Sulphur Nanoparticles: Synthesis using Prosopis Juliflora Leaf Extract, Characterization and Estimation of Antibacterial Ability

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Abstract
This work reports an eco-friendly technique of preparing sulphur nanoparticles by reducing sodium thiosulphate using aqueous extract of prosopis juliflora (PJ) plant leaves under acidic condition. A sharp peak observed at 246nm UV-vis spectral analysis revealed the formation of sulphur nanoparticles from the reaction mixture. Sulphur nanoparticles synthesized from prosopis juliflora leaf extract were further characterized by SEM analysis, FTIR analysis designates functional groups present in the phytochemicals of PJ plant leaves extract involved in stabilization of the above synthesized SNps. Dynamic light scattering technique revealed (DLS) average particles size of the SNps as 229 nm. Polydispersity index (PI) value indicates the SNps prepared in this technique were polydisperse. XRD analysis of the SNps shows characteristics peaks corresponding to the orthorhombic crystalline structure of SNps.

Synthetic SNps also showed their antibacterial ability against common pathogenic diseases causing microbe such as Escherichia coli (E. Coli) and Staphylococcus aureus (S. aureus). Hence, it is suggested that the green synthesized SNps have been used as antibacterial agents.

Keywords: Sulphur, nanoparticles, prosopsis juliflora, Antibacterial.

Introduction
Sulphur has long been recognized as an efficient antifungal and antibacterial agent treating wide range of plant diseases and dermatological infections[1]. In addition to that, sulphur is accepted as organic fertilizer and as useful nutrient for the growth of plant and microorganism[2,3]. Although, the antimicrobial efficiency of sulphur is limited in agriculture and pharmaceutical industries mainly because sulphur is required in bulk quantity and resistance power of target microbe has increased. Hence it is desirable to reduce significant amount and to minimize accrual of resistant power in target pathogen[4].

Nanoscience and technology offered appropriate bottom-up method to synthesize nano sulphur particle, which is most appropriate material to resolve the foresaid problems. Intensive research work on sulphur in past few years revealed that nano sulphur has wide applications in agricultural, industrial and pharmaceutical[5]. However, sulphur modified carbon nanotubes are used as electrochemical sensors[6], However, sulphur nanoparticles are used to prepare composites for lithium battery[7], to prepare nano-wire used for gas sensors and catalysis[8], Sulphur nanoparticles (SNps) also have anticancer, antibacterial and antifungal activates[9].

Nowadays, great effort has been taken to explore new methods to synthesize sulphur nanoparticles of anticipated size and shape. Few reports are available in literature about synthesis of nano size sulphur particles for antimicrobial activities. Deshpande et al[8] used sulphur nanoparticles by decomposition of hydrogen sulphide gas by micro-emulsion technique using a biodegradable chelate of iron catalyst. Cheng et al[10] synthesized sulphur nanoparticles using eggshell as template. Sulphur nanoparticles have also been synthesized by precipitation technique from sodium thiosulphate using surfactant in acid medium.[6,14,15]

Shamsipur et al[21] have reported that synthesis of sulphur nanoparticles by electrochemical method[15]. Most of these techniques involve many physico-chemical processes and hence, it is difficult for large scale preparation and separation of nanoparticles from microemulsion and purification. Evolving a novel green and facile method is still challenging for researchers to synthesizing sulphur nanoparticles. Recently, researchers have taken great efforts to find ecofriendly and cost-effective green method for rapid and large-scale production of SNps[16].

