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Research Article

Antibacterials Effect of Tulsi (*Ocimum sanctum*) Powder Nanoparticle and its Scientific Evaluation by Employing Modern Scientific Tools

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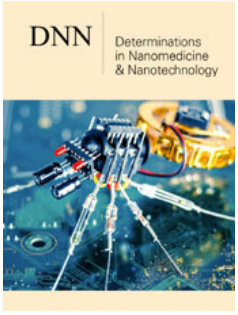

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Abstract

In recent years, nanotechnology has facilitated the development of Tulsi-based nanomedicine. This study explored the importance of Tulsi leaves Nano powder in traditional medicine and nanomedicine and its profound impact on the human body. Tulsi, also known as Holy Basil, has been recognized for its diverse therapeutic properties, including anti-inflammatory, antioxidant, and antimicrobial effects. Integrating traditional medicinal knowledge with cutting-edge nanotechnology has opened new avenues for advancing healthcare. Fresh Tulsi leaves were collected and ground using a mortar and pestle to obtain a coarse powder. The coarse powder was further ball milled for 25 hours in an ethanol medium using a high-energy ball mill method. XRD analysis revealed the crystal structure and the nanoparticle's estimated crystallite size was 21nm. The average particle size was approximately 160nm, calculated from the FE-SEM micrograph. The elements present in the Tulsi powder were estimated with the help of EDS. The antimicrobial activity of processed nanomedicine has enhanced due increase surface area of Tulsi nanoparticles and had a positive surface charge.

Keywords: Tulsi; Ayurvedic nanomedicine; XRD; FESEM; EDS; FTIR; Antimicrobial test

Introduction

Tulsi powder is a form of Ayurvedic medicine that involves the preparation of metallic and mineral substances through calcination or heating to a high temperature. The process of calcination is believed to transform the elemental composition and physical properties of substances, resulting in a medicinal powder that can be used to treat a variety of ailments [1]. The chemical constituents of medicinal plants are important because they can affect the therapeutic properties and potential toxicity of the preparation. The calcination process can alter the elemental composition and physical properties of substances, resulting in the formation of new chemical compounds and changes in the chemical structure of the original substance. These changes can impact the bioavailability, efficacy and safety of the final medicinal product. For example, ayurvedic metals such as iron, copper and zinc are believed to have anti-inflammatory and immune-boosting properties [2]. However, if the Bhasma is not prepared properly or contains toxic impurities, it may also have harmful side effects. To ensure the safety and efficacy of Bhasma, it is important to monitor and control the calcination process carefully and to test the purity, chemical composition and potential toxicity of the final product. Modern scientific tools have been employed to estimate chemical compositions. In Ayurvedic medicine, Tulsi powder treats various health conditions, including respiratory problems, digestive issues, skin disorders and infections. It is also used as a natural remedy for stress and anxiety and is believed to have a calming effect on the mind and body. Recent scientific studies have confirmed many of the traditional uses of Tulsi powder [3] (Figure 1).

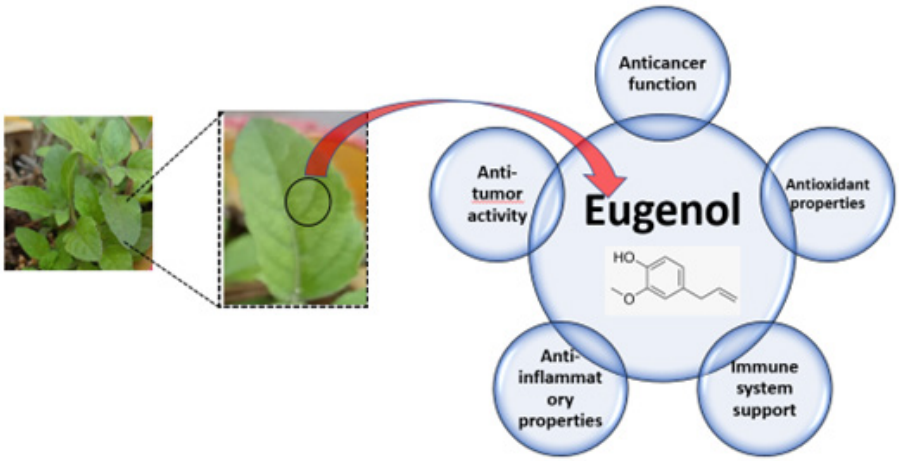

Figure 1: Photographs of Tulsi plants and leaves and the pharmacological effects of eugenol include.

