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A FACILE APPROACH FOR THE SYNTHESIS OF COBALT NANOPARTICLES FROM *PLECTRANTHUS AMBOINICUS* LEAF EXTRACT AND IT'S POTENTIAL IN BIOLOGICAL APPLICATIONS

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Abstract: **Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. This research work has aimed at achieving the synthesis of metal nanoparticles by using the leaf extract of the *Plectranthus amboinicus(Oma valli)*. Phytochemical screening of aqueous extract of the plant revealed the various bioactive compounds like Saponins, Polyuronides reducing compounds; alkaloids, flavonoids, terpenoids and glycosides. Leaf extracts of P.A. and various concentration of Cobalt chloride hexahydrate (0.02M, 0.04M, 0.06M, and 0.08M) were prepared. 25 mL of the *Plectranthus amboinicus* was added to each concentration of cobalt (II) chloride hexahydrate in 1:1 ratio. The mixture was then subjected to heating process which leads to the formation of nanoparticles in various extract concentrations. The nanoparticles formed were subjected to IR and UV studies. Antibacterial activity was carried out on well diffusion method for Pseudomonas and Streptococcus bacteria at different concentrations. Minimum inhibitory zone was found only in Pseudomonas at the concentrations 0.04M and 0.08M (at 50µL both shows zone of inhibition 0.8mm, but at 100µL 0.04M shows slightly higher inhibition of 1mm 0.9mm). Cobalt nanoparticle showed good antibacterial and antifungal properties. The metal and metal oxide nanoparticles have high surface area and high fraction of atoms which was responsible for their fascinating property of antimicrobial activity.**

Keywords: *Metal nanoparticles cobalt (II) chloride hexahydrat Plectranthus amboinicus(Oma valli) Leaf extracts Antibacterial activity*

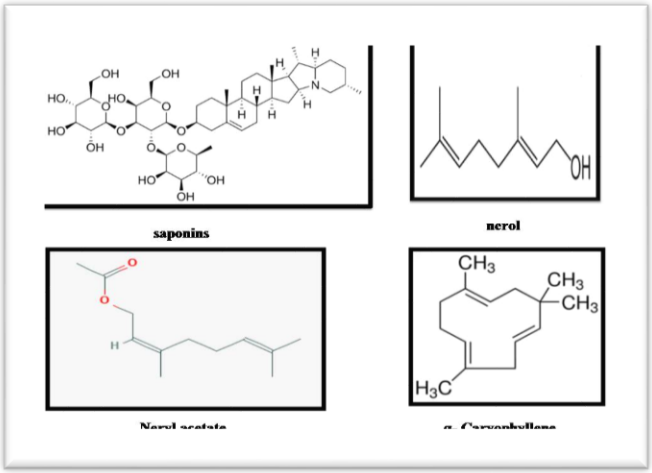
I INTRODUCTION

The COVID-19 pandemic, also known as the corona virus pandemic, is an ongoing pandemic of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).In Tamilnadu, "KabaSuraKudineer" made of numerous herbs such as Ginger (Chukku), Piper longum (pippali), Clove (lavangam), Dusparsha (cirukancoriver), Akkaarakarabha, Kokilaksha (mulliver), Haritaki (kadukkaithol), Malabar nut (adathodaielai), Ajwain (Karpooravalli), Kusta (kostam), Guduchi (seenthilthandu), Bharangi (Siruthekku), Kalamegha (siruthekku),Raja pata (Vattathiruppi), Musta (koraikizhangu). This "KabaSuraKudineer", if consumed before contracting the virus, the phyto compounds can bind to the epithelial cells of the respiratory system and boost the immune system while also stopping the virus from binding with the human cells and replicating after an individual catches the virus. Therefore, components present in the Plant synthesis hundreds of chemical compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals have been derived from phytochemicals from herbal component like *Plectranthus amboinicus*. Among the medicinal plants used in ayurveda, *Plectranthus amboinicus* is known to possess antimicrobial anti-oxidant and anti-epileptic properties. The previous literature studies had shown an interesting application of *Plectranthus amboinicus*. P.A has photocatalytic activity (B. Ajitha *etal*, 2014), anti-corrosion behavior (M. Dhanalakshmi, *etal*), used as mosquito larvicidal agent (Annadurai Senthilkumar *etal*, 2010), and it is a good bio active substance (Ramaraj Thirugnanasampandan *etal*, 2015). P.A leaves has many pharmacological properties such as respiratory diseases like congestion, bronchitis, colitis, and also has a urolithiasis, antiepileptic, antimorogenic properties

