EVALUATION, PRODUCTION OF MEDICINALLY IMPORTANT SUZYGIUM CUMINI AND IT'S BIOLOGICAL PROPERTY

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ABSTRACT

Green synthesis of plant syzygium cumini used as traditional medicine to cure diabetics was given focus's of attention. In the present study, syzygium cumini was collected and crude extract was prepared using Soxhlet apparatus and tested for its active principles by Phytochemical analysis. The presence of phenolic compounds flavonoids, saponins, glycosides, proteins, carbohydrate, steroids, threnodies, alkaloids, was recorded. The present study of biosynthesis of MgO nanoparticles treated by syzygium cumini demonstrated which illustrate a characteristic peaks with 9.5, 5.5, & 77.5 observed in the X- ray diffraction image. The X-RD paten showed 3 distinct diffraction peaks corresponding to the diffraction from 111,111, & 202 flaming of arbic spare centered magnesium oxide which are in good agreement with JCPDS (Joint Committee on Powder Diffraction Samples) card no:- 80-1916. The Fourier transform infrared spectroscopy (FTIR) reveled prominent peaks which indicated functional group changes with a band value of 3268.52cm^{-} indicates OH^{-} stretching. Another peak at wave length 1649cm^{-1} correspond to C=O stretching, and 2926cm^{-} exhibited presence of alkenes groups. FTIR study show *syzygium cumini* seed reveal presence of pheromones, and S=O group (sulphur), respectively. In order evaluate antimicrobial activity in the plant extract syzygium cumini, two bacterial pathogens such as *Staphylococcus aureus*, *Shigella Flexneri*, were studied. *Staphylococcus aureus* revealed 23mm of inhibition with plant extract as green synthesis where the chemical synthesis to determine antimicrobial activity exhibited 21mm zone of inhibition. The study further extended to assess green synthesis with clinical bacterial *Shigella flexneri and staphylococcus aureus* found to exhibited zone of inhibition.

***Keywords*:-** plant extract, syzygium cumini, nanoparticles, Phytochemical, X-RD, FTIR and antimicrobial.

I.INTRODUCTION

Nanotechnology is the ability to measure, see, manipulate, and manufacture things on an atomic or molecular scale usually between one and 100 nanometers. These tiny products also have a large surface area to volume ratio, which is most important feature responsible for the widespread use of nanomaterials in

mechanics, optics, electronics, biotechnology, microbiology, environmental remediation, medicine, numerous engineering fields and material science (Vithiya *et al*., 2014).

Green synthesis of nanoparticles has been an emerging research area now a day. The advancement of green synthesis over chemical and physical methods is, environment friendly, cost effective and easily scaled u p for large scale synthesis as nanoparticles, furthermore there is no need use high temperature, pressure, energy and toxic chemicals, Huang *et al*., 2007. Green synthesized nanoparticles offers numerous medial application in pharmaceuticals. Water purification, textile, drug designing, drug targeting, molecular imaging etc. (Virender Sharma *et al*., 2009; Rajendran *et al*., 2010; Rippka, *et al*., 2006).Silver has long been known to have strong inbitory and bactericidal effects as well as broad spectrum of antimicrobial activity even at low concentrations.

Syztgium cumini seed extract prepared in methanol and ethanol was evaluated for antibacterial by diffusion and broth dilution assay. Both extracts extracted a broad spectrum of bacteriostatic action against different gram-positive and gram-negative bacteria (Zhang *et al*., 2009). Nanotechnology is the future industrial revolution and it will be a most growing field at present in the world. Nanomaterials are very useful because of its nano size. During the last few years, synthesis of metal oxide nanomaterials has attracted the researchers due to its potential application (Jin., *et al*., 2011).

Nanocrystalline MgO is an interesting functional material due to its low heat capacity, chemical inertness, optical transparency and high thermal stability. Due to its high surface area, it is used as an efficient adsorbent for numerous toxic chemicals and acid gases. Recently, MgO nanoparticles have shown promise for application in tumour treatment and also have considerable potential as an antibacterial agent (Bindhu, *et al*., 2016).