It can be consumed in various forms, such as in tea or as a supplement [4] and is believed to have numerous health benefits, including its potential as a natural cancer treatment. A literature survey on the use of Tulsi in medicine revealed a wealth of information about its medicinal properties, active compounds and potential therapeutic applications. In 2023, Han Jiang et al. [5] synthesized cobalt nanoparticles with the help of Tulsi. They have explored its applications in wastewater purifications and biomedicals. The Tulsi-mediated Co nanoparticles exhibit strong anti-inflammatory properties, particularly at a 250µg/mL concentration. They also demonstrate potential anti-cancer properties against MDA-MB-231 human breast cancer cells, as evidenced by the MTT assay [5]. In 2011, Mondal S et al. [6] studied the immunomodulatory effects of Tulsi leaf extract in human subjects. This study provides insights into how Tulsi may modulate the immune system, making it valuable in the context of immune-related disorders [6]. In 2013, Baliga MS et al. [7] explored the potential anticancer properties of Tulsi and its phytochemical constituents. The authors discussed the mechanisms through which Tulsi compounds may prevent and treat cancer, making it a subject of interest in cancer research [7]. In 2014, Cohen MM [1] wrote a review article that provides a comprehensive overview of the medicinal properties of Tulsi, including its antimicrobial, anti-inflammatory, and adaptogenic effects. It also discusses Tulsi's role in stress management and its potential therapeutic applications in various health conditions [8]. Tulsi powder has been studied for its potential role in cancer treatment, and some studies have suggested that it may be effective in inhibiting the growth and spread of cancer cells [3]. In 2014, Cohen [1] studied Tulsi leaf powder and found that it could inhibit the growth of breast cancer cells in vitro or in a laboratory setting. It was also found that Tulsi leaf powder extract may induce apoptosis, or programmed cell death, in cancer cells [5]. In 2014, Cohen [1] wrote a brief review paper that provides a comprehensive overview of the medicinal properties of Tulsi, highlighting its diverse uses in traditional medicine. Its antimicrobial, anti-inflammatory, adaptogenic, and stress-reducing properties have been studied [9]. According to the literature, Tulsi powder has been used as ayurvedic medicine since ancient times for curing various diseases. More research is needed to explore chemical constituents and their physical properties using modern scientific tools. In this article, we studied the use of Tulsi powder in nanomedicine using modern scientific tools and its antimicrobial activity.

Materials and Methods

The fresh Tulsi leaves were collected and washed carefully under cool running water and then with sterilized distilled water. Tulsi leaves were spread in a single layer, ensuring they were not overcrowded, as shown in Figure 2. The leaves were then dried for 10 days, and the dried leaves were homogenized to a coarse powder using a mortar. The coarse powder was further ball milled for 25 hours in an ethanol medium using a high-energy ball mill method. The filtered extract was centrifuged to separate the liquid phase from any remaining solid particles. The solvent was removed from the nanoscale Tulsi extract using a vacuum evaporator. The obtained Tulsi Nano powder was stored in airtight containers for further processing [1,10]. Fifty grams of each leaf powder sample was measured and added to a separate conical flask. Then, 250 milliliters of methanol were added to each conical flask. The contents of each flask were mixed thoroughly to ensure proper extraction of the constituents from the leaves into methanol. After the cold maceration process, the mixtures were filtered to remove any solid particles or debris, obtaining a clear solution. The resulting solution was a stock solution with a 0.2g/mL concentration. This means that for every milliliter of the stock solution, there was 0.2 grams of extracted material from the leaves. The calculated volumes of the Tulsi stock solutions were measured for each desired concentration (0.2g/mL, 0.3g/mL, 0.4g/mL, 0.5g/mL, 0.6g/mL, and 0.7g/mL). If the desired final volume was 100mL, an appropriate solvent (such as methanol) was added to reach the desired volume while maintaining the desired concentration. Two bacterial cultures were used to evaluate the antimicrobial activity: *E. coli* (MTCC40) and *S. aureus* (MTCC740).