(Praveena Bhatt *etal*, 2013). Essential oil obtained from the leaves of P.A test cytotoxicity against breast (MCF-7) and colorectal (HT-29) cancel cell lines, to protect DNA from H_2O_2 induced genotoxicity through comet assay and to treat inflammation in lipopolysaccharide (LPS) induced over expression of matrix metalloproteinase-9 (MMP-9) in human peripheral blood mononuclear cells (PBMCs) by gelatin zymogram and inhibition at transcriptional level confirmed using RT-PCR (reverse transcriptase polymerase chain reaction), (Ramaraj Thirugnanasampandan *etal*, 2015)

P.A varieties contain phytochemical constituents with important pharmacological activities. A wide range of secondary metabolites including carvacrol, p-cymene, terpenoids, glycosides, flavonoids in P.A extracts possess a wide range of pharmacological activities including antibacterial, antifungal, antimicrobial, anti-inflammatory, antiplatelet, anticancer, malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, bronchitis, rheumatoid arthritis, analgesic properties (Ramaraj Thirugnanasampandan *etal*, 2015). The phytochemical studies revealed the presence of various flavonoids like quercetin, apigenin, luteolin, salvigenin, genkwanin and volatile oil in the leaves (Preeja G. Pillai, *etal*, 2011).

Figure1 Plectranthus amboinicus



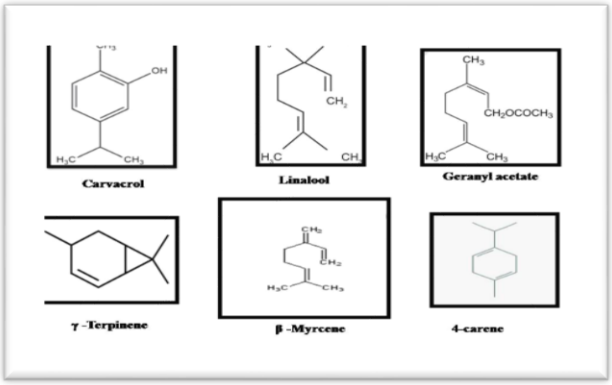


Figure2 Chemical components which are present in Plectranthus amboinicus

Application of cobalt nanoparticles

Cobalt nanoparticles prepared from the different leaf extracts (O.U. Igwe *etal*, 2018), (M. Madhukara Naik *etal*, 2019), (Rama Koyyati etal, 2016), (K.Kombaiah *etal*, 2018), shows the good antibacterial, antimicrobial, antifungal, optical, magnetic cytotoxic and photocatalytic activities. We can control the growth of carbon nanotube by using cobalt nanoparticles (Yoon Huh *etal*, 2005) and used as a good candidate for sensing organic compounds (S.O. Ranaei Siadat *etal*, 2015). Room temperature synthesis of air stable cobalt nanoparticles (Arijit Mondal *etal*, 2015) can catalyze the degradation reaction of dyes and MOF-derived cobalt nanoparticles catalyze a general synthesis of amines (Rajenahally V. Jagadeesh *etal*, 2017). Multi-walled carbon nanotubes with immobilized cobalt nanoparticles, (Saeed Shahrokhian *etal*, 2009) showed that the high sensitivity on determination of thioridazine in pharmaceutical and clinical preparations. Carbon paste electrode modified with cobalt nanoparticles (Mohammad Hadi Parvin *etal*, 2012) acts as a sensitive voltammetric sensor for fast and accurate determination of chlorpromazine in biological samples.The metal and metal oxide nanoparticles have high surface area and high fraction of atoms which is responsible for their fascinating properties such as antimicrobial, magnetic, electronic, and catalytic activity. So we are also interested to produce metal nanoparticles. Cobalt is essential to metabolism of all animals. It is a key constituent of cobalamin, which is also known as vitamin B_{12}, the primary reservoir of cobalt as an ultratrace element. Coenzyme B_{12} features a reactive C-Co bond that participates in the reactions.

