Green Synthesis of Cobalt Nanoparticles using Ethanolic Extract of *Cadiospermum halicacebium* Characterisation and its Anticancer Applications

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ABSTRACT:
In the present study, cobalt nano particles were synthesized by an ecofriendly and cost effective method using *Cadiospermum halicacebium* leaf extract. The leaf extract acts as both reducing and capping agent. The biosynthesised cobalt nanoparticles were characterized by using various techniques such as UV-visible spectro photometry, Fourier transform infrared spectrometry, XRD and Scanning electron microscopy. The spectroscopic methods confirmed the formation of cobalt nano particles and the microscopic technique confirmed the shape and size of the cobalt nano particles. The anticancer activity of samples on *Hela* and *VERO* cell line were determined by the MTT assay.

KEYWORDS: *Cadiospermum halicacebium* leaf, Cobalt nanoparticles**,** SEM, XRD, FTIR, UV-Visible Spectrophotometer and anticancer activity.

INTRODUCTION:

The *Cadiospermum halicacebium* plant act as a laxative, refrigerant, stomachic and sudorific and has antibacterial[1,2] anti-diarrheal, antioxidant activities, anticancer, vaso depressant effect[3-6]. This herb is also useful in curing of anti-inflammatory, analgesic, antibacterial and antifilarial activity[7-10]. For treatment of infections associated with the nervous system, the root alone has been employed[11]. This plant is also effective in controlling severe dermatoses[12].

Cobalt nano particles have gained significant interest in the past decade because of their unique properties which has a potential to be used in a wide range of applications. Several methods have been reported for the synthesis of nano particles, which include physical, chemical and biological systems[13,14].

There is an increasing focus on eco-friendly green routes for the production of copper nano particles, using biological entities including microorganism and plants. To fulfill the growing need to develop environmentally friendly, fast, cheap and scaable nano particles synthesis methods, it is desirable to take advantage of plant materials[15]. Characterization of Cobalt nano particles is very important for optimization of the various parameters involved during the synthesis of nano particles. This research is primarily focused on green synthesis cobalt nano particles using plants, techniques of characterization and applications.

MATERIALS AND METHODS:

Leaves from *Cadiospermum halicacebium* were collected from the local area of Chennai India. (Fig 1). The leaves were washed with distilled water to remove the dust particles and then were dried for six days to remove the moisture. The dried leaves were grind into fine powder. 5gm of powdered leaves sample was boiled with 100 ml ethanol for 30 minutes at 40°C. The aqueous extract was separated by filtration using Whatman No. 1 filter paper. The extract was stored at 4˚C to be used for biosynthesis of Cobalt nanoparticles.

Figure 1: ***Cadiospermum halicacebium leaves***

Synthesis of Cobalt NPs

10ml of *Cadiospermum halicacebium* leaf extract was added to 100ml of 1% Cobalt nitrate solution. Then the solution was magnetically stirred for 3 hours. After 2 hours, the colour of the solution changed from green to brown indicating formation of Cobalt nanoparticles. (Fig 2).

Figure 2: Solution showing the formation of cobalt nanoparticles

The nanoparticles formed were washed with double distilled water. Then, it was dried in oven at 60^0C for further analysis.

Characterization Techniques:

The reduction of cobalt nitrate to cobalt was monitored by recording UV-Visible spectrum. The measurements are recorded on Shimadzu dual beam spectrometer (model uv-1650pc) operated at resolution of 1nm. FT-IR measurement recorded using a Perkin Elmer 360 model IR double beam spectrophotometer. The Spectra were collected from 4000 to 400 cm-1 with 4 cm^{-1} resolution over 40 scans All spectra were collected against the background spectra of KBr. X-ray diffraction (XRD) measurement of the cobalt nanoparticles was carried out using powder x-ray diffractometer instrument (Seifert JSO Debyeflex-2002) in the angle range of 100 -700 operated at a voltage of 40Kv and a current of 30mA with CuKα radiation in a θ-2θ configuration. SEM images were obtained with a field emission JOEL – JSN – 6360 instrument USA.

