Green Synthesis of Sulphur Nanoparticles via *Syzygium cumini* Leaf Aqueous Extract and Study of their Antimicrobial Activity

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***Abstract* - Green synthesis of nanoparticles has many potentials application in environmental and bio medicals. Among diverse non-metal nanoparticles, sulphur nanoparticles are one of the most significant nanomaterials. A novel green approach was utilized to fabricate sulphur nanoparticles within aid of *syzygium cumini leave* extract. Plants itself contain reducing and capping agents. The effective formation of sulphur nanoparticles characterized by using UV-visible spectroscopy, Fourier transform infrared spectroscopy, X-ray diffraction, scanning electronic microscopy and high-resolution transmission electron microscopy. The major objective of this work to synthesis sulphur nanoparticles by green approach using *syzygium cumini* leaves aqueous extract and citric acid. The leaves of *syzygium cumini* aqueous extract act as a capping and stabilizing agent in the formation of sulphur nanoparticles. The synthesized sulphur nanoparticles were studied their application as antimicrobial activity.**

***Index Terms* - Green synthesis, Sulphur nanoparticles, *syzygium cumini* leaves, Antimicrobial activity.**

INTRODUCTION

Now a day's researchers develop to synthesize bio-NPs by green route. For synthesis of nanoparticles there are different methods such as physical, chemical and biological method. But according to environment issue, chemical method is hazardous, so today's researchers are focused to green chemistry. Nanoparticles synthesized by green route which is eco-friendly method[2]. Nanotechnology has different applications in different fields such as green energy, catalysis[1], agriculture, composites, antimicrobial agents[4], antifungal agents[4] industrial applications and medicines. By green route includes plants, plant parts, bacteria, fungi, yeasts, algae and as they are easily available, which is less expensive and less hazardous to environment. So, researchers are more focus on green synthesis to synthesized nanomaterials. Different methods to synthesize sulphur nanoparticles (SNPs) with well-defined shapes and nano-sizes were reported using aqueous surfactant[5], eggshell membrane[6], W/O microemulsion[8], precipitation method using sodium thiosulphate and tetra octyl ammonium bromide surfactants in hydrochloric acid media[5-9]. The non-metal sulphur has numerous uses for pharmaceutics and agriculture industry[12-17]. Also, Sulphur used as a component of fertilizers such as calcium sulphate which is the most common fertilizer in agriculture used to improve the nutritious quality of phosphorous and nitrogen fertilizers [14]. Therefore, sulphur nanoparticles have significant applications in several fields of modern technology including, lithium-Sulphur batteries [7, 15], pesticides [13], fungicides [16], carbon nanotube modification [18], gas sensor [19], catalysis and neutron capture in cancer therapy [21]. Moreover, SNPs generated significant roles in the arena of biomedical concerns such as antibacterial [9,28], reduction in antimicrobial potential[23], antitumor, antioxidant[22]. Nonmetal sulphur nanoparticles were synthesized by researchers for different plants with different morphology and size[26]. In this study we observed sulphur nanoparticle is safe non-metal.

MATERIALS AND METHODS

Materials:
All the chemicals were of AR grade and used without purification. Sodium thiosulfate pentahydrate ($Na_2S_2O_3{\cdot}5H_2O$, 99.8%) and anhydrous citric acid ($C_6H_8O_7$, 99%) was purchased from Sigma-Aldrich. The plants (*Syzygium cumini*) leaves, all glassware's

were rinsed with deionized water by drying in the oven.

Preparation of *syzygium cumini* Leaves Extract:
Fresh leaves of *syzygium cumini* were collected from the campus. Fresh leaves were washed under running tap water to remove any debris and dust attached to the leaves and subsequently with deionized water 3-4 times. After washing the leaves were separated by cutting them into small pieces and air dried for four weeks at room temperature. The dried leaves were finely powdered. The extract was prepared by taking 40 g of powdered leaves and boiled in 500 ml glass beaker along with 400 ml of deionised water for 10 minutes. The aqueous extract of leaves was separated by filtration with Whatman No. 1 and then centrifuged at 8000 rpm for 5 minutes to remove heavy materials. The filtrate was collected and stored at room temperature. This gave the final leaves extract which was used for further experimental procedure.

