International Journal of Bio-pharmacology, Biotechnology and Allied Sciences

IJBBAS, September, 2020, 1(6): 615-626 Research Article www.ijbbas.com

GREEN SYNTHESIZED NANO-ANTIBIOTICS FROM *CARICA PAPAYA* SEEDS.

[1]Priyanka, [2]Saini N

[1,]2School of Life Sciences, Banaras Hindu University, Varanasi, UP, India.

***Corresponding Author: Priyanka**

Email ID: pV23412391@gmail.com

Available online at: www.ijbbas.com.

Received 2rd March.2020; Revised 8th May.2020; Accepted 1stJune.2020; Available online September. 2020

ABSTRACT

Secondary metabolites assimilated from various plants shows numerous medicinal properties. The aim of the study is to select the active metabolite from medicinal plant which possess excellence antibacterial property provide a potential source of many unique compounds with antimicrobial and other medicinal properties. The majority of these phyto-compounds are used as medicines for combating antibacterial metabolites against various strains. The secondary metabolite of respective pant parts was extracted with polar and nonpolar solvents. Further these extracts were examined against gram positive and gram negative bacteria and phytochemical screening was also performed for identifying the phytocompounds. Nano antibiotics were prepared by using $MgSO_4$, $CuSO_4$ and the antibiogram analysis was also performed by using the nanoparticles where the effective results were obtained.

Keywords: Phyto-Compounds, Nonpolar, Phytochemical, Nanoparticles, Antibiogram.

INTRODUCTION

Phytochemical are chemical compounds produced by plants, generally to help them thrive or thwart competitors, predators, or pathogens. The name comes from the Greek word phyto, meaning plant. Some phytochemicals have been used as poisons and others as traditional medicine. as a term, phytochemicals is generally used to describe plant compounds that are under research with established effects on health and are not scientifically defined as essential nutrients. Regulatory agencies governing food labeling in Europe and the United States have provided guidance for industry limiting or preventing anti-disease claims concerning phytochemicals on food product labels. Phytochemicals generally are regarded as research compounds rather than essential nutrients because proof of their possible health effects has not been established yet. Phytochemicals under research can be classified into major categories, such as carotenoids and polyphenols, which include phenolic acids, flavonoids, and stibenes/ligans. Flavonoids can be further are classified as catechins, epicatechins, and proanthocyanidins. phytochemists study phytochemicals by first extracting and isolating compounds from the origin plant, followed by defining their structure or testing in laboratory model systems, such as cell cultures, in vitro experiments, or in vivo studies using laboratory animals.

Phytochemicals obtained from vegetables, fruits, spices, herbs and medicinal plants, such as alkaloids, terpenoids and other phenolic compounds, have been proven to suppress experimental carcinogenesis in various organs in pre-clinical models. Cancer chemoprevention by phytochemicals may be one of the most important approaches for cancer control. A majority of antioxidant activity is attributed to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, catechin etc. Antioxidant based drug formulations are used for the prevention and treatment of complex diseases like stroke, diabetes, Alzheimer's disease and cancer.

Lonicera japonica has been used medicinally for thousands of years. it is an ingredient of herbal tea and has been known for its cooling and detoxification effects. Secondary Metabolites Secondary are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism.

From the time immemorial, man has been dependent on the plant products, besides the supply of food from plants. These plant products, mostly the secondary metabolites include pharmaceuticals, flavours, perfumes, agrochemicals, insecticides and raw materials for industries. chemically, the plant products may be alkaloids, terpenoids, glycosides (steroids, phenolics) etc.

As and when available, the natural plant products are preferred to synthetic products, by man. According to a WHO survey, nearly 70-80% of the world population depends on herbal drugs. it is a fact that many chemicals with complex structures that can not be chemically synthesized can be conveniently produced in plants. The production of speciality chemicals by plants is a multibillion industry. The plant cell cultures provide laboratory managed sources for the supply of useful plant products. Although hundreds of new compounds are identified every year in plants, only a few of them are commercial importance. Attempts are made to produce them in cell culture systems.