Green synthesis of sulphur nanoparticles using plant extract as reducing and stabilizing agents has been reported in literature such as Albizia Julibrissin fruits extract,[2,3] Punica granatum peels extract[18], anti-bacterial potential of SNps synthesized from four healing plants *Azadirachta indica*, *Catharanthus roseus*, *Polyalthia longifolia* and *Mangifera indica*[16], Allium sativum extract[11] and Rosmarinus officinalis leaves extract.[1]

Thus, green synthesized of sulphur nanoparticles is a ecofriendly, cost effective best choice of mild reducing agent for synthesis sulphur nanoparticles and avoiding use of hazardous chemicals. Hence, this present work aimed to developing green synthesis of sulphur nanoparticles from sodium thiosulphate pentahydrate using Prosopsis Julifolra (PJ) plant extract as reducing agent and characterization of resultant sulphur nanoparticles using UV-Visible spectroscopy, Fourier transformer infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM). Particles size of SNps are determined using dynamic light scattering (DLS) method. Crystalize size and shape of the SNps were determined by X-ray diffraction (XRD) analysis. Antibacterial activity of the resulting sulphur nanoparticles can also be evaluated against *Staphylococcus aureus* and *E. Coli*.

Material and Methods

Preparation of leave extract in aqueous solution: Leaves of PJ plants were collected freshly from nearby area of Trichy town, Tamil nandu and cleaned thoroughly by washing with water and then dried inside a room at 30ºC over a week. The dried prosopis juliflora leaves were grinded into fine powder with mortar. 10 g of prosopis juliflora powder was boiled with half a litre of distilled water for 10 minutes and then filtered through Whatmann no. 1 filter paper. The filtrate is preserved in refrigerator to use for synthesis of sulphur nanoparticles[20].

Synthesis of sulphur nanoparticles (SNPs): A suitable amount of sodium thiosulphate pentahydrate (4.96 g) had been dissolved in 100ml of PJ leaves extract and then diluted to 500 ml with water. SNps had been formed by adding enough amount of 0.1M citric slowly into the solution of sodium thiosulphate in PJ leaves extract slowly with stirring. Then, above suspension was centrifuged to separate SNps. Biological impurities present on SNps had been removed by washing several times with distilled water. The moisture present on the SNps was removed by drying in oven at 80ºC for 6 hours. The product was light yellow in colour which was further used for the characterization and antibacterial efficacy analysis[20].

Characterization of Sulphur nanoparticles (SNPs): UV-Visible spectrophotometer (Model: Schimadzu 1601) has been used to identify the formation of sulphur nanoparticles from reduction of sodium thiosulphate in aqueous extract of PJ plant leaves. In order to identify functional groups present on PJ leaves extract involved in the stabilization of nanoparticles, FTIR spectrum (Shimaduz 84005 Fourier transform infrared spectrophotometer) of the SNPs sample has been recorded with in the range 4000 -400 cm^{-1}.

Surface morphology of SNps has been analyzed by Scanning electron microscope (model: JOEL JSM 6360 – machine). The particle size distribution of sulphur nanoparticles is determined using particle size analyzer [dynamic light scattering (DLS) spectrophotometer model: Beckman Delsa Nano C series, USA]. X-ray diffractometer was used to determine crystalline nature of SNps from X-ray analyzer. Data average size of the SNps was also determined using Debye-Scherrer equation (1):

$$D = \frac{k\lambda}{\beta COS(\theta)} \quad (1)$$

where D and λ are crystalline size of the sample and wavelength of the X-ray source (1.541 Aº) used in X-ray diffractometer respectively. In Debye-Scherrer equation, β represents full width at half maximum (FWHM) height of the peak and K is known as Debye-Scherrer constant and θ is the Bragg angle. In this present work, antibacterial potential of SNPs against multidrug resistant human pathogens has been analyzed by agar disc diffusion method[20].

Results and Discussion

UV-Visible spectral analysis of SNps: SNps formed from the solution of prosopis juliflora leaves extract by the addition of sodium thiosulphate pentahydrate are indicated by change in colour of the solution from pale green into yellow. After completion of the reaction, the mixture of prosopis juliflora leaves extract and sulphur nanoparticles solution was subjected to record UV-vis spectra.

