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

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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 nanopowder 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 21 nm. The average particle size was approximately 160 nm, 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

I. 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].

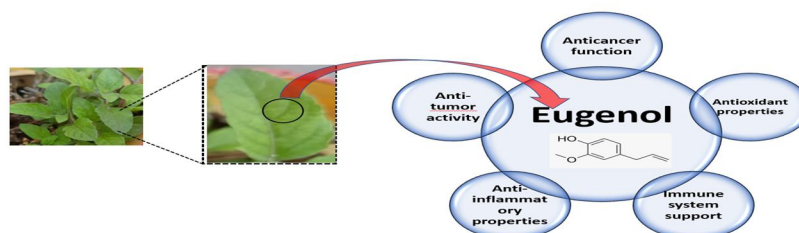


Fig. 1. Photographs of tulsi plants and leaves and the pharmacological effects of eugenol include.

It can be consumed in various forms, such as in tea or as a supplement [4-5], 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 have investigated the tulsi powder in nanoform. They have explored its applications in waste water purifications and In 2011, Mondal, S., et al. 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 [7]. In 2013, Baliga, M. S., et al. 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 [8]. In 2014, Cohen, M. M. 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 [9]. 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 [5]. In 2013, Marc Maurice Cohen 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 [6]. In 2014, Marc Maurice Cohen 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 [10]. 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.

II. 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 nanopowder powder was stored in airtight containers for further processing [11-12].

Fifty grams of each leaf powder sample was measured and added to a separate conical flask. Then, 250 millilitres 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 0.2 g/mL concentration. This means that for every millilitre 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 (0.2 g/mL, 0.3 g/mL, 0.4 g/mL, 0.5 g/mL, 0.6 g/mL, and 0.7 g/mL). If the desired final volume was 100 mL, 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).

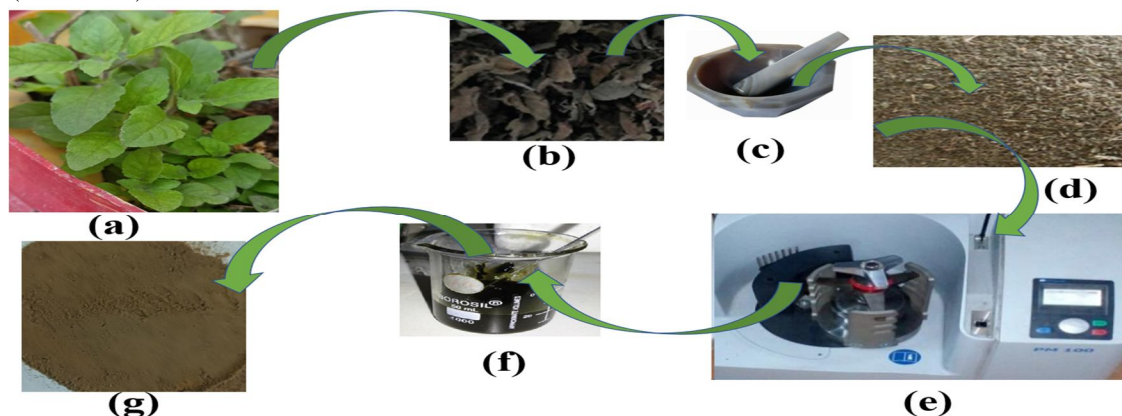


Fig. 2 (a-g). Tulsi powder stepwise preparation process.

III. RESULTS AND DISCUSSION

A. X-ray Diffraction Analysis

X-ray diffraction (XRD) analysis is a powerful tool used to determine the crystalline structure and composition of a material [13]. 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.

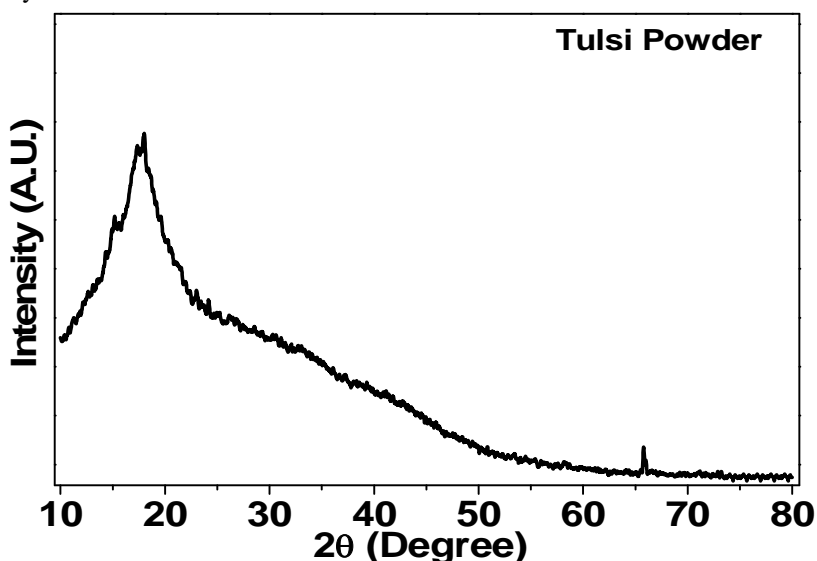


Fig. 3. X-ray diffraction pattern of Tulsi powder.