In the present investigation, much focus of attention were given to address the problem of wide spread diabetes condition and to prevent the diabetic activity with traditional, medicinally important of seed of syzygium cumini. The methanolic extract of seeds was subjected to collect medicinally important plant syzygium cumini were prepare plant extract by pulverization and purification of extract to prepare magnesium acetate tetrahydrate for synthesis of nanoparticles were perform experiments as synthesis of nanoparticle using plant as green synthesis and to study the functional group changes, using FTIR analysis, with plant extract syzygium cumini simultaneously, to evaluate the pharmacological effect of green synthesis nanoparticles for antimicrobial activity.

II. MATERIALS AND MATHODS

2.1. Identification and Collection of Sample

The investigated plant syzygium cumini were collected in Government arts college, Tiruvarur. The collected plant parts only seeds were separated from undesirable materials or plant parts. They were dried for one week. The plant parts were ground into a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place for further use analysis.

2.2.Extraction of Syzygium Cumini Seeds Powder Extract by Soxhlet Apparatus

The chemical extraction was done by following the method of SreenivasRao and Parekh 1981. 2gm of syzygium cumini was extracted in Soxhlet apparatus using methanol 100ml as solvent for 8 hours at 65°c.Soxhletation is a process of continuous extraction in which the same solvent can be circulated through the extracted several times. The process involves extraction followed by evaporation of the solvent. The vapours of the solvent were taken in a condenser and the condensed liquid will be returned to the same for continuous extraction. Soxhlet consists of a body of extractor attached with a side and siphon tube. The extractor from the lower side can be attached to distillation flask and the mouth of extractor is fixed to a condenser by standard joints. The syzygium cumini seed powder as thimble is placed in the Soxhlet apparatus. The diameter of thimble corresponds to the internal diameter of the Soxhlet extractor porcelain pieces are added into the flask to avoid bumping of solvent. The vapours of solvent pass through the side and condensed as liquid gradually increasing the levels of liquid in the extractor and siphon tube. A siphon is setup the liquid reaches the point of return and the content of the extractor chamber are transferred to the flask. The cycle of solvent evaporation and siphoning back can be continued many times without changing solvent to get efficient extraction. The resulting extracts were concentrated to dryness in rotary evaporator under pressure and were stored at 4°c.

2.3. PHYTOCHEMICAL ANALYSIS OF SYZGIUM CUMINI PLANT EXTRACTS

2.3.1.QUALITATIVE ANALYSIS

2.3.1.1.Test for Protein

To 2ml of syzygium cumini seed extract, were added with 2ml of 0.2% Ninhydrin solution, placed in baling water both for 2mintus, and the formation of purple color, suggesting the presence of free amino acids.

2.3.1.2.Test for the Carbohydrates

To 2ml of Syzygium cumini seed extract were added with 1ml of Molisch's reagent and treated with a few drops of concentrate sulpuric acid. Appearance of purple color indicated the presence of carbohydrates.

2.3.1.3.Test for Phenols/Tannins

To 2ml of syzygium cumini seed extract were added with 2ml of 5% ferric chloride was added. Appearance of greenish black color indicates the presence of phenols and tannins.

2.3.1.4.Test for Flavonoids

5ml of the diluted ammonia solution was added to a portion of the aqueous filtrate of syzygium cumini seed extract followed by addition of concentration sulpuric acid. Appearance of yellow color indicated the presence of flavonoids.

2.3.1.5.Test for Saponins

To 2ml of syzygium cumini seed extract were added with 2ml distilled water added, and shaken in graduated cylinder for 15 minutes. The formation of foam indicated the presence of saponins.

2.3.1.6.Test for Glycosides

To 2ml of syzygium cumini seed extract were added with 3ml of chloroform and 10% ammonia solution was added, the formation of pink color formation, indicated the presence of glycosides.

