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SYNTHESIS AND CHARACTERIZATION OF CR^{3+} *ALSTONIA BOONEI* LEAF EXTRACT FOR MEDICAL APPLICATION

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ABSTRACT

Metal-plant extract nanoparticles have gained tremendous attention over the last decade due to their application in medical. In this study, green synthesis of chromium-*Alstonei boonei* nanoparticles was achieved. The leaves extract of *Alstonia boonei* and synthesized Cr^{3+}-*Alstonia boo*nei were characterized by UV-Vis absorption spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR). Scanning Electron Microscopy (SEM), GC-MS spectroscopy and X-ray diffraction spectroscopy. The result revealed that CRNP has the absorption peak at 470nm and 490nm respectively. Functional groups present on the CrNPs are $C-H, C\equiv C$ *and* $N-O$ band which makes it naturally stable. X-ray diffraction analysis revealed that the synthesized CrNP has an average particle size of 89.81nm and SEM micrograph showed aggregated irregular shapes with rough surfaces. The result of GC-MS study revealed phytochemical constituents of medical importance. The results of the present study showed that synthesized CrNP is naturally stable with phytochemical compounds that can be used in various biological applications.

KEYWORDS: Cr^{3+}-NPs, green synthesis, *Alstonia boonei*, characterization, medical application.

1.0 INTRODUCTION

There is a tremendous research interest in the area of nanotechnology to develop reliable processes for the synthesis of nano materials over a range of sizes and chemical compositions

for biology, medicine and engineering applications (Saravanan, 2008). These nanoparticles have been receiving a lot of attention for potential use in therapeutics, bioengineering and therapeutics drug discovery (Abhilash, 2010).

Nanomaterials are the prominent necessity of the rapidly field of nanomedicine and bionanotechnology; and they can offer a new approach to challenge antibiotic-resistance microbes (Logeswari, 2015). Several studies have indicated that nanoparticles (NP) can be used as therapeutic tools against microbes because of their chemical, physical, magnetic and mechanical properties and these unique properties can be attributed to their defined size, shape and effective biological properties (Muhamed, 2017).

A key area in improving drug delivery is targeting of the drug to cells or tissue of choice accurately. Modern delivery technologies are far away from the design of the so called 'magic bullet', proposed by Paul Ehrlich at the beginning of the 20th century, in which the drug is precisely targeted to the exact site of action. Nanotechnology offers here another challenge to come to this goal a bit closer to drug delivery in the right place at the right time (Kayser, 2005). Targeting is the ability to direct the desired drug to the site of interest. Two major mechanisms are proposed for addressing the desired sites for drug release: (i) Passive and (ii) Active targeting. An example of passive targeting is the preferential accumulation of chemotherapeutic agents in solid tumors as a result of the enhanced vascular permeability of tumor tissues compared with healthy tissue (Bhagwat and Vailhya, 2013). A method that could allow active targeting of desired site involves the surface functionalization of drug carriers with ligands that are selectively recognized by receptors on the surface of the cells of interest. Since ligand-receptor interactions can be highly selective; this could allow a more precise targeting of the site of interest (Costas *et al.*, 2006).

Alstonia boonei is a herbal medicinal plant of West African origin popularly known as God's tree or 'Onyame dua' (John, *et a*l, 2012). All the parts of the plant are being utilized but the thick bark cut from the matured tree is the part that is most commonly used for therapeutic purposes. The bark of the tree is highly effective when it is used in its fresh form, however the dried one could equally be used. Therapeutically, the bark has been found to possess antirheumatic, anti-inflammatory (Hadi and Bremner, 2001), analgesic/pain killing, antimalaria/antipyretic, antidiabetic (mild hypoglycaemic), antihelminthic, antimicrobial and antibiotic properties (Fakae, et al, 2000). Phytochemical screening of the ethanolic leaf extract of *Alstonia boonei* indicated the presence of tannins, Phlobatannins, alkaloids, cardiac

glycosides, reducing sugar, saponins, anthraquinones and steroids. Its ethanolic leaf extract also showed varying inhibitory effect against *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Salmonella typhimurium* at 100mg/mL (Ajose, *et al.*, 2019).

