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Green Synthesis of Cobalt and Copper Nanoparticles from Extract of *Rumex Hastatus* and Their Biological Evaluation

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Abstract

The cobalt and copper nanoparticles are gaining wider attention due to their applications in medicines, chemistry, biotechnology and agriculture. In the present study, cobalt and copper nanoparticles were synthesized by green technology using leaf extract of *Rumex hastatus* and characterized by Ultraviolet-visible Spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FT-IR), X-ray Diffraction (XRD) and Scanning Electron Microscopy (SEM) technologies. The synthesis of nanoparticles were observed by colour change of reaction mixture and UV-Visible spectrum showed maximum absorption peaks at 620 nm and 570 nm for copper and cobalt nanoparticles respectively. The FT-IR spectrum of *Rumex hastatus* leaf extract showed prominent peaks at 3240-2400 cm-1 (O-H stretch), 1710 cm-1 (C=O stretch), 1654cm-1 (C=C stretch) and 1618cm-1 (C=C stretch). However, these peaks were absent in the spectrum of cobalt and copper nanoparticles, meaning that these functional groups are involved in the reduction of cobalt and copper ions into their metallic nanoparticles. The SEM result showed irregular, spherical shape crystallites with rough surface. The average size for cobalt and copper nanoparticles were found in 52 nm and 78 nm respectively. The cobalt strongly inhibits the growth of *P.aereginosa* while copper nanoparticles strongly inhibit the growth of *E.coli*. The experimental data reveals that cobalt nanoparticles have more α-amylase inhibition activity than copper. The anti-glycation activity of cobalt nanoparticles is more than copper nanoparticles while copper nanoparticles have more anti-oxidizing potential than cobalt nanoparticles.
Keywords: Green synthesis of cobalt and copper NPs • Characterization • Biological

Introduction

Green synthesis is a technique of green chemistry that concentrated on the design of different products and plans by using several plants extracts which minimizes the use and production of harsh substances. It involves the basic principle which includes environmentally friendly ways without the use of hazardous, toxic and expensive substances. It is applied for learning the knowledge of equipment at micro molecular and macro molecular level. Nanotechnology is a modern field of material science that includes synthesis, characterization, development and applications of various nano materials. The term “nanoparticles” is used to explain a particle having dimension in the range of 1 nm-100 nm. Nanotechnology involves the synthesis and development of nano sized substances for the advantage of scientific society. Exploration and development in nanotechnology are increasing day by day. Nanostructures are the subject of great interest for all applications, wherein structure and size of nanoparticles describe their characteristic properties. Increasing knowledge towards green synthesis and other biological processes, it is vital to develop synthetic methods that are cost effective and environmentally friendly. The growing desire for eco-friendly for the synthesis of nanoparticles leads to the growing fascination in biological ways which do not include the application of harsh and expensive substances. Plants supply an effective stage for the synthesis of nanoparticles as they do not involve the use of harsh chemicals as by products. The special need for structure and size of nanoparticles cannot be met with the physical and chemical methods. In this case, biological procedures that involve microorganisms and plant extract are more suitable. The nanotechnology is one of the most suitable techniques in areas of material science and engineering. Nanoparticles deal with modern, fresh and improved properties that rely on specific characteristics like structure, size,