2.3.1.7.Test for Steroids

To 2ml of syzygium cumini seed extract were added with 2ml of chloroform was added to the concentration sulpuric acid, acetic acid was poured in mixture the green color developed, indicated the presence of steroids.

2.3.1.8.Test for Terpenoids

To 2ml of syzygium cumini seed extract were added with 2ml of chloroform was added, and concentrated sulpuric acid was added carefully. The appearance formation of red brown color indicated the presence of terpenoids.

2.3.1.9.Test for Alkaloids

To 2ml of syzygium cumini seed extract were added with 2ml of concentrated hydrochloric acid was added, and the few drops of Mayer's reagent were added. Appearance formation of green color indicates the presence of alkaloids.

2.3.2.Test for Quinones

To 2ml of syzygium cumini seed extract were tread with 1ml of concentrated hydrochloric acid, appearance formation of red color, indicated the presence of quinones.

2.4. Determination of Antimicrobial Activity

The antibacterial activity of syzygium cumini extract obtained with methanol was evaluated by the well diffusion method. The 24 hours old culture was inoculated for the assay. Four bacterial pathogens are used for antimicrobial study. They are Staphylococcus aureus, Shigella flexneri. A sterile cotton swab was dipped into the bacterial suspension and then evenly swabbed over the entire surface of a sterile Muller Hinton agar plate to obtain uniform inoculums. The disc was punched on the seeded plates using a sterile cork borer and plates were allowed to dry for 5 minutes.

The solvent extraction of methanol extract was dispensed in to each well using a sterile micro pipette. The Nalidixic acid disc was used as positive control. The plates were incubated for 24 hours at 37^0c. The antimicrobial activity was determined by measuring the diameter of zone of inhibition (mm).

2.5.Magnesium Nanoparticles Synthesis

For the synthesis of magnesium oxide nanoparticles, 1Molarity of magnesium acetate tetrahydrate and syzygium cumini seed extract were taken in conical flask. It was kept in shaker for through mixing for 2 hour. After a short gap of 10 minutes, the 1% of NAOH solution ass added to the solution. It was mixed well and keeps in hot air oven for 2 days at 130°c, after the dried particles were collected and the sample was packed for analysis.

2.6. X- RAY Diffraction Analysis

In order to study the nanoparticles of the synthesized compound and its optical properties x-ray diffraction study was carried out. X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, homogenized, and average bulk composition is determined. X-ray diffraction is based on constructive interference of monochromatic X-ray and crystalline sample. This x-ray is generating by cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference. When conditions satisfy Bragg'a law ($n\lambda=2d \sin \theta$). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample.

2.7.Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a powerful tool for identifying types of chemical bonds in molecules by producing an infrared absorption spectrum that is like a molecular "fingerprint". FTIR can be used to identify chemicals from spills, paints, polymers, coatings, drugs and contaminants. FTIR is perhaps the most powerful tool for identifying types of chemicals bonds (functional groups). The wavelength of light absorbed is characteristics of quantitative analysis. Usually these are rather simple types of tests in the concentration range ofa few ppm upto the percent level.

2.7.1.Sample Preparation for FTIR

Syzygium cumini seed extract was collected after 20 days of incubation. The sample was air dried and used for FTIR analysis. Sample for FTIR can be prepared in a number of ways. For liquid sample, the easiest is to place one drop of sample between two plates of sodium chloride(salt).Salt is transparent to infrared light. The drop forms a thin line between the plates. Solid chromium sample were milled with potassium bromide (KBr) to form a very fine powder. This powder was then compressed into a thin pellet which can be analyzed. KBr is also transparent in the IR. Alternatively, solid sample dissolved in a solvent such amethylene chloride and the solution placed in to a single salt plate.

