for efficient extraction of phytochemicals. Solvent for extraction are chosen based on polarity, it was found that only ethanol gave maximum yield (large amount of drug) with a much smaller quantity of ethanol. The ethanolic crude extract was submitted for fractionation by Column Chromatography method. Nine different colored fractionates were generated and those with similar color and Rf value were combined. According to column chromatography elution, Toluene: Acetone (9:1) proportion was determined to be suitable solvent system to be used as it runs through the glass column without dissolving the solid with maximum elution (90%) of polyphenols.

Screening processes

Preliminary phytochemical screening was done for qualitative analysis of crude extract. The presence of phenols, alkaloids, flavonoids, glycosides, tannins and reducing sugars was revealed while there is no trace of saponins as shown in Table 1. While the Table 2 shows the results obtained from thin layer chromatography and the calculated Rf values. The presence of Phenols and Flavonoids was indentified with the help of the Rf value i.e. 0.965 and 0.975 respectively by using different solvent system as mobile phase. The obtained Rf values of the respective extract were in correlation with the Rf value of polyphenolic compounds like rosmarimic acid, protocatechuic acid, caffeic acid, and umbelliferon; and flavonoids like flavonols and flavan-3-ols.After fractionation, the obtained fractionates were subjected to TLC. The Rf value of each fractionate was calculated and the results obtained were tabulated in Table 3. The TLC investigation showed the presence of phenols in fractionates F1, F3, F4, F5, M1 and M2 with Rf value lying within the standard value i.e. 0.965 whereas the TLC analysis showed the absence of the phenol in fractionates F2, M3, M4 with substandard Rf value.

Spectroscopy techniques

All fractionates were analyzed under UV-Vis spectroscopy at 200-500 nm. Maximum absorption peaks were recorded at 270-274nm, specifically for phenolic compounds. Factors such as particle size and shape, silver metal and the media affect the absorption and scattering efficiency.Figure 4 depicts spectrum obtained from FT-IR studies. The main perspective of FT-IR studies to identify and characterize specific phenolic molecular structures. Major peaks were observed at 3434.28 cm^{-1};2989.61 cm^{-1};2882.29 cm^{-1};1706.60 cm^{-1};1478.33 cm^{-1};1376.61 cm^{-1};1249.04 cm^{-1}; 1044.35 cm^{-1};880.35 cm^{-1}.The absorbances at 3434.28 cm^{-1}; 2989.61 $cm^{-1}$and 2882.29$cm^{-1}$are typically associated with –OH, –CH and $-CH_2$ stretching vibrations. These results indicate the presence polyphenols which is responsible for stabilizing nanoparticles.

Evaluation of Silver Nanoparticles

The prepared silver nanoparticles were evaluated and characterized for particle size, size distribution, zeta potential and surface morphology. The results recorded by the Horiba analyzer were shown in Table 4 and Figure 5.These measurements are influenced by preparation method and chemical reactions. F1 showed average particle size of 145.4±2.4nm, a zeta potential of -39.1 ± 2.4 mV, with a PDI of 0.358 ± 0.02, which indicates formation of a monodispersed Nanosuspension, thus good physico-chemical stability. F1 was therefore selected as best formulation. The size and morphology of F1 particles were further visualized by SEM analysis as shown in Figure 5, showed particles with mean size ranging from 45.6-150.4±2.4 nm and spherical in shape. The percent entrapment efficiency (%EE) is the percentage of plant extract successfully entrapped or absorbed into nanoparticles. It was calculated and found to be 87.23% ± 0.25. hence, it was concluded that maximum herbal drug (polyphenols) was entrapped into the nanoparticles and drug loss was minimum during the synthesis of

the formulation F1.

Antioxidant activity

Polyphenols in nanoceuticals act as reducing, capping and stabilizing agent. Their antioxidant effects involve the phenolic functional group which donates hydrogen, accepts free radical or interrupts the oxidation reaction chain. The total phenolic content of each formulation was estimated it showed large variations, between 12.67 $\pm$ 1.47 and 65.21 $\pm$ 3.721 mg GAE/g extract. The total phenolic content value of fractionates F1, F2, F3, F4, F5, M1,M2 ,M3, M4 was found to be 65.25 $\pm$ 3.712, 16.11 $\pm$ 2.1, 17.48 $\pm$ 1.67, 18.23 $\pm$ 1.98, 9.17 $\pm$ 1.125, 17.99 $\pm$ 1.77, 18.32 $\pm$ 1.11,18.18 $\pm$ 1.61, 12.67 $\pm$ 1.47 and DPPH assay (IC50) μg/ml value was found to be 10.07$\pm$2.12, 18.4 $\pm$2.14, 17.96 $\pm$ 3.82, 25.94 $\pm$ 4.79, 31.67 $\pm$ 7.14, 29.67 $\pm$ 8.70, 29.09 $\pm$ 6.90, 27.24 $\pm$ 5.30, 23.78 $\pm$ 4.01 and the results are shown in Figure 6. Maximum phenolic content was found in F1 (65.21 $\pm$ 3.721 mg equivalent GAE/g of extract); in another terms, 2g of crude extract contain 65.21 $\pm$ 3.721 mg of phenol equivalent of Gallic acid. Hence, F1 is expected to exhibit better DPPH scavenging activity when compared to ascorbic acid. On comparing the IC_{50}values**,** F1 and F5 exhibited the lowest and highest values respectively. Previous studies reported that the lower the IC_{50}, the higher the antioxidant activity. Therefore, when compared to others, F1 possesses higher DPPH radical scavenging potential.

Antimicrobial activity

The antimicrobial activity of prepared silver nanoparticles was assayed by well diffusion method. Figure 7 shows the inhibition efficiency of nanoparticles against both *E. coli* and *Salmonella species.* The diameter of inhibition was measured at 04 mm, 08mm, 12mm and 18 mm and 05 mm, 10 mm, 15 mm and 20 mm for *E. coli* and *Salmonella species* respectively with 15μg/ml, 30μg/ml, 45μg/ml and 60μg/ml of nanoparticles. From that observation, we can conclude that prepared nanoparticles possess higher antimicrobial efficiency against *Salmonella* species than *E. coli.*

CONCLUSIONS

This study described a simple and biological approach for synthesizing AgNPs under mild condition by using extract from the mixture of Red rose petals, Orange peels and Potato peels. Spherical shaped AgNPs were prepared with moderate particle size of 145.4 nm and more zeta potential of -39.1 mV, indicating a good stability for colloidal suspension and with adequate polydispersity index. The synthesized AgNPs were extensively characterized and evaluated in terms of particle size, zeta potential and SEM analysis. The presence of polyphenols triggering the antioxidant activity was demonstrated by TLC, UV spectrophotometer and FT-IR spectral studies. Furthermore, the total phenol content was determined and the prepared AgNPs (F1) exhibited an excellent in vitro free radical scavenging property by DPPH assay. Therefore, biosynthesis of AgNPs is simple, safe, efficient and ecological. Hence, it has been concluded that polyherbal silver nanoparticles could find important potential antioxidant effect which plays a major role in anti-ageing property.

Declaration of Competing Interest

The authors report that there is no any conflict of interest to declare.

Human and Animal Rights

The authors ensure that there was no experimentation with animal or human subjects.

Consent for Publication

The corresponding author taken authorized consent from all the authors. All the authors read the manuscript thoroughly and given consent for publication.

Availability of Data and Material

Data are collected from PK solver (PharmPK); Design

Expert 9 Software (Stat-ease, Inc. USA); Graph pad Prism (Version 7 software — USA).

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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