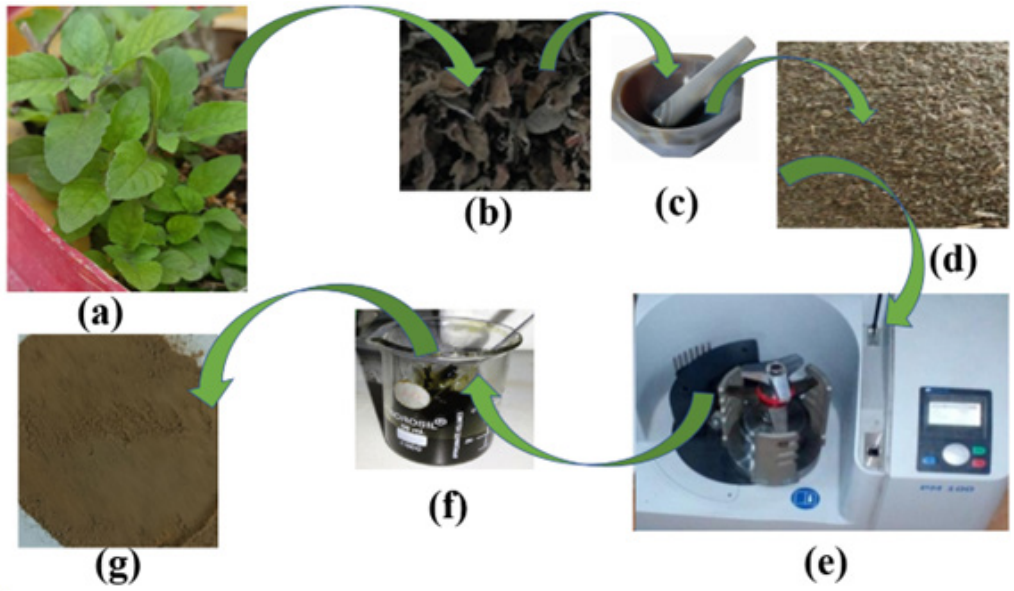

Figure 2: (a-g). Tulsi powder stepwise preparation process.

Results and Discussion

X-ray diffraction analysis

X-Ray Diffraction (XRD) analysis is a powerful tool used to determine the crystalline structure and composition of a material [11]. Tulsi powder, also known as Holy basil powder, is derived from the leaves of the *Ocimum sanctum* plant and has been used for medicinal purposes in Ayurvedic medicine for centuries. XRD analysis of Tulsi powder can provide valuable information about the crystal structure and composition of the powder. The analysis involved bombarding the sample with X-rays and measuring the angle at which the crystal lattice of the sample diffracted the X-rays. This produces a diffraction pattern that can be used to determine the arrangement of atoms in the crystal lattice.

The XRD pattern was recorded using a Bruker D8 Advance X-ray diffractometer with CuKα radiation at a standard voltage and current of 40kV and 40mA, respectively. The scanning rate was 2° per minute in the range of 10°-80°, as shown in Figure 3. The crystallite size of the Tulsi powder was calculated using Debye-Scherer's formula, as shown in Equation 1 [12,13].

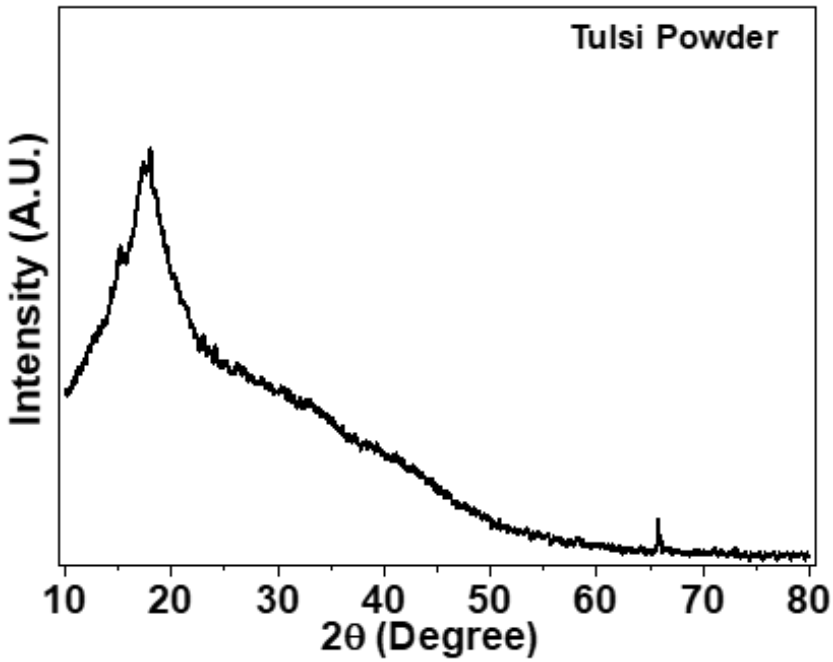

Figure 3: X-ray diffraction pattern of Tulsi powder.