The solvent was then evaporated off, leaving a thin film of the chromium residue on the plate.Solution can also be analyzed in a liquid cell. This is a small container made from NaCl (or other IR transparent material) which can be filled with liquid, such as the extract for syzygium cumini seed 4.427 analysis. These create a

longer path length for the sample, which leads to increased sensitivity. Sampling method include making a mull of a powder with syzygium cumini or pyrolyzing insoluble polymers and using the distilled pyrolyzate to cast a film.

III.RESULTS AND DISCUSSION

Green chemistry and its advantage are generating interest of research towards eco-friendly, bio synthesis of nanoparticles. In the present study green synthesis of magnesium oxide nanoparticles, were performed using aqueous extracts of syzygium cumini, which as inferred by changes in color form green to brown color which indicates the formation of magnesium oxide nanoparticles an intensity of colour was directly processional to formation of nanoparticles. The results were showed in Figure:1, 2 and 3. In order to check the efficiency of magnesium oxide nanoparticles antimicrobial activity was performed which reveal strong potential of biological activity such as antibacterial activity. Besides, considerable progress in management of diabetic mellitus by synthetic drugs the research for indigenous natural antidiabetic agents with the use of medicinal herbs for preventing and treatment of disease using syzygium cumini as traditional remediation was studied. Treatment as plant extracts reduced blood glucose their by preventing the onset of diabetics. Biosynthesis of magnesium acetate tetrahydrate nanoparticls by syzygium cumini seed was analyzed by XRD and confirmed the nature of nanoparticles.

Figure: 1 Collection of Plant Syzygium Cumini Seeds

Figure: 2. Soxhlet Apperatus

Figure:3. Methanolic Extraction of Syzygium Cumini Seed

3.1.Phytochemical Analysis of Nanoparticles

Phytochemical characterization of methanolic extract of syzygium cumini was summarized in Table1. The present investigating on methanolic extract of syzygium cumini revealed the presence of CHO, phenols, Saponins, Glycosides, Alkaloids, and Steroids. Whereas the protein, Flavonoids, where absent in seed, fruits, syzygium cumini. The results were recorded in Table.1 and 4 and 5.

Table: 1. Phytochemical characterization of methanolic extract of Syzygium cumini

S.No	Test	Result
1	Protein	Negative
2	Carbohydrates	Positive
3	Phenols/ tannins	Positive
4	Flavonoids	Negative
5	Saponins	Positive
6	Glycosides	Positive
7	Steroids	Positive
8	Terpenoids	Negative
9	Alkaloids	Positive
10	Quinones	Negative

