RESEARCH ARTICLE

Green Synthesis of Zinc Nanoparticles using Medicinal Plant Extract

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ABSTRACT
Azoospermia is a common problem that leads to the diagnosis of infertility in males. This is a global problem, treatments or aids such as therapies related to hormone-related surgeries, but the results are very low scale or the results are temporary. This enhances the urgency for safe, effective, and accessible medicines and treatments. Zinc is a vital micronutrient element needed for testis maturation, spermatogenesis, hormone related control. In the last few years, zinc nanoparticles have grasped the attention for their control of absorption rate and precise effort compared to formal zinc addition. green production of zinc nanoparticles utilizing medicinal plants extract ion gives unharmed and nature-friendly techniques, which also provides growth. Current research states that zinc nanoparticles, while this also amplifies the biological effect due to the proximity of the bioactive compounds. In this current research, zinc nanoparticles are combined with fenugreek seeds, fenugreek leaves, pumpkin seeds, and shilajit. Fenugreek has the presence of diosgenin, alkaloids, and antioxidants that play a major role in male reproductive health. pumpkin seeds accord zinc, omega fatty acids, and phytosterols, which guard the testes and enhance the hormonal balance. The mixture of fenugreek seeds and pumpkin seeds gives both nutritional and antioxidant uphold possibly offering collaboration advantage for the formation of sperm (spermatogenesis). Zinc nanoparticles amongst these phytopharmaceuticals may generate a global benefit fulfilled by decreasing oxidative stress, modulating reproductive hormones such as luteinizing hormones and follicle-stimulating hormone, enhancing sperm cell growth, and guarding the testicular tissues. This kind of pharmaceutical vision, nanomedicine access could open a rising prospect in coping with azoospermia while enduring cost-effective, biocompatibility, and sustainable futuristic animal studies and clinical research will be important to authenticate these outcomes.

Keywords: Azoospermia, Medicinal Plants, Reproductive Hormones, Zinc, Nanoparticles
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INTRODUCTION

Nanotechnology is an interdisciplinary field that involves the manipulation, study, and application of matter on the nanometer scale, typically defined as structures between 1 and 100 nanometers (nm) (Garg, 2021). At this scale, materials often exhibit properties very different from their bulk counterparts, due to quantum mechanical effects and a very high surface-to-volume ratio (Britannica, 2025). These distinct behaviors open up possibilities for creating novel materials, devices, and systems with tailored electrical, optical, mechanical, and chemical properties (Picraux, Britannica, 2025). Historically, the conceptual roots of nanotechnology can be traced back to physicist Richard Feynman's seminal lecture, "There's Plenty of Room at the Bottom" (1959), in which he mused on the possibility of manipulating individual atoms (Garg, 2021; Wikipedia, n.d.). However, the term "nanotechnology" was formally coined much later, and the field began to crystallize during the 1980s and 1990s with the invention of critical experimental tools such as the scanning tunneling microscope and the discovery of fullerenes (Molecules, 2020; Wikipedia, n.d.). These advances enabled scientists to directly image and manipulate matter at the atomic scale, giving birth to modern nanoscience (Tolochko, 2021). Nanotechnology research spans a broad spectrum of disciplines, including physics, chemistry, biology, materials science, and engineering (Milojević, 2012). This multidisciplinary nature reflects how nanoscale phenomena do not belong to a single traditional domain; instead, they emerge from the convergence of multiple fields. For instance, materials scientists design nanocomposites with enhanced strength, while biologists explore nanoparticle interactions with cells, and engineers develop nanosensors and nanoelectromechanical systems (Picraux, Britannica, 2025; Milojević, 2012). One of the most promising areas of nanotechnology application is medicine and healthcare. Nanoparticles can be engineered to deliver drugs with high precision, targeting specific tissues or cells while minimizing side effects (Huang et al., 2004). In addition, nanoscale diagnostic devices—such as biosensors and quantum dots—enable early detection of diseases, improving diagnostic accuracy and patient outcomes (Picraux, Britannica, 2025). The capacity to control matter at the molecular level also opens up opportunities for regenerative medicine, imaging, and even molecular machines. Beyond biomedicine, nanotechnology holds

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transformative potential in energy, environment, and manufacturing. Nanostructured materials contribute to more efficient solar cells, lightweight yet strong composites, and advanced coatings that resist wear and corrosion (Padiya et al., 2013). Furthermore, nanomaterials are being developed for water purification, pollution control, and environmental remediation—offering sustainable solutions to some of the most pressing global challenges (Britannica, 2025; Garg, 2021).Despite its promise, nanotechnology also raises important challenges and risks. The very properties that make nanoparticles useful—such as their small size and reactivity—can lead to toxicity, unpredictable biological interactions, and environmental impacts (Picraux, Britannica, 2025). Regulatory frameworks are still evolving, and researchers must carefully assess the safety, ethical, and societal implications of nanotechnology (Gupta, 2024). Addressing these challenges is critical to ensuring that the benefits of nanotechnology can be realized in a responsible and sustainable way. A recent generation that with the continuation of nanotechnology development, integration with biotechnology, artificial intelligence (AI) and innovative manufacturing introduces smart nanomaterials is emerging. Such hybrid systems can sense the current conditions of the surrounding and react to the stimuli and also the active evolution of their characteristics (Gupta, 2024). Predicting the nanoparticles behavior of a nanoparticle can now be done in an accurate manner through AI assisted modelling and this has made the process of coming up with new nanostructure to use in biomedical, industrial and environmental purposes much easier. It is such an interdisciplinary fusion that is giving rise to the age of intelligent nanotechnology that can make decisions independently at molecular scale. Nanotechnology has revolutionized electronics, agricultural and textile sciences, as far as the industries are concerned. To further the frontiers of computational speed and energy efficiency, the semiconductor industries started to resort to nanoscale transistors and quantum dots (Padiya et al., 2013). Nanoparticles provide water resistance and UV protection, antimicrobial protection as well as reducing comfort and breathability in the textile industry. In the farming sector, nanofertilizers and nanopesticides enhance delivery of nutrients and are washed into the runoff, which will be part of the sustainable measures in agriculture (Garg, 2021). All these factors point to the fact that nanotechnology has heavily penetrated the industries. However, the scalding of the nanotechnology market demonstrates the severe need for the proper application of ethical checks and norming tests, and policies. The harmfulness of the nanoparticle, the long-term environmental filtration, and the lack of understanding of how humans are exposed to them are also part of the leading issues of concern to the entire globe (Picraux, Britannica, 2025). The science institutions and international bodies are developing the guidelines to evaluate the safety of A nanoparticles, responsible innovation, and to make sure that the technological advances are going to adopt the same line with the environmental and population-health priorities (Gupta, 2024). Once made, such structures will be very important to the safe, scalable, and equitable application of nanotechnology to all of the world.

Nanotechnology has become one of the most revolutionary scientific disciplines in the 21st century, with new solutions found in medicine, agriculture, materials science, and environmental engineering. One of the biggest improvements in this domain concerns the creation of metal-based nanoparticles because of their peculiar physicochemical characteristics on a programmable scale (Khan et al., 2019). The zinc oxide (ZnO) and zinc-based nanoparticles have a very significant place concerning their biocompatibility and stability, as well as their widespread usage. The green synthesis of nanoparticles has become an extremely popular alternative method of nanoparticle production in recent years as a promising approach towards a more sustainable production of nanoparticles compared to the traditional chemical and physical methods. Conventional synthesis tends to make use of hazardous reducing agents, temperatures, and toxic solvents that cause environmental and biological hazards (Iravani, 2011).

In green nanotechnology, on the other hand, natural plant extracts, microbes, or biomolecules are used, and they serve as reducing, capping and stabilizing agents at the same time. This reduces toxicity in addition to enhancing the efficiency and biocompatibility of nanoparticles. Zinc acetate is a highly utilized precursor substance in the green production of zinc oxide nanoparticles because it is both soluble and commercially feasible and can give highly pure nanostructures (Abdelmoneim et al., 2020). Zinc acetate can be reduced under a controlled condition in the presence of plant extract phytochemicals to produce nanoparticles with desirable morphology and improved functionality properties. These green-synthesized nanoparticles are also likely to be more stable, smaller, and high in biological activity. The importance of plant extracts in the green synthesis is critical in the formation of nanoparticles. The processes that regulate the particle size and shape of particles and distribution are bioactive compounds that include polyphenols, flavonoids, alkaloids, terpenoids, and proteins, which can reduce and capping (Ahmed et al., 2016). Both have been shown to stay on the surface of the nanoparticle giving them other antioxidant, antimicrobial and anti-inflammatory functions. Consequently, the biosynthesized nanoparticles are superior to chemically synthesized. Nanoparticles made through green methods based on zinc use have amazing antimicrobial, antioxidant, anticancer, and photocatalytic characteristics (Singh et al., 2018). Their high antimicrobial potential has rendered them very useful in bio-medical uses such as in wound healing, drug delivery and antibacterial surfaces. The smaller size of the nanoparticles at the nanoscale enables higher reactivity and surface area and thus, enables zinc nanoparticles to penetrate microbial membranes more easily. Green-synthesized zinc nanoparticles can also be useful in green chemistry in the environmental sector to purify water, eliminate contaminants, and get rid of poisonous dyes (Hameed et al., 2020). Their ability to decompose harmful organic substances in the presence of UV or sunlight is facilitated by their photocatalytic ability, which helps in supporting the use of environmentally friendly technologies of treating wastewater. These properties can be used to prove the environmental appropriateness and efficiency of zinc nanoparticles synthesized greenly. The other area to offer promise to zinc nanoparticles synthesized by zinc acetate is in agriculture. They increase the deliverability of nutrients, enhance soil health, increase the germination and growth of seeds and plants (Rizwan et al., 2019). It is due to their gradual release nature and concentration delivery of the nutrients that nano-fertilizers cause less loss of nutrients and prevent environmental contamination, unlike conventional fertilizers. Therefore, zinc nanoparticles produced in a green way can be used as a sustainable instrument in the precision agriculture of the contemporary world. Green synthesis is also in line with cost-effective, eco-friendly, and scalable production methods, which is one of the biggest advantages of green synthesis. Instead of using high-pressure reactors and the use of toxic chemicals, plant-mediated nanoparticles synthesis does not require them, which is why it can be utilized at a large-scale industrial level. In addition, the method is easy, which is why it can be reproducible, the energy consumption is limited, and the amount of waste is decreased (Iravani, 2011). Although these have their benefits, a number of challenges still lie in the field. There is also the lack of expressional stability in plant metabolites, the absence of homogenous synthesis protocols, and the lack of control of morphology, which does not always lead to uniform nanoparticles (Ahmed et al., 2016). Also, the pathway of the nanoparticle formation during green synthesis has not been fully understood yet and requires additional research that is entailed by combining analytical and biochemical methods. All in all, the green synthesis of zinc nanoparticles with zinc acetate is a prospective area of research that should allow filling the gaps between sustainable chemistry and developed nanotechnology. It is environmentally friendly, low in preparation costs, and has strengthened functionalities, which make it suitable for various applications in the field of healthcare, industry and agriculture. Continued studies are focused on the optimization of the synthesis parameters, the description of the nanostructures in a more appropriate manner, and their application in new scientific fields.