$$D = K\lambda / \beta cos\theta \quad (1)$$

where D represents the crystallite size, K is the Scherrer constant, λ is the wavelength of the X-rays used (in nm), β is the full width at half maximum (FWHM) of the peak in radians, and θ is the Bragg angle in degrees [14]. The average crystallite size was calculated to be approximately 21nm.

Morphology analysis

An FE-SEM micrograph and the corresponding particle size distribution of the Tulsi powder are shown in Figure 4. The average particle size was 160nm. Although the particle sizes are not uniform, the particles are uniformly distributed in the Tulsi powder. It should be noted that while the particle sizes are not uniform, the particles are uniformly distributed throughout the powder [15].

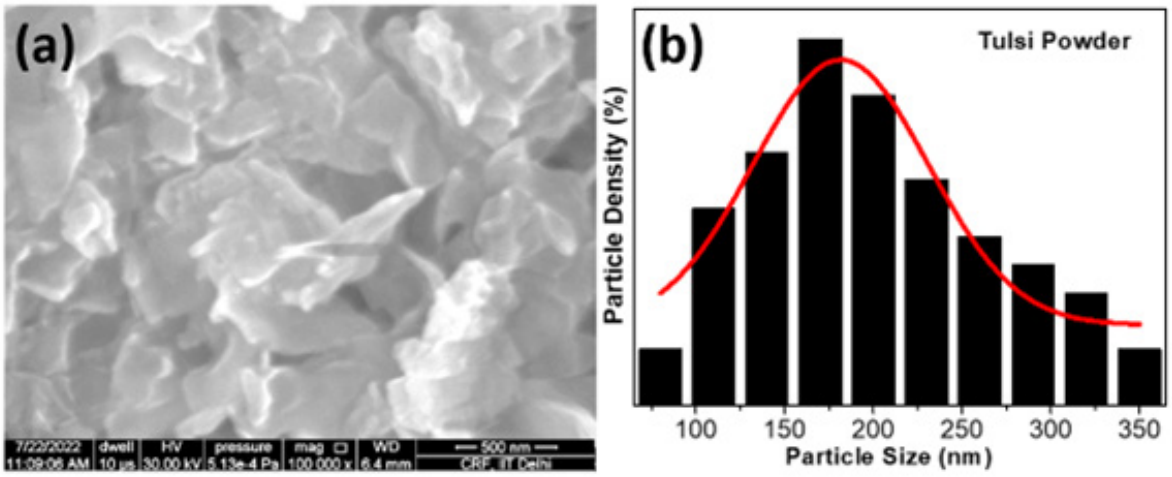

Figure 4: (a) FE-SEM micrograph and (b) histogram corresponding to the particle size distribution of Tulsi powder.

The particle size distribution analysis of the Tulsi powder showed a range from 70nm to 200nm. This indicates that the majority of particles fall within this size range, although there may be a few outliers with larger or smaller sizes. The FE-SEM micrograph provides a visual representation of the Tulsi powder, allowing for the observation of its particle size distribution and the overall distribution of particles within the powder [16]. The average particle size of 160nm suggests the presence of nanoscale particles in the Tulsi powder, which may have implications for its physicochemical properties and potential applications. The FE-SEM micrograph and corresponding particle size distribution analysis offer valuable insights into the size characteristics and distribution of particles in Tulsi powder, contributing to a better understanding of its physical properties and potential applications in various fields. The difference in size between the particle size (160nm) and the crystallite size (21nm) estimated by FESEM and XRD, respectively, suggested that the observed particles were composed of smaller crystalline domains or nanoparticles. The particles may have agglomerated or aggregated together, forming larger clusters while maintaining their individual crystalline structure [17].