Chromium is an essential trace element that is used by some people as a supplement. It is an interesting metal to be studied that has two forms namely; trivalent and hexavalent chromium. Its hexavalent form is a known toxin that causes skin problems and lung cancer while the trivalent form that is found in foods and supplements is safe for humans and has medical applications. (Althius *et al.*, 2002). The trivalent chromium forms a compound in the body that seems to enhance the effects of insulin and lower glucose levels. Some studies have shown that chromium supplements may be helpful for people with type 2 diabetes and insulin resistance (prediabeties) (Wang *et al.*, 2007).

Some of the most studied metallic nanoparticles include silver(Ag) (Ajitha et al, 2012; and Feng et al, 2013); gold (Au) (Moreira et al, 2012) platinium (Pt) (Aritonang, et al, 2014, 2015 and 2017), palladium (Pd) (Raut et al, 2013) iron (Fe) and zinc (Zn) (Saravanan, 2008); but there is paucity of information on chromium-synthesized nanoparticle. Synergizing the medical potentials of *Alstonia boonei* with that of chromium forms the focus of this paper. In addition, the synthesized drug was characterized using Fourier Transform Infra Red spectrophotometer (FTIR), Ultra violet spectrophotometer (UV), Scanning Electron Microscope (SEM), X-ray Diffraction spectrometer (XRD) and Gas Chromatography (GC).

2.0 MATERIALS AND METHODS

2.1 Collection of Plant Materials

Fresh leaves of *Alstonia boonei* were collected from the tree plant from Umuowa, Ngor Okpala, Imo State. It was identified by a botanist; Dr Duru of the Federal University of Technology, Owerri, Imo State. After identification, it was then subjected to ethanolic extraction.

2.2 Preparation of Ethanolic Plant Extract

The collected flowers were thoroughly washed under running tap water and rinsed severally with distilled water, followed by air drying to remove the residue moisture. The dried materials were cut into fine pieces and ground into fine powder. 400g of the fine powder was extracted in 2500mL of ethanol using soxhlet extractor. The extract was concentrated using rotary evaporator.

2.3 Synthesis of Cr^{3+} - *Alstonia boonei* Extract Complex

For the synthesis of Cr^{3+}-*Alstonia boonei* extract complex, 10g of the leaf extract was added to 90mL of 1mMol Cr^{3+}-solution into 250mL Erlenmeyer flask and mixed thoroughly. The content was then transferred quantitatively into a 100mL volumetric flask and solution of 1mMol Cr^{3+} was then used to make up to the mark of the volumetric flask (Igwe and Nwamezie, 2018). The Cr^{3+}-*Alstonia boonei* complex was dried using water bath at a temperature of 37^{o}C and was stored in a container.

2.4 Ultra Violet –Visible Spectroscopy Analysis

The bio-reduction process of Cr^{3+}-ions in aqueous solution was measured by sampling 1mL aliquot compared with 1mL of pyridine used as blank and subsequently measuring UV-Visible spectrum of the solution. UV-Visible spectrums of the samples were monitored on Shimadzu dual beam spectrophotometer (model, UV-2500PC series) operated at a width slit of 2nm within the wavelength range of 200 to 980nm.

2.5 Fourier Transform Infra Red Spectroscopy Analysis

This was carried out on *Alstonia boonei* ethanolic leaf extract and on Cr^{3+} - *Alstonia boonei* synthesized complex. FTIR measurement of the samples was performed using FTIR-8400S Fourier Transform Infra Red Spectrophotometer, Shimadzu, Japan, in a diffused reflectance mode at a resolution of 4cm^{-1} in potassium bromide (KBr) pellets in the wave number range of 4500-250cm^{-1}.

2.5 Scanning Electron Microscopy (SEM) Analysis

Morphology of the nano particles was studied using SEM analysis (MODEL PHENOM Prox Scanning Element Microscope manufactured by Phenom World Eindhoven, the Netherlands).