MATERIALS AND METHODS

Phytochemical Screening

Fenugreek Seeds

Another key step in bioactive compound identification to help in enhancing the male reproductive system is the screening of fenugreek (Trigonella foenum-graecum) seeds with phytochemicals. The use of fenugreek seeds is an ancient tradition to promote vitality and fertility, and, according to scientific evidence, its effects cannot be explained by the absence of antioxidant and hormone-regulating phytochemicals. Diluted ethanol is an effective extraction solvent, giving the possibility to recover not only polar but also moderately non-polar compounds of interest to male infertility (Srinivasan, 2006; Basch et al., 2003). Collection of plant material is the first stage in phytochemical screening. Fenugreek seeds are washed using pure water, and then dust and other impurities are wiped off. Shade-drying of the seeds is followed by their fine-grinding by use of a mechanical grinder to obtain fine powdered seeds to ensure that the bioactive compounds are preserved from heat-sensitive compounds. The surface area is enhanced in the fine powder, and this makes them more penetrative and solvent-based during extraction (Harborne, 1998; Kokate, 2005). In the extraction process, the solvent that is chosen is diluted ethanol (70-80) due to the solubility of alkaloids, flavonoids, phenolics, saponins, and glycosides. Ethanol is too relatively safe, and it is widely utilized in reproduction and pharmacology studies. This is an ideal solvent to use in extracting antioxidant compounds that are related to the prevention of oxidative stress-induced infertility in males (Pandey and Tripathi, 2014; Srinivasan, 2006). Using the maceration method, a known amount of fenugreek seed powder is immersed in diluted ethanol. The mixture is stored in an airtight vessel and swirled periodically within 48-72 hours at room temperature. This process enables slow but effective diffusion of bioactive substances into the solvent without destroying phytochemicals of supportive fertility (Sofowora, 1993; Evans, 2009). The mixture is filtered under the muslin cloth and then Whatman No. 1 filter paper to allow the elimination of solid residues. A rotary evaporator or water bath at a controlled temperature is used to create the filtrate, and the excess solvent is then evaporated. This concentrated ethanolic extract is kept at 4 deg C pending additional phytochemical assays (Harborne, 1998; Kokate, 2005). The initial qualitative analysis in the phytochemical screening is a qualitative analysis of alkaloids, whereby reagents like Mayer reagent, Wagner reagent, and Dragendorff reagent have been used.When a cream or reddish- brown precipitate forms, it is an indication that there are alkaloids. The alkaloid trigonelline in the seeds of fenugreek alters metabolic regulation and indirectly aids the spermatogenesis of infertile males (Gupta et al., 2001; Yadav and Baquer, 2014). The detection of flavonoids is performed by the use of the alkaline reagent test and lead acetate tests. The flavonoid is confirmed by the presence of a yellow-coloured colour upon the addition of acid. They are essential in alleviating oxidative stresses in the sperm cell and enhancing sperm motility, viability, and membrane integrity of the sperm in the conditions of male infertility (Dixit et al., 2005; Agarwal et al., 2014). Phenolic compounds are observed under ferric chloride and lead acetate tests, to give a deep blue or green color. Fenugreek seeds are effective antioxidants that have phenolics in their chemical composition. They are necessary to inhibit lipid peroxidation and oxidative DNA damage of testicular tissues related to male infertility malpractices (Srinivasan, 2006; Sharma et al., 2016). Saponin screening is conducted with the aid of the foam test, in which the formation of persistent foam is a positive result. The saponins of fenugreek seed are especially those that are referred to as steroidal saponins, like diosgenin. They have been identified as having an influence on the testosterone synthesis and hormonal regulation that is necessary to support normal spermatogenesis and male reproductive function (Yadav and Baquer, 2014; Ramasamy et al., 2019). Tannins are identified in terms of ferric chloride or gelatin tests, and a dark green or precipitate structure is obtained. Tannins are antioxidants and antimicrobial which aid in the preservation of the integrity of reproductive tissues. Their existence backs the effectual action of the fenugreek seed extract in the protection of infertility caused by inflammation (Trease and Evans, 2009; Sofowora, 1993). One uses the Keller-Killani and Borntrager tests to verify the presence of glycosids.

Glycosides increase the bioavailability and stability of active phytochemicals in the biological systems. Glycosides have been shown to aid in the prolonged release of fertility-enhancing compounds to enhance the therapeutic efficacy of the glycoside use in male infertility research (Kokate, 2005; Evans, 2009). The terpenoids are determined by utilizing the Salkowski test, in which a reddish-brown interface is a positive result. Terpenoids contain anti-inflammatory and immunomodulatory effects to promote a healthy testicular environment. This is especially necessary in infertility cases being linked to chronic inflammation (Srinivasan, 2006; Abarikwu et al., 2020). Each of the qualitative phytochemical methods provides initial evidence of the presence of certain bioactive groups in the ethanolic extract. Though these tests do not measure the compounds, they are necessary in the process of correlating chemical composition and biological effects that are observed. These correlations will be useful in studying the role of fenugreek seeds as fertility-enhancing supplements (Harborne, 1998; Pandey and Tripathi, 2014). Considering the infertility in men, multiple phytochemicals are found in fenugreek seeds, and this indicates that they might act synergistically. The protection of the sperm dodges oxidative harm, hormonal regulations are carried out through saponins, and the DNA structural integrity is preserved with the help of phenolics. This is a multi-targeted intervention, which is especially useful in complicated cases like oligozoospermia and non-obstructive azoospermia (Agarwal et al., 2014; Sharma et al., 2016). Summing up, the screening of fenugreek seeds with diluted ethanol demonstrates that the given plant contains such significant bioactive substances as alkaloids, flavonoids, phenolics, saponins, tannin, glycosides, and terpenoids. These substances work together in the antioxidant defense system, hormonal levels, and cell protection, which are essential to male reproduction. This screening is a scientific basis of further experimental research to assess the use of fenugreek seed extract as a natural therapeutic agent in male infertility treatment (Srinivasan, 2006; Yadav & Baquer, 2014).

Fenugreek Leaves

The screening of fenugreek leaf in the form of phytochemical investigation is a task of critical importance in gaining insight into the therapeutic potential of leaves, especially when reproductive biology is investigated. Fenugreek leaves contain abundant natural bioactive compounds that could affect spermatogenesis, hormonal homeostasis, and the antioxidant defense system. In azoospermia, where oxidative stress and damaged testicular