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morphology, variation and division [1]. In the current years, there has been impressive research in the area of nanotechnology, with various processes functioning to synthesize nanoparticles of specific size, structure and shape based on specific demands. Metal nanoparticles have a large special surface and specific fraction of surface atoms. Due to unique physical and chemical properties of nanoparticles like magnetic properties, optical properties, catalytic properties, antibacterial properties and electronic properties, they are fascinating researchers for their novel methods of synthesis. Nanoparticles are being used for various purposes including medical treatment, industry production like oxide fuel and solar batteries for energy storage and light generation, sensor technology, treatment of cancers, clothes, cosmetics and biological labelling. Nanoparticles are composed of variety of chemical nature like metals, metal oxides, carbon, biomolecules, silicates, and non-oxide ceramics. Nanotechnology has the potential to generalize a number of medical and biotechnological devices and methods, so that they are safer, portable, cheaper and easier to handle. Metallic nanoparticles have vast uses in different industries like silver, gold, magnetic, alloys etc. Biosynthesis of nanoparticles using microorganisms introduces numerous benefits of eco-friendliness and compatibility for remarkable applications as toxic substances are not involved. Metallic nanoparticles such as gold, silver, palladium, platinum, cadmium, iron, zirconium and numerous metal oxides are synthesized by microorganisms including both prokaryotes and eukaryotes. These microorganisms are bacteria, fungi, algae and actinomycetes. The metallic nanoparticles can be synthesized extracellular or intracellular. Metal nanoparticles can be synthesized by various physiochemical methods such as photochemical reduction, chemical reduction, heat of vaporization and electrochemical reduction. The reagents can be inorganic compounds or organic compounds. These compounds have potential to oxidize. Recently, different nanoparticles are being synthesized using gold, silver, titanium, zinc, copper, alginate and magnesium. There is great demand to develop noble metal nanoparticles by environmentally friendly techniques that do not involve harsh chemicals in their synthesis. The exploration has led to several biological processes, one of the fundamental proposals of which involves bioreduction. Keeping in view that green synthesis of metallic nanoparticles and their immense applications described above, it was proposed to synthesize the nanoparticles of medicinal plant botanically classified as Rumex hastatus. It belongs to polygonaceae family. Its local name is khatimal, khatebul / yellow sock. It is a bushy perennial small shrub, mostly grows in waste lands. It is found in Pakistan, India, China, Bhutan and Afghanistan. It is up to 2 ft tall. It has soft stem but base of the shrub is woody. It has small numerous pink flowers and small green leaves. Its flowering season is June-October. Rumex hastatus includes the treatment of cough, asthma and fever. Young leaves are used in chutneys and spinach [2].

Material and Methods

Rumex hastatus leaves were collected from different areas of district Sudhnoti AJ& K in the month of April 2018. The leaf sample was identified and authenticated by a taxonomist Dr. Prof. Hamayun Shaheen at the department of Botany, University of Azad Jammu and Kashmir Muzaffarabad.

Preparation of *Rumex hastatus* extract

Relatively fresh leaves of *Rumex hastatus* were collected from district Sudhnoti, Azad Jammu and Kashmir. Fresh leaves were thoroughly washed under running tap water first time to remove all the remains of dust and unwanted visible particles and then several times with distilled water followed by sun drying to remove residual moisture. The leaves were placed in open air for 3-6 hours. The dried leaves were cut into fine small pieces. Weighed about 90 g of dried leaves and boiled with 500ml of sterile distilled water in a 1000 ml glass beaker and boiled at 75oC for 25 minutes on hot plate and were allowed to cool. After that, the aqueous mixture was double filtered through Whatman No.1 filter paper and the filtrate was immediately used for the formation of cobalt and copper nanoparticles. The leave extract was kept at 4oC inside a refrigerator for further experimental work [3].

Preparation of copper nanoparticles

For the synthesis of copper nanoparticles, 20 ml of aqueous leaf suspension was added to 80 ml aqueous solution of 1 mM aqueous solution of CuSO4.5H2O in a 500 ml Erlenmeyer flask. The leaf mixture was warmed on a heating mantle by adding magnetic bar till the colour of the solution changed to light green. This change in colour showed the formation of copper nanoparticles. The reaction mixture was then centrifuged at 5000rpm for 20 minutes. After getting rid of the supernatants, pellets formed at bottom was re-dispersed in deionized water followed by centrifugation for 20 min at 5000 rpm [4]. This procedure was repeated four times. By centrifugation, stable copper salt and leaf extract were removed by pelleting and washing. The prepared nanoparticles were finally washed with alcohol, dried in a laboratory at 75oC and then kept to cool. The copper nanoparticles were stored in an air tight container and saved the samples for further experimental work.