Synthesis of Sulphur Nanoparticles (SNPs):
In present work an appropriate amount of sodium thiosulfate pentahydrate ($Na_2S_2O_3.5H_2O$) (1.935gm) was dissolved in 40 ml of leaf extract under mild stirring for 5 minutes at room temperature and then diluted with deionized distilled water to 100 ml. Subsequently citric acid (20%) was added drop by drop under stirring for allowing the sulphur precipitations uniformly. The suspended sulphur particles obtained were separated by centrifugation at 8000 rpm for 5 minutes and then repeatedly washed with sterile distilled water to remove any biological materials. Sulphur nanoparticles after purification were dried in a vacuum at 80 ºC for 6 hours. The product was finally dried and used for characterization.

RESULT AND DISCUSSION

The synthesized SNPs were characterized by UV-visible spectrophotometer, FT-IR, XRD, SEM and HRTEM and SAED. The absorbance measure for SNPs by UV-visible with Jascov 300 using a quartz cuvette. Fourier transform infrared spectra were recorded on Schimadzu IR-Affinity-1 using KBr pellets. The crystallinity of the SNPs was studied using XRD on Rigaku Miniflex 600. The surface morphology of the prepared SNPs was characterized by scanning electron microscopy (SEM) on Model JSM 6100 (Jeol). High resolution electron microscopy (HRTEM) were carried out on JEOLMODEL JEM 2100 plus. It revels structural morphology, polycrystalline nature and selected area electron diffraction (SAED).

UV– visible spectroscopy:
UV– visible spectroscopy mentions to absorption spectroscopy in the region of UV-visible spectrum. Light frequencies of the 200 – 800 nm is commonly used. UV– visible spectroscopy is a crucial technique to ascertain the stability and formation of sulphur Nanoparticles visible absorption measurement of the wavelength ranging from 232 nm - 365 nm is utilized to characterize SNPs.

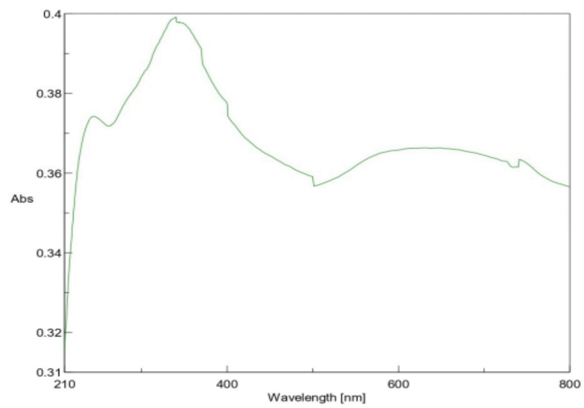


Fig.1 UV-visible spectrum for Sulphur nanoparticles

FT-IR Analysis
FT-IR spectrum of *syzygium cumini* leaves aqueous extract is illustrated in Fig. 2. FT-IR measures aqueous leaf extract and synthesized sulphur nanoparticles were carried out to identify the possible secondary metabolites responsible for the reduction, capping and stabilization of sulphur nanoparticles. A strong and a broad absorption bands at 3564 cm^{-1}- 3298 cm^{-1} could be described to the stretching absorption band of amino (-NH), hydroxyl (–OH) stretching, H-bonded alcohols and phenol and inter molecular bond formation. The shoulder peak at 1747 cm^{-1} due to C=O stretching ester. The band at 1681 cm^{-1} and 1608 cm^{-1} is characteristic of conjugated alkene and amide formation. The band 1456 cm^{-1} is nitro group are present. In C-N stretch of aromatic amines and carboxylic acids gives rise to band at 2200 cm^{-1}. The strong and sharp peak at 468 cm^{-1} indicate S-S linkage in sulphur nanoparticles.

