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Comparative analysis of natural sample and synthesis of silver nanoparticles (NPs) using medical plant caesalpinia bonducella seed

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ABSTRACT

Nanostructured noble metal has unusual physicochemical properties compared to their bulk parent materials. Thus in recent years a number of physical, chemical and biological techniques were applied for the development of metal nanoparticles (NPs). Here we have silver nanoparticles were synthesized using rapid, single step and completely green biosynthetic method employing aqueous Caesalpinia bonducella seeds. Silver ions were rapidly reduced by the aqueous Caesalpinia bonducella seed seeds leading to the formation of highly crystalline silver nanoparticles. The preparation of stable, uniform silver nanoparticles by reduction of silver ions is reported in the present paper. It is a simple process of recent interest for obtaining silver nanoparticles. The samples have been characterized by Fourier Transform Infrared (FTIR) Spectroscopy, UV-Visible spectra, Scanning Electronic Microscopy (SEM) and Energy Dispersive X-ray (EDX) spectra of the medicinal plants Caesalpinia Bonducella Seeds were recorded. The vibrational assignments, intensities and wave number (cm^{-1}) of dominant peak were obtained from FTIR spectra. Probable assignments of the bands were made with respect to the components present in the samples. The microphotograph obtained from SEM and weight percentage of specific elemental concentration obtained from EDX spectrometer attached to SEM are reported and analyzed sample wise and the results are discussed. The UV/Vis spectra shows that an absorption peak, occurring due to Surface Plasma Resonance (SPR).

KEYWORDS

UV-visible;
FT-IR spectra;
SEM-EDS;
Caesalpiniabonducella seed;
$AgNO_3$.

INTRODUCTION

Plants have been used in traditional medicine for several thousand years. Medicinal plants as a group comprise approximately 8000 species and account forabout 50% of all the higher flowering plant species in India[1]. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. In a large number of countries, human population depends on medicinal plants for treat in various illnesses as well as a source for livelihood. The World Health Organization (WHO) estimated that 80 % of populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs[2,3]. The objective of this study was to identify various chemical groups present in the important medicinal plants caesalpinia bonducella seed.

Caesalpinia bonducella

Caesalpinia bonducella (L.) Fleming (Syn. Caesalpinia bonduc (L.) Roxb, Syn. Caesalpinia crista Linn.), belonging to the family Fabaceae/ Caesalpiniaceae, is a prickly shrub widely distributed all over the world. Specially, in India, Sri Lanka and Andaman and Nicobar Islands, in India specially found in tropicaland subtropical regionsof the world[4,5]. "Bonducella" the name of the species is derived from the Arabic word "Bonduce" meaning a "little ball" which indicated the globular shape of the seed[6]. The seeds are grey coloured and resemble eyeballs, which explains the Sanskrit name kuberakshi, meaning eyes of kubera, the Hindu God of wealth[7,8]. Seed coat is hard, glossy, and greenish to ash grey in colour. The plant was much confused with Caesalpiniabonducella (Syn. C. bonduc) and was described under the same[9-15]. Beside this species like C. nuga[16-18] and C. jayoba are also sometimes wrongly designated as synonyms for C. crista[13]. Seed coat is hard glossy and greenish to ash grey in colour and is traversed by circular and vertical faint markings of the cracks, forming uniform rectangular to squarish rectulations all over the surface Seeds 1-2 oblong, lead-coloured, 1.3 cm long. A raised hilum with remains of the stalk lies in the centre of the dark spot, at the narrow edge of the seed. Adjacent to the hilum, lays a faint coloured circular to oval elevated micropyle. In dry seed, kernel gets detached from the testa. Testa is about 1-1.25 mm in thickness and is composed of three distinct layers, the outermost - thin and brittle, the middle one - broad, fibrous and dark – brown and the innermost – white and papery.