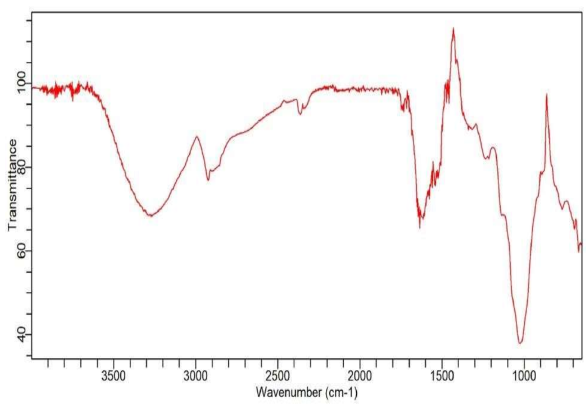

Figure 1. FTIR Peak analysis of fenugreek seeds crude extract

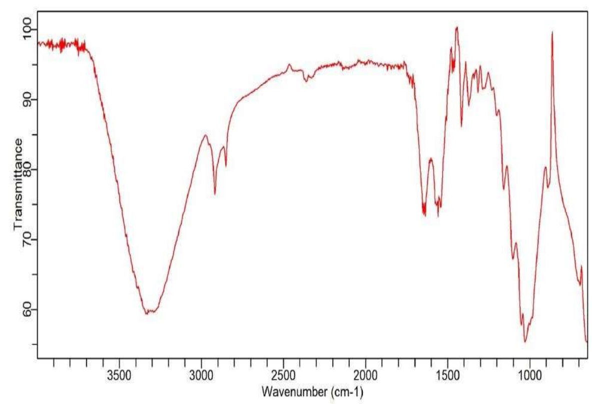

Figure 2. FTIR Peak analysis for fenugreek seeds zinc nanoparticle

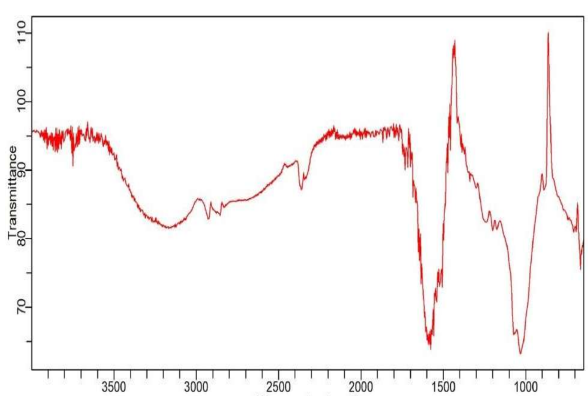

Figure 3. FTIR Peak analysis for fenugreek leaves crude extract

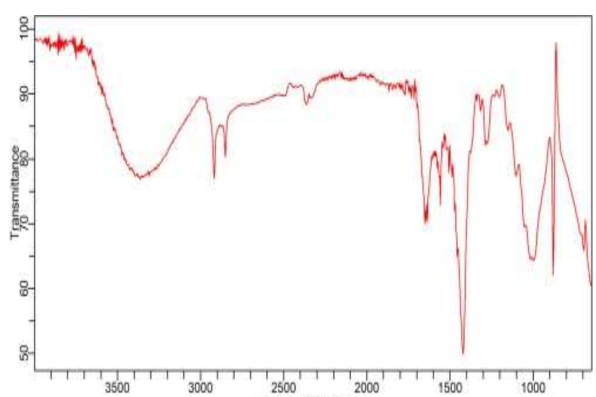

Figure 4. FTIR Peak analysis for fenugreek leaves zinc nanoparticle

functionality are prevalent, the identification of these phytochemicals will offer scientific rationale for their use as support treatment (Srinivasan, 2006; Agarwal et al., 2014). Fenugreek leaves were chosen in the screening of phytochemicals since a leafy portion of the medicinal plant usually has higher concentrations of antioxidants than the seed. The antioxidants play a significant role in guarding germ cells against oxidation, which is a significant cause of non-obstructive azoospermia. Fenugreek leaf usage is consistent with the findings regarding the importance of plant-based interventions to enhance the state of male reproductive health (Basch et al., 2003; Sharma et al., 2016). Fresh leaves of fenugreek were gathered, washed in a lot of distilled water, and dried in the shade at ambient temperature. Shade drying was used to avoid the degradation of bioactive compounds that are sensitive to heat. Effective drying guarantees the maintenance of phytochemicals that could be used to guarantee testicular protection and enhancement of fertility (Harborne, 1998; Kokate, 2005). The dried leaves were carefully powdered by using a sterile mechanical grinder and kept in air-tight containers. Surface area of plant material is made larger through powdering, thus helping in the penetration of the solvent during extraction. The step is essential in getting a phytochemically enriched extract to be used in research studies concerning reproductive studies (Evans, 2009). Dilution ethanol (700mL ethanol in distilled water) has been chosen as the extraction solvent because the compound is suitable in dissolving polar and moderately non-polar compounds. It is also believed that ethanol is safe, biocompatible, and a broadly used substance in fertility-related phytochemical research works. The given solvent will make sure that it extracts antioxidants of interest to azoospermia models (Harborne, 1998; Pandey & Tripathi, 2014). Fennugreek leaf powder was weighed at its measured amount and soaked in the diluted ethanol in a clean conical flask. Constant agitation was maintained on the mixture in rotary shaker of 24-48 hours at the temperature of the room. Maceration gives ample interaction among the solvent and the plant matrix to facilitate maximum extraction of bioactive compounds (Sofowora, 1993; Kokate, 2005). The mixture was filtered with Whatman No. 1 filter paper after extraction in order to remove plant debris. The transparent filtrate was that of the unrefined ethanolic extract of fenugreek leaves. To get a clean extract that could be used in the qualitative tests of phytochemicals and other biological assessments, filtration is necessary (Evans, 2009). A low temperature rotary evaporator was used to remove surplus solvent in the filtrate. The concentrated extract was frozen at 4 o C before analysis. The concentration is beneficial in increasing the detection of phytochemicals and their retention of biological activity in infertility-related research (Harborne, 1998). The preliminary phytochemical screening was conducted so as to identify the presence of dominant secondary metabolites. The qualitative tests allow obtaining a quick idea about the chemical nature of the extract, as well as correlating the phytochemical composition to the biological effects in the research on azoospermia (Trease and Evans, 2009; Sharma et al., 2016). To examine the presence of alkaloids, Mayer and Wagner reagent were used.

The positive result was obtained due to the formation of a creamy or reddish-brown precipitate. Alkaloids are also reported to control metabolic and cellular activities indirectly to promote spermatogenesis in infertilities (Kokate, 2005; Basch et al., 2003). The alkaline reagent and the lead acetate tests were used to detect the presence of flavonoids. Their presence was demonstrated by the appearance of yellow coloration. Flavonoids are also important as they minimize the effect of oxidative stress on the testicular tissue, and they are the most applicable in the treatment of azoospermia (Dixit et al., 2005; Agarwal et al., 2014). Phenolic compounds were also observed by redundant use of ferric chloride test which gave a dark green or blue color. Phenolics are effective antioxidants, which suppress oxidative damage of the sperm DNA and testicular cells commonly observed in azoospermic men (Srinivasan, 2006; Sharma et al., 2016). Saponins were detected by froth test in which the creation of persistent foam was regarded as the positive game. Saponins are linked to hormonal changes and testosterone levels, which are critical to the normal spermatogenesis (Yadav and Baquer, 2014; Ramasamy et al., 2019). The concluding tannins were tested by gelatin and ferric chloride tests. Tannins also have antimicrobial and antioxidant effects designed to prevent reproductive tissues against inflammation and oxidative damages in cases of infertility (Trease and Evans, 2009; Sofowora, 1993). The identification of terpenoids was done by the Salkowski test and the coloration of the interface was reddish-brown which signified a positive result. Terpenoids also have anti-inflammatory effects and are useful in preserving a healthy microenvironment in the testicles to ensure the survival of germ cells (Harborne, 1998; Abarikwu et al., 2020). Keller-Killani and Borntrager tests were used to identify Glycosides. Their bioavailability and stability in the active compounds are enhanced by the presence of glycosides to promote enduring antioxidant and hormonal activity in azoospermia models (Kokate, 2005; Trease & Evans, 2009). Phytochemical screening results established that ethanol extract of the leaves of fenugreek is diluted with several bioactive compounds of relevance in supporting the fertility of plants. They are justified by the presence of antioxidants and hormone-regulating phytochemicals, which will help in the management of azoospermia caused by oxidative stress (Agarwal et al., 2014; Sharma et al., 2016). Genetic screening of phyto-chemicals is a platform on which advanced testing including FTIR, GC-MS as well as HPLC is done. These methods have the ability to offer structural and quantitative validation of compounds observed in the initial screening. These studies enhance the scientific knowledge of the fenugreek leaves with regard to reproduction studies (Evans, 2009; Pandey and Tripathi, 2014). Applying to the context of azoospermia, the phytochemicals found in the leaves of fenugreek have a hand in the description of their protective properties on the testicular tissues and germ cells. These substances could promote a better spermatogenic response, lessen oxidative stress, and raise hormonal homeostasis when administered as a dietary or experimental agent (Agarwal et al., 2014; Ramasamy et al., 2019).

Bioactive Components

Fenugreek Seeds

The seeds of Fenugreek (Trigonella foenum-graecum) are known to have very wide therapeutic use in traditional medicine, and their therapeutic use by the ancient has been supported by modern scientific studies. Fenugreek can be considered as being largely possessing medicinal properties due to rich phytochemical composition. It was found that ethanol can be used effectively in extracting polar and moderately non-polar compounds, leading to an extract containing biologically active compounds to be used in pharmacological and reproductive research (Srinivasan, 2006; Basch et al., 2003). Trigonelline, an alkaloid that is antidiabetic and antioxidant, is one of the most important bioactive agents that exist in ethanolic extract of fenugreek seeds. Trigonelline contributes to relieving oxidative stress and enhances the metabolism of cells, which is essential especially in the case of disorders related to the imbalance of the metabolite and reproductive dysfunction (Yadav and Baquer, 2014; Gupta et al., 2001). The flavonoids found in the ethanolic extracts of fenugreek seeds are also quercetin, kaempferol, vitexin and isovitexin. These substances are very potent antioxidants; they suppress any free radicals as well as shielding the components of cells against oxidation. It is their antioxidant ability and this ability is important in maintaining reproductive cell integrity and enhances the overall health of the cell (Dixit et al., 2005; Srinivasan, 2006). The other significant group of compounds obtained after the use of ethanol is phenolic acids, including gallic acid, caffeic acid, ferulic acid, and vanillic acid. Such phenolic compounds are the one that add to the free radical scavenging capacity of fenugreek seed extract. They also have antimicrobial and anti-inflammatory effects, in addition to antioxidant activity, which facilitate the formation of tissue protection as well as physiological stability (Billaud et al., 1981; Dixit et al., 2005). The saponins in particular the steroidal saponins feature prominently in the fenugreek seeds and they are removed effectively using ethanol. One of the most prominent among them is diosgenin which is extensively researched regarding its use as a precursor in the production of steroid hormones. These saponins have also been related to enhanced hormonal regulation and complemented spermatogenic activity with a controlled dosage (Srinivasan, 2006; Yadav, & Baquer, 2014). Glycosids (particularly flavonoid and saponin glycosides) are also present in the ethanolic extract and have the effect of increasing the stability and bioavailability of active phytochemicals. These polymers enable a slow release of the bioactive molecules, thus having a prolonged biological action and enhancing the effectiveness of the therapeutic outcome (Acharya et al., 2008; Basch et al., 2003). Fenugreek seed ethanolic extract has moderate amounts of tannins. Tannins are astringent and antimicrobial in nature and prevent oxidative and microbial destruction of tissues. Their existence is an additional indication of the antioxidative defense system of the extract and in favor of its utilization in both the experimental and treatment settings (Billaud et al., 1981; Dixit et al., 2005). Ethanol may also be used to extract some terpenoids in fenugreek seeds. These compounds have been known to have anti- inflammatory and immunomodulatory effects. Terpenoids co-operate with flavonoids and phenolic compounds and increase the ultimate pharmacological enhancing of the extract (Srinivasan, 2006). Despite the presence of large quantities of fixed oils in the form of fenugreek seeds, only little amounts of fatty acids like linoleic acid and oleic acid, are extracted in the presence of ethanol. These fatty acids have been known to cause stability of the membrane and protection of cells even when they occur in low levels and aid normal metabolic and reproductive processes (Acharya et al., 2008). Finally, the ethanolic extract of fenugreek seeds is rich in diverse utilization of bioactive phytochemicals such as alkaloids, flavonoids, phenolics, saponins, glycosides, tannins, terpenoids and fatty acids. The amalgamated and synergistic actions of these compounds generate good antioxidant, anti-inflammatory, antimicrobial, and fertility-promoting actions. This richness in phytochemicals explains why the ethanolic extract of the fenugreek seed has been widely used in these biomedical, pharmacological, and reproductive studies, including male infertility and azoospermia studies (Basch et al., 2003; Yadav and Baquer, 2014).