Preparation of cobalt nanoparticles

For the synthesis of cobalt nanoparticles, 15 ml of aqueous leaf extract was added to 85mL aqueous solution of Co (NO)3.6H2O in a 500ml Erlenmeyer flask. Now the solution was allowed to warm in heating mantle in the presence of magnetic bar till the colour of the solution changed to dark brown. This variation in colour pointed out the formation of cobalt nanoparticles. The reaction mixture was then centrifuged at 5000 rpm for 15 min. After discarding the upper solution, pellets appeared at bottom of the centrifugation tube were allowed to redisperse in deionized water, followed by the centrifugation for 15 minutes at 5000 rpm. The whole process was repeated four times [5]. By centrifugation, unreactive copper salt and leaf extract were separated by pelleting and washing. The cobalt nanoparticles were dried in a laboratory at 75oC and then allowed to cool. The cobalt nanoparticles were stored in an air tight container and stored the samples for further experimental activity.

Biological Activities

The green synthesized cobalt and copper nanoparticles were checked for different biological activities. These bioactivities include various assays like anti-bacterial, anti-diabetic, anti-glycation and anti-oxidant.

Anti-bacterial activity

The antibacterial activities were tested by agar well diffusion technique, performed in the presence of cell suspension of approximately 1.5x106 CFU/ml, organized from McFarland turbidity standard No. 0.5. Holes of 6 mm diameter were bored on (8 mm thick) the Mueller Hinton agar (MHA) plate and filled with specific quantities of cobalt and copper nanoparticles. After inoculation, the plates were kept for 1 day at 37oC temperatures. For the evaluation of antibacterial potential, inhibition zone was determined. The average diameter was calculated by repeating the assay three times. The imipenem was used in this bioassay as standard antibiotic.

Anti-glycation activity

Appropriate quantity of test samples approximately 20ml each of nanoparticles was acquired by adding solvent DMSO with the sample (10µl BSA+ 10 µl of anhydrous glucose and trial substance 10µl). This activity control consisted of 10 µl glucose, 10µl Na¬3PO4 buffer and 10µl BSA, and the blank control composed of 10 µl BSA which is buffered with 20µl Na¬3PO4. Examined samples were kept in incubator by taking 39 well plates at 37.4°C for 7 days and are taken away from incubator and kept at 25oC. After that 40µl of absolutely pure TCA was poured into each well and centrifuged (10000 rpm) for 5 min at 5°C.Later on, the supernatant are separated consisted of glucose, inhibitor, interfering sample, AGE-BSA pellet and PBS [6]. Rutin was employed as standard inhibitor in this activity. The percentage inhibition was determined as given:

Inhibition (%)=100–[OD (test)/OD (blank)] × 100

Anti-oxidant activity

The anti-oxidant activity was determined in serial dilution as per thiocyanate approach. Each sample (500 µg) in 0.5 ml methanol was added in 5 ml DMSO (2.5 ml, 0.02 M, pH 7) and phosphate (2 ml, 0.2 M, pH 7) in a flask and kept in dark at room temperature. Spectrophotometer determined the peroxide value showing absorbance at 745 nm followed by addition of ferric chloride and thiocyanate at regular moments during incubation.

Assay for α-amylase inhibition

Measured quantity of reducing sugar mainly maltose is released during the assay which was utilized for the measurement of anti-diabetic potential. The inhibition activity of enzyme was estimated by the amount of maltose released during the chemical reaction was estimated by alternative DNS. After incubation, α-amylase having concentration of 1U/ml was added to every sample along with 1ml of starch solution. 1ml of DNS was mixed and mixture was boiled for 10 minutes. After that, two tests were performed, one blank test was done in absence of nanoparticles and other test was performed in absence of α-amylase enzyme, and being modified by buffer (20 mM sodium phosphate+ 6.7 mM NaCl, pH 6.8, Temperature 15oC) [7]. The absorbance obtained was 530 nm. Maltose equivalent which was liberated from starch was calculated by standard plot. Acarbose function as a positive control. The final conc. was obtained by using different concentrations of 1mg/ml, 3mg/ml and 5 mg/ml of nanoparticles diluted in buffer. The α-amylase inhibition potential was calculated by the following two equations.

% reaction=(maltose) test/(maltose)control × 100

% inhibition=100% reaction

Techniques Applied

The synthesized nanoparticles were described by using different techniques like Ultraviolet-Visible spectroscopy (UV-Vis), Fourier Transform Infrared spectroscopy (FTIR), X-ray Diffraction (XRD) and Scanning Electron Microscopy (SEM).

