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BIOSYNTHESIS OF SILVER NANOPARTICLES USING MINT LEAVES EXTRACT PHYTOCHEMICAL ANALYSIS AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT:

Mentha spicata is an essential aromatic and medicinal plant.The family of mint is Lamiaceae. The medicinal value of plants lines in bioactive of phytochemical constituents that produce different Physiological action on the human body. The most important bioactive phytochemical constituents are Tannin, Alkaloids, Saponins, flavonoids, carbohydrates ,terpenoid, glycosides. The aim of the study was to develop the synthesis of silver nanoparticles using mint plant leaves extracts. The fresh suspension of plant extract was yellowish green in colour. However after the addition of silver nitrate solution, the suspension showed a colour change in Yellowish green to dark brown in colour after 24 hours of incubation at room temperature. The antibacterial activity of the mint leaves extract against the gram positive and gram negative bacteria synthesized silver nanoparticles from *Mentha spicata* were studied for its antibacterial activity against pathogenic microorganism by disc diffusion method , synthesized AgNPs colloidal solution better antibacterial activity against the pathogen like *E.coli, Staphylococcus*. The synthesized silver nanoparticles from menthe spicata act against the antifungal activity.

INTRODUCTION:

Pudina are often refer to as mint it is used to flavor for food and beverages because it is cool andrefreshing flavor. These leaves are also used as a common element in chewing gum mouth wash toothpaste and breath fresheners .Mint leaves provide numerous health advantages in ayurvedic medicine to treat a number of condition including headaches sinus infections digestive issues and common cold and also treat respiratory disorders .Mint leaves have been consumed in a variety of the ways including fresh, dried, and essential oil. Pudina is also known as the family of lamiaceae since the occurrence of infection by antibiotic resistant microbes is rising dramatically. Pudina contain alkaloids so it provide investigated. The phytochemical and antibacterial screeningof pudina leaves against several pathogenic microbes. Mint leaves produce the Silver nanoparticles , antibacterial activity and antifungal activity

SYNTHESIS OF SILVER NANOPARTICLES:

The synthesis of silver nanoparticles by aqeous ethanolic and methanolic leaf extract of menthespicata as reducing and stabilizing agent Silver. It is the most widely used metal in nanotechnology. This metal has been effective in medicine due to the antibacterial antifungal, antiseptic, antiviral,and anti oncogenic effect. The aqeous extract of menthe spicata to add silver nitrate solution to change the colour greenish yellow to dark brown the redaction of silver nitrate to silver nanoparticles.

PHYTOCHEMICAL ANALYSIS OF MINT :

The medicinal value of these plant is bioactive phytochemical constituents that produce different physiological actions on the human body. Some of the most important bioactive phytochemical constituents are Flavonoids, Saponins, Alkaloids Tannins ,Carbohydrates ,Terpenoid Glycosides. Phytochemicals from the plants have shown great promise in the treatment of infectious human diseases including viral infections (Cowan, 1999)

ANTIMICROBIAL ACTIVITY OF MINT:

A mint extract was found to be the most active extract of the test samples showed considerable antibacterial activity against the bacterial strains used in the study .Antibacterial activity of silver nanoparticles was tested against multidrug resistantance *staphylococcus, E.coli,* The antibacterial properties of silver particles are size dependent with smaller particles exhibiting a greater effect. The antibacterial properties of silver elements from their ability to relese silver ions with smaller particles have great effect of pathogenic microorganism.

MATERIALS AND METHODS:

PREPARATION OF PLANT EXTRACT:-

- Fresh leaves of M.spicata were collected from region of Tiruvannamalai and washed several times in distilled water then dried and grinded to form fine powder. The plant extract was prepared by suspending 10g of using ultrasonication for 1 hour at filtered by what man filter paper is the (cold extract) 10 g of the powder in 250ml water were boiled for 10 minutes is the (hot extract) and then the complete the same steps

mentioned.

PREPARATION OF SOLVENT EXTRACTION:

AQUEOUS EXTRACT:

- Solvent extraction is the most widely used method. The plant material was thoroughly washed several times in distilled water .then the leaves are dried and form fine powder .10g of powder is mixed with 250ml of aqueous solution and the extract was filtered by whatman no 1 filter paper. The filtrate was concentrated in a rotary evaporator at 40▫ c and freeze dried for 48 hrs. The freeze dried extracts was stored at 20▫ c

ETHANOL EXTRACT:

- The fresh leaves are washed several times in distilled water .then the leaves are dried to form fine powder .10g of powder is mixed with 250 ml ethanol solution and the extract was filtered by whatman no 1 filter paper .the filtrate was concentrated in a rotary evaporator at 40▫ c and freeze dried extract was stored at 20▫ c

PHYTOCHEMICAL ANALYSIS:

TEST FOR FLAVONOIDS:

- Take 1ml of solvent extract on a test tube. Add 1ml 10 %lead acetate solution .Then shake well formation of yellow precipitate it indicates the positive result for flavonoids.