Fenugreek Leaves

The consumption of fenugreek leaves use as a leafy vegetable intake is listed in traditional medicine as a medical herb and as a female reproductive health enhancer medication, and the most recent scientific research has also pointed out that it can also be used to male reproductive health. Antioxidants, vitamin, and phytochemicals abundant in the leaves and unlike fenugreek seeds, specifically enhance the direct effects of the testicles and sperm quality. These are some of the bioactive substances that assist in overcoming the oxidative stress that is among the main causes of male infertility (Srinivasan, 2006; Basch et al., 2003). Among the most crucial categories of bioactive compounds found in the leaves of fenugreek, there are flavonoids, such as quercetin, kaempferol, luteolin and apigenin. These flavonoids are strong antioxidants which prevent the oxidative damages on sperm cells. As sperm membranes contain a lot of polyunsaturated fatty acids, they are very susceptible to free radicals, and flavonoids can be used to maintain sperm motility and viability (Dixit et al., 2005; Yadav and Baquer, 2014). Phenolic compounds like gallic acid, caffeic acid and ferulic acid are also found in large amount in fenugreek leaves. These phenolics play important part in the antioxidant activity of the leaves and the protective effect against the lipid peroxidation of the testicular tissues. Phenolic compounds enhance normal spermatogenesis by minimizing the effects of oxidative stress, enhance sperm concentration, and morphology (Billaud et al., 1981; Srinivasan, 2006). The saponins are another significant bioactive substance in the leaves of fenugreek although the concentration values of saponins in leaves are lower than the seed contents. These saponin steroids are recognized to interfere with the hormonal balance aiding in production of testosterone. It is also necessary that testosterone levels are adequate to produce typical amounts of sperm, and that saponins play a role in keeping endocrine functions normal in males who have exhibited subfertility (Yadav and Baquer, 2014). Fenugreek leaves are also high in alkaloids, one of them being trigonelline, which has been related to better metabolic and cellular activity. Trigonelline indirectly boosts the male fertility by increasing the glucose metabolism and lowering the oxidative stress which is significantly correlated with the absence of spermatogenesis in infertile men (Gupta et al., 2001; Basch et al., 2003). The vitamins found in the leaves of fenugreek, vitamin C, vitamin A, and folic acid especially are very significant in the male reproductive health. Vitamin C enhances sperm count and motility by supplying the sperm DNA against oxidative damage whereas vitamin A is necessary in the spermatogonial differentiation process. The folic acid aids in the formation of DNA and stability of chromatin in the developing sperms (Srinivasan, 2006). Fenugreek leaves contain also the necessary minerals of zinc, iron, magnesium, and calcium. Zinc plays a role especially in male fertility by being involved in production of testosterone, maturation and stabilization of the sperm chromatin of the male body. Zinc deficiency has been well linked to the decrease in sperm quality and male infertility (Acharya et al., 2008). The terpenoids that are in the fenugreek leaves also increase their capacity to support the fertility. The terpenoids are anti-inflammatory and immunomodulatory agents, which are used to and decrease inflammation in the reproductive tissues. Testicular inflammation may be chronic leading to the impairment of spermatogenesis and the terpenoids assist in maintaining the healthy testicular microenvironment (Srinivasan, 2006). Fenugreek leaves are also anti- stress and adaptogenic which is healthy to the male fertility. Stress may cause adverse impact on the hormonal balance by influencing the hypothalamic-pituitary- gonadal axis, which can be either psychological or physiological. Bioactive components in the leaves of fenugreek can mediate the response to stress and indirectly maintain the normal secretion of reproductive hormones (Yadav & Baquer, 2014). To sum it up, the fenugreek leaves are rich in the variety of bioactive elements, such as flavonoids, phenolics, saponins, alkaloids, vitamins, minerals and terpenoids, which can be used to support the enhancement of male reproductive health. Fenugreek leaves have a high potential as a natural treatment agent in controlling infertility in men through their antioxidant, hormonal, anti- inflammatory, and metabolic regulatory effects. Their properties can be used in models that are designed to undertake experiments, dietary interventions and also complementary to the treatment of infertility related disorders (Basch et al., 2003; Srinivasan, 2006).

Zinc Nanoparticle

Fenugreek Seeds

Fenugreek (Trigonella foenum-graecum L.) is a herb that is an annual plant of the Fabaceae family, and is commonly grown in South Asia, the Mediterranean region as well as part of Africa (Saikia, 2012).

Its trifoliate leaves, small yellowish flowers, and seed which have the typical appearance of hard and rhomboid are also known. Fenugreek is also one of the most comprehensive and researched medicinal plants because of the value of its nutritional, therapeutic, and industrial uses (Saikia, 2012; Revisiting Trigonella foenum-graecum, 2022).The botanical and physicochemical peculiarity of the seeds of fenugreek is that they have a distinct structure and composition. They have a structure of their seed comprising a hard center embryo and on them a layer of transparent endosperm and a husk that is made of fibers. The seeds are often 3-6 mm in length, 2-5 mm in width, and have rather bitter and maple-like taste (Revisiting Trigonella foenum-graecum, 2022; Pharmacological effects, 2013). These characteristics of the anatomy bear repercussions on processing and extraction of bioactive compounds.Fenugreek seeds are nutritionally good in macronutrients and micronutrients. They have large proportions of soluble and insoluble dietary fiber, considerable protein selenium content, and minerals, including iron and calcium, as well as essential amino acids (Journal of Survey in Fisheries Sciences, n.d.; Therapeutic and protective valuation, 2023). This is an addition to their nutritional content, besides the basis of many of their physiological effects.The phytochemical composition of fenugreek grains is exceptionally varied among which galactomannans (mucilage), steroidal saponins (instead, diosgenin), alkaloids like trigonelline, or the amino acid 4-hydroxyisoleucine could be present in significant amount (Saikia, 2012; Bioactive Potential, 2023). The compounds are the key focus of the pharmacological effects of the seed, which interact in many ways to mediate the health effects in a synergistic manner.The antidiabetic effect of fenugreek seeds is one of the most widely researched pharmacological activities of the latter. It is revealed that the presence of 4-hydroxyisoleucine leads to the stimulation of insulin secretion whereas galactomannans reduce the absorption of carbohydrates, which results in the enhancement of glucose tolerance (Bioactive potential, 2023; Pharmacological effects, 2013). The effect of fenugreek in the glycemic control is supported in both animal and human studies, hence making it a good botanical adjunct in diabetes management.Fenugreek seeds are also hydrolipidemic and cholesterol lowering in addition to glycemic effects. The mucilaginous fiber couples the bile acids and it results in the decreased cholesterol absorption and escalated excretion. Saponins that are steroidal like diosgenin also act as thought to aid in the effect of lowering the lipid (Bioactive Potential, 2023; Longdom, n.d.). These lipid-regulating actions also increase the possibility of fenugreek in the metabolic health.The other important aspect of the pharmacologic profile of fenugreek is that it is an antioxidant and anti-inflammatory agent. The seeds are also rich in polyphenols and flavonoids which are free radical scavengers hence they prevent oxidative stress (South African Journal of Animal Science, n.d.; Therapeutic and protective valuation, 2023). All these antioxidant activities have been demonstrated in in vitro studies and in animal models, which implicates them in preventing chronic diseases.Fenugreek was reported to possess anticancer properties besides being metabolically healthy. A subset of in vitro investigations have shown that extracts of fenugreek cause cell apoptosis, prevent migration, and disrupt products of tumor-suppressor gene expression (MDPI Plants, 2022). The cytotoxicity to be dose-dependent and in vivo studies in animal models have reported insignificant acute toxicity at the therapeutic doses thus fenugreek is a good candidate to be investigated further.Fenugreek is reproductive and lactation reproductive as well. The seeds have historically been used as a galactogogue a substance that stimulates lactation and both traditional and contemporary nutritional supplements have used them to stimulate milk production in lactating women (Saikia, 2012). Also, it has modulatory effects on fertility and hormonal balance, which should be investigated in the context of clinical trials of more rigor, and some of them report it (Trigonella foenum-graecum, 2022).Although it has encouraging advantages, fenugreek does not have no threats and adverse effects. Others might develop gastrointestinal disturbances and large doses might affect blood sugar or hormonal sensitive diseases (Journal for Research in Applied Sciences and Biotechnology, n.d.; A range of bioactive compounds, 2025). Also, it is not as safe as commonly thought, and standard dosing and long-term use is still being studied and there are no established regulatory structures on the use of these medicines that are completely compatible across regions.To conclude, the Trigonella foenum-graecum (fenugreek seeds) is the complex botanical system with high nutritional and phytochemical qualities. The seeds have a broad biological spectrum of activities which include antidiabetic, hypolipidemic, antioxidant, anticancer type with lactation support applications. Their potential in terms of nutraceutical and pharmaceutical applications can be well supported by the level of current research but additional clinical and mechanistic research would be necessary in order to maximize safe and evidence-based implementation of these tool.

Fenugreek Leaves

Fenugreek (Trigonella foenum-graecum L.) is a genetically significant medicinal and culinary her to a large extent used medicinally in Asia, Africa, and the Mediterranean region due to nutritional, therapeutic, and aromatic values. A distinctive combination of bioactive compounds makes the leaves a unique dietary source of bioactive compounds that facilitate the medicinal potential of the leaf (Basch et al., 2003). Due to the growing attention within the global sphere to plant-based health solutions, the relevance of the use of fenugreek leaves in the field of pharmacological or nutraceutical studies grows. Fenugreek is a botanically an annual herb which is a member of the Fabaceae family, and is trifoliate, small white flowered plant with characteristic aromatic compounds (Srinivasan, 2006). Its leaves contain high levels of chlorophyll and minerals, Vitamins and phenolic constituents which have crucial functions in nutrition and prevention of diseases. The flexibility to different climatic conditions and the historical importance in the traditional systems of medicine as an herb-Ayurveda, Unani, traditional Arabic processes has resulted in the herb being the subject of scientific interest. Fenugreek leaves have a good nutritional value in terms of proteins, dietary fibre, carotenoid and essential micronutrients, which are iron, calcium and potassium (Mehrafarin et al., 2010). These nutrients serve to make the plant effective towards the fight against malnutrition and other health conditions amid populations where the frequency of the consumption of fenugreek is common. The high number of fibers also boosts the health of the digestive system, encourages the regularity of the bowels, and regulates the absorption of glucose. The list of secondary metabolites in the phytochemistry of fenugreek leaves is quite long: flavonoids, alkaloids, tannins, saponins, and steroidal diosgenin (Petropoulos et al., 2016). These bioactive compounds give the leaves a powerful antioxidant activity, thus, able to counterfree radicals and alleviate oxidative stress. Fenugreek has a promising future as a natural drug because of the complexity of the phytochemical profile. The use of the fenugreek leaves has traditionally been applied to treat diseases that include GIT impairment, inflammation and respiratory conditions. According to Ayurvedic medicine, they are said to balance the doshas, help in metabolism and rejuvenate the tissues (Srinivasan, 2006). Most of these ancestral claims are upheld by modern studies, as it is shown that fenugreek leaf has anti-inflammatory, antimicrobial, antidiabetic, and lipid-lowering effects (Nagulapalli Venkata et al., 2017). Recent scientific reports emphasise the therapeutic use of fenugreek leaves in the metabolic disorders especially diabetes and obesity. Insulin sensitivity is increased, as well as postprandial glucose and lipid profiles, with the use of the leaves because of the fiber, polyphenols, and trigonelline (Basch et al., 2003). The results confirm the use of fenugreek leaves in herbal formula and functional foods that aim at metabolic health. The leaves of fenugreek also showed considerable antimicrobial properties against the pathogenic bacteria and fungi. Their bioactive constituents especially phenolics and alkaloids interfere with microbial cell membrane and prevent the activity of enzymes and as a consequence, fenugreek would be a good natural preservative and antimicrobial agent (Mehrafarin et al., 2010). This antimicrobial possibility is more and more applicable in the perspective of increasing antimicrobial resistance. Besides the medicinal use, the leaves of fenugreek are important in farming and the sustainability of the environment. Fenugreek is a type of leguminous herb able to enhance the soil fertility and aid sustainable agricultural production (Petropoulos et al., 2016). It has low chemical requirements in cultivation and thus suitable to be grown in agricultural systems that are environmentally friendly and organic farming. Recent developments in uses of fenugreek has been found in nanotechnology, food chemistry and biotechnology. For the green synthesis of nanoparticles, extracts of fenugreek leaves are used, which demonstrate increased antimicrobial, antioxidant and catalytic effects (Vimala and Jacob, 2020). The present environmental friendly strategy is in line with the current worldwide initiatives of producing sustainable nanomaterials to be used in biomedical and industrial products. Though it has a vast set of advantages, additional studies should be conducted to clearly understand the pharmacokinetics, toxicity, and effective therapeutic doses of the constituents of the fenugreek leaf. Though it is considered to be safe, the change in chemical composition under the impact of environmental and genetic influences may affect its effects (Nagulapalli Venkata et al., 2017). It should be noted that the potential of fenugreek leaves, as a useful food and a medicine plant, remains unexplored due to continued interdisciplinary research.