Diverse methods are currently being used for nanoparticle production, including physical, chemical, and hybrid systems (e.g., chemical reduction in aqueous or non-aqueous solutions (Petitetal.,1993), sono chemistry (Poletal.,2002), micro emulsions (Solanki and Murthy, 2010), and microwave-based systems Li et al., 2010.

Biosynthesis of silver nanoparticles has been reported from bacteria, fungi, yeast, plants, and fruits (Jha et al., 2009). Gold and platinum nanoparticles have been widely utilized as novel antiviral, antimicrobial, anticancer, and anti-inflammatory agents (Hu et al., 2006; Jain et al., 2008).

Nanoparticles have received a tremendous attention for their optimistic impact in many sectors of research and development. In particular, CuO NPs are being used and marketed as antifouling paints for boats, commonly utilized by ink, plastic, ceramic, electronic and chemical industries (Cioffi et al. 2005; Saison et al. 2010; Pan et al. 2010). Similarly, TiO2 NPs are used in medicinal formulations due to germicidal and antimicrobial properties. It is also well-known for making corrosion-proof surfaces of metals (Wold 1993; Ellsworth et al. 2000).

The ongoing production and application of metal oxide NPs have increased, due to which possibility of exposure to plants via aerial or root path has been elevated. Once adsorbed to plant surfaces, exact behavior of the NPs inside plant system is still not well-explored because different NPs can lead to either positive or negative effects on the plant system. Toxic response of NPs has been induced by changes in the plant metabolism (Limbach et al. 2007). Retardation in growth and other harmful effects on plants could be correlated with the generation of reactive oxygen species (ROS) in the plant system which results into oxidative stress. Plants may respond to oxidative stress by enzymatic ROS scavenging systems including ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD). Among ROS compounds, H2O2 is the central hub of various reactions within a plant system and has a relatively long half-life (1 ms) and its small size allows traversing through cellular membranes and migration in different compartments, which facilitates its signaling functions (Bienert et al. 2006). Nanotechnology is expected to be the basis of many main technological innovations in the 21st century. Research and development in this field is growing rapidly throughout the world. A major output of this activity is the development of new materials in the nanometer scale, including nanoparticles. These are usually defined as particulate materials with at least one dimension of less than 100 nanometers (nm), even the particles could be zero dimension in the case of quantum dots. Metal nanoparticles have been of great interest due to their distinctive features such as catalytic, optical, magnetic, and electrical properties.

METHODOLOGY

Sample collection

Papaya fruit was collected from local market. The Seeds were then washed and kept for drying in the sun and hot oven. The dried leaves and Stem were then ground to powder using a mixer and grinder.

Microbial strain and culture preparation

Bacterial Gram +ve, *staphylococcus aureus (sa)* and Gram -ve, *E coli* (Ec) and *Klebsiella pneumonia (kp)* available at the MRD lifesciences as my test pathogen. Initially be used pre- culture plates of the pathogen and streaked them in new agar plates of revive them. The revived culture worked as a source of pathogen broth.

Preparation of antimicrobial extract by solvent extraction method

The powdered samples were dipped into samples in 1:10 ratio and incubated for 48 hours further the samples were filtered by using whatsman filter paper.

Phytochemical screening

Due to the presence of certain bioactive components or secondary metabolites, the various activities possessed by the plant. A standard procedure was provided by Treaseand Evans et al., 1989, to identify these secondary metabolites.

- **Test for Terpinoids**(Salkowiski test) :

  1ml of the extract was blended with 2ml of chloroform and carefully applied concentrated H2SO4 (3ml) to form a sheet. To indicate positive results for the presence of terpinoids, a reddish brown coloration of the interface was created.

- **Test for Flavonoids**

  In diluted NaOH, 1ml of extract was dissolved and HCl was added. The existence of flavonoids is shown by a yellow solution that becomes colourless.

- **Test for Tannins :**

With a few drops of 1 percent lead acetate, 1ml of extract was added. A yellowish precipitate suggests that tannins are present.

- **Test for Steroids :**

1ml of the extract was dissolved in 10ml of chloroform and the sides of the test tubes were applied to the same amount of concentrated sulphuric acid. The upper layer turns red, and the layer of sulphuric acid shows yellow with green flurosis. This means that steroids are present.