II EXPERIMENTAL

Fresh *Plectranthus amboinicus* leaves were collected in and around Madurai district of Tamilnadu, India. The plant leaves were cleaned with tap water and then with double distilled water about 2 to 3 times. Then it was used for making the water extract of *Plectranthus amboinicus* (P.A). The aqueous leaf extract were prepared by collecting thoroughly

washed leaves of 50g, followed by boiling 250 mL distilled water about 6 0 ^{0}C for 4hours. Then the extract was collected filtered through Whatmann no.1 filter paper and preserved at room temperature in order to use it for further experiments.

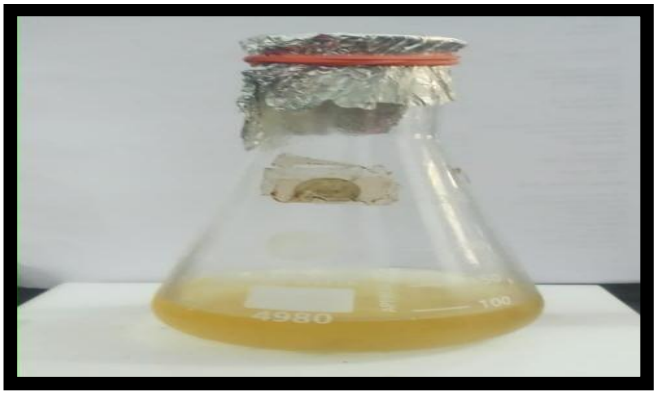


Figure. 3water extract of P.A

An aqueous 0.1M cobalt (II) chloride hexahydrate solution was prepared with 100 mL distilled water at room temperature. Various concentration of Cobalt chloride hexahydrate (0.02M, 0.04M, 0.06M, and 0.08M) were prepared. 25mL of the plectranthus amboinicus was added to each concentration of cobalt (II) chloride hexahydrate in 1:1 ratio. The mixture was boiled for about 4 hours. Then we had observed the colour change from reddish brown to brown giving evidence of nanoparticle formation. The synthesized nanoparticles were then separated by centrifugation. Through drying, a dried powder of nanosized cobalt was obtained.

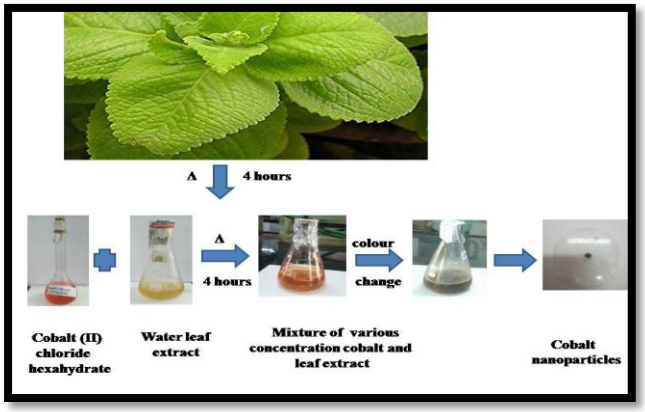


Figure4 Synthetic process of Co nanoparticle

Qualitative analysis of phytochemical test for the identification of carbohydrates, resins, tannins, flavonoids, alkaloids, steroids, phenols and glycosides were carried out for water extract of "plectranthus amboinicus" leaves (PA) as per the methods described by Trease and Erans (1983), Harbone (1998), Sazada etal (2009) and Thakur etal (2018). As per the biological tests conducted by Plectranthus amboinicus showed the presence of the tested phytochemicals phenolic compounds, flavonoids, carbohydrates, resins.

Table-1 phytochemical analysis of P.A

No. of phyto	Phyto Chemical Tests	Leaf
1	Alkaloids	+
2	Terpinoids	+
3	Phenolic compounds	+
4	Flavonoids	+
5	Carbohydrates	+
6	Steroids	-
7	Glycosides	-
8	Tannins	+
9	Resins	+

III CHARACTER STUDIES AND RESULTS

UV spectral study of Cobalt nanoparticles

UV spectral study of cobalt nanoparticles of both concentrations 0.04M and 0.08M shows absorbance spectram at ~400 nm. These absorptions confirm the formation of Cobalt nanoparticles.

