SYNTHESIS AND ANTIBACTERIAL STUDY OF NICKEL OXIDE NANO PARTICLE USING AQUEOUS EXTRACTS OF CARICA PAPPAYA LEAVES

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ABSTRACT:
Nanoparticles are important role drug delivery systems in the medical fields. The present study was synthesis of nickel oxide nanoparticles using aqueous extracts of Carica papaya leaves. The nanoparticles was characterized by X ray diffraction method and its pharmacological studies was carried out against the bacterial strains of Strephylococcus aureus, Strephylococcus epidermidis, Proteus mirabilis and Klebsiella pneumoniae. The zone of inhibition in the petriplates and the graphical representations indicates that antibacterial activity has been dose dependant mode because increase the concentration the activity also increases.
Key words: Carica papaya, nickel oxide nanoparticles, aqueous extracts, XRD, Antibacterial activity.

INTRODUCTION
The margin of biological and inorganic materials represents one of the fastest growing and most trust areas of nanotechnology. The nano bio boundary comprises dynamic, kinetics, physicochemical interactions and thermodynamic interactions between nanomaterial surfaces and surfaces of the biological components [1]. Synthesis of nanomaterials in bulk by physical and chemical methods using strong as well as weak chemical reducing agents and some of the shielding agents such as alcohols, $NaBH_4$ and sodium citrate etc. [2]. NiO_2 has lots of uses in the developed alkaline battery cathodes, dye-sensitized solar cells, semiconductors, heterogeneous catalytic materials, magnetic materials, electrochromic films, solid oxide fuel cells, p-type opaque conducting films and antiferromagnetic layers gas sensors [3]. In current years, the significance in the antimicrobial materials has full-grown due to numerous applications such as medicinal, food packaging, military items and sanitary materials [4].
The natural phytochemicals contained generally electron rich oxygen species or nitrogenous functions and have outstanding potential to anchor noble metal ions, diminish them to corresponding nano particles in a toxic free environment subsequent green passageway and to end with stabilized by capping [5]. In general, there are two techniques are usually used for the synthesis of nano particles, top-down and bottom-up processes. These preparation methods approaches can be categorized as physical, chemical and biological approaches [6]. Recent activities have been devoted to use such synthetic route which allowed the researchers to manage the physical parameters of the nanoparticles such as size, morphology, structure, solubility, functionality stability etc [7]. The continuous use of such chemical pathway could enlarge the environmental hazards. Therefore render their medical applications despite having gorgeous properties of nanomaterials [8]. Medicinal plant extracts are simpler for maximum production applications and proved to be environmental friendly, cheaper and secure for human beings and the environment due to utilize of biodegradable organic substances [9]. The usage of plant parts

 https://doi.org/10.36893/JK.2025.V15I05.010

extracts are ordinary preparation procedures for a different types of nanoparticles of metals, non-metals and quite a lot of of their compounds. Mostly plant extracts holds polyphenols, proteins, terpenoids, enzymes, peptides, phenolic acids, sugars, alkaloids are involved in nanoparticle formation [10]. Plant extracts are mostly used for the following nanoparticles synthesis Au, Cu, Ag, CuO, ZnO, TiO2, Fe, Fe2 O3, Se and Pb [11]. Nanoparticle size ranges can vary significantly Au nanoparticle size ranges from 5–85 nm, CdS nanoparticle size ranges form ultrasmall 2–5 nm to large 200 nm and Ag (5–400 nm. Factors influencing the NPs size and shape are concentrations of the plant extract, metal salts, reaction time, pH and temperature [12]. Natural medicines are the origin of about 75 to 80% of the world population and it is reported to least side effects [13].

Ayurveda is early medicinal system and is practiced extensively in India and some of the other countries. The importance of herbal remedies in the treatment of human illnesses cannot be overstated. Approximately 20% of the plants found in the world have been subjected to pharmacological or biological testing, and a significant percentage of novel antibiotics launched to the market are derived from natural or semi-synthetic sources. The research of certain phytochemicals for their antibacterial properties can be very important in medicinal treatments [14].