TEST FOR SAPONINS:

- Take 2ml solvent extract on the test tube ,add a small amount of HCL, shake well on the test tube and decant the aqueous layer ,Finally add a two drops of Mayer s reagent .The formation of intense colour the forming lather was taken as a positive test for saponin.

TEST FOR ALKALOIDS:

- Head 2ml solvent extract ,to adding 10 % NaOH solution in a test tube ,to form the white precipitation. It is a positive result for alkaloids.

TEST FOR TANNINS:

- For tannins ,2ml of solvent extract are taken from the test tube ,heat the solvent extract ,adding concentrated HNO3 with excess ammonia, to form white precipitation ,the development of white precipitation shows the presence of tannins.

TEST FOR CARBOHYDRATES :

- To take a 2ml of solvent extract on the test tube ,add few drops of Molisch reagent ,then mixed with concentrated Sulphuric Acid corrosion ,the formation of reddish violet ring shows the presence of carbohydrates.

TEST FOR TERPENOID:

- 5ml of leaves extract are take a test tube ,then mixed with 2ml of chloroform,3ml of concentrated sulphuric

acid was carefully added ,to form a layer .A reddish brown coloration at the interface demonstrated the presence of the terpenoids.

TEST FOR GLYCOSIDES:

- 0.5 ml of solvent extract are diluted by 5ml refined water ,add 2ml of glacial acetic acid , add one drop ferric chloride solution ,then add 1ml concentrated sulphuric acid ,to form brown ring .A brown ring showed the presence of glycosides.

SYNTHESIS OF SILVER NANOPARTICLES:

- Synthesis Silver nanoparticles by the preparation of plant extract to add the silver nitrate solution 1mm,2mm,3mm,4mm was added to 5%,10%,15% 20% of the leaf extract after addition the solution was fixed with rotary shaker for 15 mins. Reduction of silver nitrate to silver nanoparticles to change a colour from greenish yellow to dark brown.

SPECIMEN COLLECTION AND BACTERIAL ISOLATION:

- Using sterile screw-capped containers as a midstream urine collection method is to take aurine samples were taken from UTI patients in Thiruvannamalai Government Hospital. These samples were cultured under streaking on Blood and Macconkey agar the culture medium is incubated at 37°c for 24hrs. A single colony of bacteria that grow on meida was-recultured three times to produce pure culture. Bacterial isolate were identified using bio-chemical test. Morphological and cultural testing of the Biochemical test is used to identify the shape and colour of the colonies on the selected media were observed in order to perform morphological & cultural characterictics to isolate stained by gram stain to identify the gram positive (or) Gram Negative bacteria and biochemical test were carried whether it is positive or negative to conform the given sample.

ANTIMICROBIAL ACTIVITY:

ANTIBACTERIAL ACTIVITY OF MENTHA SPICATA:

- The antibacterial activity of silver nanoparticles was tested against multidrug rsistance *staphylococcus* , *Escherichia coli* . It is isolated from urinary tract infection patents Antibacterial activity of Mentha spicata was prepared by using disc diffusion method .The nutrient broth is used for antibacterial test . The test bacterial strains were saturated within the Nutrient broth and incubated at 37 °C for 24 hrs. For the incubation the culture tube was inspect with the turbidity level. Fresh bacterial isolates of 0.1 ml were dispersed on Muller Hinton agar (MHA) plate using sterile swab on the Petri plates Place the synthesized silver nanoparticles extract on the filter paper disc. The plates were incubated in an incubator at 37 °C for 24 hrs. The antibacterial activity was assessed by measuring the zone of inhibition.

ANTIFUNGAL METHOD:

- Were the fungi is isolated from the department of mycology ,by using lacto phenol cotton blue staining to observing the fungi

- Potato dextrose Agar was prepared by dissolving 65 gm of the medium in 1 liter of distilledwater. The tube containing media is autoclaved at 121 ºC for 15 minutes.Pour the agar in to Petri plate Allow to solidify
- The nutrient broth used for antifungal test . the fungal strains were saturate with in the nutrient broth and incubate 37▫ c for 24 hrs. For the incubation the culture tube was inspect the turbidity level fresh fungal isolates of 0.1 ml were dispersed on potato dextrose agar plate using sterile swab. Place the filter paper disc. Then incubation at 28 ºC for 7-10 days and compared with control. (cultured on a lonely of Potato dextrose agar).After incubation from seven to 10 days. Estimation rate of inhibiting diameter of fungi after incubated according to the following equationaccording to Wang et al. (2007).

$$\text{Rate inhibit (\%)} = \frac{\text{Diameter of fungi(control)-diameter of fungi}}{\text{Diameter of fungi (control)}} \times 100$$

RESULT AND DISSCUTION:

PHYTOCHEMICAL ANALYSIS:

- The world is rich in natural and unique medicinal plants. Medicinal plants are now getting more attention than ever because they have potential of large benefits to society or indeed to all mankind,especially in the line of medicine. The medicinal value of the plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body.