Effects of Herbal Plants on Azoospermia Semen

The most egregious type of male infertility, which is called azoospermia and the total absence of spermatozoa in the ejaculate, has a prevalence of about 1 percent among the male population and up to 15 percent of infertile men (Esteves et al., 2021). It can either be pre-testicular, testicular or post- testicular and testicular azoospermia is most frequently linked to compromised spermatogenesis, oxidative stress, a hormonal imbalance, and structural destructions of the seminiferous tubules. The rising male infertility problem world-wide has elicited a rising curiosity in plant-based therapeutics which could help to support reproductive repair. Fenugreek (trigonella foenum-graecum), a well- recognized traditional Ayurvedic and herbal medicine ingredient that has a provisional clinical indication of value in the management of male reproductive disorders. The leaves and seeds of fenugreek have a good profile of bioactive compounds and are characterized by saponins, alkaloids, flavonoids, diosgenin, and other necessary micronutrients (Srinivasan, 2006). These phytochemicals have been described as being antioxidant, anti-inflammatory, and hormone-modulating and thus fenugreek is an interesting choice in the treatment of infertility-related diseases. These distinctive chemical reactions of the fenugreek seeds, especially protodioscin, steroidal saponins, and galactomannans, have been associated with the effects of improved testicular functionality and enhanced androgenic activity (Wankhede et al., 2016). In the meantime, the leaves of the fenugreek offer more antioxidant molecules, vitamins, and minerals that could have a protective effect on the testicles. It has been widely acknowledged that oxidative stress is one of the key factors of azoospermia and impaired development of sperm. Too much reactive oxygen species (ROS) may damage the Sertoli cells, the Leydig cells, and the germinal epithelium, which helps in hindering spermatogenesis (Agarwal et al., 2014). The antioxidant activity of fenugreek has been endorsed by multiple studies that have proved its characteristic to decrease lipid peroxidation, enhance catalase and superoxide dismutase activity, and reestablish reproductive tissue redox in response (Kamenan et al., 2020). Under these antioxidative activities, fenugreek could help rescue testicular tissue against oxidative damage which is a common cause of the azoospermia. The other significant pathophysiology of azoospermia is that of hormone dysregulation and inadequate production of testosterone. Testosterone plays a crucial role in Sertoli cell activities and activation of meiosis in the germ cells. The studies show that fenugreek seed extract has the potential to improve total and free testosterone levels, which may happen due to its saponin composition and the impact it has on the hypothalamic-pituitary-gonadal axis (Maheshwari et al., 2017). Optimal testosterone can lead to the germ cell maturation and regain spermatogenic functions in azoospermic men. Furthermore, fenugreek has also demonstrated a possibility in enhancing the metabolic health, which is strongly associated with the reproduction performance. These disorders, including obesity, metabolic syndrome, and insulin insensitivity have been identified as providing a contribution to azoospermia through altering endocrine regulation and augmenting oxidative stress (Jensen et al., 2017). Fenugreek seeds also showed antidiabetic and hypocholesterolemic effects which have an indirect regulatory effect that can enhance metabolism competence and foster a healthy hormonal environment in the system to support spermatogenesis (Srinivasan, 2006). Little has been done concerning it, but leaves of fenugreek have been reported to contain large quantities of ascorbic acid, beta-carotene, and flavonoid-compounds--compounds that have been linked with cellular protection as well as anti-inflammatory. Certain experimental researchers note that leaf extracts are capable of maintaining the testicular architecture and decreasing inflammation in chemically induced reproductive toxicity (Kamenan et al., 2020). These results are indicative of a therapeutic action of fenugreek leaf in facilitating testicular healing and enhancing microenvironment that is necessary to sustain spermatogenesis. A number of animal researches have also confirmed the action of fenugreek in improving sperm parameters; such as sperm count, substance of sperm, motility, viability and morphology. The rodent model study has demonstrated positive improvements in the testicular weight, seminiferous tubules integrity, and density of the germ cells after fenugreek administration (Wankhede et al., 2016). The above observations indicate that not only the leaves but seeds may also play a role in the spermatogenic stages between proliferation of spermatogonial to spermiation, especially in the case of azoospermic models. In addition to its direct action on the testicular tissue, fenugreek has antinflammatory and immunodulatory effects which can potentially prevent autoimmune-induced damage to the testicles which is a minor but not insignificant causative agent of azoospermia (Agarwal et al., 2014). Chronic inflammation may interfere with the blood-testis barriers, affect the functioning of Sertoli cells, and cause germ cells apoptosis. Anti-inflammatory effects of fenugreek (flavonoid and polyphenols) can also help in alleviating these pathologic alterations and improving recovery of testicles. Regardless of the encouraging signs, the comparative studies of the usage of the leaves of fenugreek and fenugreek seeds in the conditions of azoospermia are absent. The initial evidence indicates that seeds can have a greater endocrine and spermatogenic influence because of the saponin profile and leaves can potentially be better antioxidant and anti-inflammatory. The difference in bioactivity of both regions of the plants needs to be understood to enable the creation of specific therapeutic approaches. Due to the growing attention to the application of plant-based medicines to treat male infertility, more studies should be conducted on the impact of the fenugreek leaves and seeds on azoospermia. Their role as individuals and as a combination towards the restoration of spermatogenesis can be explored as a way of providing new ways of therapy particularly in situations where standard medical interventions fail to be accessible or effective. The aim of the research is to determine the relative and synergistic advantages of fenugreek leaves and seeds on the recovery of male reproductive system with emphasis to be given to the hormonal control, testicular morphology and the maintenance of oxidative stress.

Plant Material

Samples of leaves and seeds of Trigonella foenum-graecum L. were obtained from the Botanical Garden of NIMS University in Jaipur, Rajasthan. Following collection, the samples were washed thoroughly with distilled water, and air-dried at room temperature.

Preparation of the leaf extract

The leaves and seeds of Trigonella foenum-graecum L. were dried, finely powdered, and stored under refrigeration. Five grams of the powdered sample were mixed with diluted ethanol, and kept at room temperature for 24 hours. The sample was filtered through Whatman No. 1 filter paper. The resultant filtrate was stored for future preparation of ZnO nanoparticles at 4 °C.

Bioactive Components

The Trigonella foenum-graecum also includes a high range of phytochemicals which have historically been linked to male reproductive health. The biochemical profile of seeds and leaves is different as seeds have more steroidal saponins and alkaloids, whereas leaves have more vitamins and antioxidants. The applications of these bioactive classes have also been investigated in regard to the possible effects in the reduction of oxidative stress and hormonal control, which are topical in male infertility and in some types of azoospermia (Maheshwari et al., 2017). Some of the most studied bioactive constituents of fenugreek seeds are the saponins which are sexa-, hexa-, and octagenic steroidal steroidal saponins mainly diosgenin, a sapogenin, which is a chemical analog of steroid hormones. Diosgenin has demonstrated endocrine-modulating and antioxidant effects on models, which is an indication that this substance could have an indirect effect on the testicular biology (Tak et al., 2024). Though scientific attention is paid to structural relations to precursors of testosterone, human data on the topic is limited and inconclusive. Another saponin known to be commonly found in commercial extracts of fenugreek, protodioscin, has been tested in terms of its possible capacity to positively effect testosterone levels and semen morphology and quality. Protodioscin-standardized extracts including Furosap(r) were used as part of clinical trials and were found to increase the sperm count and free testosterone levels of subfertile men (Maheshwari et al., 2017). But the results are not generalized to raw fenugreek seeds as bioactive composition can be considerably differentiated on the basis of cultivar, preparation and extraction. A number of alkaloids can be found in fenugreek seeds, among them trigonelline that has antioxidant, hypoglycemic and cell-protective activity. They demonstrated that the radical-scavenging property of trigonelline could benefit the metabolic and oxidative stress on tissues, which are potentially indirectly favoring spermatogenesis (Visuvanathan et al., 2022). Nevertheless, in clinical literature, there is no direct evidence indicating the connection of trigonelline and the reversal of azoospermia. Fenugreek seeds and leaves possess an apt supply of flavoring substances (Fenugreek) like quercetin glycosides and phenolic acids like caffeic and gallic acid. These are potent antioxidants that have the capacity of decreasing reactive oxygen species (ROS) in biology. Animal models indicate that fenugreek extracts dotted with antioxidants have the capability to protect testicular cells against oxidative stress which is a significant cause of regressive sperm production (Salam et al., 2023). The soluble fiber fraction of the fenugreek seeds which are galactomannans is mostly associated with metabolic properties of glycemic regulation and better digestion, but the indirect effects on hormone balance and nutrient absorption could also aid in improving reproductive health (Srinivasan, 2006). Though it is not directly related to spermatogenesis, it is crucial to keep the metabolic conditions stable because insulin