The seed is exalbuminous. The kernel surface is furrowed and ridged, hard, pale yellowish – white,circular to oval, flattened and about 1.23- 1.75 cm. in diameter as shown in Figure 1. A scar of the micropyle lies at one end of the kernel, from where arises a prominent ridge demarking the two cotyledons of the embryo. Plumule – radical axis is thick, cylindrical and straight. Taste is very bitter and odour is nauseating and unpleasant. All parts of the plant have medicinal properties so it is a very valuable medicinal plant which is utilized in traditional system of medicine. The plant has been reported to possess anxiolytic, antinociceptive, antidiarrhoeal and antifilarial activities. Phytochemical analysis of seeds of Caesalpinia bonducella has revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and triterpenoids. Despite the progress in conventional chemistry and pharmacology in producing effective drugs, the plant kingdom might provide a useful source of new antiulcer compounds for development as pharmaceutical entities or, alternatively, as simple dietary adjuncts to existing therapies[6-9]. Caesalpinia bonducella seed as shown in Figure 1 can be used as environment friendly and sustainable insecticides to control mosquito. The caesalpinia bonducella of the leaf juice is used astonic for jaundice and leaf paste is applied on the affected area for toothache[4]. It is also good for the diseases of the spleen, stomatitis, toothache, hemicrania, fever, pain in the liver. It is juice in combination with honey that is administeredfor catarrh.

Figure 1 : Caesalpiniabonducella seeds.

Ayurvedic description[26-28]

Properties

Rasa: Tikta (bitter), kashaya (astringent)
Guna: Laghu (light), ruksha (dry), tikshna (sharp);
Dosha: Pacifies tridosha

Action and uses

Kapha, vat samak, sothahar, badanasthapan, dipan, anuloman, krimighan, raktsodhak, swashar, mutral, jwaraghan

Traditional and modern uses

The seed is claimed to be styptic, purgative and anthelmintic and cures inflammations, useful in colic, malaria, hydrocele, skin diseases and leprosy. In Madras (Chennai) an ointment is made from the powdered seeds with castor oil and applied externally in hydrocele and orchitis. The seeds are considered tonic, ferifuge, anthelmintic, antiblennorrhagic, and specific in the treatment of hydrocele. The oil from the seeds is used in convulsions and paralysis[29,30].

In Guinea, the pounded seeds are considered vesicant. The powdered seeds were mixed with equal part of pepper powder to malaria patients and were found to posses feeble antiperiodic properties. In malignant malaria, they did not do any good. The seeds are ground in water and given internally in snake-bite. The seeds are not an antidote to snake-venom. Seed and long pepper powders have taken with honey gives good expectorant effect. Burnt seeds with alum and burnt areca nut are a good dentifrice useful in spongy gums, gum boils, etc.

In West Indies, the roasted seeds are used as anti diabetic. The kernel of the seed is very useful and valuable in all ordinary cases of simple, continued and intermittent fevers. The kernel powder mixed with equal parts of black pepper is taken thrice a day in a dose of 15-30 grains by adults and 3-4 grains by children. It was made official in the Indian Pharmaceutical Codex 16 the dose of the powder being 15-18 grains.

It is said to produce lots of perspiration, leading to the reduction of fever. Kernel powder with sugar and goat milk gives good result results in liver disorder. Decoction of roasted kernels was used in asthma. Children unable to digest mother's milk were given the extract of the kernel or its powder along with ginger, salt and honey to get good stomachic effect. Paste prepared from kernel gives relief from boils and other such swellings[31-34].

MATERIALS AND METHODS

Preparation of silver nanoparticles by precipitation method

The Fresh Caesalpinia bonducella seeds were collected from Agaram village, Pochampalli Taluk, Krishnagiri, Tamil Nadu, India. The seeds were carefully collected from the plant. These cleaned plant parts were placed separately in polythene bags. Then, these are shade dried in a clean environment to avoid the contamination for 10 days and oven dried at 60 °C for four hours, to remove the moisture content. The oven dried seeds were ground into a fine powder by using an agate mortar. Nanoparticles are being considered as fundamental building blocks in nanotechnology. The most important and distinct property of nanoparticles is that they exhibit larger surface area to volume ratio[36]. Metal nanoparticles have tremendous applications in science and technology. In the present investigation the synthesis of silver nanoparticles by chemical route is discussed, which is an easy, simple and convenient route for preparing metal particles in nanometer range[37-38]. $AgNo_3$ was obtained from sigma Aldrich chemicals. All glass wares have been washed with deionised water and dried in oven before use. All reagents were used as received without any further purification. To prepare Nanocomposites by taking 50 g Caesalpinia bonducella Seed powder dissolved in 200 ml of Deionised water and the solution is stirred for 2 hours at room temperature. Then the extract was filtered and stored at 4^0 C for further experiments as reducing and capping agent. The 4 ml of extract was added to 20 ml and 0.1 mM. The 4 ml of extract was added to 20 ml of 10^{-4} m $AgNO\text{-}\text{-}_3$ aqueous solution and kept at room temperature. After the stirring process, the colourless solution is slowly changes to brown colour indicate the formation of silver nanoparticles. Subsequently, the solution was kept for 5 hours for the deposition of the nanocomposites. The particles were collected in a Petri dish as a white precipitate subsequently the materials were dried at 80° C for 2 hrs. The Brownish nanocomposites are collected. Finally the nanocomposites were grained and preserved in an air tight container.