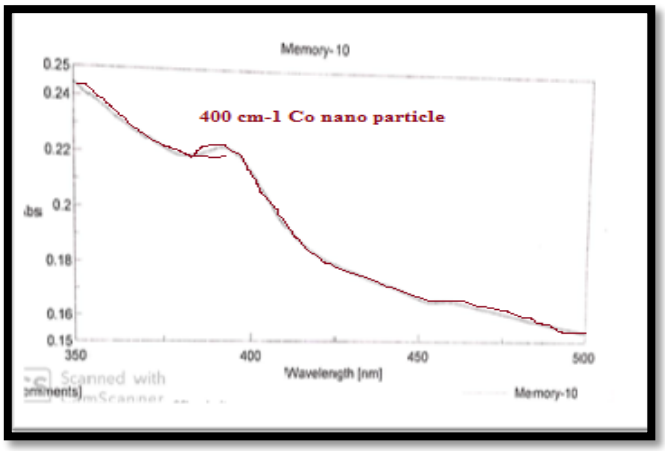


Figure 5 UV spectra of 0.02M Cobalt nanoparticles

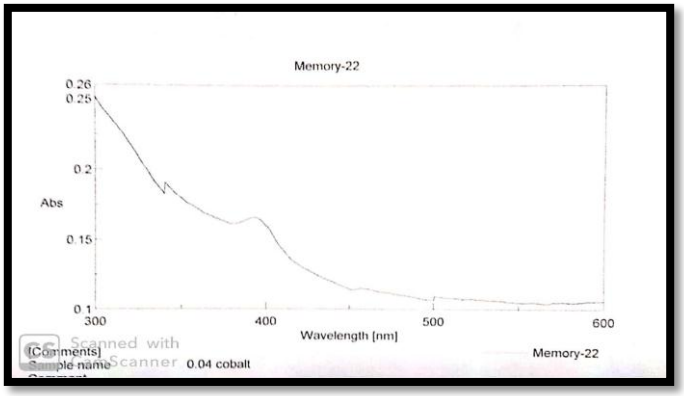


Figure 6 UV spectra of 0.04M Cobalt nanoparticles

The nanoparticles formed from the*plectranthus amboinicus* leaf extract were characterised by IR spectral studies and the results are shown in figure 7

The spectral values at wavenumbers cm^{-1} 669.04, 709.92, 758.96, 884.35, 892.76, 952.65, 1021.71, 1164.60, 1302.16,

1456.83, 1632.18 (finger print region) confirmed the presence of C-H bending, strong 1,3- disubstituted C-H bending, strong C=C bending vinylidene, medium C-N stretching amine, strong S=O stretching (sulfonic acid, sulfonamide, sulfone), strong C-O stretching (ester,tertiary alcohol), strong C-F stretching fluoro compound, strong C-N stretching aromatic amine, strong C-O stretching aromatic ester, medium C=C stretching (disubstituted (cis) alkene,conjugated alkene, cyclic alkene), N-H bending amine.

The spectral values (functional group region) at wave numbers cm^{-1} 1927.75, 2004.29, 2118.43, 2143.95, 2239.78, 2316.72, 2362.98, 2428.40, 2533.91, 2629.68, 2740.10, 2803.85, 2884.69, 3285.66, 3323.22, 3749.87, 3807.27 confirmed the presence of strong N=C=S stretching isothiocyanate, weak C≡C stretching monosubstituted alkyne, strong S-C≡N stretching thiocyanate

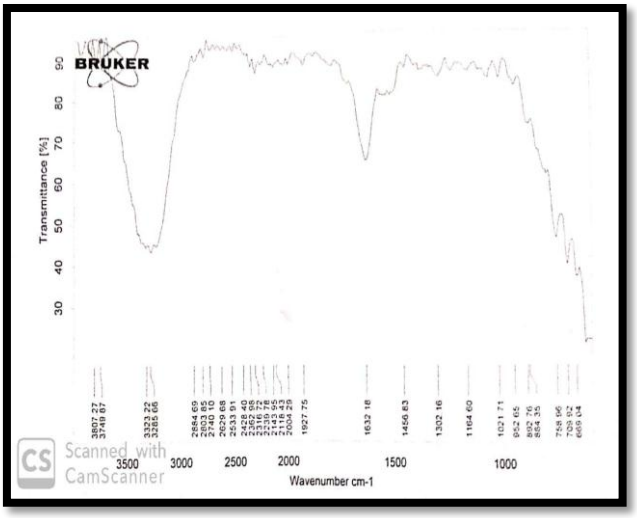


Figure 7 IR spectra of *Plectranthus amboinicus* leaf extract

IV BIO APPLICATIONS

Plant extract of aqueous P.A cobalt nanoparticles was used in the antibacterial activity on pseudomonas – gram negative bacteria, and Streptococcus – gram positive bacteria.