Energy Dispersive X-Ray Spectroscopy (EDS) microanalysis

The chemical composition of the Tulsi powder used for medicinal purposes in Ayurveda is of utmost importance because it may not contain any harmful elements. Energy Dispersive X-ray Spectroscopy (EDS) microanalysis was used to investigate the chemical composition of different parts of the sample [18], as illustrated in Figure 5. EDS analysis revealed the presence of various elements, including carbon (C), oxygen (O), sodium (Na), magnesium (Mg), silicon (Si), sulfur (S) and chlorine (Cl). The concentrations of these elements observed in the sample are provided in Table 1.

Table 1: Weight % and atomic % of all the elements present in the Tulsi powder.

Element	Weight%	Atomic%
C K	51.54	59.17
O K	46.22	39.83
Na K	0.15	0.09
Mg K	0.19	0.11
Si K	0.93	0.46
S K	0.11	0.05
Cl K	0.07	0.03
K K	0.74	0.26
Cu K	0.05	0.01
Totals	100	

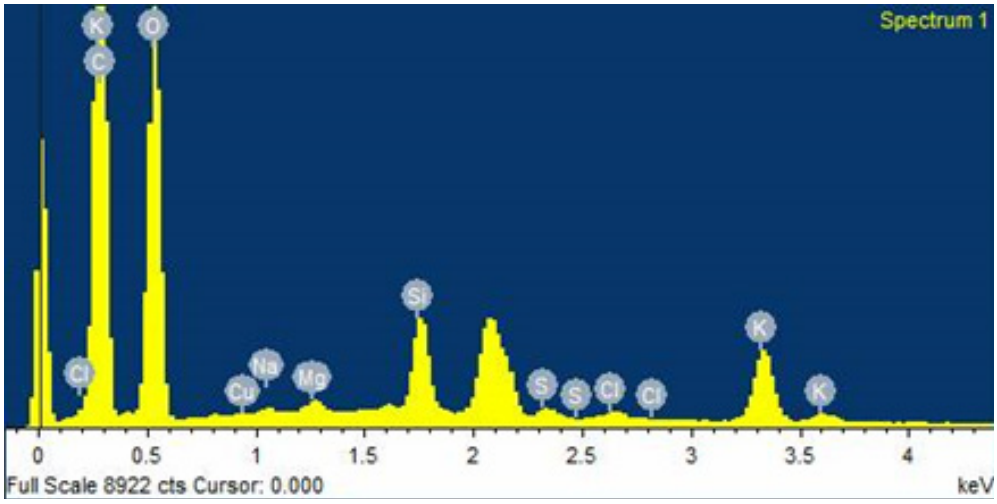

Figure 5: EDS image of Tulsi powder

EDS microanalysis of the Tulsi powder allows for a quantitative assessment of the elemental composition of the sample. Table 1 shows the concentrations of carbon, oxygen, sodium, magnesium, silicon, sulfur, and chlorine in the Tulsi powder. The absence of harmful elements and the presence of desirable elements at appropriate concentrations are essential considerations for ensuring the quality and safety of Tulsi powder used in Ayurvedic medicine [18]. These EDS microanalysis findings contribute to the understanding of the chemical composition of Tulsi powder and its suitability for medicinal purposes.

FTIR analysis

FTIR analysis of Tulsi powder may provide valuable insights into its molecular composition, functional groups, and chemical properties, contributing to a better understanding of its potential medicinal applications in Ayurveda. The mixture of Tulsi powder and KBr was then compressed under high pressure to form a pellet. The prepared pellet was placed in the sample holder of the FTIR spectrometer [19]. The spectrometer emits infrared radiation, typically over a range of wavenumbers, which passes through the sample. The interaction of the infrared radiation with the sample results in the absorption or transmission of specific wavelengths, generating an infrared spectrum. The obtained spectrum was analyzed to identify the characteristic peaks and functional groups present in the Tulsi powder. The peaks are compared to reference spectra or databases to determine the chemical components and structural features of the sample [20].