Characterization of silver nanoparticles (NPs)

The reduction of metallic silver ions was monitored by measuring the UV-Vis spectrum after about 48 hours off reaction. The absorption signal was measured for the wavelength from 200-800 nm on UV-Vis spectrometer (Systronics Double beam Spectrometer). The FT-IR spectra were recorded using BRUKER IFS 66 model FT-IR spectrometer in the region 4000–400 cm^{-1} by employing standard KBr pellet technique. The microphotographs of these samples were recorded using SEM

JEOL model, JSE-5610 LV with an accelerating voltage of 20 kV, at high vacuum (HV) mode and Secondary Electron Image (SEI). Typically setting at a magnification at ×15,000 (1 μm) for a sample at study. The semi quantification elemental analyses to identify the weight percentage of major and minor elements present in the samples were done using energy dispersive X-Ray spectrometer (EDS), JEOL model, JSD-5610 LV with an accelerating voltage of 20 kV.

RESULTS AND DISCUSSION

UV-vis spectral studies

The UV-Vis spectralanalysis of pure and synthesized NPs were shown in Figures 2(a) & 2(b).

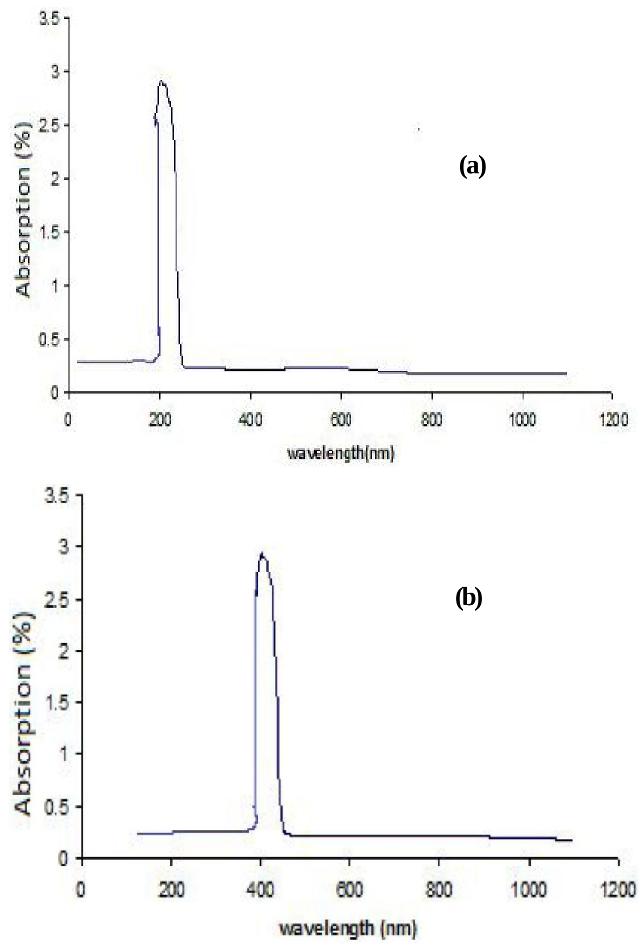


Figure 2 : UV-Vis spectra of (a) pure and (b) synthesized silver NPs.