Anticancer Activity:

The anticancer activity of samples on Hela and Vero was determined by the MTT assay. Cells (1 × 105/well) were plated in 1ml of medium/well in 24-well plates (Costar Corning, Rochester, NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide cells (MTT) solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 570 nm was measured with a UV-Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of HT-29 was expressed as the % cell viability, using the following formula: % cell viability = A570 of treated cells/A570 of control cells × 100%

Phytochemistry:

To know the presence of secondary metabolites like Tannins, saponins, flavonoids, alkaloids, protein, steroids and anthraquinones were detected using the method described by Evans (1994). The methodology adapted is briefly described below:

***Cardiospermum halicacabum*:**

Test for Alkaloids: To the 1ml of extract, add 1ml of Dragendroff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Test for Saponins:

Take small quantity of alcoholic and aqueous extract separately and add 20ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins.

Test for Glycosides:

Dissolve the extract in pyridine and add sodium nitro prusside solution to make it alkaline. The formation of pink to red colour shows the presence of glycosides

Test for Carbohydrates:

To 2ml of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence of carbohydrates.

Test for Tannins and Phenolic Compounds:

Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

Test for Flavonoids:
The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light; formation of fluorescence indicates the presence of flavonoids.

Test for Proteins:
Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color is produced, and then add to the 1ml of the extract. Formation of pinkish or purple violet color indicates the presence of proteins.

RESULTS AND DISCUSSION:

UV-Visible Spectroscopy

The UV visible spectrum for the cobalt nanoparticles in aqueous solution showed the absorption peak at 448nm (Fig.3) which is due to the surface Plasmon vibration.

Figure 3: UV Absorption Spectra of cobalt nanoparticles biosynthesised from *Cadiospermum halicacebium* extract

Fourier Transform Infra-Red Spectroscopy:

The chemical groups present in the biosynthesied ZnO NPs are identified by FTIR analysis and the obtained spectrum is represented in the Figure 4, peak values at 3760.0, 3735.0, 3355.0 and 1630.0 cm^{-1} was observed. Peak at 1630.0 and 3355.0 cm^{-1} corresponds to C=O stretching of amides and O-H stretching of phenolic compounds respectively. The other peaks obtained in the cobalt nanoparticle sample are 3760.0 and 3735.0 cm^{-1} due to O-H stretching of hydrogen bonded of alcohols and phenols. The very strong band at 1073.0 cm^{-1} arises C-O-C symmetric stretching and C-O-H bending vibration of protein present in plant extract.

Figure 4: FTIR Spectra for Cobalt nanoparticles biosynthesized from *Cadiospermum halicacebium* extract

XRD Analysis:

Using XRD spectrum analysis, the two different diffraction peaks at 27.81,32.11, 39.1,46.11 68.3 and 77.20 which is shown in figure 5. The average crystalline size according to Scherrer equation calculated using the highest peak of the 46.11 is found to be 50nm.

Figure 5: XRD Pattern for Cobalt nanoparticles biosynthesized from *Cadiospermum halicacebium* extract

Scanning Electron Microscopy of Cobalt Nanoparticles:

The biosynthesized cobalt structure by employing *Cardiospermum halicacabum* aqueous extract was further demonstrated and confirmed by the structural view under the scanning electron microscope (Figure 6). Size of the cobalt nano particles synthesized was the range of 40 to 200µm. The SEM image showing the high density cobalt particles synthesized by the extract of *Cardiospemum halicacabum.*

Figure 6: SEM image for Cobalt nanoparticles biosynthesized from *Cadiospermum halicacebium* extract

ANTI Cancer Activity:

The invitro cytotoxicity activity of zinc nanoparticles was performed against *Hela* cell line (breast cancer cell line) and compared against the *Vero* cell line (normal cell line). The result and corresponding figure are shown in table 1 and 2 and figure 7 and 8.

Table 1: Cytotoxicity effect of Sample on HeLa cell line

S. No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.225	18.11
2	500	1:1	0.286	23.02
3	250	1:2	0.346	27.85
4	125	1:4	0.405	32.60
5	62.5	1:8	0.466	37.52
6	31.2	1:16	0.529	42.59

7	15.6	1:32	0.586	47.18
8	7.8	1:64	0.657	52.89
9	Cell control	-	1.242	100

Figure 7: Cytotoxicity activity of biosynthesised Cobalt nanoparticles on HeLa cell line

Table 2: Cytotoxicity effect of Sample *on Vero* cell line

S. No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.322	43.51
2	500	1:1	0.371	50.13
3	250	1:2	0.420	56.75
4	125	1:4	0.469	63.37
5	62.5	1:8	0.517	69.86
6	31.2	1:16	0.565	76.35
7	15.6	1:32	0.613	82.83
8	7.8	1:64	0.661	89.32
9	Cell control	-	0.740	100

Figure 8: Cytotoxicity activity of Biosynthesised Cobalt nanoparticles on Vero cell line

The result showed that the cobalt nano particles show moderate activity against the cancer cell but seems to have less toxicity towards normal cell. The selectivity index of the synthesized cobalt nano particles was found to be 32. This is indicative of the fact that the biosynthesized nanoparticles show considerable activity with less side effects.