CARICA PAPAYA:

The Carica Papaya tree is a perennial and flowering medicinal plant that belongs to the Caricaceae family. This Plant is known by a number of common names in India such as Pappali in Tamil, Boppayipandu in Telugu, Pharangi in Kannada, Papita in Punjabi and Hindi Amrutbhanda in Oriya, Papaya in Gujarati, Omakai in Malayalam, Eerankari in Rajasthani, Pepe in Bengal and apai in Marathi. [15]. The Carica Papaya tree is widely cultivated for its ripe pleasant fruit, which provides high-quality nutritional value and simple digestion. It is cultivated in lot of the countries and exceedingly abundant in India. [16]. C. Papaya leaf extracts have long been used as medication for cancer and some of the infectious diseases. The leaf aqueous extract have the wound healing activity, whereas the leaf methanol extract has exhibited antioxidant activity, vasodilating effects and both being related with cardiovascular risk reduction [17].

Fig:1 Carica Papaya leaf

The phytochemical components of alkaloids, flavonoids, glycosides, vitamins, saponins, phenolic compounds, tannins, resins, proteins, provitamin a carotenoids, ascorbic acid, lycopene, fiber and minerals were present in the fruit juice and leaf extracts of Carica papaya [18].

MATERIAL AND METHODS

Collection of sample:

The leaves of Carica papaya was collected in the villages of Mangalakudi, Kalayarkoil and Kannagudi in Sivagangai district on the month of February.

Preparation of Carica papaya leaf extract:

The C. papaya leaf was washed with running tap water followed by deionized water then cut and socked in deionized water. After that, the leaf was dried under sunlight for 2 days. The dried leaves was

 https://doi.org/10.36893/JK.2025.V15I05.010

ISSN: 2278-4632
Vol-15, Issue-05, No.02, May: 2025

collected and stored. In this study, 5 g of dried leaves was weighed and soaked in 50ml of deionized water for one day. The resulting extract was filtered through Whatman number – 1 filter paper and the filtrate stored for further experimental use.

SYNTHESIS OF NICKEL OXIDE NANOPARTICLES USING CARICA PAPAYA LEAF EXTRACT:

$NiCl_2.6H_2O$ was purchased from Nice Chemicals Cochin Kerala state. 0.1N of $NiCl_2.6H_2O$ solution was prepared and used for the synthesis of nanoparticle preparation. 10 ml of prepared C. papaya extract was dissolved in 90 ml of aqueous Nickel ion solution. The reaction mixture have the colour of pale green and it was taken in a 250 ml conical flask and started the reaction between the temperature of 80ºC for vigorously stirred at 2 hours. The synthesis of nanoparticles was measured the effect of pH range is 7. Subsequently, spread 30 minutes reaction mixture was immediately changed to the colour of dark green. The colour change was referred to formation of Nickel nanoparticles. The Carica papaya extract act as a reducing agent so reduce the Nickel ion to Nickel nanoparticles at the same time act as a stabilizing agent. The reaction mixtures of obtained nickel nanoparticles so attained were centrifugation strongly at 4000 rpm at 30 minutes. The collected nanoparticles have dried at 100ºC for air oven. The final product was calcinated at 500ºC temperature in air for 4 hours to obtain a pale green powder of NiO. After dried sample of nanoparticles grind and it is used for the further characterization purposes.