The reduction of silver ions during reaction Caesalpiniabonducella seeds have easily followed by UV-Vis spectroscopy. The absorption spectrum of pure and silver NPs exhibits a well defined absorbed peak at 205 nm and 404 nm respectively. It is observed that the silver SPR band is centred at about 404 nm and the reduction of silver ions. The band due to π-π^* transition in a compound with conjugated π system is usually intense and frequency referred to as the K-band (German konjugierte).

FTIR studies

The FTIR analysis of the untreated and silver NPs treated C-B seeds are shown in Figures 3(a) & 3(b). The absorption bands, the wave number (cm^{-1}) of dominant peak obtained from absorption spectra are presented in TABLE 1.

TABLE 1 : Wave number (cm^{-1}) of dominant peak obtained pure and synthesized silver NPs from FTIR spectra.

Functional groups	Pure Sample		Synthesised Nanoparticles (NPs)	
	Wave number (cm^{-1})	Visible intensity	Wave Number (cm^{-1})	Visible intensity
Carboxylic Acid				
O-H stretching	2841	Strong	2975	strong
O-H stretching	1403	very strong	1400	very strong
Amine				
N-H stretching	3408	very strong	3450	very strong
N-H bending	1640	strong	1642	strong
C-N stretching	1017	Weak	1050	Weak
Amide				
N-H stretching	3408	very strong	3450	very strong
C-O stretching	1640	very strong	1642	very strong
Amino Acids				
N-H stretching	2841	very strong	2975	Strong
C-O stretching	1403	very strong	1400	very strong
Polysaccharide				
C-O-C stretching	1017	very strong	1050	very strong
Carbohydrate				
N-H wagging	707	Weak	695	Weak
Aldehydes				
C-Hstretching	2841	medium	-	-
C-H bending	1403	medium	1400	medium

The very strong absorption band observed around 3408–3450 cm^{-1} may be due to the presence of bonded N–H/C–H/O–H stretching of amines and amides. The very strong absorption at 3408 cm^{-1} shows the presence of amino acids in the seeds. The very strong absorption band appearing in the region 2841, 2975 cm^{-1}