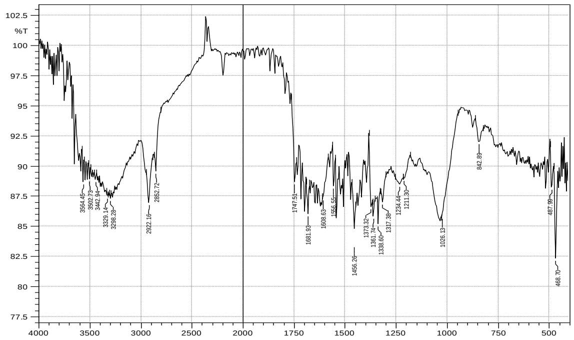


Fig. 2: FT-IR analysis of sulphur nanoparticles

XRD analysis

XRD is a helpful tool for receiving information about the atomic and crystal structure of nanomaterials. XRD is a precious characterization technique to confirm the formation of sulphur nanoparticle, analyse the crystal structure. The XRD pattern of green synthesized sulphur nanoparticles by s*yzygium cumini leaves* aqueous extract is illustrated in Fig. 3. The XRD pattern shows sulphur nanoparticles are crystalline in nature. There is a formation of crystalline S_8 with orthorhombic structure (JCPDS N-96901364). The average particle size of the synthesized sulphur nanoparticles was calculated using Debye Scherrer formula. D = (kλ/βcos θ). The average particle size was found to be 68 nm.

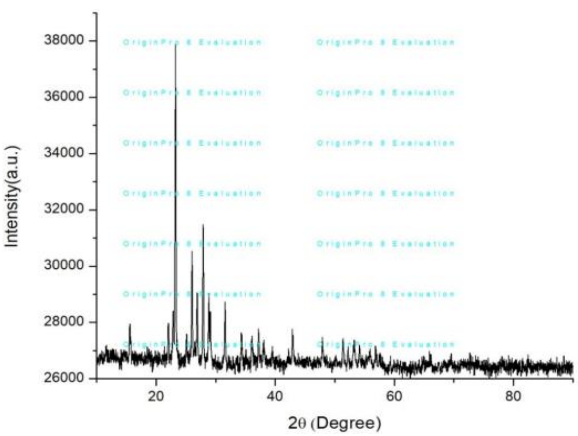


Fig. 3: XRD pattern of the SNPs

SEM analysis

SEM is an essential analysis method that utilizes electrons instead of light to form a micro-image as an output. The SEM investigation is utilized to describe the shape, size, morphology and distribution of bio synthesized SNPs (Fig. 4). The SEM micro photographs also evidence the polydispersity and purity of resulting sulphur nanoparticles.

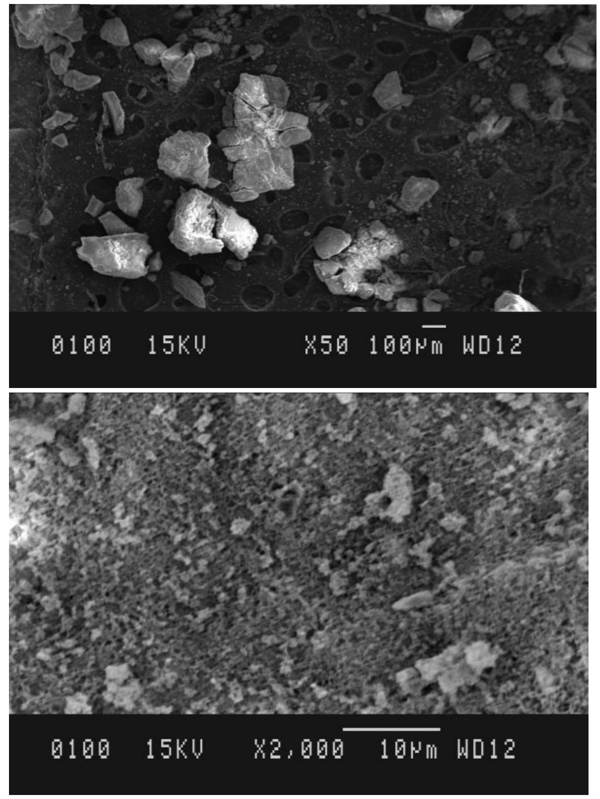
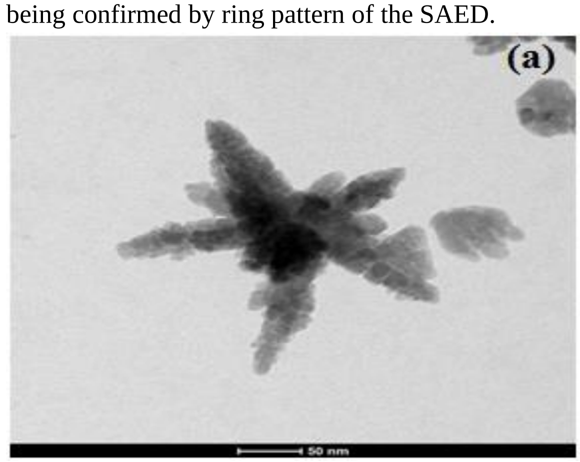