- Flavonoids:Positive,Saponins:Negative,Alkaloids:Negative,Tannis:Positive,Carbohydrates:Negative, Terpenoid:Positive,Glycosides:Negative.

SYNTHESIS OF SILVER NANOPARTICLES:

- This experience is showed that the silver nanoparticles cannot be synthesized in darkness in caseof preparing in cold extract while boiled aqueous extract can be synthesized in darkness after 24 hr.Aqueous, Ethanol and methanol extract are prepared from leaf of M. spicata was used for rapid biosynthesis of silver nanoparticles under dark conditions without direct sunlight radiation.

- In addition to chemical synthesis of nanoparticles do not induced by sunlight, biosynthesis of nanoparticles not induced light have been studied by many researches Among the various biosynthesis methods, dark condition induced plant extract biosynthesis nanoparticle figure 7.

- Biosynthesis of silver nanoparticles using the aqueous extract of mint . However, the mechanism of biosynthesis of nanoparticles by induced light is not clear (Jena et al., 2016).

- Recently, silver nanoparticles were synthesized using aqueous mint extract utilizing the dark condition . Nanoparticles in this case were synthesized at the end of 15 min when placed in dark place .

- The oxidation of phenols by pH change to phenolates were depicted as being more readily oxidized than phenols in extract of plant (Zuman and Holthuis, 1988).

- The study was quite different from Kajani et al. (2014) who used the different change of pH adjusted and temperature after adding silver nitrate to mint extract, while Kahrilas et al. (2013) prepared silver nanopartcles by organ peel extract using microwave- assisted, by mint leaf extract (Parveen et al., 2016

ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES

- In the persent study mentha spicata aqueous extract are used to activate against the multidrug resistant .The mentha spicata extract act against various pathogenic organism are tabulated in **table 2** it shows the colony morphology of isolated organism .the total number of UTI isolates shown in **chart 1** .Morphology characteristic feature of UTI isolates are performing by gram stainig and it was shown in **figure13** and **14** . biochemical test such as Indole test ,Methyl red test ,Voges proskavers test,Citrate utilization test ,Urease test , oxidase test and triple sugar iion for H2S production. The result of biochemical test were tabulated in table 2 the figure shows the biochmical characteristic of UTI isolates the biochemical test were carried whether it is positive or negative to conform the given sample.the reagents are used to observe the colour change during reaction

- Antimicrobial activity analysis of M. spicata was done using broth dilution assay. The antimicrobial action of leaf removes was analyzed against pathogenic microscopic organisms by estimating the zone of a hindrance. The results revealed that the high antimicrobial activity of leafextracts . pudina was observed for aqueous solvents against *Staphylococcus, Escherichia coli, salmonella, Candida albicans, Aspergillus Niger, and Aspergillus clavatus.*

- The maximum zone of inhibition was found insynthesized silver nanoparticles from Mint actagainst **,** *staphylococcus , E.coli.*

ANTIBACTERIAL ACTIVITY:

- Biosynthesized silver nanoparticles by this method were studied for antimicrobial activity against pathogenic bacteria by disc diffusion method; it was observed that silver nanoparticles have antibacterial activities at concentration of 50 µl/well.

- AgNO3 was used as a control. The silver nanoparticles biosynthesized from plant extracts showed inhibition zone against microorganisms *E. coli ,staphylococcus*. A maximum zone of inhibition (MZI) is listed in **Table 3** and **figure 15 and 16** . it is evident that the nanoparticles synthesized are good candidates for their usage as an antibacterial agent.

- The mechanism of inhibitory action of silver nanoparticles on microorganisms, still not very clearly understood. Several possibilities could be nanoparticle adhesion to the cell membrane and further penetration inside or by their interaction with phosphorus containing compounds like DNA and hampering the normal replication process, loss of cell viability and eventually resulting in celldeath. It is also preferable for nanoparticles to attack on the respiratory chain. It has also been suggested that a strong reaction takes place between the silver ions and their groups of vital enzyme sultimately inactivate them.

- The synthesized AgNPs colloidal solution has shown better antibacterial activity against both Gram-positive and Gram-negative bacterial strains and they showed the inhibition zone on the petri plates using the disc diffusion method **figure15 and16**

- The diameter of the inhibition zones of AgNPs against the bacterial strains such as, Staphylococcusaureus (25 mm) , Escherichia coli (20 mm) at 50 µg/ml concentrationas shown on Table3 Zone of inhibition of antimicrobial activity of silver nanoparticles (Mint)

ANTIFUNGAL ACTIVITY:

- The study of antifungal activity of silver nanoparticles for aspergillus,penicilium cause dermatophytoses.the result show in the **table 4** it shows antifungal activity of silver nanoparticles . the rate inhabitation of diameter of fungi increased in the **figure 21 and 22** along with increase in the concentration of silver nanoparticles at 5mg/ml have antifungal 100 percentage of *Aspergillus ,Penicilium* rate of inhabitation of the diameter at 5mg/ml was 80 percentage.that have antidermatophytic activity .

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