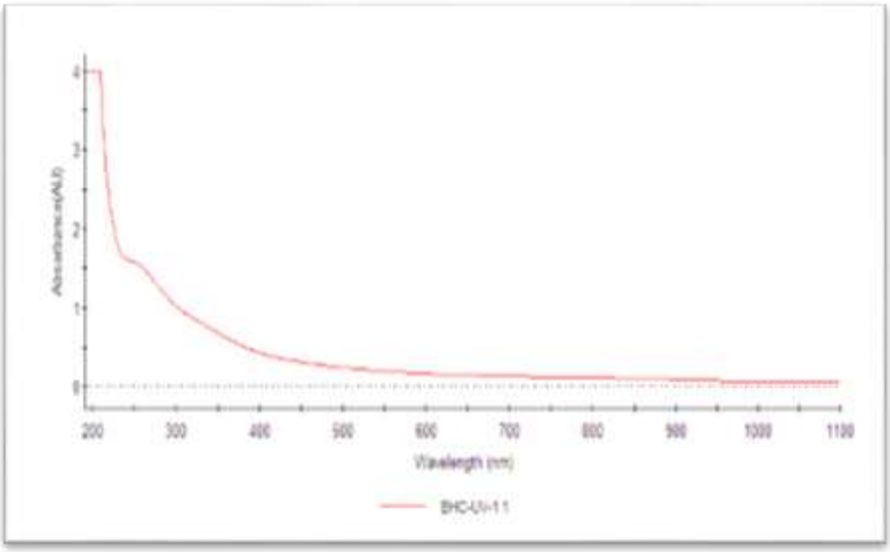

Figure 1: UV-vis spectrum of SNps synthesized using extract of PJ leaves

The figure shows adsorption band at around 246 nm which is corresponding to surface plasmon resonance peak of colloidal sulphur nano particles[16,21]. Thus, figure 1, indicates that sulphur nanoparticles are synthesized using prosopis juliflora leaves extract as reducing agent.

Scanning electron microscopy (SEM) analysis: SEM analysis of SNps is shown in the figure 2. It shows that SNps prepared from reduction of sodium thiosulphate using PJ leaves extract are spherical in shape. The larger size of SNps may be due to the agglomeration of the particles with phytochemicals present in the PJ leaves extract which act as caping and stabilizing agent for SNps[22].

FTIR Analysis: FTIR spectrum of prosopis juliflora leaves extract (Figure 3a) shows a broad band of absorption peak strongly at 3398 cm^{-1} attributed to the stretching vibration of -NH and –OH functional groups in amino and hydroxyl compounds involved in hydrogen bonding[16]. The peaks at 2922 $cm^{-1}$and 2845 cm^{-1} may be due to the asymmetric and symmetric stretching of C-H bond in methyl or methylene functional groups present in the alkyl chain. In FTIR analysis, PJ leaves extract also shows a characteristics absorption peak at 1654 cm^{-1} corresponding to the stretching vibration of -C=O bond of amide. An absorption band corresponding to N-O stretching vibration of nitro compounds appears at 1570 cm^{-1} in FTIR spectrum of PJ leaves.

The band for scissoring vibration of methylene groups in protein chain of the plant extract is present at 1418 cm ^{1}. A sharp peak at 1327 cm^{-1} may be due to stretching vibration of C-O bond in carboxylic group. A strong peak at 1240 cm^{1} can be assigned to C-O vibrations [9]. The sharp band at 1038 cm^{1} can be assigned to C-N bond in amine. In addition to that, the peaks at 843 cm^{-1} and 549 cm-^{1} are due to bending modes vibration of aromatic compounds[18].

Figure 3b shows the FTIR spectrum of the sulphur nanoparticles synthesized using leaves extract of prosopis juliflora plant. FTIR spectra of SNPs indicate formation of chemical bonds on the surface of sulphur nanoparticles and phytochemicals present in the propsis juliflora leaves extract. Distinguishing FTIR peaks of aqueous extract of prosopsis juliflora leaves have been presented in FTIR spectra of SNps with little shift in frequency of absorption. Thus, it could be recommended that functional groups such as carbonyl, amine, alcoholic group in aqueous extract of prosopis juliflora leaves caping the surface of SNps help to stabilize synthesized SNps without aggregation[19].

Particles determination by DLS method: Size of SNps had been determined by using DLS particle size analyzer (Figure 4). Histogram shows average size SNps as 227.0 nm. The polydispersity index (PDI) value is found to be 0.513. The sulphur nanoparticles are highly monodispersed in water and used as solvent to take DLS histogram.