UV-vis (Ultraviolet-Visible Spectroscopy)

The UV-Vis spectroscopy is applied to detect the optical characteristics of the synthesized nanoparticles. UV-Vis spectroscopy is used to confirm the synthesis of metallic nanoparticles. The colour change indicates formation of nanoparticles.

FTIR (Fourier Transform Infrared Spectroscopy)

It also has potential to identify the existence of nanoparticles with various ranges. FTIR measurements were performed to identify the biomolecules responsible for capping of nanoparticles synthesized by plant extract.

XRD (X-Ray Diffraction)

XRD is used to find out the collection of particles in diverse range. X-rays diffraction was performed to ratify the crystalline state and average range of metallic nanoparticles.

SEM (Scanning Electron Microscopy)

It is applied to measure the particle size in micrometer range. SEM provides direct visualization of biosynthesized nanoparticles. The structural study and size of nanoparticles were observed by scanning electron microscopy (SEM).

Results and Discussion

UV-visible spectroscopy

UV-Vis spectroscopy is used to confirm the synthesis of metallic nanoparticles. The colour change from light to dark indicates formation of copper and cobalt nanoparticles. The synthesis of cobalt and copper nanoparticles indicates colour change from light yellow to dark brown and green within 30 minutes, respectively. UV-Vis spectrum of cobalt and copper nanoparticles is shown in the (Figure 1, Figure 2).

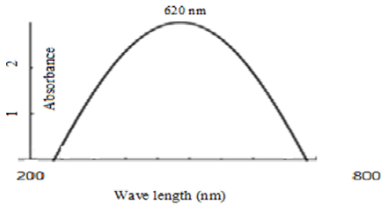

Figure 1. UV spectrum of cobalt nanoparticles.

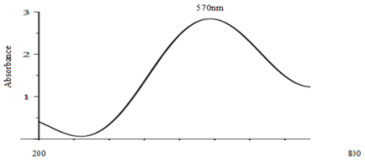

Figure 2. UV-Vis spectrum of copper nanoparticles.

The maximum absorption curve was noticed at 620 nm confirmed the synthesis of CoNPs. For the copper nanoparticles highest absorption curve was noticed at 570 nm wavelength, as shown below.

Fourier transform infrared analysis

The FTIR spectrum of *Rumex hastatus* plant extract is represented in (Figure 3).

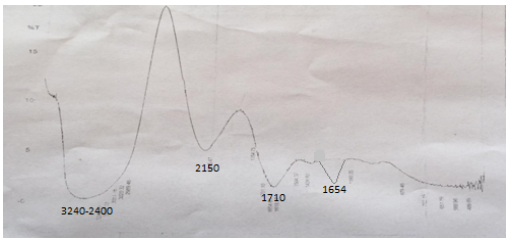

Figure 3. FTIR spectrum of *Rumex Hastatus* extract.

Very Broad band absorption occurs at 3240-2400 cm-1 shows presence O-H stretching vibration of carboxylic acid. The peak at 2150 cm-1 revealed C ≡C stretching. The peak at 1710 cm-1 revealed C=O stretching The band 1654 cm-1 arises due to the presence of C=C stretching. The presence of carboxyl group –COOH is accountable for the synthesis and stabilization of cobalt and copper nanoparticles.

FTIR measurements were performed to identify the biomolecules responsible for capping of cobalt and copper nanoparticles synthesized by *Rumex hastatus* leaf extract. The FTIR spectrum of Co nanoparticles is represented in (Figure 4).

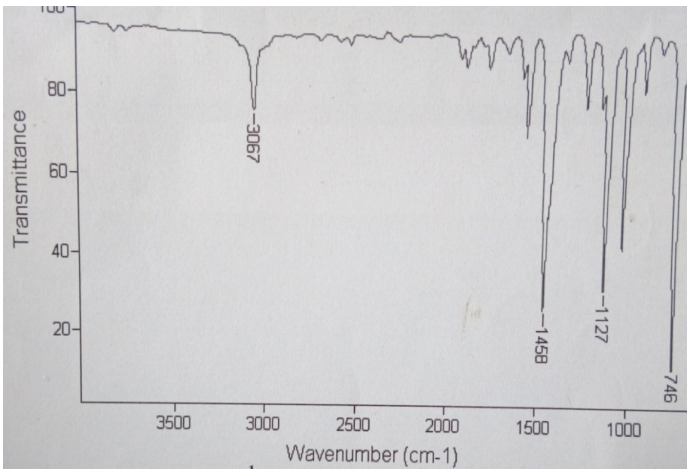

Figure 4. FTIR spectrum of CoNPs using *Rumex Hastatus* extract.