Fig 2: $NiCl_2$ Solution

Fig 3: $NiCl_2$ Solution + Plant extract **Fig 4: NiO Nanoparticles**

RESULTS AND DISCUSSION:

XRD analysis of synthesized NiO nanoparticles using Carica papaya

XRD analysis ofthe crystal structure nickel oxide nanoparticle prepared from the aqueous extract of Carica papaya was carried out by the X-ray diffraction, the obtained patterns are presented in Figure **1**. XRD analysis showed a series of diffraction peaks at 2θ of 20.27°, 21.08°, 31.42°, 37.94°, 41.95° and 53.95° can be assigned to (300), (250), (195), (175) (220) and (140) planes, respectively. All the diffraction peaks were readily indexed to a pure cubic phase of NiO (JCPDS Card no. 65-2901) no impurity peaks were observed. Furthermore, the strong and sharp diffraction peaks confirm the high

https://doi.org/10.36893/JK.2025.V15I05.010

ISSN: 2278-4632
Vol-15, Issue-05, No.02, May: 2025

crystallinity of the synthesized nanoparticles. The average crystallite size of the obtained NiO nanoparticles was 34.4 nm.

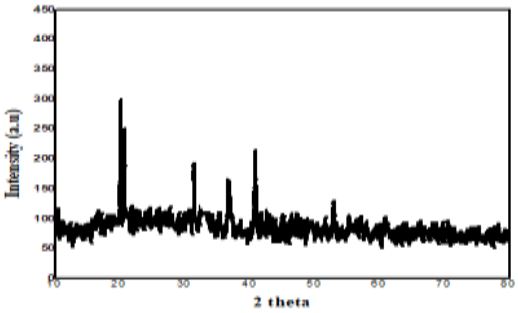

Figure 5: X-ray diffraction pattern of the nickel oxide nanoparticle prepared from the aqueous extract of Carica papaya

ANTIBACTERIAL STUDIES:

Screening of antibacterial activity:

The gram positive bacterial strains, Strephylococcus aureus, Strephylococcus epidermidis and gram negative bacterial strains Proteus mirabilis, Klebsiellapneumoniae, were used throughout the investigation. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

PREPARATION OF INOCULUMS:

Stock cultures were maintained at 4^0C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton Broth (MHB) that were incubated without agitation for 24 hrs at 37^0C. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to $2.0x10^6$ colony forming units (CFU/mL).

ANTIBACTERIAL SUSCEPTIBILITY TEST:

The disc diffusion method was used to screen the antibacterial activity. In-vitro antibacterial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The Muller Hinton Agar plates were prepared by pouring 15 mL of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes**.** The synthesized nanoparticles were loaded on 6 mm sterile disc. The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^0C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. Standard antibiotic Amikasin of concentration 1mg/mL was used as positive control [19].

S. No	Bacterial strains	Standard control Amikasin (1 mg/mL)	Inhibition Zone (mm)
1	Strephylococcus aureus	18	15
2	Strephylococcus epidermidis	18	16
3	Proteus mirabilis	17	15
4	Klebsiella pneumoniae	17	14

https://doi.org/10.36893/JK.2025.V15I05.010

ISSN: 2278-4632
Vol-15, Issue-05, No.02, May: 2025

Table 1: Antibacterial activity of the nickel oxide nanoparticle prepared from the aqueous extract of Carica papaya