- **Test for Saponins :**

Add 0.1ml of extract to 1ml of distilled water and shake vigorously and observe for persistent froth. Combine three drops of olive oil. Emulsion formation reveals the presence of saponins.

- **Test for Glycosides :**

In 1ml of glacial acetic acid containing 1 drop of ferric chloride solution, 0.1ml of extract was dissolved. It was layered with 0.1 ml of sulphuric acid underneath. The appearance of a brown ring at the interface suggests that glycosides are present.

- **Test for Saponins :**

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- **Test for Glycosides :**

In 1ml of glacial acetic acid containing 1 drop of ferric chloride solution, 0.1ml of extract was dissolved. It was layered with 0.1 ml of sulphuric acid underneath. The appearance of a brown ring at the interface suggests that glycosides are present.

- **Test for Alkaloids:**

Stir in 3 g of ethanol extract containing 3% tartaric acid. The philtre was divided into three breakers and screened as follows for alkaloids:—

Connect Hagar's reagent to the first beaker, and connect Mayer 's reagent to the second beaker and Marquin's reagent to the third beaker. The presence of alkaloids suggests precipitation in each of the three test tubes.

Nanoparticles preparation

The plant extract was taken and kept at 80^0C temperature and continuously stirring by adding the salts at 1:4 ratio and 1:1 ratio. Further the nanoparticle solutions were transferred in weighed bowl and allowed to evaporate the solution, then the remaining residue were dissolved into dimethyl sulphoxide.

Antibiogram analysis

The samples were screened for their antibacterial activity by using agar well diffusion method. Then 50µl samples were loaded into well and then incubated at 37^0C for 24 hours. Where highest zone of inhibition, indicates the potency of antibacterial property of respective nanoparticles.

RESULTS

Collected Sample

The seeds were collected from papaya and then rinsed with distilled water. The seeds were sun dried and converted into powder.

Antibiogram analysis

The antibacterial analysis were carried out by performing the antibacterial analysis of Ethyl acetate and propanol extracts of seed powder and the nanoparticles with MgSO4 and CuSO4 against *E coli, S. aureus & K. pneumoniae.*

Table 1: Antimicrobial sensitivity test of nanoparticles of Ethyl acetate extracts with MgSO4 at 1:1 & 1:4 ratio.

Pathogen	Zone of Inhibition (mm)			
	DMSO	$MgSO_4$	Nano particle	Tetracycline
Carica papaya **extracts and $MgSO_4$ (1:1)**				
E coli	0	10 mm	20mm	23.5mm
S. aureus	0	9mm	18mm	22.3mm
K. pneumoniae	0	11mm	19mm	22.5mm
Carica papaya **extracts and $MgSO_4$ (1:4)**				
E coli	0	11 mm	22mm	21.5mm
S. aureus	0	10mm	18mm	20.3mm
K. pneumoniae	0	9mm	29mm	25.5mm

Table 2: Antimicrobial sensitivity test of nanoparticles of propanol extracts with MgSO4 at 1:1 & 1:4 ratio.

Pathogen	Zone of Inhibition (mm)			
	DMSO	$MgSO_4$	Nano particle	Tetracycline
Carica papaya **extracts and $MgSO_4$ (1:1)**				
E coli	0	9 mm	10mm	23.5mm
S. aureus	0	19mm	18mm	22.3mm
K. pneumoniae	0	11mm	19mm	22.5mm
Carica papaya **extracts and $MgSO_4$ (1:4)**				
E coli	0	10 mm	12mm	21.5mm
S. aureus	0	10mm	18mm	20.3mm
K. pneumoniae	0	9mm	19mm	23.5mm

Table 3: Antimicrobial sensitivity test of nanoparticles of Ethyl acetate extracts with CuSO4 at 1:1 & 1:4 ratio.