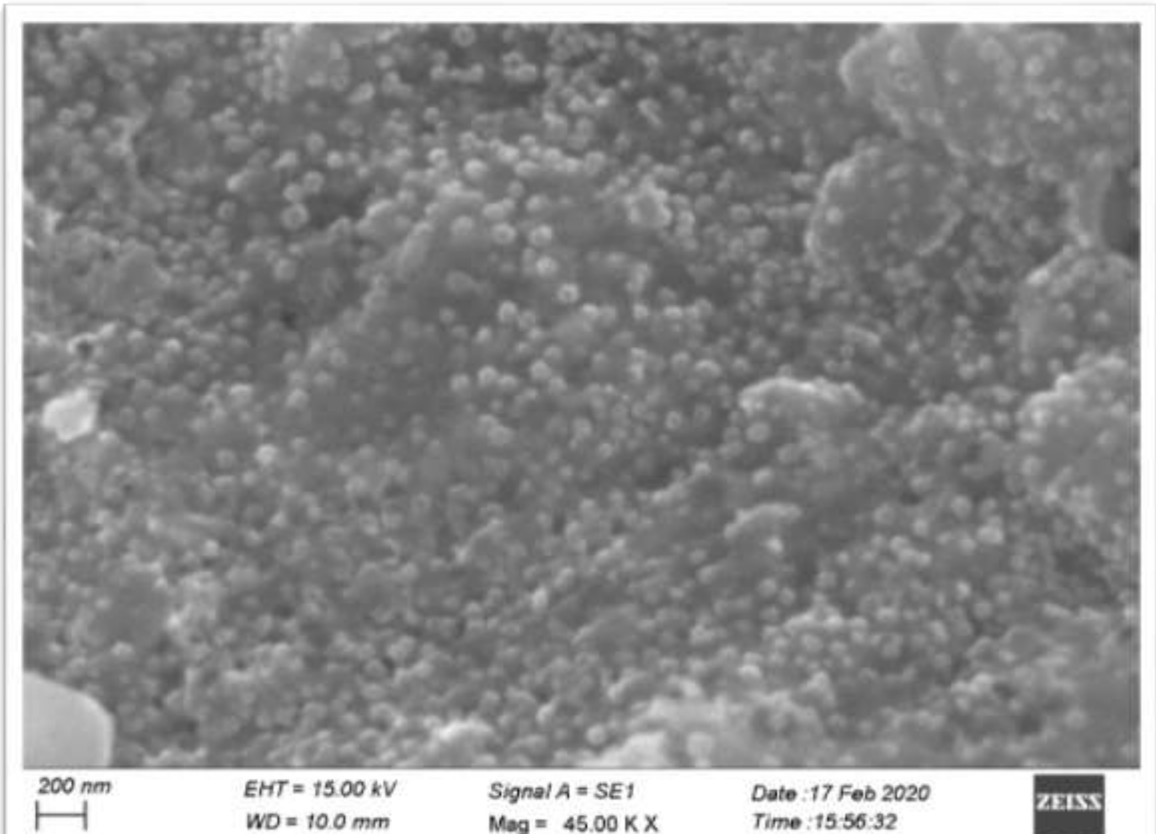

Figure 2: SEM photo of SNps synthesized using extract of PJ leaves

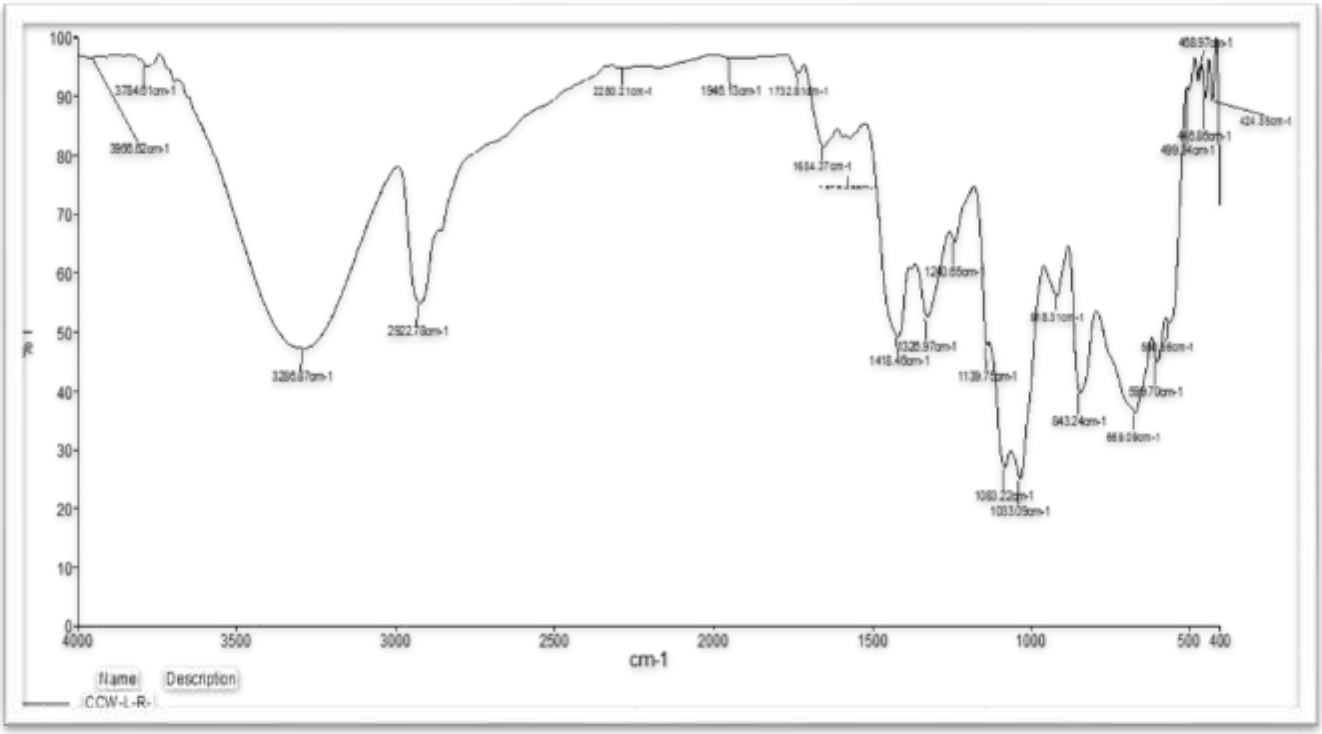

Figure 3a: FTIR spectrum of aqueous extract of PJ leaves

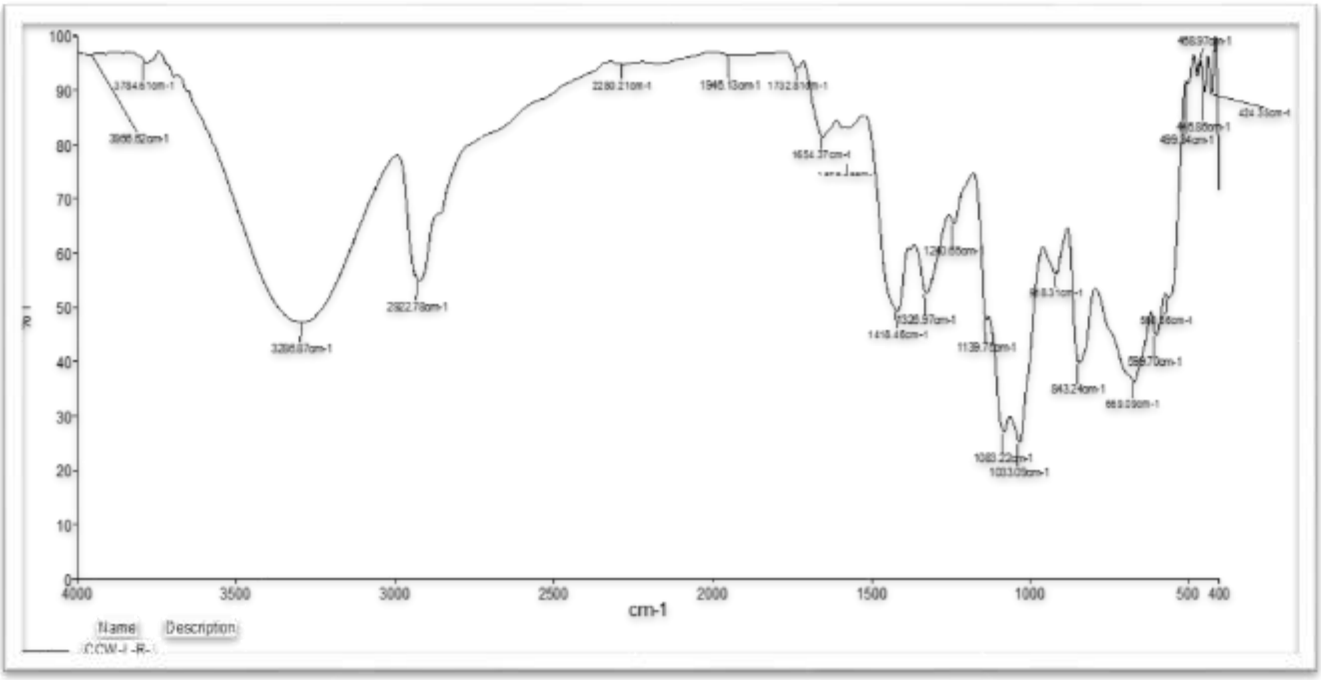

Figure 3b: FTIR spectrum of SNps synthesized using extract of PJ leaves

Characterization using X-ray diffraction analysis: Figure 5 shows X-ray diffraction pattern of SNps synthesis from aqueous extract of PJ plant leaves. Figure 5 shows peaks at 15.25, 21.65, 22.90, 27.87, 28.87 and 29.62 corresponding to the SNps crystal plans of 113, 220, 222, 040, 311 and 044 respectively. Hence, SNps may have orthorhombic crystal structure. These values agree with Joint Committee on Powder Diffraction, standard (JCPDS). The position and intensities of the diffraction peaks were compared with standard sulphur particle diffraction pattern (JCPD). The undispersed peaks in X-ray diffractogram may be due to organic biomolecules present in prosopis juliflora leaves extract. Average particle size of SNps has been calculated using Debye-Scherrer equation from the data obtained in X-ray diffractogram and found to be 152.03 nm [16-18].