CONCLUSION:

The present study deals with the bio extraction of cobalt nanoparticles from leaf extract of *Cadiospermum halicacebium* which provides cost effective, easy and proficient way for synthesis of nanoparticles. *Cardiospermum halicacabum* L possess certain significant properties that support its role in medicinal field. The anticancer activity of cobalt nano particles were investigated. The result showed that the nanoparticles have considerable activity with less side effects.

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REFERENCES:

1. Dhar LM, Dhar MM, Dharwan NB, et al. Screening of Indian plants for biological activity. Indian J. Exp. Biol.1968; 6: 232–247.
2. Raman N, Radha A, Ravi M. Antibacterial activity of *Cardiospermum halicacabum* against human and plant pathogens. Indian Drug.1998; 35: 29–31.
3. Rao V, Chandra PNK, Shanta KSM. Pharmacological investigation of *Cardiospermum halicacabum* (Linn) in different animal models of diarrhoea. Indian J. Pharm. 2006; 38(5): 346-349.
4. Kumaran A, Karunakaran RJ. Antioxidant activities of the methanol extract of *Cardiospermum halicacabum*. Journal of Pharm. Biol.2006; 44(2): 146–151.
5. Sheeba MS, Asha VV. Effect of *Cardiospermum halicacabum* on ethanol induced gastric ulcers in rats. J. Ethnopharmacol.2006; 106(1): 105– 110.
6. Gopalakrishnan C, Dhannanjayan R, Kameshwaran L. Studies on the pharmacological actions of *Cardiospermum helicacabum*. Indian J. Physiol. Pharmacol. 1976; 20: 203–208.
7. Babu V, Krishnakumari KC. Anti-Inflammatory and Antioxidant Compound, Rutin in *Cardiospermum halicacabum* Leaves. Ancient Sci. Life.2005; 25(2): 47-49.
8. Muthumani P, Meera R, Venkatraman S, et al. Study of phytochemical, analgesic and antiulcer activity of extracts of aerial parts of *Cardiospermum halicacabum* Linn. I.J.P.S.R.2010; 1(10): 128-137.
9. Jigna P, Darshana J, Sumitra C. Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. Turk. J. Biol.2005; 29: 203-210.
10. Khunkitti W, Fujimaki Y, Aoki Y. In vitro antifilarial activity of extracts of the medicinal plant *Cardiospermum halicacabum* against Brugiapahangi. J. Helminthol. 2000; 74(3): 241-246.
11. Subramanyam R, Newmaster SG, Paliyath G, et al. Exploring ethnobiological classifications for novel alternative medicine: a case study of *Cardiospermum halicacabum* L. (Modakathon, Balloon Vine) as a traditional herb for treating rheumatoid arthritis. Ethnobotany. 2007; 19(2): 1-16.

12. Shakhtmeister Y, Sharipova V. Treatment of chronic dermatoses with plant pharmaceuticals. Vestnik Dermatologii Venerologii, I.M. Sechenov Mosc. Med. Acad., Moscow, Russia. 1997; 6:33-35.
13. T Varaprasad1, Boddeti Govindh, B. Venkat. Green synthesized Cobalt Nano particles Using Asparagus and Evaluation of Antibacterial Activity. International Journal of Current Research and Academic Review.2016; 10(9), 337-345.
14. M.Yarestani1, A.D. Khalaji, A. Rohani et al. Hydrothermal Synthesis Of Cobalt Oxide Nano particles and Optical And Magnetic Properties. International of Biological of Chemistry. 2014; 29(3), 84-90.
15. Gayathri. V, Nivedha. S, Pujita. V, et al. Green synthesis of copper nanoparticles using bracts of *Musa paradisiaca* (Monthan) and study of its antimicrobial and antioxidant activity. Research Journal of Pharmacy and Technology, 2020; 13(2), 781-786.

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