Pathogen	Zone of Inhibition (mm)			
	DMSO	$CuSO_4$	Nano particle	Tetracycline
Carica papaya **extracts and $CuSO_4$ (1:1)**				
E coli	0	9 mm	22mm	23 mm
S. aureus	0	8 mm	28mm	22 mm
K. pneumoniae	0	11mm	19mm	22.5mm
Carica papaya **extracts and $CuSO_4$ (1:4)**				
E coli	0	13 mm	13mm	23.5mm
S. aureus	0	11 mm	18mm	25.3mm
K. pneumoniae	0	10 mm	19mm	21.5mm

Table 4: Antimicrobial sensitivity test of nanoparticles of propanol extracts with CuSO4 at 1:1 & 1:4 ratio.

Pathogen	Zone of Inhibition (mm)			
	DMSO	$CuSO_4$	Nano particle	Tetracycline
Carica papaya **extracts and $CuSO_4$ (1:1)**				
E coli	0	10 mm	26mm	23.5mm
S. aureus	0	9mm	28mm	22.3mm
K. pneumoniae	0	11mm	26mm	22.5mm
Carica papaya **extracts and $CuSO_4$ (1:4)**				
E coli	0	11 mm	23mm	24.5mm
S. aureus	0	10 mm	18mm	20.3mm
K. pneumoniae	0	11mm	17mm	22.5mm

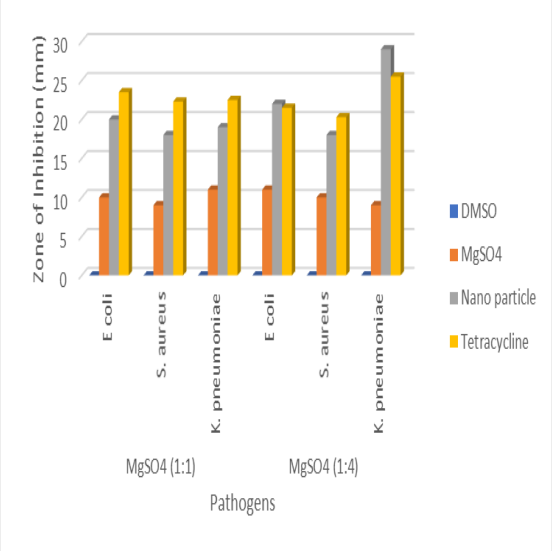

Figure 1: Antimicrobial sensitivity test of nanoparticles of Ethyl acetate extracts with MgSO4 at 1:1 & 1:4 ratio.

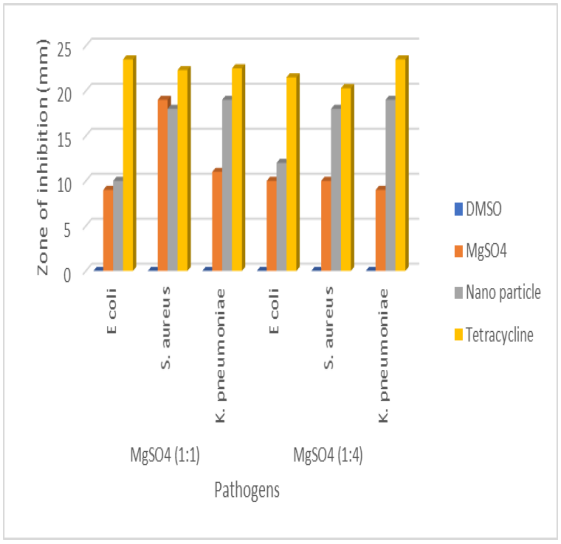

Figure 2: Antimicrobial sensitivity test of nanoparticles of propanol extracts with MgSO4 at 1:1 & 1:4 ratio.

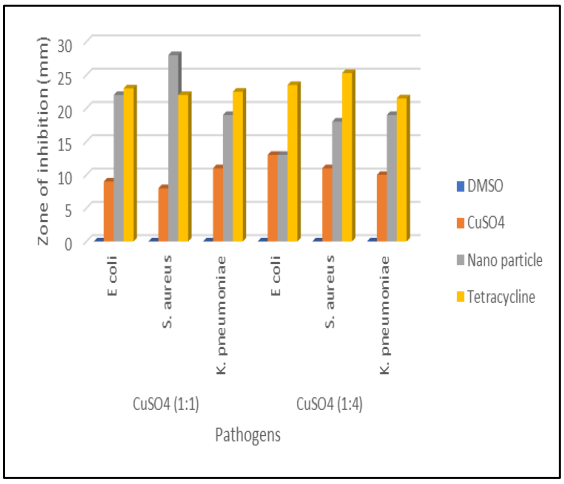

Figure 3: Antimicrobial sensitivity test of nanoparticles of Ethyl acetate extracts with CuSO4 at 1:1 & 1:4 ratio.