2.7 X-Ray Diffraction (XRD) Analysis

XRD (PAN analytical, Netherlands) patterns were obtained with a diffractometer (Empyrean model, Netherlands) using Cu-Kr radiation of wavelength (λ) = 1.541A. The sample was made smoother and was imparted on a slide which was then charged into the machine after adjusting the machine parameters and was operated in a monitor.

2.8 Gas Chromatography Analysis

The quantification of the phytochemicals present in the leaves extract of *Alstonia boonei* and the chromium nanoparticles was done using BULK M910 GC equipped with a flame

ionization detector. The injector was set at split less injection mode and 2µL of sample was injected at velocity of 150cm^{-1}, the carrier gas used was Helium 5.0pa.S with a flow rate of 40mLmin^{-1}. The oven operated from initial temperature of 70^{o}C and was held at this 70^{o}C for 10mins after which it moved to 170^{o}C at the rate of 10^{o}Cmin^{-1}. It was held at 170^{o}C for another 5mins after which the temperature was allowed to reach 300^{o}C at a rate of 5^{o}Cmin^{-1}. The concentration of the different phytochemicals was expressed in percentage.

3.0 RESULTS AND DISCUSSION

Synthesis of Chromium nanoparticles (CrNPs) was confirmed by the characteristics changes of the extract and aqueous $CrCl_3$ mixture colour to dark greenish. This indicated the reduction of aqueous chromium ions to CrNPs when added to the extract of *Alstonia boonei* and the colour change is the result of excitation in the metal nanoparticles.

3.1 Electronic Spectra

The bio-reduction of Cr^{3+} ions in aqueous solutions was monitored by measuring UV/Visible spectra. Electronic spectral analysis of the plant extract and CrNPs were done at a wavelength range of 200-980nm to study their absorption peaks. The electronic spectrum of *Alstonia boonei* extract showed two close peaks at the UV and Visible region of 350 and 450nm. The peaks at 350nm are due to $n \rightarrow \pi^*$ transition while the spectrum at 450nm is assigned to $n \rightarrow \pi^*$ transition (Al-Jebouri and Noorikhaleel, 2019). Chromium-*Alstonia boonei* nanoparticles showed high intensity band of visible region of 470nm and 490nm which is due to $^4A_{2g} \rightarrow {}^4T_{2g}$ and $^4A_{2g} \rightarrow {}^4T_{1g}$ transition of octahedral symmetry of Cr^{3+} complex.

3.2 FT-IR Analysis

FT-IR spectroscopy was used to study the functional groups that participated in the reduction of chromium ions to chromium nanoparticles. FT-IR spectra of *Alstonia boonei* leaf extract and that of the chromium nanoparticles are shown in figures 1 and 2 while the interpretations of the peaks are in tables 1 and 2 respectively. Some absorption found in the spectrum of the extract.

Table 1: FT-IR Absorption Peaks of *Alstonia boonei* leaf Extract.

Peak (cm^{-1})	Functional Group
3805.91	$O-H$ stretch of alcohol or phenol
3448.38	$O-H$ stretch of alcohols/phenols
3336.93	$N-H$ stretch of amines
3009.40	$C-H$ stretch of CH_3, CH_2 or CH
2780.30	$C-H$ stretch of CH_3, CH_2 or CH
2687.47	$O-H$ stretch of carboxylic acid
2574.01	$O-H$ stretch of carboxylic acid
2450.00	$O-H$ stretch of carboxylic acid
2249.89	$C\equiv N$stretch due to nitriles
2088.19	$C\equiv C$ stretch of alkynes
1963.87	$C-H$ stretch of aromatic compound
1610.91	$C\equiv C$ bend of amines.
1397.24	$N=O$ bend of nitro compounds
1238.74	$C-O$ stretch of alcohols or phenols or carboxylic acid.
883.00	$C-H$ bend of alkenes or aromatic compound
732.86	$C-H$ bend of alkenes or aromatic compound

Table 2: FT-IR Absorption Peaks of Chromium *Alstonia boonei* Nanoparticle.