The XRD pattern was recorded using a Bruker D8 Advance X-ray diffractometer with CuK_{α} radiation at a standard voltage and current of 40 kV and 40 mA, 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-Scherrer's formula, as shown in Equation 1 [14-15].

$$D = K\lambda/\beta\cos\theta \quad \text{-----}1$$

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 [16]. The average crystallite size was calculated to be approximately 21 nm.

B. 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 160 nm. 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 [17].

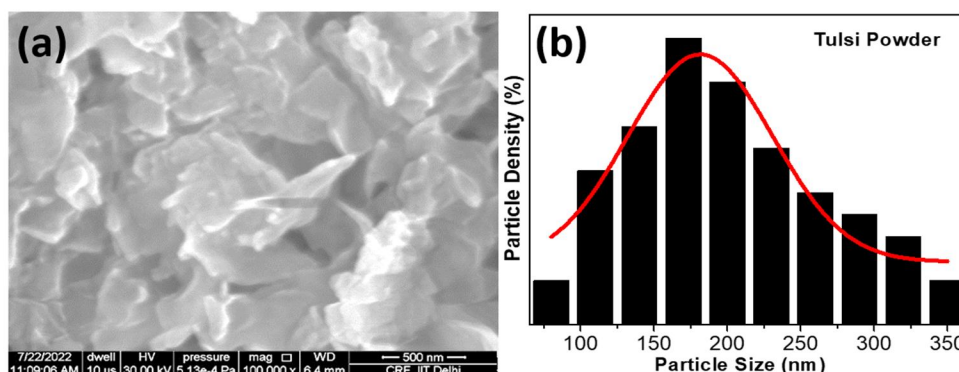


Figure 4. (a) FE-SEM micrograph and (b) histogram corresponding to the particle size distribution of Tulsi powder.

The particle size distribution analysis of the Tulsi powder showed a range from 70 nm to 200 nm. 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 [18]. The average particle size of 160 nm 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 (160 nm) and the crystallite size (21 nm) 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 [19].

C. 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 [20], 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 I.

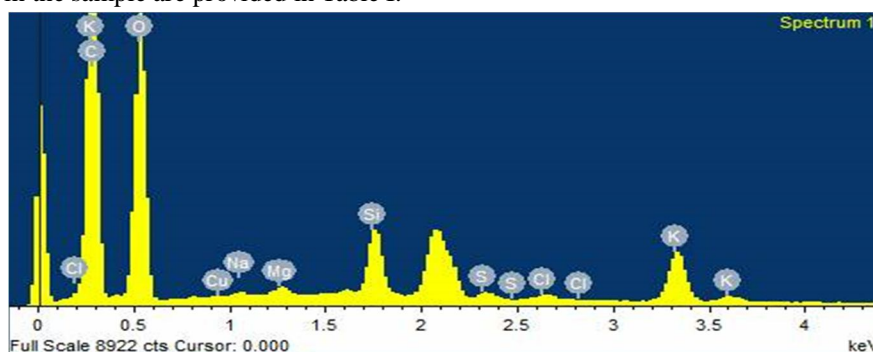


Figure 5. EDS image of Tulsi powder.

EDS microanalysis of the Tulsi powder allows for a quantitative assessment of the elemental composition of the sample. Table I 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 [20]. These EDS microanalysis findings contribute to the understanding of the chemical composition of Tulsi powder and its suitability for medicinal purposes.