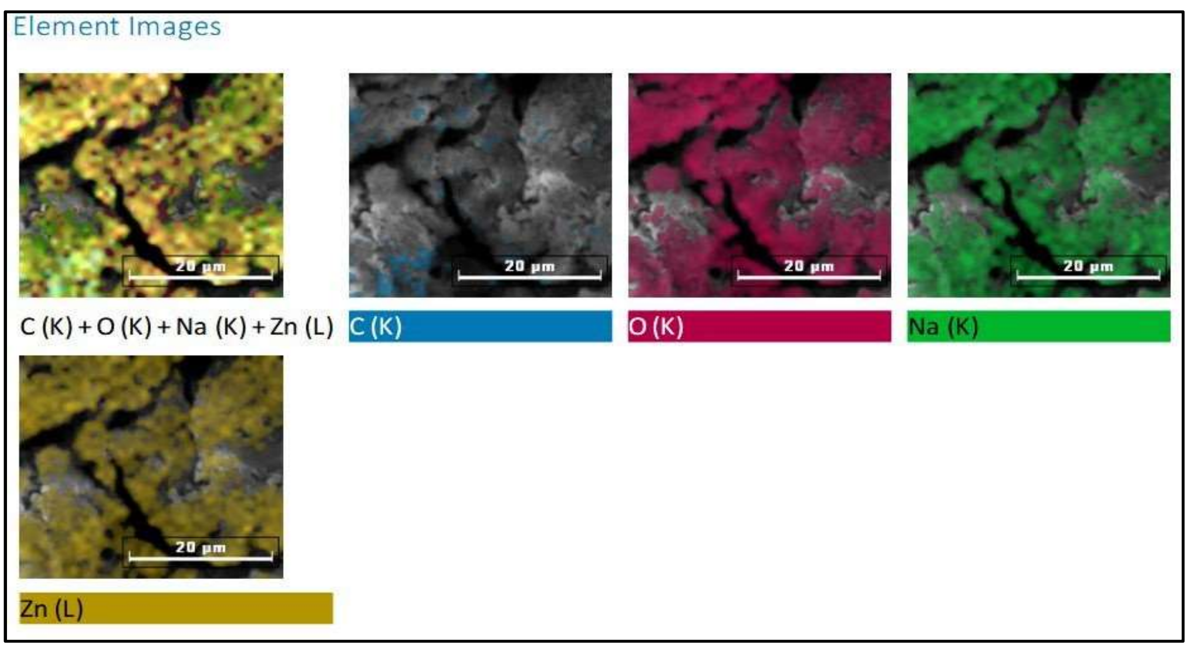

Figure 5. SEM Analysis for nanoparticle and crude extract

resistance and metabolic disorders have been pointed out to be correlated with male infertility. The muscular leaves of fenugreek are relatively rich in vitamin C, b-carotene, and other antioxidants that prevent activities of cells, immunity, and oxidative stress. These antioxidant-containing plant-based matrices can be useful in the reproductive health sector, as too much ROS may negatively affect sperm DNA quality and Sertoli and Leydig cell damage (Salam et al., 2023). These antioxidative functions are not curative but supportive to azoospermia which is due to structural or genetic abnormality. Trace minerals containing zinc, magnesium and iron are also provided by fenugreek and are also very necessary in enzyme processes and hormone production. Zinc is already known to be also a potent essential factor in spermatogenesis and sperm stabilization of chromatin, and low levels of zinc are associated with low-quality semen (Agarwal et al., 2014). Even though fenugreek contains moderate amounts of minerals, they can be used as supplements to diet but not as the only solution to azoospermia. The mechanistic clues of animal studies are beneficial. As an example, Hamza et al. (2016) showed that the

S. C. africanus seed extract prevented the testicular toxicity induced by cisplatin by enhancing sperm concentration, morphology as well as antioxidant enzyme activity. The results highlight the promise of the role of saponins and polyphenols found in the fenugreek in preserving the functioning of the testiscles once subjected to oxidative or chemical stress. Further preclinical findings propose that the ingredients of fenugreek have the ability to stimulate the activity of antioxidant systems that already exist within the body e.g., glutathione, superoxide dismutase etc. This boosts cellular resilience to testicular apoptosis, one of the major processes involved in the improper development of the sperm (Visuvanathan et al., 2022). Nonetheless, animal model can never mimic the multifaceted etiologies of human azoospermia. Fenugreek and male fertility Human research is still in small scale. The application of standardized extracts of fenugreek in clinical trials has demonstrated positive effects on the level of free testosterone, the number of fertilized eggs, and sexual desire (Sankhwar et al., 2023). However, these were found mainly in men who had oligospermia and not azoospermia and this reveals that the fenugreek has the potential to improve compromised spermatogenesis but is not able to produce sperm where there is no testicular germinal epithelium. The difference between obstructive and non-obstructive azoospermia is vital; azoospermia obstructive (OA) and non-obstructive azoospermia (NOA). Mechanical obstruction, like vas deferens blockage, is something taken care of by the fenugreek phytochemicals. In the case of NOA, results are determined by leftover spermatogenic ability. Fenugreek has been suggested to have antioxidant effects and if further endocrine regulating effects, which in turn could be beneficial in men with partial spermatogenic activity, but not in those with full Sertoli cell-only syndrome or genetic defects (Agarwal et al., 2021). We can mention, it is also applicable that the increase in free testosterone levels due to the consumption of fenugreek extracts can be observed slightly as various clinical studies report (Maheshwari et al., 2017). Leydig cell activity can be assisted by hormonal changes even in case of the preservation of testes germ-cells and the microenvironment may change determined potentially by proliferating sperm. Another application of fenugreek is in the inhibition of inflammation by modulating the synthesis of the cytokines of TNF-a and Il-6. Persistent inflammation may result in weakening of the blood flow in the testicles and hampering the hypothalamic-pituitary-gonadal axis. Anti-inflammatory regulation, thus, can have secondary advantages in men with azoospermia of inflammatory or oxidative etiology (Visuvanathan et al., 2022). The hypoglycemic effect of fenugreek which is largely due to alkaloids and soluble fibers might have an indirect effect of enhancing reproductive health as metabolic syndrome is closely linked with low levels of testosterone and defective spermatogenesis. Thus, metabolic gains can be able to provide a better environment where sperms can be produced (Srinivasan, 2006). The leaf of fenugreek induces tannins carotenoids and chlorophyll which are involved in the cell protection and the detoxification mechanisms. These ingredients aid the liver removal processes, which indirectly promotes hormone metabolic activity and elimination of ROS, and it can lead to an improvement in the general fertility (Salam et al., 2023). Although these are possible mechanisms, fenugreen is not a reported intervention in azoospermia. The positive outcomes of it can be best described in the instances of subfertility caused by oxidative stress, mild hormonal disorientation, or poor quality of sperm. Depending on the cause, azoospermia may be fixed by surgery, hormone treatment, or assisted reproduction (Agarwal et al., 2021). There are standardized extracts which offer more consistent levels of bioactive than the raw seeds e.g. Furosap(r) extracts. A variety of clinical reports on such extracts indicated an improvement in semen volume and semen motility but none of them mentioned recovered sperm in patients with the condition of azoospermia (Sankhwar et al., 2023). Here, the standardization of dosage, formulation and extraction are important. There is a general safety because fenugreek has a high degree of tolerance with mild adverse effects commonly being experienced in the gastrointestinal tract. Nonetheless, its hypoglycemic action may be combined with antidiabetic drugs, and its hormonal effect should be raise concerns in men under endocrine treatment (Visuvanathan et al., 2022). These are the issues that should be put into consideration when incorporating fenugreek into fertility management. On the whole, the seeds and leaves of fenugreek contain abundant levels of bioactive compounds - steroidal saponins, alkaloids, flavonoids, phenolic acids, vitamins, and minerals, which have the potential to maintain antioxidant protection, metabolic and hormonal stability. Although these properties give reason to find the supportive role of male reproductive health, fenugreek cannot be referred to as a single therapy in azoospermia with either severe or structural cases. It still needs clinical validation to ascertain any beneficial therapeutic effects (Agarwal et al., 2021).

Preparation of zinc nanoparticles

The eco-friendly synthesis of zinc oxide nanoparticles commenced by dissolving 1.1g of zinc acetate (0.1M) in 100 mL of deionized water and stirring it for 10 min at room temperature, allowing the precursor solution to homogenize (Khan et al. 2019). Next, 2g of sodium hydroxide was added in a stepwise fashion to facilitate controlled nucleation of naked zinc hydroxide particles, which is a well- established method of alkaline-mediated ZnO synthesis (Talam et al. 2012). The reaction mixture was then titrated with 5 mL of Trigonella foenum graecum leaf, followed by seed extracts, acting as natural reducing and stabilizing agents, which have been used widely in green nanoparticle synthesis (Anbukkarasi & Vasantharaj 2021). This mixture was continuously stirred for 2 hours and, during that time, the pH was maintained at 12 to facilitate the proper conversion of zinc complexes to ZnO precursors (Ramesh et al. 2020). After 2 hours of reaction, the sample was centrifuged at 5600 RPM for 30 min to separate the solid fraction, which was subsequently boiled for 60 min. Based on appearance, the material went through progressive phases converting from a watery suspension to a light white paste, demonstrating dehydration and particle formation over time.