The band at 3067 cm-1 represents C-H stretching frequency of aromatic compounds. The bands at 1458 cm-1 corresponds to aromatic C=C bending vibration frequency. The band 1127 cm-1 shows the presence of –CH2 bending frequency. The FTIR spectrum of Cu nanoparticles is represented in (Figure 5).

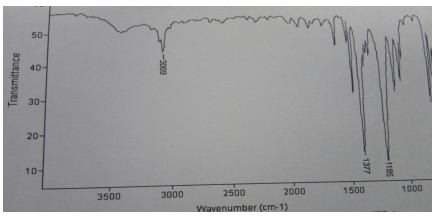

Figure 5. FTIR spectrum of CuNPs using *Rumex Hastatus* extract.

The band at 3069 cm-1 shows stretching frequency vibration of aromatic compounds. The peak at 1500 cm-1 is due to C=C vibration of aromatic compounds. The prominent peak 1377 arises because of –CH3 bending vibration. The assignment at 1185 cm-1 represents C-O-C stretching vibration. The spectroscopic data given above revealed that IR spectra of the synthesized nanoparticles are completely different from crude extract of *Rumex hastatus*.

XRD analysis

X-rays diffraction was performed to ratify the crystalline state and average range of metallic nanoparticles. XRD spectra of biofabricated cobalt and copper nanoparticles from extract of *Rumex hastatus* is shown in (Figure 6, Figure 7).

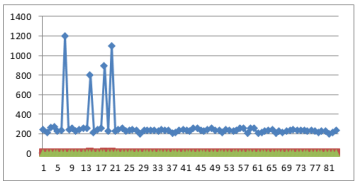

Figure 6. XRD spectrum of cobalt nanoparticles.

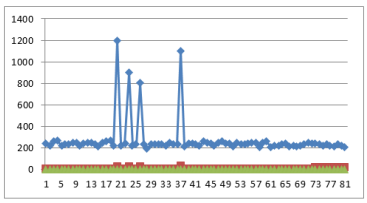

Figure 7. XRD spectrum of copper nanoparticles.

The XRD spectrum points that cobalt and copper nanoparticles are naturally crystalline when we compare our XRD spectra with the standard XRD patterns. The measured 2θ values for cobalt and copper nanoparticles are 10, 17, 21, 23 and 23, 26, 29, 40 respectively. The size of metallic nanoparticles were calculated by applying Debye-Scherer equation given below

$$D=K\lambda/\beta\cos\theta$$

Where K=Scherer's constant

λ=wavelength of applied X-rays (0.9 nm)

β=Half width of XRD peak

The average size of cobalt and copper nanoparticles were measured 52 nm and 78 nm respectively.

SEM analysis

The SEM images points that cobalt and copper nanoparticles are naturally crystalline .These were observed as irregular, spherical shape with rough surface and exhibited average size of 50 nm and 70 nm respectively (Figure 8, Figure 9).

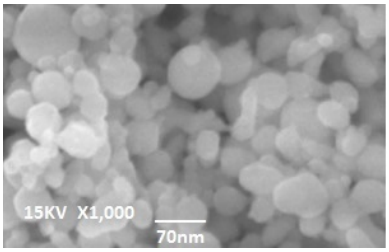

Figure 8. SEM image of copper nanoparticles.

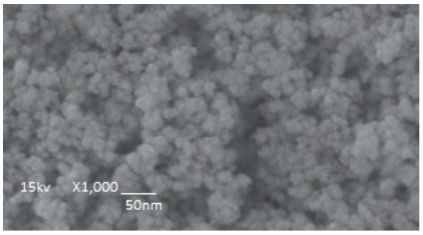

Figure 9. SEM image of cobalt nanoparticles.