Peak (cm^{-1})	Functional Group
2831.03	$C-H$ stretch due to CH_3, CH_2 or CH
2056.63	$C\equiv C$ stretch of alkynes
2055.25	$C\equiv C$ stretch of alkynes
1374.21	$N=O$ stretch of nitro compounds
1373.02	$N=O$ stretch of nitro compounds

Some absorption wave numbers were absent in the spectrum of chromium nano particles. The absorption in the extracts that disappeared in the spectrum is at 3805.91, 3448.38, 3336.93, 2687.47, 2574.01, 2450.00, 2249.89, 1963.87, 1610.91, 1238.74, 883.00 and 737.85cm^{-1} respectively. The absence of these functional groups in the spectrum of chromium nano particles (fig. 2) is an indication that a chemical change took place and these functional groups were involved in the bio-reduction of chromium ions to chromium nano particles. The existence of C−H, C≡C and N=O groups in the nano particles indicates that the surface of the nanoparticles are associated with compounds whose chemical nature comprised these groups. Presence of organic molecules on the surface of metal nano particles have been reported to have an influence on the FT-IR peaks (Saranya *et al*, 2017), successful aiding in the formation and stablisation of the nano particles (Senthilkumar, *et al*, 2018).

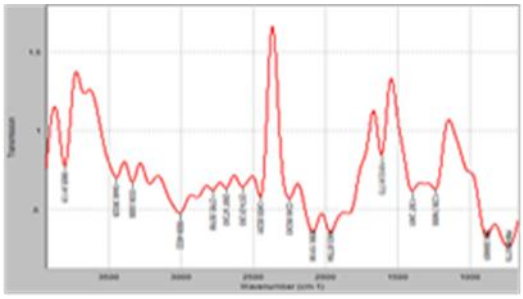

Fig 1: FTIR spectra of *Alstonia boonei* extract

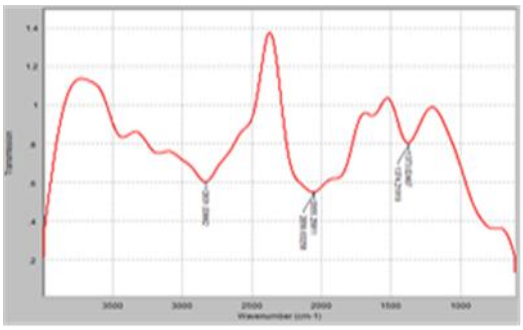

Fig. 2: FTIR spectra of chromium nanoparticle

3.3 Scanning Electron Microscopy (SEM) Analysis

The formation of CrNPs and the morphological structure were analyzed using SEM (fig. 3). SEM micrographs revealed that the synthesized chromium nanoparticles were highly aggregated as irregular cluster shapes with rough surfaces. The clusters were formed in the diameter below 100nm.

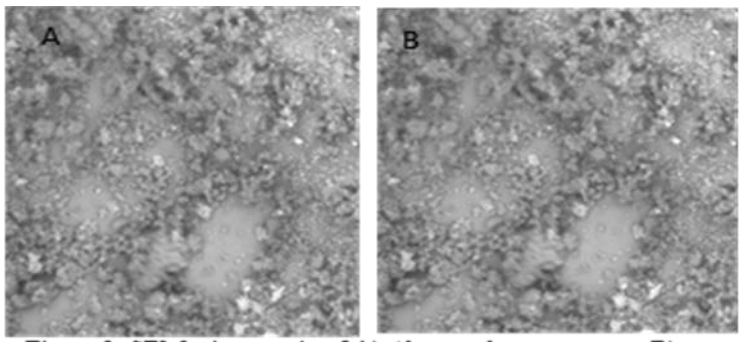

Figure 3: SEM micrograph of A) *Alstonia boonei* extract B) Cr^{3+} - *Alstonia boonei* nanoparticle

3.4 X-ray Diffraction Analysis

The XRD patterns of Chromium ions and chromium nano particles as shown in Figs. 4 and 5 revealed a distinct diffraction peaks at 2θ, 15.75^{o} and 15.69^{o} respectively, suggesting the non amorphous nature of the studied samples. The crystalline sizes of the chromium nano particles were calculated from Debye-Scherrer formula (Eqn 1) (Nleonu, 2020).