RESULTS AND DISCUSSION

Characterization

The green synthesis of zinc oxide nanoparticles began by dissolving 1.1 g of zinc acetate (0.1 M) in 100 mL of double-distilled water under continuous stirring for 10 minutes, facilitating complete homogenization of the precursor solution (Khan et al., 2019). Next, two g of sodium hydroxide was added in a controlled manner to aid in controlled nucleation. Zinc oxide nanoparticles (ZnO NPs) have gained considerable attention because of their superior optical, antimicrobial, and catalytic properties; many of these properties are dramatically improved when the material is beneficially scaled to the nanoscale (Agarwal et al., 2017). Given the increased interest in safer and more sustainable approaches to nanotechnology, there is growing interest in environmentally friendly nanotechnology, and green synthesis process can also be implemented as a sustainable and non-toxic approach to synthesis. The green synthesis of nanoparticles typically utilizes commonly available plant extracts that are rich in phytochemicals (flavonoids, terpenoids, tannins, phenolics etc.) that act as reducing and stabilizing agents during nanoparticles formation (Anbukkarasi & Vasantharaj, 2021). These biomolecules are capable of converting zinc ions into the formation of ZnO nuclei and naturally capping its surfaces thereby minimizing aggregation and it improves biocompatibility making green biosynthesized ZnO nanoparticles ideal for biomedical, agricultural, and environmental applications (Faizan et al., 2021). However, characterization methods are essential to establishing successful synthesis and ensure quality of the nanoparticles. Fourier Transform Infrared Spectroscopy (FTIR) is one of the first characterization techniques used to identify functional groups in plant extracts and to assess the nature of the interaction of the functional groups to the surface of the nanoparticles. The presence of the Zn–O stretching vibrations ranging from 400–600 cm^{-1} indicates successful formation of particles, and additional peaks reveal the organic compounds that served as capping agent (Agarwal et al., 2017). Scanning Electron Microscopy (SEM) is another essential characterization technique that serves an important role in analyzing nanoparticle morphology, which are able to provide high-resolution images and reveal the shape, surface texture, aggregation behaviour of the particles (Suresh et al., 2018). Typically, green synthesized ZnO NPs will have spherical, rod, or hexagonal morphology depending upon the type of plant extract. In addition to SEM, characterization can also confirm the elemental composition using Energy-Dispersive X-ray Spectroscopy (EDX). In EDX characterization, an elemental peak is detected from zinc and oxygen that confirms the purity of the nanoparticles while carbon signal peaks usually indicate capping agents (Ramesh et al., 2020). Dynamic Light Scattering (DLS) is another important characterization tool to measure hydrodynamic particle size and polydispersity index in suspension media to assess colloidal stability, which is important for biomedical and aqueous applications (Khan et al., 2019). Nanoparticle aggregates in their dry state usually reflect higher size measurements as compared with their effective size in environments under physiological conditions (e.g., in water). The DLS analysis is stated to provide a more accurate measure of the effective size of nanoparticles in physiologic, aqueous dispersions. Finally, Zeta potential provides insight of surface charge and stability, whereas the description denotes potential dispersion over time for nanoparticles synthesized. A high positive or negative zeta potential introduces electrostatic repulsion and a greater extent of stability and enhanced suspension of nanoparticles, which is relevant to the fields in drug delivery, antimicrobial coatings, and catalysis (Ramesh et al., 2020). Overall, the five characterization - SEM, EDX, DLS, FTIR, and zeta potential - provides a full picture of the structural, chemical and functional attributes of green synthesized ZnO. A full picture of characterization is useful in improving the synthesis conditions to improve sintering, adding stabilizers, and better understand how nanoparticles are safely and reliably applied in scientific, technological and other areas.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is among the most crucial analytical techniques utilized for examining the chemical structure, surface chemistry, and functional groups of zinc oxide nanoparticles (ZnO NPs), especially those produced from green or plant-mediated synthesis. FTIR is instrumental in investigating the role of plant phytochemicals in the formation, stabilization, and functionalization of the ZnO NPs. During green synthesis, some biomolecules, including phenolics, flavonoids, terpenoids, saponins, and organic acids, engage with the zinc precursors and facilitate the reduction of zinc ions, along with the capping of the nanoparticle surfaces to avoid aggregation (Agarwal et al., 2017). Because these interactions occur on a molecular scale, FTIR is therefore a very important method for validating the contributions of plant compounds and the hypothesis around nanoparticle formation. FTIR is a method whereby infrared radiation interacts with the vibrational energy levels of chemical bonds, and each functional group will produce a different absorption band, which means that FTIR can be used to provide a molecular "fingerprint" of any material. As pertains to ZnO nanoparticles, FTIR will normally display a strong absorption band between 400 and 600 cm^{-1} that corresponds with a Zn-O stretching vibration, which confirms the successful creation of zinc oxide (Talam et al. 2012). The appearance, shift, or disappearance of peaks compared to the original plant extract spectrum can indicate the binding of phytochemicals onto the surface of the nanoparticles, which will provide information to researchers about which functional groups (hydroxyl (-OH), carbonyl (C=O), amine (-NH), or aromatic rings) acted as reducing and stabilizing agents (Suresh et al. 2018. FTIR spectrometry serves a prominent role in green nanotechnology because they provide a spectrum of organic compounds that assist with the synthesis of nanoparticles in potentially divergent methods. For example, broad peaks around 3200-3500 cm^{-1} typically indicate O-H stretching vibrations from alcohols or phenolic compounds, indicating that topical plant material contains polyphenolic groups that are capable of reducing zinc ions into zinc based nanoparticles. Similarly, peaks over the range of 1600-1700 cm^{-1} indicates a C=O stretching from flavonoids or proteins that are used as capping molecules (Anbukkarasi eet al., 2021). These bands indicate the organic compounds are holding fast to the nanoparticle and creating an organic shell surrounding the nanoparticle that supports stability and deters unnecessary agglomeration. The shits in bands in these ranges also indicates that there is a chemical bound between the plant molecules and the nanoparticle core. Another key feature of FTIR analysis involves detecting impurities or residual functional groups coming from plant extracts, which can affect the biological activity of ZnO nanoparticles. Additional peaks between 1000-1400 cm^{-1} might indicate C-O, C-N, and C-H vibrations attributed to carbohydrates, amino acids, or other organic molecules (Faizan et al., 2021). The identification of such groups is important because these phytochemical residues often enhance antibacterial or antioxidant activities. FTIR thus relates chemical composition to functional benefits, especially when nanoparticles are destined for biomedical applications. It acts as a comparative tool for assessing nanoparticle purity, chemical modifications, or surface oxidation states, as green-synthesized ZnO nanoparticles often perform differently than those synthesized chemically. The presence of well-defined Zn-O bands and correctly assigned organic peaks ensures that the nanoparticles have appropriate chemical characteristics. Furthermore, FTIR is nondestructive, with minimal sample preparation, and can be applied on powders, films, and suspensions, thus possessing great versatility in nanoparticle research. Characterization techniques such as XRD, SEM, DLS, and zeta potential supplement the FTIR data and provide complete information about the physicochemical properties of the nanoparticle. In summary, FTIR remains essential in the confirmation of nanoparticle formation, understanding molecular interactions, and functional stability of zinc oxide nanoparticles synthesized via eco-friendly green methods.

The Fenugreek crude extract 'FTIR spectrum' includes a wide range of functional groups indicative of the diverse phytochemicals present in the fenugreek seeds (Fig1). Notably, there is a broad band (3600–3200 cm-1) in the O–H stretching region, which consists mainly of alcohols, phenolics, and water associated with hydrogen bonding. This broad intensity suggests that the fenugreek crude extract contains significant amounts of flavonoids, polyphenols, and saponins that are important for biological activities including antioxidant properties and reducing power. At approximately 1650-1550 cm-1 in the range of mid-frequency for the crude extract, you will see several peaks corresponding to amide I and amide II vibrations representing protein and amino acid residue. You will also observe additional peaks in the range of 1400-1000 cm-1 corresponding to C-N and C-O stretching vibrations, which represent carbohydrates, glycosides and plant-based organic acids. All of these different peaks indicate that fenugreek extract contains a complex mixture of biomolecules that can both bind to stabilised and interact with metal ions during nanoparticle synthesis. If you examine the fingerprint region of the crude extract more carefully, you'll notice a series of small, well-defined peaks—these are strong indicators of the presence of aromatic rings, alkaloids, and a diverse array of other organic molecules. While these signals may initially appear subtle, they actually underscore the remarkable chemical complexity found within fenugreek seeds. The functional groups associated with these compounds are not only responsible for the extract's notable antioxidant and medicinal properties—they also play a crucial role as the foundational elements in the synthesis of green nanoparticles. These bioactive compounds have the unique ability to donate electrons, thereby enabling the reduction process, and they persist afterward to stabilize and cap the newly formed nanoparticles, preventing their aggregation. This dual action is precisely what makes the crude extract such a potent and efficient natural medium for the formation of ZnO nanoparticles. By leveraging both the electron-donating and stabilizing capabilities of the various functional groups present, the extract ensures that the nanoparticles are produced in a controlled and eco-friendly manner, highlighting the significant potential of plant-based materials in advanced nanotechnology applications.

The FTIR spectrum observed for the synthesized ZnO nanoparticles is distinctly different from that of the crude plant extract, which strongly indicates that the nanoparticles have successfully formed (Fig2). Notably, the broad O–H stretching band typically found near 3400 cm^{-1} either diminishes in intensity or undergoes a slight shift in the nanoparticle sample. This change suggests that phenolic and hydroxyl functional groups present in the extract were actively involved in the reduction of zinc acetate during the synthesis process. As these bioactive compounds participate in the reaction, the characteristic O–H absorption peak decreases, providing clear evidence of their consumption and confirming their crucial role as reducing and stabilizing agents in the formation of ZnO nanoparticles. This transformation not only highlights the involvement of specific functional groups but also demonstrates the effectiveness of the green synthesis approach, where natural phytochemicals facilitate both the reduction of metal ions and the stabilization of the resulting nanoparticles. In the mid-infrared (mid-IR) region, distinct peaks appear that are associated with the presence of proteins and polysaccharides—specifically, a strong peak is observed near 1650 cm^{-1}, which is characteristic of amide bonds in proteins, and a broader region between 1400 and 1100 cm^{-1} that is typically linked to carbohydrate structures found in polysaccharides. When these peaks shift position or exhibit a decrease in intensity, it is a clear indication that biomolecules from the plant extract are interacting with and adhering to the surface of the newly formed nanoparticles. This interaction is significant because the attachment of these biomolecules leads to the formation of a natural capping layer on the nanoparticles. Such a capping layer is crucial; it serves to prevent the nanoparticles from aggregating or clumping together, thereby maintaining their stability and dispersity in solution. The observed spectral shifts underline an important point: the compounds extracted from fenugreek are not merely acting as reducing agents to convert metal ions into ZnO nanoparticles. Instead, they also play an essential role in coating and stabilizing the nanoparticles by forming a protective and functional biological shell around them. This biologically derived coating not only imparts stability, preventing the ZnO nanoparticles from aggregating, but may also introduce additional beneficial properties, such as enhanced biocompatibility or functional activity, to the nanoparticles, making them more suitable for various applications in fields like biomedicine, catalysis, or environmental remediation. Ultimately, the spectral evidence makes it clear that the fenugreek- derived biomolecules are integral not only to the synthesis of ZnO nanoparticles but also to their subsequent stabilization and functionalization. The standout feature appears prominently in the lower wavenumber region, specifically between 500 and 400 cm^{-1}. In this area, there is a strong and distinct absorption band, which serves as a definitive marker for the Zn–O stretching vibration. The presence of this single, bold peak is a clear indication that zinc oxide nanoparticles have indeed formed during the synthesis process. This finding confirms that zinc ions have successfully undergone transformation into ZnO as a result of the plant-mediated method. When you observe this characteristic Zn–O band in conjunction with noticeable alterations in other functional groups within the spectrum, it becomes evident that the intended chemical reaction has taken place as planned. Not only do these results verify the presence of the nanoparticles, but they also highlight the important role played by biomolecules from the fenugreek extract, which act to stabilize and support the newly formed nanoparticles at a chemical level. This synergy between the plant-derived compounds and the inorganic particles underlines the efficiency of the green synthesis approach, demonstrating how the natural constituents not only facilitate the reduction process but also enhance the stability and function.

The FTIR spectrum for the crude extract of fenugreek leaves highlights the remarkable complexity and diversity found within these leaves (Fig3). When analyzing the spectrum, distinct traces of various bioactive compounds become evident—phenolics, flavonoids, alkaloids, proteins, carbohydrates, and essential oils—all of which exhibit their own unique spectral signatures. By scanning across the broad range from 4000 to 650 cm^{-1}, the FTIR analysis provides a comprehensive molecular fingerprint of the extract, capturing both the subtle and pronounced features of its chemical composition. This detailed fingerprint enables researchers to discern how the multitude of compounds in fenugreek leaves interact and coexist within the extract. Such interactions are crucial, as they collectively contribute to and influence the extract's antioxidant, reducing, and capping properties. Moreover, by understanding these molecular relationships, scientists can better appreciate how the synergy between these compounds enhances the overall bioactivity and therapeutic potential of fenugreek leaf extracts, paving the way for more targeted applications in pharmaceuticals, nutraceuticals, and functional foods. The spectrum is particularly notable for its wide, intense band stretching from 3600 to 3200 cm^{-1}. This region is well-known as the signature range for O–H stretching vibrations—a hallmark of functional groups such as alcohols, phenols, or even water molecules, especially when they are actively involved in hydrogen bonding. In the case of fenugreek leaves, this pronounced peak is commonly observed when there is an abundance of polyphenols and flavonoids, both of which are rich in hydroxyl groups capable of strong hydrogen bonding interactions. The impressive size and broad nature of this peak leave little doubt: these leaves are not chemically inert. Instead, they are highly dynamic, brimming with molecules that are able to readily donate hydrogen atoms or electrons. Such reactivity is not only a testament to their biochemical richness but also plays a crucial role in processes like green nanoparticle synthesis. The presence of these active compounds means that fenugreek leaf extracts can effectively reduce metal ions and stabilize nanoparticles, making them highly valuable in eco-friendly nanotechnology applications. This inherent chemical activity underscores why plant extracts, particularly those from polyphenol- and flavonoid-rich sources like fenugreek, are increasingly favored as green reducing and capping agents in sustainable synthesis methods. In addition to the prominent O–H stretching, you'll frequently encounter subtle signals from N–H stretching vibrations, which are characteristic of functional groups like amines and amides, as well as C–H stretching arising from aliphatic hydrocarbon chains. These absorption peaks generally appear in the 3300–3000 cm^{-1} region for N–H stretching and in the 2920–2850 cm^{-1} range for C–H stretching. When these features are present in a spectrum, they point to the existence of biomolecules such as proteins, amino acids, lipids, and plant waxes—constituents that are typically abundant in leaf tissue. The presence of these molecules is more than a mere fingerprint of composition; they play crucial roles in the life processes of the plant. For example, proteins and amino acids are actively involved in enzymatic and metabolic pathways, while lipids and waxes contribute to cellular structure and protection. Importantly, these compounds engage in natural reduction and oxidation reactions, underlining the dynamic and reactive nature of the leaf extract. This biochemical activity is essential for the plant's growth, defense, and adaptation, and it explains why leaf extracts are often harnessed as bioactive agents in various applications, from green synthesis to medicinal uses.