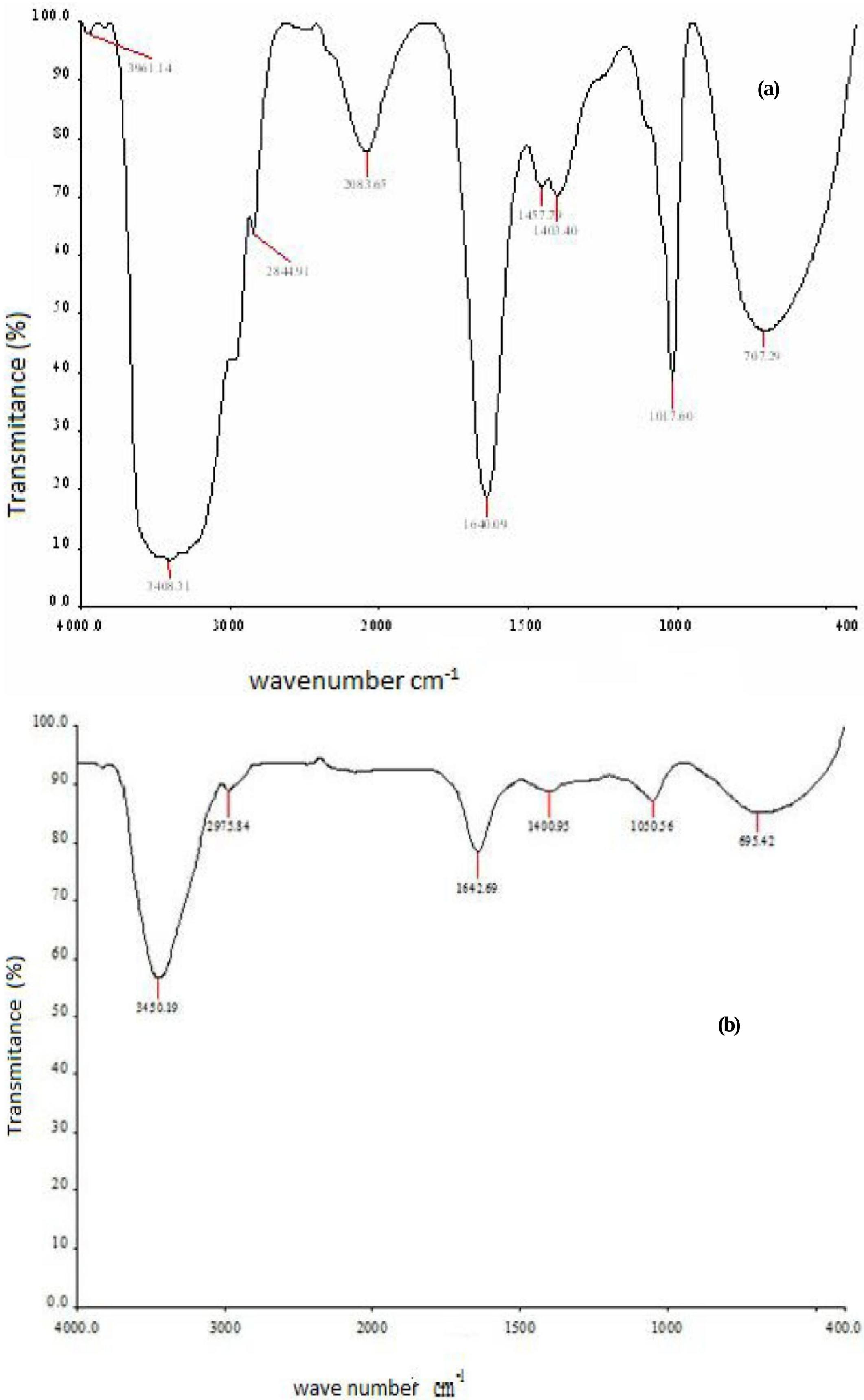


Figure 3 : FTIR spectra of (a) pure and (b) synthesized silver NPs.

for the sample due to N–H stretching vibration of NH_3 group shows the presence of primary amines. The C-H stretching methylene group appears near 2927 cm^{-1}. A symmetrical stretching of NO_2 group results in strong absorption in the region 1640-1642 cm^{-1} indicates the presence of amines (protein). This gives the evidence that the seeds are rich in protein. The observed very strong absorption band between 1403 and 1400 cm^{-1} in seed is due to the presence of bonded C–O/O–H bending. The strong band occurring at 1017 cm^{-1} and 1050 cm^{-1} is due to the presence of bonded C–O stretching vibration in secondary alcohol[1,2,9]. The very strong band occurring at 1050 cm^{-1}, 1017 cm^{-1} shows the presence of symmetrical C–O–C stretching in vinyl ether. The absorption at 1050 cm^{-1} indicates the presence of polysaccharides. Many C–O–C groups exhibit characteristic bands in the 1050 -1017 cm^{-1} spectral range and generally the strong band at 1050 cm^{-1} is assigned to the vibration of C–O in alcohol hydroxyl group.

The stretching vibrations assigned to the C–S linkage occur in the region at 700–600 cm^{-1}. The weak absorption band of 695, 700 cm^{-1} indicates the presence of sulphate. The brominates compound shows an infrared band region 600–500 cm^{-1}[1,9]. The more intense bands occurring at 3410 cm^{-1}, 2927 cm^{-1}, 2841 cm^{-1}, 1637 cm^{-1}, 1400 cm^{-1}, 1050 cm^{-1}, 1017 cm^{-1}, 695 cm^{-1} and 675 cm^{-1} corresponding to O–H/N–H, C–H, C–O and C–Cl/C–S stretching/bending vibrations respectively indicate the presence of amino acids, alkenes, nitrates, ethers, organic halogen compounds and carbohydrates in Caesalpiniabonducella seed.

SEM analysis

The SEM analyses of the untreated and silver NPs treated C-B seeds are presented in Figures 4(a) & 4(b). Drops of the sample were placed on carbon coated copper grids. The films on the grids were allowed to dry prior to record on JEOL-SEM model, JSM-5610LV microscope. The morphology and size of the particles are determined by the SEM images. The nanoparticles were observed in a particle magnification of 15,000X operating at 20 kV. It can be seen that the obtained product has mixture of tip nanocluster with the length of 50 nm to 300 nm.