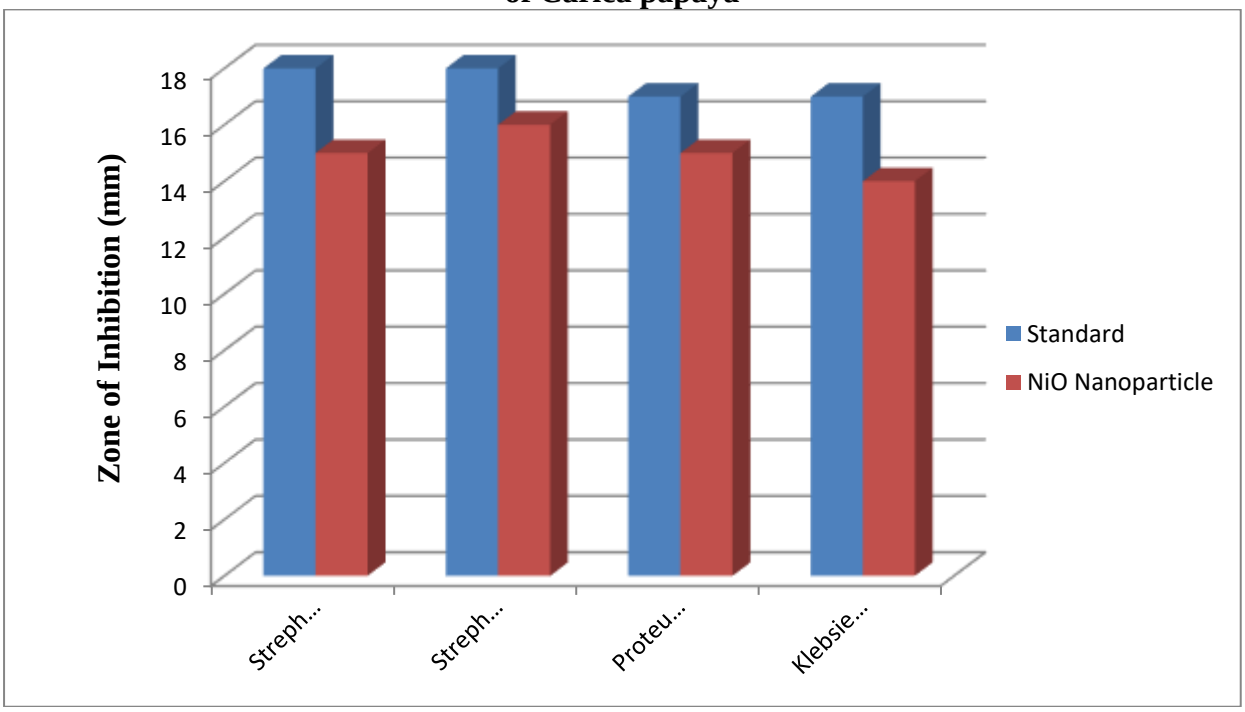

Figure 6: Graphical representation of antibacterial activity of the nickel oxide nanoparticle prepared from the aqueous extract of Carica papaya

Strephylococcus aureus

Strephylococcus epidermidis

Proteus mirabilis

Klebsiella pneumoniae

Figure 7: Antibacterial activity of the nickel oxide nanoparticle prepared from the aqueous extract of Carica papaya

https://doi.org/10.36893/JK.2025.V15I05.010

CONCLUSION:

The Nickel oxide nanoparticle screening confirms that, presence of phytochemicals such as carbohydrates, saponins, tannins, flavonoid, alkaloids, and steroids in the aqueous extract of Carica papaya for X- Ray Diffraction studies reveals that the structures and size of the nanoparticles.

The aqueous extract of Carica papaya possess moderate antimicrobial activity against gram positive bacterial strains Strephylococcus aureus, Strephylococcuse pidermidis and gram negative bacterial strains Proteus mirabilis, Klebsiella pneumonia when comparison with standard control Amikasin. The data existing in table 1 and 2 represents the nickel oxide nanoparticle prepared from the aqueous extract of Carica papaya plays an important antibacterial agent.

The disc diffusion assay reports exhibited all the concentrations of the nickel oxide nanoparticle prepared from the aqueous extract of Carica papaya possess antibacterial activity against above said bacterial strains of Strephylococcus aureus, Strephylococcus epidermidis, Proteus mirabilis and Klebsiella pneumoniae. The zone of inhibition in the petriplates and the graphical representations indicates that antibacterial activity has been dose dependant mode because increase the concentration the activity also increases.

Various infectious diseases are arising due to the pathogenic bacteria, fungi and viruses etc. From these studies a little amount of green synthesized nanoparticles products have the screening effect against pathogenic micro organisms. This antibacterial analysis revealed that the nickel oxide nanoparticle prepared from the aqueous extract of Carica papaya effective against both gram positive and gram negative bacterial diseases.

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