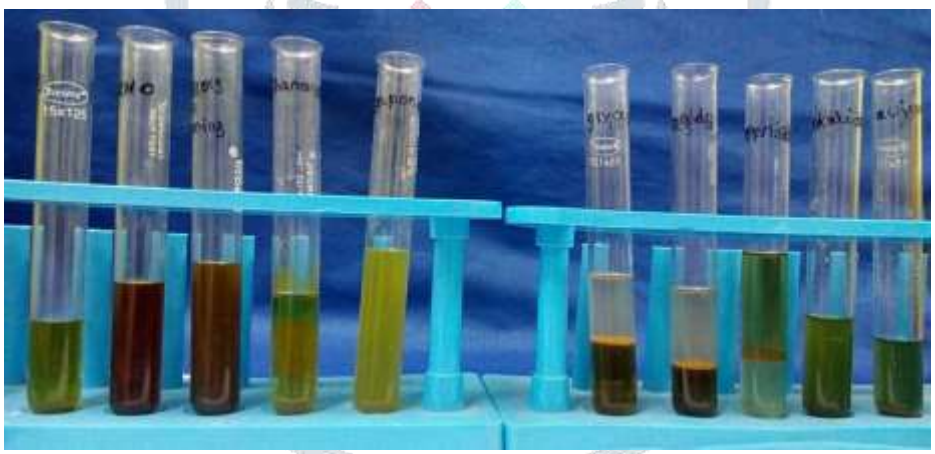

Figure: 4. Phytochemical Analyses Of Syzygium Cumini Seed

Figure: 5.Megnesium Acetate Tetrahydrate as Nanoparticles Synthesized Compounds

3.2.Antimicrobial Activity

In nano the check for the efficacy of magnesium oxide and syzygium cumini plant extract synthesized nanoparticles, were studied. The antibacterial activity, against the grambacteria *Shigellaflexneri* and *Staphylococcus aureus*were carried out. The study was carried out as green synthesis and chemical synthesis separately to evaluate antibacterial activity. Evaluation of antibacterial activity of green synthesis against *Staphylococcus aureus* showed zone of inhibition of 23mm. The nanoparticles amended plant extract as green synthesis exhibited an increase in zone of inhibition, found to be 29mm treated against *Shigella flexneri*. The antibacterial activity was performed for standard in order compare the result nted in figure:6.

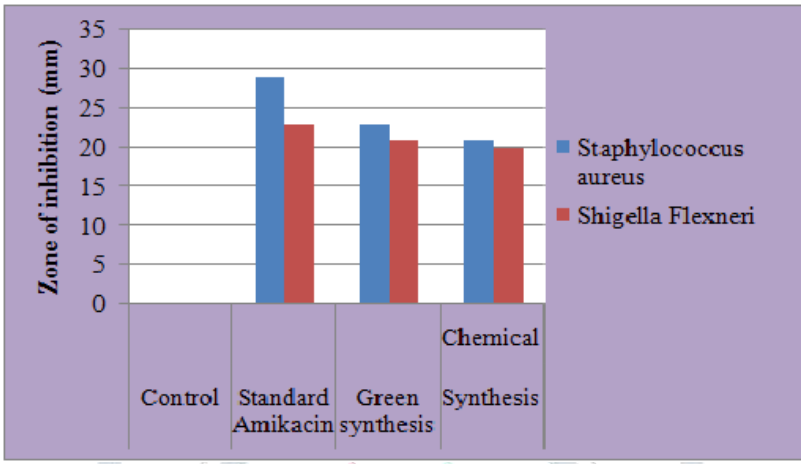

Figure: 6.Antimicrobial activity of MGO nanoparticles synthesis compound, green synthesis and chemical synthesis of plant extract syzygium cumini.