Fig. 4: SEM image of SNPs

HRTEM analysis

HRTEM analysis was carried for evaluation of particle size, crystallinity and morphology of the sample. In particle size measurement microscopy is the only method in which the individual particles are directly observed and measured. Fig. 5 (a) represents the TEM image of SNPs were starfish like shape and average dimensions found to be 65 – 70 nm. Lattice fringe image Fig. 5 (b) exhibits the regular spacing of the lattice plane. The selected area electron diffraction (SAED) image in fig. 5 (c) with polycrystalline nature being confirmed by ring pattern of the SAED.



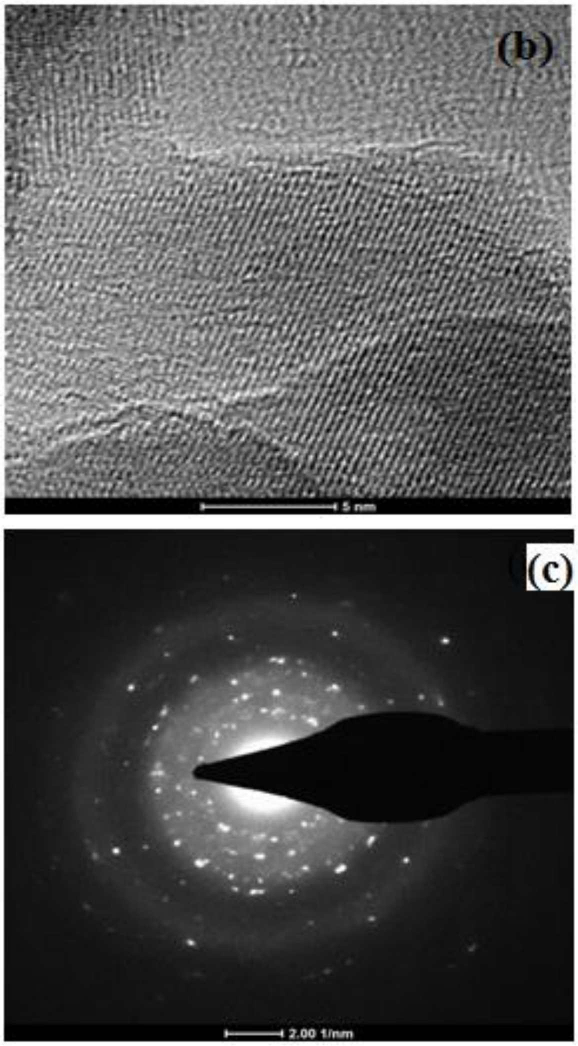


Fig.5 HRTEM micrographs (a), lattice scale fringes (b) and SAED pattern (c) of SNPs