$$D = \frac{0.94\lambda}{\beta cos\theta} - - - \quad (1)$$

Where λ is the wavelength of the X-ray (1.5406), β represents full width at half maximum of the peak in radians, and θ is diffraction angle. The average crystalline sizes of Chromium and chromium-*Alstonia boonei* nano particles were obtained to be about 89.83 and 89.81nm respectively. The XRD result demonstrated that the average size of the NPs was below

100nm. The diffraction peaks correspond to the hkl value of 210 denoting face centered cubic (FCC) phase structure of the nano particle.

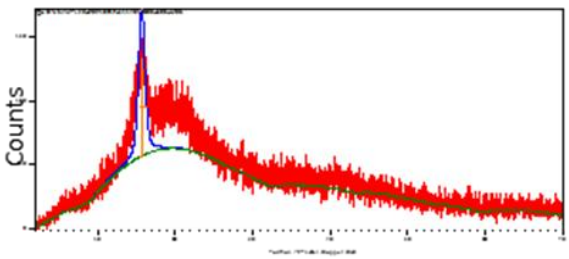

Fig 4: XRD patern of Alstonia boonei extract

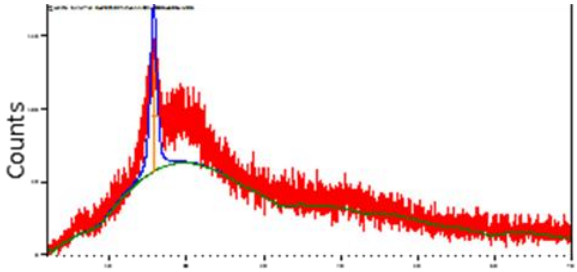

Fig. 5: XRD patern of chromium nanoparticle

3.5 GC-MS Characterization of Leaves Extract of *Alstonia boonei* and its Chromium Nanoparticles

The GS-MS analysis of the leaves extract and chromium nano particles identified a number of organic compounds. The identified phytochemicals with their retention time (RT), molecular weight, molecular formular and peak area in percentage are presented in table 3 and table 4 while Figs. 6 and 7 shows the GC-MS chromatogram respectively.

The analysis of ethanolic leaf extract of *Alstonia boonei* showed the presence of twenty (20) known compounds and chromium extract nano particles showed eighteen (18) compounds belonging to different chemical classes. The studied sample was rich mainly in alkaloids and flavonoid compounds. Alkaloids and flavonoids are known for their biological activities which include anti-oxidant activity, muscle relaxant property, anti-microbial, anti-cancer and anti-diabetic activities (Onuah *et al*., 2019).

The result of the present study showed disappearance of the following phytochemical constituent present in ethanol leaves extract of *Alstonia boonei*; Naringin, spartein, epicatechin and oxalate in chromium nano particles. Two new phytochemicals; rutin and ephedrine were identified from chromium nano particles.

A lot of research has shown that alkaloids and flavonoids compounds possess anti-diabetic activity (Gaikwad *et al*., 2014).

Table 3: Phytochemical constituents identified in the ethanolic extract of *Alstonia boonei*.