The SEM ocimum santum encapsulated nano particles reveals that the synthesized particles were in nano size. The particles were roughly spherical in shape. The particle size was found to be approximately in the range of 50 nm to 300 nm. The even distribution of the nano particles on the surface was visualized. It is clearly revealed that the particle size is decreased for untreated to $AgNO_3$ nanoparticles tread of C-B seeds.

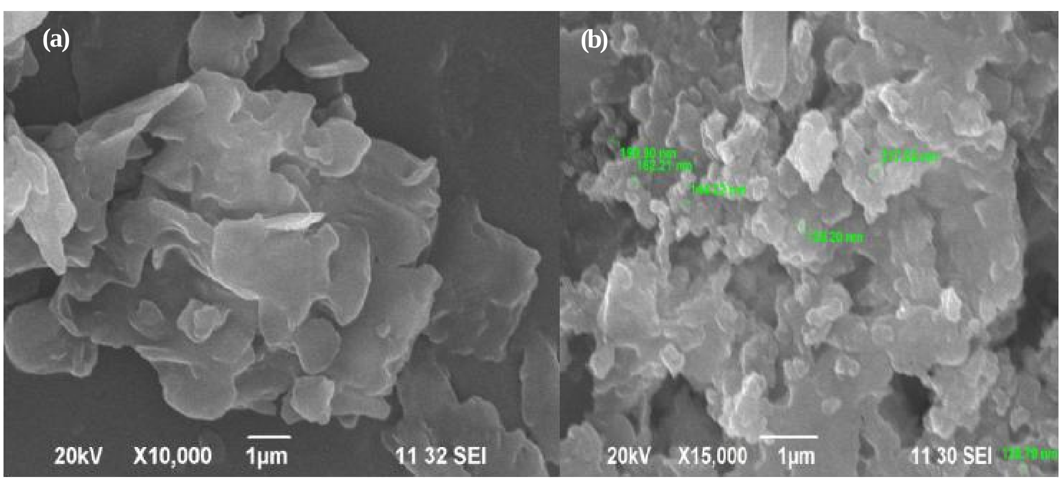
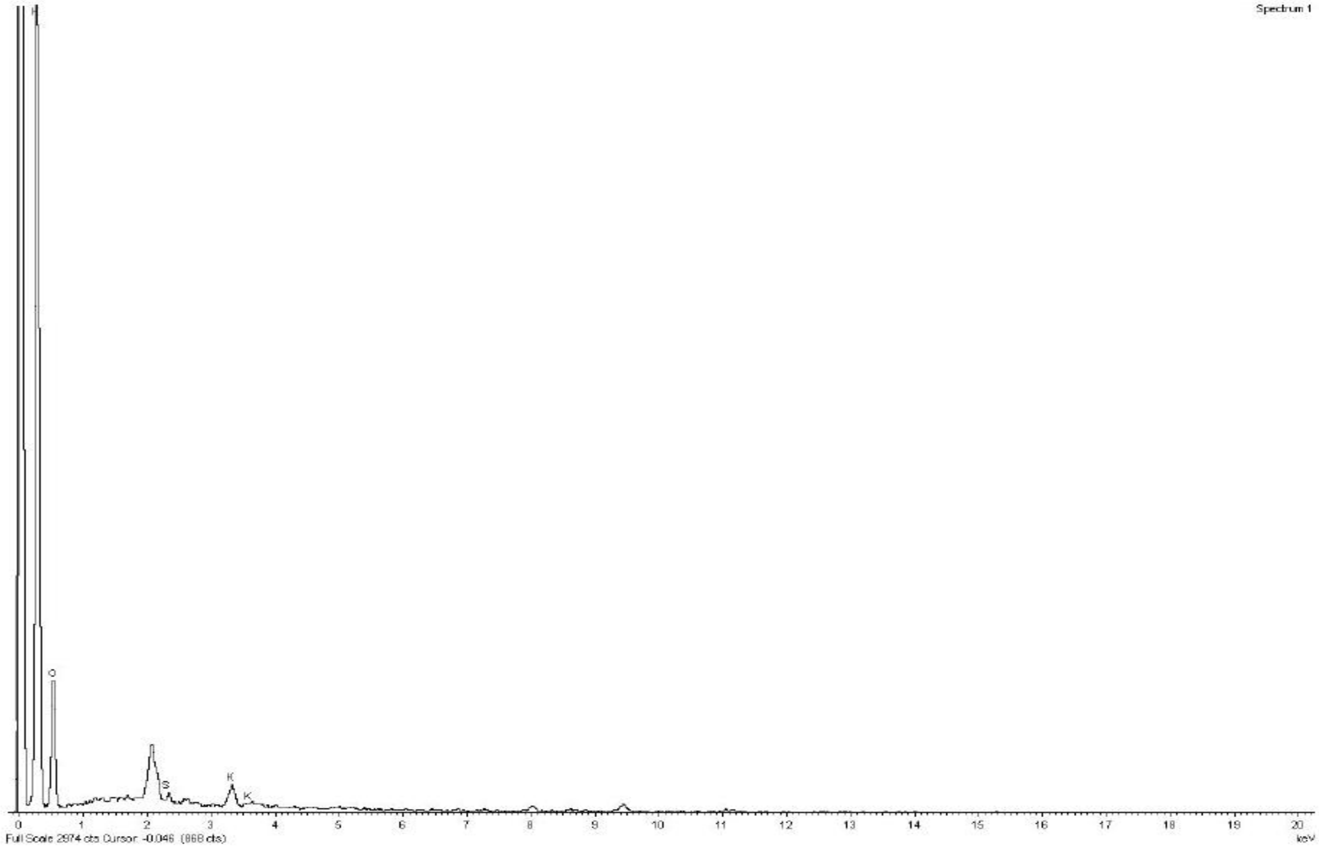