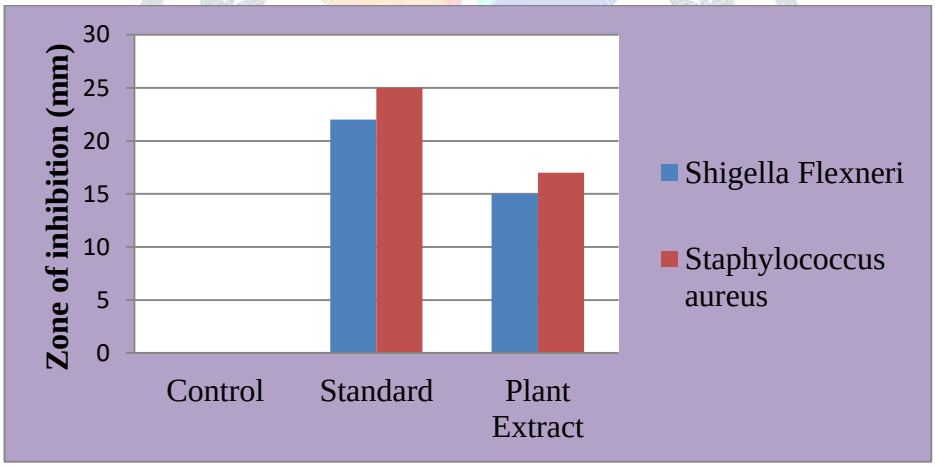

Figure: 7.Antimicrobial Activity of Plant Extracts Syzygium Cumini

Amikacin was used as reference standard. In which nanoparticles synthesized plant extract was found to exhibit maximum activity against *Staphylococcus aureus* with zone of inhibition 29mm respectively. Similarly on observing the result of standard *amikacin* against *Shigella flexneri*, revealed a zone of inhibition 23mm. In order to assess antimicrobial activity of plant extract syzygium cumini treated against *Shigella Flexneri* showed 15mm zone of inhibition, compared to standard value exhibited 22mm zone of inhibition. Similarly the plant extract treated against *Staphylococcus aureus* revealed slightly increased zone of inhibition with 17mm anther compared to standard which showed 25mm zone of inhibition, depicted Fig:7, 8 and 9.

Figure: 8. Antimicrobial Activity of Syzygium Cumini Seeds Treated against Clinical Pathogens

Figure: 9. Antimicrobial Activity of Green Aynthesis and Chemical Synthesis against Clinical Pathogens

3.3.FTIR Analysis

FTIR spectrum of the compound from syzygium cumini showed sharp strong vibration band at 3268.52cm^{-1} which indicates presence of OH- stretching. Another prominent wavelength at 1649cm^{-1} indicates C=O stretching and N-O asymmetric stretching. FTIR spectrum indicates presence of another big at 2926cm^{-1} indicated the presence of Alkenes groups. This charactertic functional group indicates presence of Pheromones. FTIR spectrum showed broad strong absorption bond with the range of 626-6049cm^{-1} this absorption or assigned to different stretching vibration, another prominent with wave length of 1416.78cm^{-1} correspond to C=O stretching, which indicates presence of carboxyl acids. Another important band appeared at a wave length of 1042.7 assigned to be sulphur group an indication of S=O groups. In conclusion, the methanolic extract of medicinally important plant syzygium cumini has primary important compounds with unique metabolites appear to play an important role in green synthesis (Fig:10).

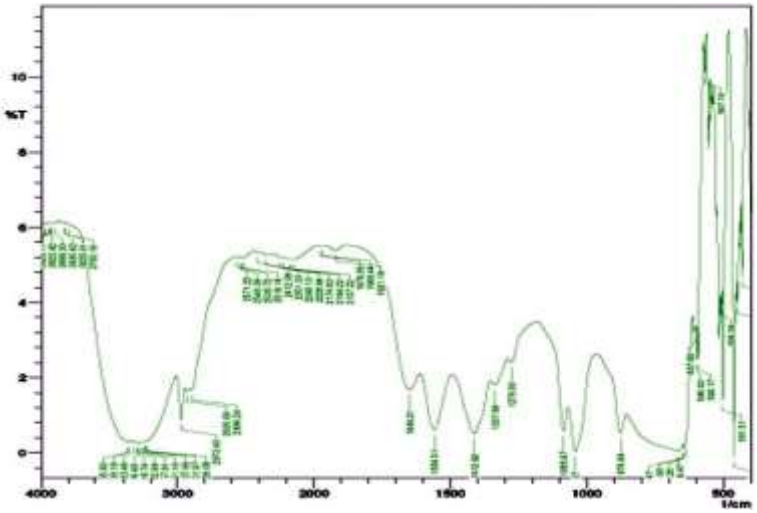

Fig: 10. FTIR analysis in plant sysygium cumini extraction

3.4.X-RD Analysis

Biosynthesis of magnesium acetate tetrahydrate nanoparticles by Syzygium cumini seed, was analysis for XRD study to confirm nature of nanoparticles. The XRD pattern reviled three distinct diffraction peaks 2θ at of X- Ray, at 9.5, at 5.5, 17.5, that will point and 28.5 point 44.4°. The 100% intensity as found at 2θ value with 28.2°. (Fig: 11).