ANTIMICROBIAL ACTIVITY

The Antimicrobial activity of pathogen was established using well diffusion method. At a different concentration of samples, the distinct zone of inhibition was formed around the wells. DMSO and Tetracycline were used as a negative and positive controls respectively to compare the zones. To cultivate the bacteria, nutrient agar media was used. About 20 ml of sterile molten agar was poured into the sterile petri dishes. After solidification of medium, the petri dishes were inoculated with the 24 hours old bacterial cultures by spread plate method and then the wells were prepared with 9 mm cork borer. Further the Sulphur nanoparticles with 0.05 g/ml concentration in DMSO were prepared and 100 µl volume was added into the well. Petri dishes were incubated for 24 hours at 37^{o}C. Antibacterial capacity of the sulphur nanoparticles was studied by measuring the zones of inhibition. Significant results were observed in *E. coli* and *Bacillus subtilis* showed less inhibition as shown in Fig .6. This result indicates that SNPs can be used as an antimicrobial agent.

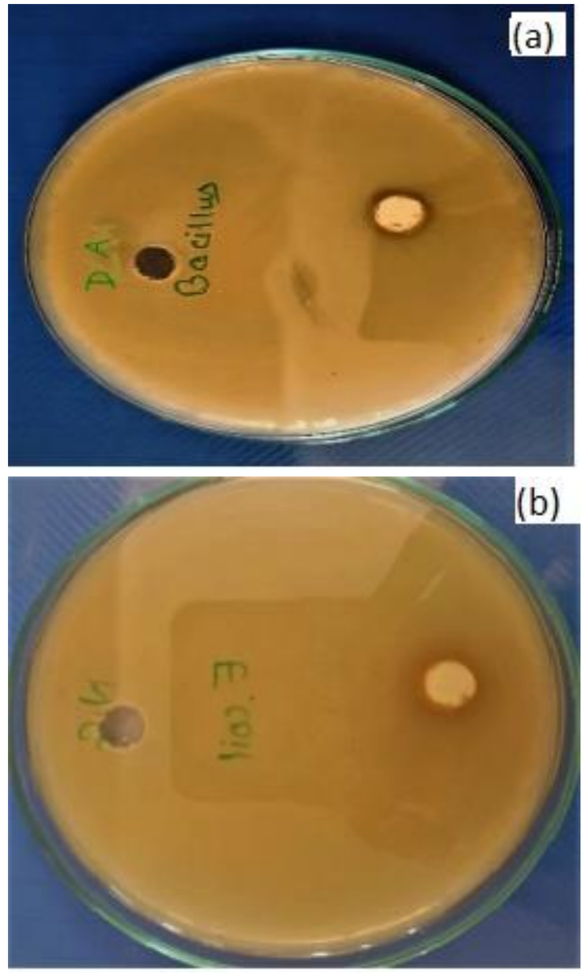


Fig. 6: Antimicrobial activity of SNPs (a) *Bacillus subtilis* (b) *Escherichia coli*

Bacteria	Zone of inhibition (Outer Zone-Inner zone) (in mm)
Escherichia coli	4
Bacillus subtilis	8

The aqueous extract of *syzyium cumini* L. leaves were added to the aqueous solution of sodium thiosulphate. Sulphur nanoparticles were rapidly synthesized. The aqueous sodium thiosulphate changes a colour with time. The UV–vis spectrum of *syzyium cumini* L. leaves extract is shown in (Fig.1). The results showed a strong absorption peak at 365 nm, which increased in intensity with time.

Qualitative Phytochemical screening:

The results of phytochemical screening were represented in Table (1)

Table 1 Results of Phytochemical analysis.

Test Number	Phytochemical	Test	Observation	Result
1	Alkaloid	Mayer' test	No Appearance of yellowish precipitate	Negative
		Wagner test		Negative
2	Flavonoid	Lead Acetate teat	Yellow precipitate	Positive
3	Tannin	Ferric chloride test	Appearance of bluish black precipitate	Positive
4	Phytosterol	Salkowski's test	Golden brown colour (Ring form)	Positive
5	Saponin	Foam test	Yellow precipitate	Positive
6	Carbohydrate	Molisch test	No formation of violet ring at the junction	Negative

In present work a new green protocol for the synthesis of SNPs using extract of *Syzygium Cumini* L. leaves is developed, which is eco-friendly method and a promising, low cost and without using any toxic chemicals. Average size of synthesized SNPs 68 nm.

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