Pseudomonas causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systematic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immune suppressed. The most infects include malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia, and septicemia.

Streptococcus can cause many disorders including pharyngitis, pneumonia, wound and skin infections, sepsis, and endocarditis.

Table 2 Antibacterial activity of different concentration of cobalt nanoparticles

Micro organisms		50µl (mm)	100µl (mm)	Standard (amphicillin) in (mm)	Control
pseudomonas	Sample1 (0.04M)	0.8	1.0	1.4	0.0
	Sample2 (0.08M)	0.8	0.9	1.5	0.0
Streptococcus	Sample1 (0.04M)	-	-	1.0	0.0
	Sample2 (0.08M)	-	-	1.0	0.0

V CONCLUTION

Cobalt nanoparticles were prepared through water leaf extract of P.A by novel method. Phytochemical screening tests were conducted for water extract of P.A such as alkaloids, terpenoids, phenolic compound, flavonoids, carbohydrates, steroids, tannins, glycosides, Resins and observed the results as the occurrence of phenolic compound, flavonoids, carbohydrates, resins. The structure was confirmed by an absorption at ~ 400 nm in the UV spectrum and functional groups were studied by IR spectral data. The spectral values at wavenumbers cm^{-1} 669.04, 709.92, 758.96, 884.35, 892.76, 952.65, 1021.71, 1164.60, 1302.16, 1456.83, 1632.18 (finger print region) , the spectral values (functional group region cm^{-1}) at wave numbers cm^{-1} 1927.75, 2004.29, 2118.43, 2143.95, 2239.78, 2316.72, 2362.98, 2428.40, 2533.91, 2629.68, 2740.10, 2803.85, 2884.69, 3285.66, 3323.22, 3749.87, 3807.27 confirmed the presence of medium weak C-H bending aromatic compound (Overtone), strong N=C=S stretching isothiocyanate, weak C≡C stretching monosubstituted alkyne, strong S-C≡N stretching thiocyanate.The above information confirms the structure of the plant.Antibacterial activity was carried out on well diffusion method for Pseudomonas and Streptococcus bacteria at different concentrations. Minimum inhibitory zone were found only in Pseudomonas at the concentrations 0.04M and 0.08M (at 50µL both shows zone of

inhibition 0.8mm, but at 100µL 0.04M shows slightly higher inhibition 1mm than 0.08M shows 0.9mm)

Cobalt nanoparticle showed good antibacterial, antimicrobial, antifungal properties. The metal and metal oxide nanoparticles have high surface area and high fraction of atoms which was responsible for their fascinating property such as antimicrobial activity. The method of preparation of cobalt nanoparticles was a novel method and used to achieve the goal of research.

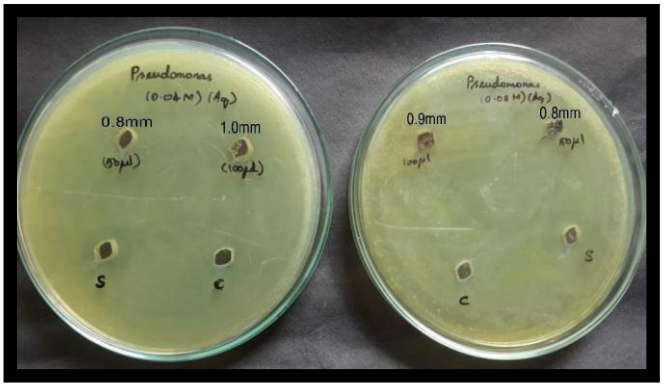


Figure 7Cobalt nanoparticles on Pseudomonas

Aqueous P.A cobalt nanoparticles showed the higher inhibitory zone for 0.04M at 100µL on pseudomonas than 0.08 M. but there is no inhibition on streptococcus for both 0.04M and 0.08M concentrations. The results were shown in the **Table 3**.

Figure 7 shows the different concentration of cobalt nanoparticles on *pseudomonas* and *streptococcus*. This confirms the antibacterial activity of cobalt nanoparticles *in vitro* studies. The cobalt nanoparticles showed the fair potential of antibacterial activity on pseudomonas when compared to *streptococcus*.

In future, if the amount of cobalt nanoparticles substance is increased, it may show better results than this and will also show inhibition on streptococcus. It will be of great use to treat pseudomonas infections.

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