Fourier Transform Infrared (FTIR) analysis of the Tulsi powder provided valuable information about its molecular composition and functional groups. By analyzing the infrared spectrum, characteristic peaks can be identified, allowing for the identification and characterization of various organic compounds present in the Tulsi powder. FTIR analysis of Tulsi powder typically revealed peaks corresponding to different functional groups, such as hydroxyl groups (OH), carbonyl groups (C=O), aromatic compounds, aliphatic compounds, and various other bonds [21]. The FTIR spectrum of Tulsi powder typically consists of a range of wavenumbers, typically from 400cm to 4000cm, as shown in Figure 6. The peaks observed in the spectrum may be assigned to specific functional groups based on their characteristic frequencies. For example, the presence of a peak at approximately 3300-3500cm^{-1} indicates the presence of hydroxyl (OH) groups, while peaks in the region of 1650-1750cm^{-1} are indicative of carbonyl (C=O) groups [22,23]. The FTIR absorption peaks of specific chemical bonds, such as C-H stretching, C-O stretching, or C-C stretching, and the corresponding vibrational modes are listed in Table 2. By analysing the FTIR spectrum of Tulsi powder, valuable information can be obtained about its chemical composition, allowing for the identification and characterization of important organic compounds present in the powder. This information can contribute to a better understanding of the medicinal properties and potential applications of Tulsi in Ayurvedic medicine.

Table 2: Wavenumbers of the corresponding functional groups and vibrational modes determined by FTIR analysis of Tulsi powder [22].

Wavenumber (cm^{-1})	Appearance	Compound Name	Vibrational modes
478.32	Medium	Alkyl halides	C-Cl
582.16	Strong	Halogen compound	C-H
766.55	Medium	Halogen compound	C – H bending
924.98	Strong	Alkene	C = C Bending
1029.49	Strong	Ether	S = O stretching
1084.082	Strong	Alkyl amine	

1148.652	Strong	Ether	C-O
1213.222	Medium		C – O stretching
1422.242	Medium		C–O/C–H bending
1640.581	Medium	Amine	C = C stretching
2097.895	Medium	Silicon compounds	
2858.088	Medium	Alkene	C – H stretching
3419.246	Strong	Alcohol	O – H stretching

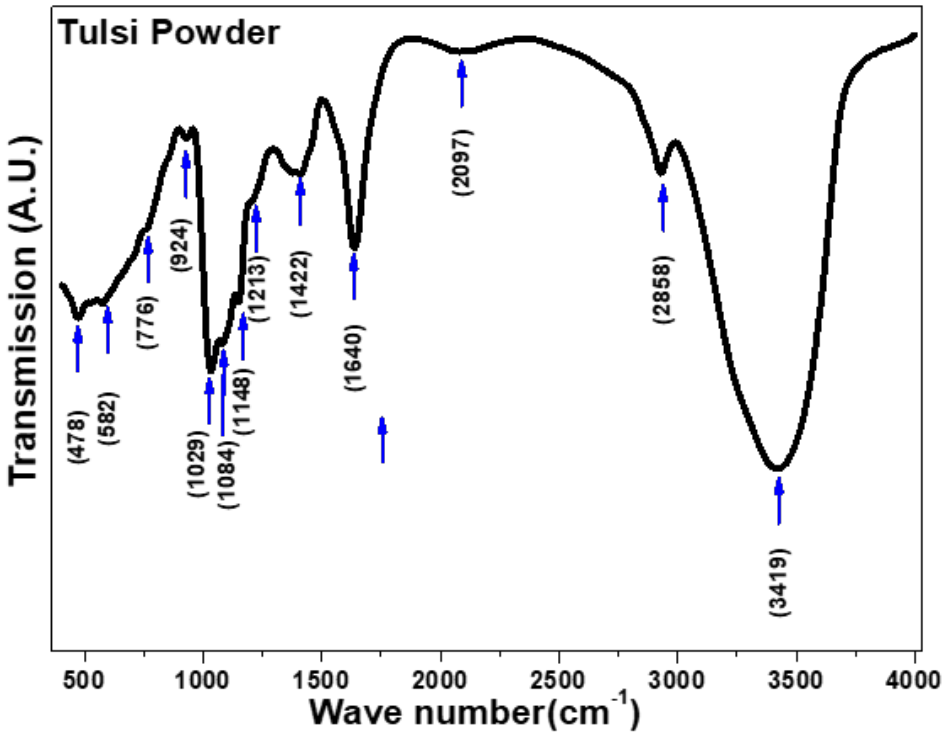

Figure 6: FTIR spectrum of Tulsi powder.

Antimicrobial activity

The Tulsi powder extract was prepared by mixing a specific weight or volume with a suitable solvent. Typical solvents used for extraction include water, ethanol, or methanol [24]. The concentration of the extract may depend on the desired test concentration. Different microorganisms may be chosen for assessing the antimicrobial activity of Tulsi powder (Figure 7).