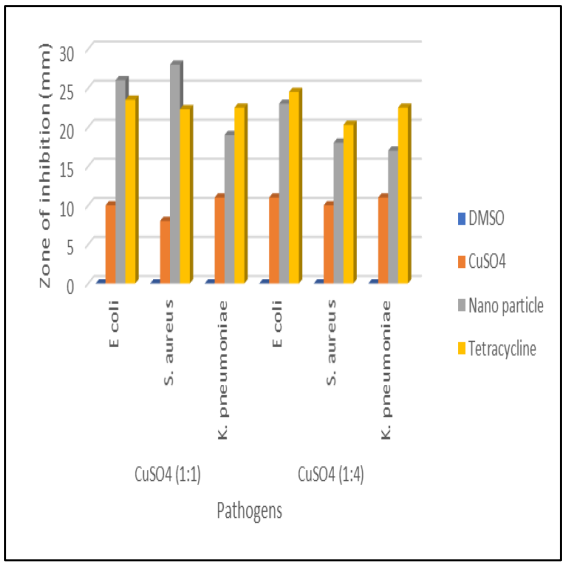

Figure 4: Antimicrobial sensitivity test of nanoparticles of propanol extracts with CuSO4 at 1:1 & 1:4 ratio.

Table 5: Test for different phytochemical analysis was done and results were observed.

Phytochemical	Acetone	Chloroform	Propanol	Ethyl acetate
Steroids	Positive	Positive	Negative	Negative
Flavonoids	Positive	Positive	Positive	Negative
Terpenoids	Positive	Positive	Negative	Negative
Saponin	Positive	Positive	Positive	Positive
Tanin	Negative	Positive	Positive	Negative

DISCUSSIONS

The last few years have witnessed a change, with the modernization of the world and advancement in technology we have a great increase in medicines. The disease which were non- curable some year back are new no longer a threat. In fact human raced is totally immune to some of disease. New pathogen which is multiple drugs resistant has come into existence. To combat such a scenario we consume loss of medicines antibiotics through they show instant result and we get cured in immune possible time, but these medicines have an adverse effect on us. Prolonged use of such highly dose medicines which are the cure us today make us ill today.

Nature has a blessed us with a vast treasure of medicinal plants. Despite giving us fruit to eat wood for fuel lowers for aesthetic beauty all what nature gives us is the gateway to herbal medication it is we who need to discover it and implement in the benefit of human beings and entering in to this gateway I choose four different plants the evaluate its antimicrobial activity and didn't obtained the good results. Then I have prepared the nanparticles with metal ions and plants extract combination and obtained great results with respect to normal metal salts and crude plant extract.

The nanoparticles formed in the ratio of 1:1, shows good antimicrobial properties so then the minimum concentration of that nanoparticle was calculated, which is responsible for the maximum inhibition of the growth.

For finding the phyto-compounds which are present in the plants are qualitatively analyzed with phytochemical tests regarding the phytochemicals.

CONCLUSIONS

Wintering up the work done throughout this project, we come up to a point where we can state that *Carica papaya* are good source of antimicrobial compound and can serve to be a good source of herbal drug.

They yield of antimicrobial can be enhanced by using more sophisticated procedure and can be tested in various other solvent. It has proved to be effective against both fungus and bacteria and used at elevated temperatures. So we can conclude that drugs made out of would not be dependent on what conditions that are stored, this gives an advantage in handling the drugs made out of this fruits.

Future aspect of my project work would be to, firstly lay emphasis on the specific components rendering it with the prolific it has shown. Secondly to develop a more sophisticated protocol for the extraction of secondary metabolites from the plant materials.

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