Figure 4 : SEM spectra of (a) pure and (b) synthesized silver NPs.

EDS analysis

The EDX analysis of the untreated and silver NPs treated C-B seeds are presented in Figures 5(a) & 5(b). Trace elements are estimated by determining the percentage abundance (%) of elements O, Si, S, Cl, Ca and K in the samples collected. The concentrations of such elements are reportedin TABLE 2 and Figure 5. Indeed quite a large number of heavy metals are essentialto plant and animal (including human) life. These include, naming a few oxeye, sulphur, silicon, calcium, potassium, manganese, copper, nickel, zinc, cobalt, chromium, molybdenum and vanadium. Ifany of these



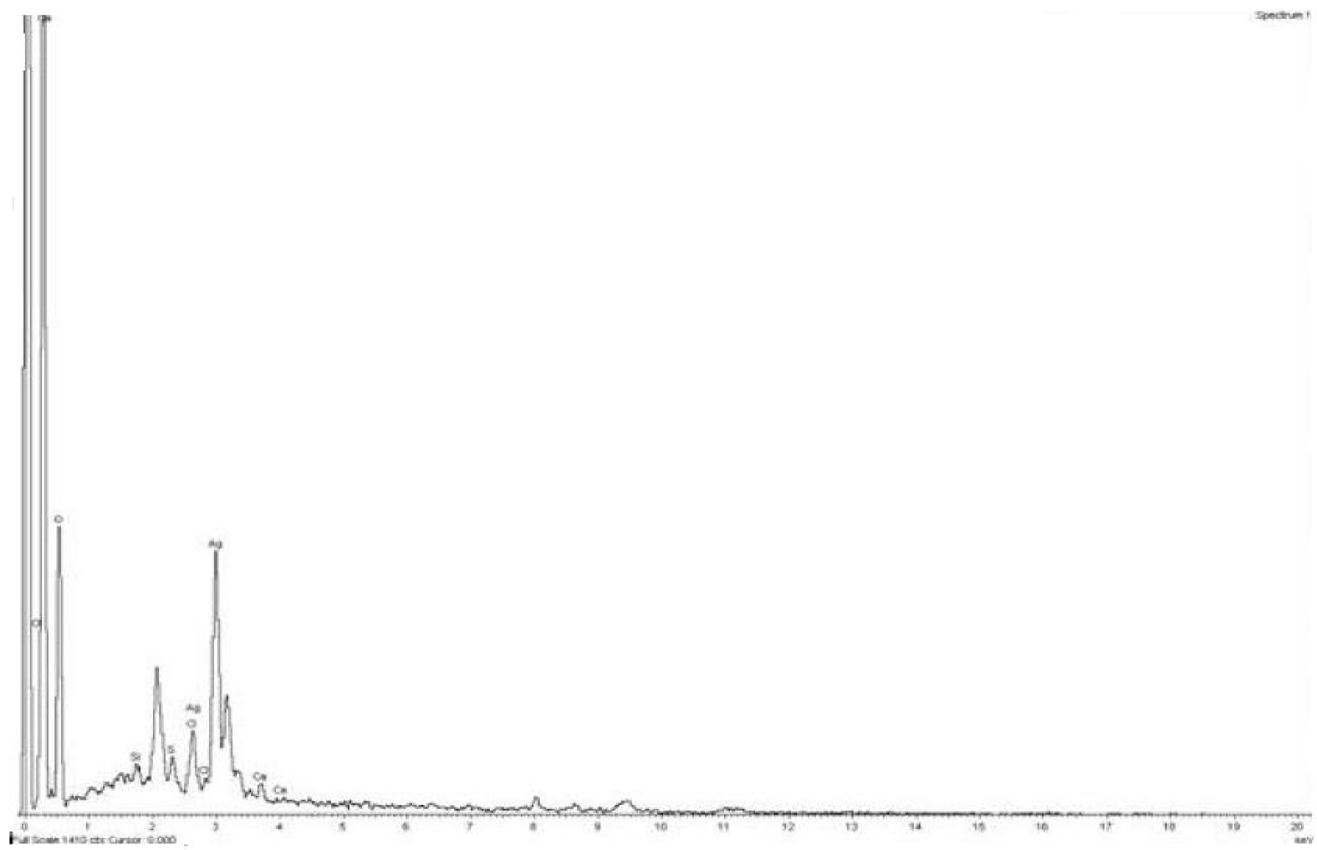


Figure 5 : EDX spectra of (a) pure and (b) synthesized silver NPs.

metals is eliminated from our nutrition we would be suffering from one or the other disease. They are indeed like vitamins and if we are deficient witheven one of them, we would suffer from one or other health problem. It is observed from TABLE 2 that in. Sodium was considered to be non-essential for plants. The concentration of oxen's is 85.55 % pure sample and 52.91 % of synthesised sample respectively.

TABLE 2 : Percentage of element in pure and synthesized silver NPs.

Element	Weight (%)	
	Pure sample	Synthesized NPs
O	85.55	52.91
Si	-	1.24
S	-	1.78
Cl	-	3.13
Ca	2.81	1.54
K	11.67	-
Ag	-	39.41