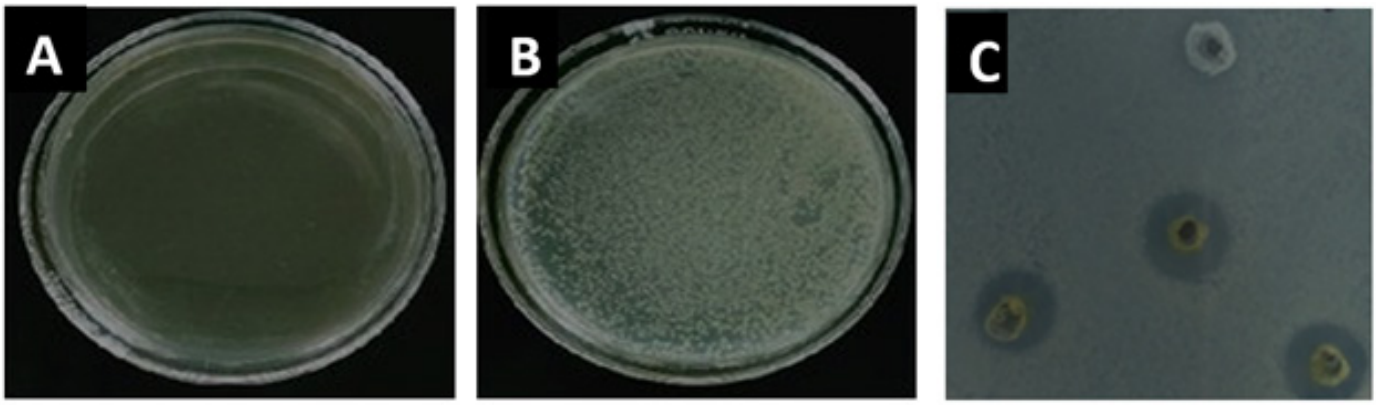

Figure 7: Antimicrobial activity of Tulsi powder: (a) agar plate, (b) test microorganism, and (c) zones indicating the degree of inhibition or growth inhibition caused by the Tulsi powder extract.

As discussed, in the present study, *Staphylococcus aureus* and *E. coli* were used as model organisms to assess the antimicrobial activity of the Tulsi extract [25]. *Staphylococcus aureus* and *E. coli* were cultivated in appropriate growth media until they reached the desired growth phase. The microbial density was then adjusted to achieve the required inoculum size. This can be determined by measuring the optical density or performing a viable count (colony-forming units/mL) using agar plates [26-31]. The antimicrobial susceptibility of the selected microorganisms was determined using methods such as agar well diffusion or disc diffusion. In these methods, agar plates are inoculated with the test microorganism, and wells are created on the agar. The Tulsi powder extract was added to the wells, and the plates were incubated under optimal conditions for the specific microorganism being tested.

As above discussed in material and method section of this article, nutrient agar plates were prepared and inoculated with the specific microorganisms mentioned earlier using the spread plate technique. Nutrient agar plates were prepared according to the standard protocol. The plates were inoculated by spreading the bacterial inoculum evenly on the surface of the agar using a sterile spreader. The wells on the agar plate were created using a sterile punch, resulting in wells with a diameter of 6mm. These wells serve as the sites where the extracts will be introduced. Tulsi powder extracts were prepared at various concentrations (0.2g/ml, 0.3g/ml, 0.4g/ml, 0.5g/ml, 0.6g/ml, and 0.7g/ml), as mentioned earlier. Using a micropipette, 50 µl of the Tulsi powder extract of each concentration was transferred to separate wells on agar plates [32,33]. The extract was allowed to diffuse into the agar medium by incubating the plates at 37 °C for 24 hours. During this incubation period, the antimicrobial compounds present in the Tulsi extracts diffused into the surrounding agar. After incubation, the plates were observed, and Zones of Inhibition (ZOIs) were identified around the wells. The ZOIs appeared as clear circular regions where bacterial growth was inhibited due to the antimicrobial activity of the Tulsi extract. The diameter of the ZOIs was measured using a Vernier scale or a ruler with millimeter markings. The widest point of the clear zone was measured, and the diameter was recorded in millimeters. The observed Zone of Inhibition (ZOI) provided an indication of the effectiveness of the extracts in inhibiting the growth of microorganisms [34,35]. The larger the diameter of the ZOI was, the stronger the antimicrobial activity of the extract against the tested microorganisms. Statistical analysis was performed to evaluate the significance of the results, as shown in Figure 8.