Figure 4: DLS histogram of SNps synthesized using extract of PJ leaves

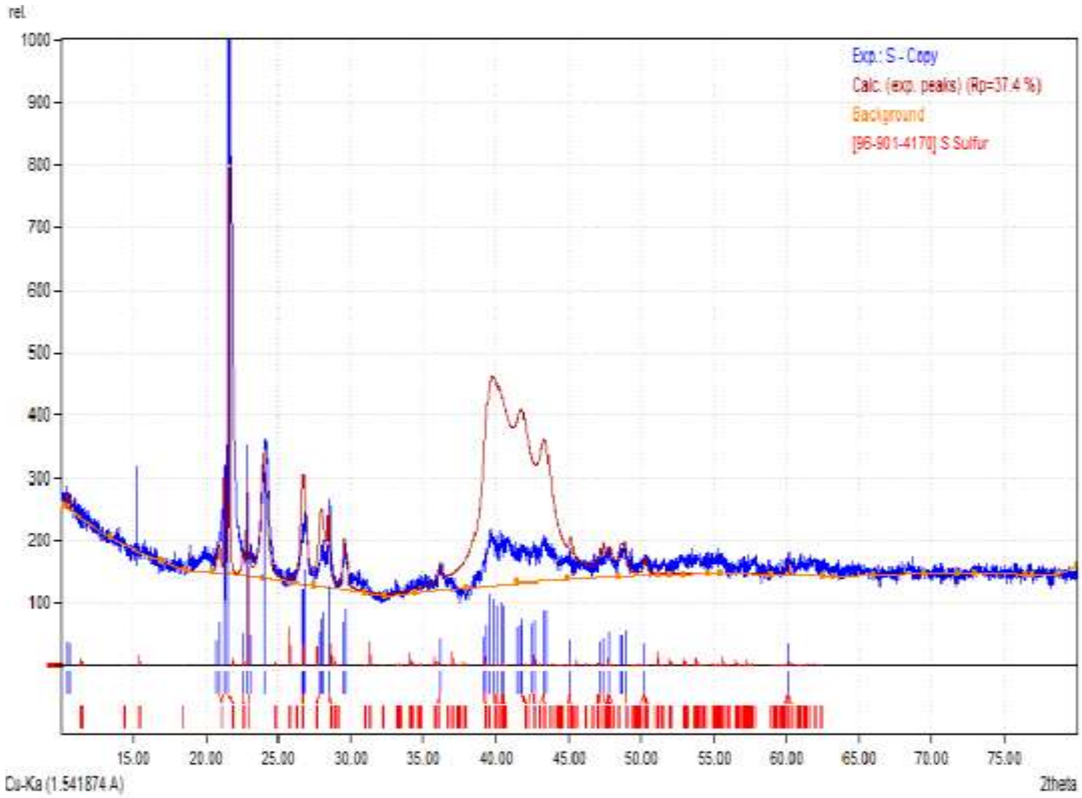

Figure 5: X-ray diffraction pattern of SNps synthesized using extract of PJ leaves

Antibacterial activity of synthesized nanoparticles: Antibacterial effect of SNps against gram negative bacteria (*E.Coli*) and gram positive bacteria (*S.aureus*) is examined by exposing the disc containing 10µl of the sulphur nanoparticles of concentration 200 ppm in aqueous solution for 24 h. The antibacterial efficiency of SNps against *E. coli* and *S. aureus* has been shown in the fig. 6. The results revealed sulphur nanoparticles synthesized in presence of aqueous extract of PJ leaves inhibiting the growth of both the microbes *E.Coli* and *S. aureus* effectively. Hence, SNps synthesized in this method can be used as antibacterial agents[19].

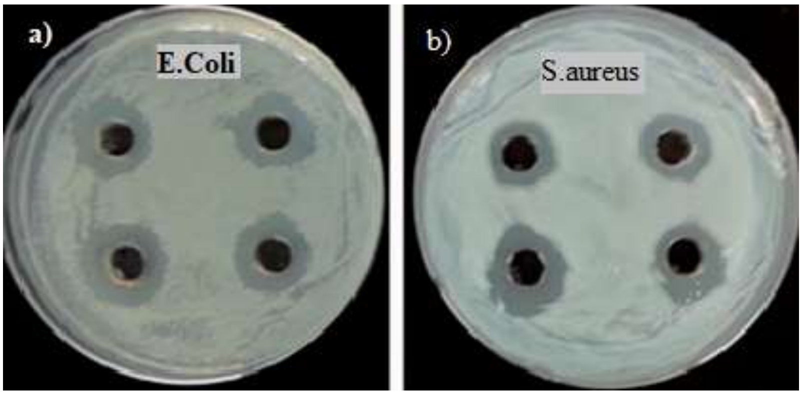

Figure 6: Anti-bacterial activity of SNps synthesized using extract of PJ leaves

Conclusion

A novel approach for green synthesis of sulphur nanoparticles by using prosopis juliflora leaf extract has been reported. This gives highly crystalline pure sulphur nanoparticles with uniform shape and average particle size of 50-200 nm. The phytochemicals present in prosopis juliflora leaf extract play important role in the green synthesis of sulphur nanoparticles. XRD, SEM and FTIR analysis support the formation and stability of the biosynthesized SNPs. The sulphur nanoparticles had an antibacterial activity against bacterial pathogens.

Therefore, prosopis juliflora leaf extract can be used as simple, cost effective and environmentally friendly biomaterial acting as stabilizing and dispersing agent during synthesis of SNPs with antibacterial activity. Hence, sulphur nanoparticles synthesized using prosopis juliflora leaves extract can be used as antibacterial agents.

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