Retention time	Phytochemicals	Class of Phytochemicals	Peak Area (%)	Molecular Formular	Molecular Weight
0.366	Proanthocyanin	Flavonoid	1.38	$C_{31}H_{28}O_{12}$	576.50
2.390	Naringin	Flavonoid	6.79	$C_{27}H_{32}O_{14}$	580.00
4.116	Quinine	Alkaloid	3.30	$C_{20}H_{24}N_2O_2$	324.42
6.023	Flavon-3-ol	Flavonoid	10.25	$C_{15}H_{14}O_2$	226.27
7.430	Anthocyanin	Flavonoid	3.99	$C_{15}H_{12}O$	208.24
10.366	Ribalinidine	Alkaloid	10.12	$C_{15}H_{17}NO_4$	275.30
12.966	Naringenin	Flavonoid	2.88	$C_{15}H_{12}O_5$	272.25
15.460	Spartein	Alkaloid	2.23	$C_{15}H_{26}N_2$	234.00
17.966	Sapogenin	Saponin	5.67	$C_{30}H_{50}O_5$	410.00
20.313	Phenol	Phenol	6.56	C_6H_6O	94.11
22.730	Flavonones	Flavonoid	4.91	$C_{15}H_{12}O_6$	224.25
25.650	Steroids	Saponin	5.27	$C_{19}H_{28}O_2$	288.40
27.536	Epicatechin	Flavonoid	6.14	$C_{15}H_{14}O_6$	290.00
29.860	Kaempferol	Flavonoid	2.84	$C_{15}H_{10}O_6$	286.23
32.996	Phytate	Anti nutrient	7.88	$C_6H_{18}O_{24}P_6$	660.04
34.583	Flavone	Flavonoid	3.04	$C_{15}H_{10}O_2$	222.00
36.876	Oxalate	Anti nutrient	3.73	$C_2H_2O_4$	90.00
39.200	Catechin	Flavonoid	5.48	$C_{15}H_{14}O_6$	290.26
42.280	Resveratol	Flavonoid	1.80	$C_{14}H_{12}O_3$	228.25
44.170	Tannin	Tannin	5.74	$C_{76}H_{52}O_{46}$	1701.20

Table 4: Phytochemical Constituents identified in Chromium Nanoparticle.

Retention time	Phytochemicals	Class of Phytochemicals	Peak Area (%)	Molecular Formular	Molecular Weight
0.246	Proanthocyanin	Flavonoid	7.01	$C_{31}H_{28}O_{12}$	576.50
1.640	Rutin	Flavonoid	3.01	$C_{27}H_{30}O_{16}$	610.50
3.190	Quinine	alkaloid	5.37	$C_{20}H_{24}N_2O_2$	324.42
4.096	Epihedrine	alkaloid	5.42	$C_{10}H_{15}NO$	165.23
5.233	Flavon-3-ol	Flavonoid	3.68	$C_{15}H_{14}O_2$	226.27
5.613	Anthocyanin	Flavonoid	5.35	$C_{15}H_{12}O$	208.24
7.090	Ribalinidine	alkaloid	12.66	$C_{15}H_{17}NO_4$	275.30
9.026	Naringenin	Flavonoid	7.12	$C_{15}H_{12}O_5$	272.25
17.033	Sapogenin	saponin	7.63	$C_{30}H_{50}O_5$	410.00
19.113	Phenol	Phenol	11.17	C_6H_6O	94.11
23.306	Flavonones	Flavonoid	2.62	$C_{15}H_{12}O_6$	224.25
25.080	Steroids	saponin	7.06	$C_{19}H_{28}O_2$	288.40
28.593	Kaempferol	Flavonoid	2.51	$C_{15}H_{10}O_6$	286.23
30.986	Phytate	Anti nutrient	4.34	$C_6H_{18}O_{24}P_6$	660.04
34.116	Flavone	Flavonoid	5.14	$C_{15}H_{10}O_2$	222.00
39.026	Catechin	Flavonoid	3.19	$C_{15}H_{14}O_6$	290.26
43.876	Resveratol	Flavonoid	3.56	$C_{14}H_{12}O_3$	228.25
44.450	Tannin	Tannin	3.12	$C_{76}H_{52}O_{46}$	1701.20

Fig. 6: GC-MS chromatogram of Alstonia boonei extract

Fig. 7: GC-MS chromatogram of chromium nanoparticle

4.0 CONCLUSION

The present work reported green synthesis of chromium nanoparticles from leaves extract of *Alstonei boonei*. The FTIR and GC-MS results showed the functional group and phytochemical constituents present in the CrNPs. XRD and SEM results showed that homogeneous sized CrNPs with irregular shapes can be obtained with particle size of less than 100nm. The green synthesis method used is cost effective, environmentally friendly and gives a stable product which makes Cr^{3+} - *Alstonei boonei* nanoparticle a promising material for medical application.

Conflict of Interest

The authors have not declared any conflict of interest regarding funding and publication of this work.

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