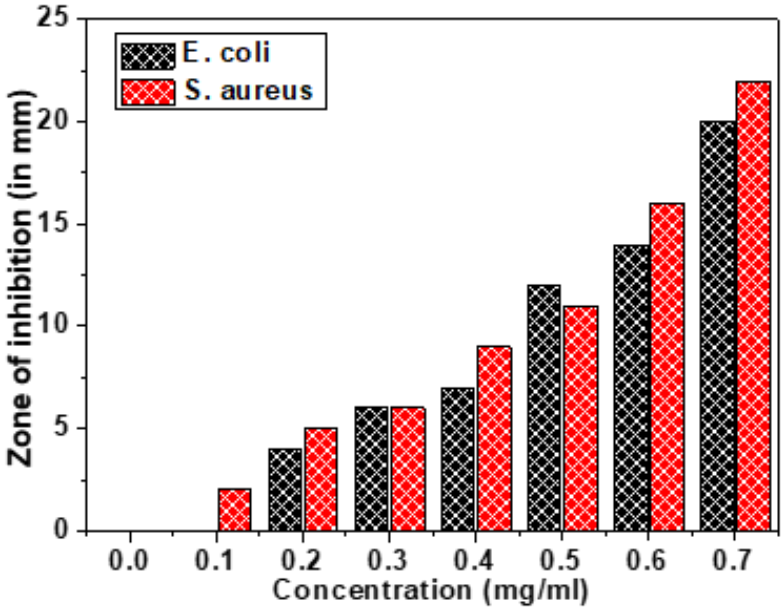

Figure 8: These zones (mm) indicate the degree of inhibition or growth inhibition caused by the Tulsi powder extract.

Table 3 compares current work on Tulsi nanoparticle nanomedicine antimicrobial activity with traditional Tulsi powder medicine. The abrupt enhancement of antimicrobial activity of Tulsi nanoparticle due increase in surface area. The antimicrobial study of Tulsi powder nanoparticles shows effectiveness on bacteria and, may be useful to control the bacterial infection disease.

Table 3: Comparison of antimicrobial activity of Tulsi powder present study with literature.

Plant Name	Extract Solvent. (µ)L	Diameter of the Inhibitory Zone (mm)		References
		E. coli	*S. aures*	
Tulsi (*Ocimum Sanctum*)	100	19.12	14.23	[9]
	100	11	10	[10]
	100	16	18	[11]
	100	21	22	This study

Discussion

X-Ray Diffraction of the Tulsi powder showed its semicrystalline nature, with an average crystallite size in the nanometer range. FESEM micrograph images of the Tulsi powder revealed a close-packed microstructure with average particle 150nm. The chemical composition estimated by EDX of the Tulsi powder such as Carbon (C), oxygen (O), sodium (Na), magnesium (Mg), silicon (Si), sulfur (S), chlorine (Cl) and no trace of any harmful elements. The combination of these elements gives rise to various functional groups listed in Table 2. The Minimum Inhibitory Concentration (MIC) for both microorganisms was 0.3g/ml in the antimicrobial test. Tulsi powder extract was shown to have a more powerful effect on *S. aureus* than on *E. coli*.

Conclusion

Modern scientific research tools have provided insights into the physical properties of Tulsi nanoparticles as well as their medicinal properties. XRD analysis of the Tulsi nanoparticles revealed their crystalline phases and average crystal size of 21nm. The FESEM micrograph showed that the surface morphology of the Tulsi powder had a uniform particle distribution throughout the sample. The average particle size is 160nm. EDS detected and quantified the elements present in the Tulsi powder, namely, carbon (C), oxygen (O), sodium (Na), magnesium (Mg), silicon (Si), sulfur (S), and chlorine (Cl). Harmful elements are not detected in the EDX spectrum. FTIR analysis of Tulsi can help identify various compounds, such as phenols, flavonoids, terpenes, and other organic molecules, which contribute to its medicinal properties. Hence, the present experimental results on Tulsi powder can help Ayurvedic doctors in the treatment of different diseases. The abrupt enhancement of antimicrobial activity of Tulsi nanoparticle due increase in surface area. The antimicrobial study of Tulsi powder nanoparticles shows effectiveness on bacteria and, may be useful to control the bacterial infection disease.

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