Biological activities

The synthesized nanoparticles were tested for different biological activities.

Antibacterial activity: In order to check anti-bacterial potential, green synthesized nanoparticles were tested against different strains of bacteria like *S.aureus, B. cereus, P. aereginosa, K. pneumoniae and E. coli.* The acquired results are depicted by measuring inhibition zones (mm) in the (Table 1).

NPs Sol	S.aureus	B.cereus	P.aeregino sa	K.pneumo niae	E.coli
(Co)	18	16.5	20	19.5	18
(Cu)	15	16.5	18	18.5	19
Imipenem	20	18	25	22	24

Table 1. Anti-bacterial activity of nanoparticles.

In the table, it is prominent that cobalt nanoparticles shows maximum antibacterial activity against *P. aereginosa* while copper shows maximum antibacterial activity for E. coli.

α-Amylase inhibition: Green synthesized nanoparticles were used to investigate inhibition potential for α-amylase enzyme. The obtained data is shown in (Table 2 a).

% Inhibition					
Nanoparticles Fractions	1mg/ml	0.5mg/ml	0.25mg/ml	0.125mg/ml	0.0625mg/ml
Hexane	1.726	0.741	0.42	0.2	0.14
Ethyl Acetate	1.856	1.245	0.72	0.48	0.141

Table 2a. α-amylase inhibitory potential of cobalt nanoparticles.

It is apparent that 1 mg/ml conc. of cobalt nanoparticles in ethyl acetate and *n*-hexane exhibit high inhibition activity 1.856 & 1.726 and minimum activities at concentration 0.0625mg/ml are 0.141 and 0.140 (Table 2 b).

% Inhibition					
Nanoparticles Fractions	1mg/ml	0.5mg/ml	0.25mg/ml	0.125mg/ml	0.0625mg/ml
Hexane	1.615	0.742	0.45	0.21	0.131
Ethyl Acetate	1.745	0.135	0.71	0.38	0.12

Table 2b. α -amylase inhibitory potential of copper nanoparticles.

Table 2 b shows that copper nanoparticles in ethyl acetate and *n*-hexane exhibit high inhibition activity 1.745 & 1.615 and minimum activities at concentration 0.0625 mg/ml are 0.120 and 0.131. From experimental data, it is clear that these biosynthesized nanoparticles have great potential for carbohydrate's absorption and have effective role for diabetes control.

Anti-glycation activity: Anti-glycation activities of nanoparticles were examined by using various concentration which lies from 1µg/ml to 0.007734 µg/ml range prepared via serial dilution procedure. The acquired results are shown in the (Table 3).

1	2	Mean	3	4	Mean	5	6	Mean	7	8	Mean	9	10	Mean
11	12	11.5	5	6	5.5	12	7	9.5	8	9	8.5	12	12	12
13	12	12.5	7	8	7.5	15	13	14	12	11	11.5	14	15	14.5
10	8	9	7	6	6.5	10	10	10	10	9	9.5	11	10	10.5
8	8	8	12	10	11	10	10	10	7	7	7	10	10	10
8	6	7	9	10	9.5	9	10	9.5	6	10	8	10	9	9.5
7	8	7.5	10	11	10.5	8	9	8.5	6	6	6	10	10	10
6	6	6	11	9	10	6	8	7	5	11	8	11	10	10.5
7	8	7.5	9	10	9.5	8	9	8.5	5	10	7.5	12	8	10
n-Hexane			Ethyl Acetate			n-Hexane			Ethyl Acetate			Standard		
Cobalt Nanoparticles						Copper Nanoparticles								

Table 3. Anti-glycation activity of nanoparticles.

From the data given below, it is clear that cobalt nanoparticles dispersed in *n*-hexane solvent displayed maximum anti-glycation potential of 12.5 and minimum potential of 6, while copper nanoparticles dispersed in *n*-hexane solvent displayed maximum anti-glycation potential value of 14 and minimum value of 7 by using 1µg/ml concentration. By using concentration of 0.5µg/ml, it was

observed that cobalt nanoparticles dispersed in ethyl acetate solvent displayed anti-glycation activities 11 and minimum anti-glycation potential 7, while copper nanoparticles dispersed in ethyl acetate displayed anti-glycation activity 11.5 and minimum anti-glycation potential 6 at conc. of 0.25µg/ml (Figure 10).