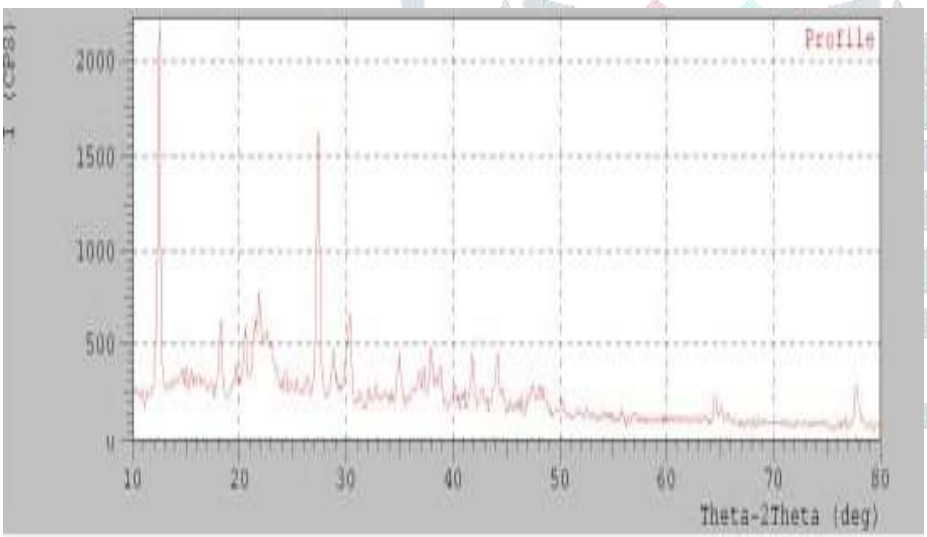

Fig:11. X- RAY Diffraction Structural Studies

Magnesium oxide (MgO) is the versatile metal oxide having numerous applications, such as of catalyst, and as adsorbent to remove dyes metals further an important application as antimicrobial material. Nowadays green chemistry using plant extracts *Syzygium cumini* as green synthesis with magnesium nanoparticles has many advantages such as safe, eco-friendly synthesis.

The result of the phytochemical analysis *Syzygium cumini* having suitable metabolites with hydroxyl functional groups and presence of CHO, Phenols, Glycosides, Alkaloids, and Steroids. The *Syzygium cumini* has potential to consider as antimicrobial agents and anti diabetic agents. The result clearly show that medicinally plant is growing in interest to be used as adjuvant in the treatment of diabetics was studied (Manish, *et al.*, 2016).

The FTIR analysis for characterization of seed extract *Syzygium cumini* magnesium oxide nanoparticles showed presence of various polyphenols in the seed of *Syzygium cumini*. Further *Syzygium cumini* revealed the presence of alkaline groups which indicates in presence of phelenones. Present study revealed with biosynthesis of magnesium acetate tetrahydrate nanoparticles by *Syzygium cumini* seed revealed charactertic peaks at 9.5, 5.5, 17.5, when observed of the large size of *Syzygium cumini* magnesium nanoparticles was due to present in plant seed extract. The morphology and nano crystallite size determined from peaks untainted from XRD image (Ali, *et al*., 2011).

The present investigating referred with variety verity of dispense ranging from basic material science to personal care application using medicinally important seed extract to reduce as antimicrobial agents. This magnesium acetate tetrahydrate along with green synthesis of sliver nanoparticles have potent application. Antimicrobial activity of plant extract revealed strong potential of biosynthesis nanoparticles another compare to chemical synthesis. It was suggested that magnesium nanoparticles produce free radicals and this radicals create pores in bacterial cell changing the membrane permeability. Our study coincides with report of Soni *et al.*, 2004.

CONCLUSION

Nanotechnology is the ability to measure, manipulate and manufacture at an atomic scale by using plant extract the inorganic nanoparticles using $MgNo_3$ synthesized from plant extract to cure metabolic disorder of endocrine system caused by disease diabetic mellitus. In conclusion magnesium acetate tetrahydrate nanoparticles with syzygium cumini received an increased attention in green synthesis to curb the problem of diabetes. Besides, syzygium cumini also possessed antimicrobial agents when treated against clinical pathogens.

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