The concentration of silicon is 1.24 %. The concentrationof chloride is 1.78 %. The concentration of potassium is 11.67 % in pure sample. It is used as ash, manure andfertilizer. In the present investigation, the concentration of calcium is 2.81 % pure sample and 1.54 % of synthesised sample respectively. The concentration of silicon, sulphur and chlorine is 1.24 %, 1.78 % and 3.13 % of synthesised sample respectively. It is absence of the pure sample. The concentration of silver is 39.41 % it is conform for synthesised silver nanoparticles.

CONCLUSION

The bio-synthesis of silver NPs using C-B seeds are simple, non toxic and efficient. This green chemistry approach is amenable to large scale commercial production. The use of environmental benign and renewable plant material offers enormous benefits of eco-friendliness and compatibility for biomedical and pharmaceutical applications. Thus, the synthesized silver NPs could have a high potential for use in biological applications. This method is inexpensive and highly recommended to be used in large scale production of silver NPs. The reduction of silver ions during reaction Caesalpiniabonducella seeds has easily followed by UV-Vis spectroscopy. The main functional group of these plants is wedelolactone which is confirmed by FT-IR study. The presence ofcharacteristic functional groups of carboxylic acids, amines, amides, polysaccharides, nitrates and carbohydrate are responsiblefor various medicinal properties of both herbal plants. The various functional groups and trace elements are identified using FTIR and EDX analysis. The SEM images reveal the nano nature of the prepared samples.

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