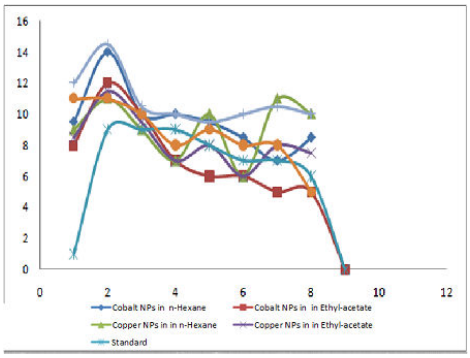

Figure 10. Anti-glycation activity of nanoparticles.

Anti-oxidant activity: This biological activity of nanoparticles was determined on the peroxidation of DMSO displayed in (Figure 11a-11d).

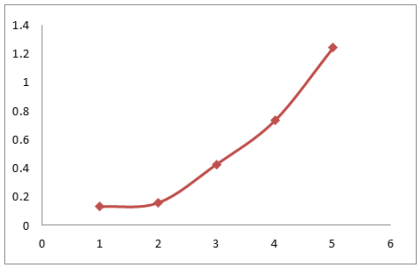

Figure 11a. Anti-oxidant activity of cobalt nanoparticles in n-hexane.

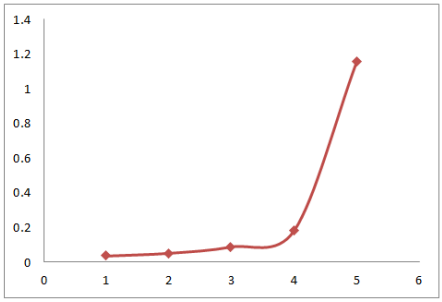

Figure 11b. Anti-oxidant activity cobalt nanoparticles in ethyl acetate.

It was observed that cobalt nanoparticles dispersed in n-hexane showed anti-oxidant potential which was recorded as 1.236 % and the cobalt nanoparticles dispersed in ethyl acetate solvent exhibit 1.157 % anti-oxidant activity, similarly the copper nanoparticles dispersed in n-hexane solvent displayed anti-oxidant activity 0.887 % and those dispersed in ethyl acetate solvent exhibit 1.873 %, inhibition of DMSO peroxidation. This suggests the presence of polyphenols in the extract of Rumex hastatus and responsible for the anti-oxidant potential.

Conclusion

Cobalt and copper nanoparticles were prepared successfully using extract of Rumex hastatus by green technology. The plant extract reduces cobalt and copper ions to respective metallic nanoparticles. The prepared metallic nanoparticles were interpreted by using latest spectroscopic and microscopic techniques. Firstly, UV-Vis spectrum indicates maximum absorption for cobalt and copper nanoparticles at 620 nm and 570 nm respectively. It was observed that the experimental data obtained was matched with the reported data and certified the synthesis of nanoparticles. The FT-IR spectrum of Rumex hastatus extract displayed intense peaks at 3240-2400 cm-1 (O-H stretch), 1710 cm-1 (C=O stretch), 1654 cm-1 (C=C stretch) and 1618 cm-1 (C=C stretch in conjugation). The spectrum of cobalt and copper nanoparticles demonstrated the absence of above mentioned peaks indicating that these functional groups were included in the reduction of cobalt and copper nanoparticles. The XRD spectrum points that cobalt and copper nanoparticles are irregular, spherical shape when we compare our XRD spectra with the standard XRD patterns. The sizes of cobalt and copper nanoparticles are 52 nm and 78 nm respectively. The SEM reported irregular, spherical shape with rough surface. The cobalt strongly inhibits the growth of P.aereginosa while copper nanoparticles strongly inhibit the growth of E.coli. The experimental data reveals that cobalt nanoparticles have more α-amylase inhibition potential than copper. The anti-glycation activity of cobalt nanoparticles is more than copper nanoparticles while copper nanoparticles have more anti-oxidizing potential than cobalt nanoparticles.

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