Table I: 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	100.00	

D. FTIR Analysis

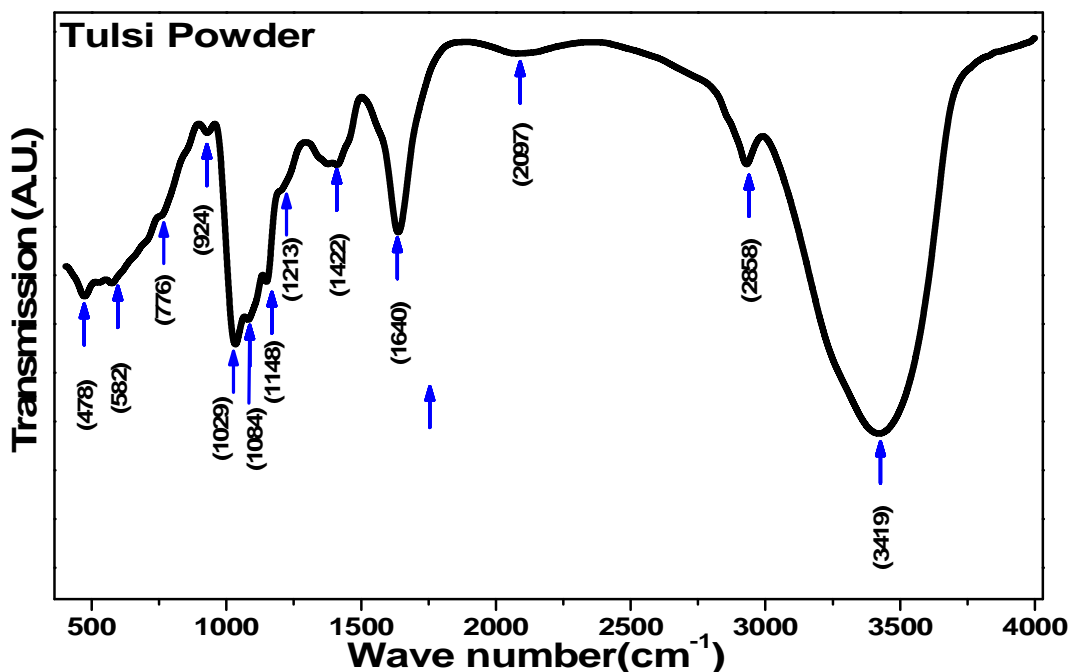


Figure 6. FTIR spectrum of Tulsi powder.

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. [21]. 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 analysed 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 [22].

Table II: Wavenumbers of the corresponding functional groups and vibrational modes determined by FTIR analysis of tulsi powder [24]

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		C-O
1213.222	Medium	Ether	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

Fourier transform infrared (FTIR) analysis of the Tulsi powder provided valuable information about its molecular composition and functional groups. By analysing 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 [23]. The FTIR spectrum of Tulsi powder typically consists of a range of wavenumbers, typically from 400 cm to 4000 cm, 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 3300-3500 cm⁻¹ indicates the presence of hydroxyl (OH) groups, while peaks in the region of 1650-1750 cm⁻¹ are indicative of carbonyl (C=O) groups [25]. 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 II. 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.

E. 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 [26]. 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.

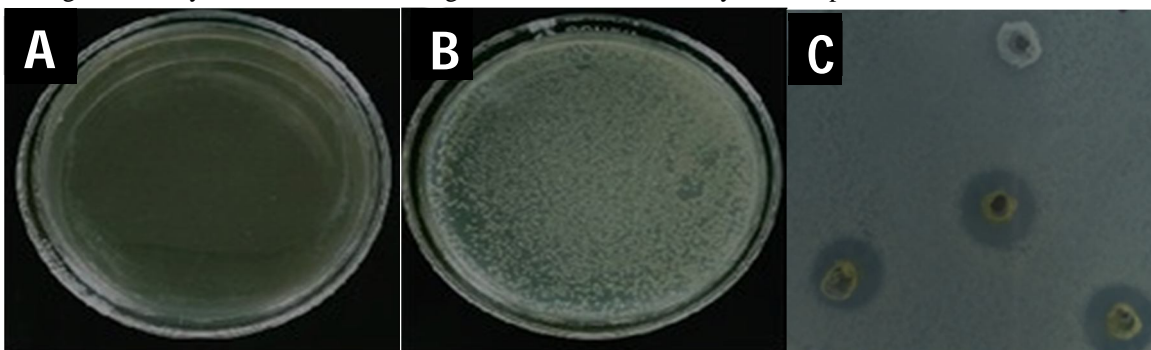


Fig. 7. Antimicrobial activity of Tulsi powder: (a) agar plate, (b) test microorganism, and (c) zones indicating the degree of inhibition or growth inhibition caused by the Tulsi powder extract.

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 [27]. *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 [28-33]. 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.

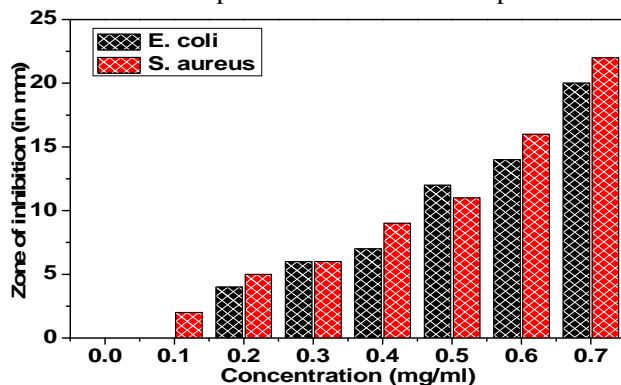


Fig. 8. These zones (mm) indicate the degree of inhibition or growth inhibition caused by the Tulsi powder extract.

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 6 mm. These wells serve as the sites where the extracts will be introduced. Tulsi powder extracts were prepared at various concentrations (0.2 g/ml, 0.3 g/ml, 0.4 g/ml, 0.5 g/ml, 0.6 g/ml, and 0.7 g/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 [34, 35]. 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 [36-38]. 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.

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

Plant Name	Extract solvent. (µL)	Diameter of the inhibitory Zone (mm)		References
		E. coli	S. aures	
		Tulsi (Ocimum Sanctum)	100	
	100	11	10	[10]
	100	16	18	[11]
	100	21	22	This study

Table III 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.

IV. DISCUSSION

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

V. 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 21 nm. 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 160 nm. 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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Nil.

B. Conflicts of Interest

There are no conflicts of interest.

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