You observe a distinct and prominent absorption band near 1650 cm^{-1}, which corresponds to the C=O stretching vibration characteristic of amide I. This serves as a clear indicator of the presence of proteins or peptides within the extract. Additionally, there is another noticeable peak appearing around 1550 cm^{-1}, associated with the amide II band, which arises mainly from N–H bending and C–N stretching vibrations. The detection of both these amide I and amide II peaks provides strong evidence supporting the existence of proteinaceous materials in the sample. Fenugreek leaves are well known to be rich in various plant proteins and enzymes, and the appearance of these specific IR absorption bands directly reflects their abundance in the extract. The identification of these amide bands is particularly significant, as the protein molecules they indicate are not merely passive components; they actively participate later in the synthesis process of nanoparticles. During nanoparticle formation, these proteins function as stabilizing and capping agents, helping to control particle growth, maintain colloidal stability, and prevent aggregation. Their presence, as revealed by the spectral peaks, underscores their crucial role in ensuring that the nanoparticles maintain the desired size and shape, making the extract especially suitable for green synthesis applications. Distinctive cluster of peaks have been observed in the 1400 to 1000 cm^{-1} region of the spectrum. This range is a hallmark zone for vibrations such as C–N stretching, C–O stretching, and O–H bending. These characteristic signals are strong indicators of the presence of carbohydrates, glycosides, and organic acids—compounds that are notably prevalent in fenugreek leaves. The distinctiveness of these peaks effectively serves as a signature, signaling the presence of polysaccharides within the extract. The appearance of these peaks is not merely a chemical detail; rather, it has significant implications for the properties and functionality of the material. These biomolecules, especially polysaccharides, play a critical role by stabilizing nanoparticles. They achieve this stabilization through direct interactions with the surfaces of metal ions, often forming a protective layer that prevents aggregation. This capacity to interact and bind with metal surfaces makes them essential contributors in nanoparticle synthesis and stability. Consequently, the identification of these spectral features not only provides chemical evidence of specific functional groups but also underscores the practical importance of these natural compounds in facilitating and enhancing nanoparticle formation and durability.

Look closer at the fingerprint region, and you'll notice a dense network of peaks—each one a subtle clue revealing the complex mixture of plant metabolites present within the extract. Alcohols, ester groups, aromatics, sugar derivatives—all make their appearance in this region, each characterized by its own distinct vibrational signature. This particular stretch of the spectrum is so uniquely tailored to individual compounds that it effectively becomes the crude extract's identification card, capturing its chemical uniqueness. The sheer diversity and degree of overlap among these signals convey an important message: fenugreek leaf extract is anything but simple. Instead, it's a rich reservoir brimming with a vast array of bioactive molecules, many of which are capable of binding to and stabilizing metal ions or nanoparticles through various chemical interactions. Shifting focus to the lower end of the spectrum, specifically between 700 and 650 cm^{-1}, the data reveals faint but significant peaks—easy to overlook at first glance, yet highly informative. These peaks correspond to out-of-plane bending vibrations of aromatic C–H bonds, as well as backbone vibrations inherent to phenolic structures. Though these signals may be small in amplitude, they serve as reliable markers, pointing to the presence of plant-derived phenolics and alkaloids. Together, these subtle and prominent spectral features paint a comprehensive picture: the FTIR profile leaves no room for doubt—fenugreek leaf crude extract is densely packed with multifunctional biomolecules. These compounds collectively endow the extract with notable antioxidant properties, an enhanced capacity to chelate or bind metal ions, and the structural integrity needed to support and stabilize nanoparticle frameworks. This biochemical versatility is not only crucial for the extract's natural role within the plant but also makes it an exceptional candidate for use in green synthesis and stabilization of nanoparticles. The presence of such a diverse set of bioactive constituents means that fenugreek leaf extract can perform multiple functions simultaneously, from reducing metal ions to preventing nanoparticle aggregation, all while providing an environmentally friendly alternative to synthetic chemical reagents. In summary, the detailed spectral evidence underscores the extract's multifaceted potential, confirming its value as a robust, natural resource for advanced material synthesis and nanotechnology applications.

The FTIR spectrum of zinc nanoparticles which have been synthesized by the synthesis of fenugreek seed extracts presents various organic and inorganic signals which indicate the creation of nanoparticles as well as plant-based approaches to stabilizing nanoparticles (Fig4). The scan is carried out within the region of 4000- 650 cm- 1 in order to identify all the important functional groups that have been involved in the synthesis process. The broad dispersion of the absorption bands in the spectrum is an indicator that the nanoparticles are closely involved with various bioactive components that are naturally present in fenugreek. The spectrum has one of the most prominent features in the broad band at the 3200-3600 cm -1 region. The table represents this region to OH stretching frequency ranges and the range generally indicates hydrogen bonding. In this regard, it indicates the existence of the phenolic and flavonoid extracts of the fenugreek, as well as the absorbed water molecules. These hydroxylated biomolecules are very important during the manufacturing process, as they assist in the reduction of zinc ions and they are a stabilizing shell of the newly formed nanoparticles. The other area of significance is found between 2870-2960 cm-1 and weaker peaks that are associated with aliphatic C-H stretch are observed. Such signals are formed by organic remnants i.e. methoxy groups and long-chain plant compounds. The fact that they form makes it clear that, they retain debris of these natural compounds embedded on the surface after the nanoparticles are formed, which prevents clumping and enhances the stability of the particles A better and more chemically informative peak is found at 1640–1650 -1, or amide carbonyl (C=O) stretching. The seeds of fenugreek are known to have proteins that have a binding capacity to metals and this observation is very much an indication of their role in the synthesis. Such protein-based molecules have a tendency of being natural, mild stabilizers meaning that the zinc oxide nanoparticles are formed in a controlled state and dispersed well. Peaks of medium intensity that are related to aromatic C=C vibrations also appear in the spectrum at the range of 15001600 cm -

1. This spectrum is typical of polyphenols - potent antioxidant of the plants that are abundant in fenugreek. Those are also indicative of their involvement in decreasing the Zn 2 + ions given that these spheroids are established in giving electrons in processes of green synthesis. Moving further into the spectrum, the range of 1010- 1270 cm -1 has a few different absorption bands which are associated with the C -O

-C and C -O vibrations. These are mostly derived out of plant carbohydrates, sugars and glycosides. Fenugreek seeds contain a good amount of these compounds and when they appear on the surface of the nanoparticle, it is a sign that they are stabilizing and capping the particles forming a natural biocompatible type of coating. The strongest indication of the nanoparticle is obtained about the intense absorption spectrum at 430-560 cm -1. This area is what is denoted by the ZnO stretch- vibration the indisputable fingerprint of the zinc oxide. What makes this obvious, sharp signal is that the matter synthesized is, without a doubt, ZnO, or a transformation of zinc ions was successful and resulted in a crystalline metal oxide structure. Taken collectively together, the FTIR spectrum draws a distinctive and coherent image of zinc nanoparticles which have been not only produced successfully but also naturally stabilized with nad Fenugreek seed extract. All of the major functional overlapping groups present, including the hydroxyls, carbonyls, aromatics, and carbohydrate- based C -O stretches, play a role in either reducing the zinc ions or protecting the resulting nanoparticles. Combining the above characteristic Zn O peak, these results are a solid confirmation of the structural identity and green synthesis pathway of the ZnO nanoparticles.

SEM: Scanning Electron Microscope

SEM came in handy to give in-depth information about the surface and structural properties of the zinc nanoparticles that had been synthesized. SEM has been identified to be highly popular to provide high-resolution of surface imaging through scanning of materials with focal electron beam, which is very suitable when looking at nanoscale morphology. The SEM micrographs, in this scenario, have given a clear indication of how developed zinc nanoparticles were, which validates the use of green synthesis strategy to make clearly defined nanostructures, which are in line with other eco-friendly approaches already reported (Agarwal et al., 2017).The analysis, through Scanning Electron Microscopy (SEM) allowed specific information on the surface appearance and the structural properties of the produced nanoparticles (zinc). It has been well known that SEM is an ideal technique used to analyze nanoscale morphology by providing high-resolution surface images through scanning the material with a focused beam of electrons. In the paper, the SEM micrographs were able to display the achievement of the formation of zinc nanoparticles successfully, attesting that the green synthesis methodology has yielded clear and defined nanostructures in agreement with already reported environmentally friendly techniques (Agarwal et al., 2017). Looking at the SEM images under higher magnification, we finally saw the particles clearly—their rough, uneven surfaces caught the light like tiny grains of sand. Most of the zinc nanoparticles looked like round beads, though a few had small, uneven bumps. You'll often see these shapes in plant-based synthesis because the plant's own chemicals that drive reduction and growth shift naturally—like how a leaf's scent deepens after rain. Because the nanoparticles' surfaces were mostly smooth, the formation process was likely steady, and the plant extract's organic compounds that clung to them helped keep the structure firm—almost like a thin, even glaze holding everything in place. (Alagumuthu & Kirubha, 2020). The particles measured in these sharp, high-resolution images fell squarely in the nanoscale range usually seen for biosynthesized zinc nanoparticles, each one barely larger than a speck of dust. Most particles measured between 20 and 80 nm—a sweet spot for stronger surface activity, like fine dust catching light on glass. Because of their huge surface-to-volume ratio, ZnO nanoparticles perform far better at the nanoscale—boosting antimicrobial, photocatalytic, and biosensing functions much like sunlight intensifies through a magnifying glass (Rao & Biswas, 2009). SEM images also showed a thin organic film draped over many nanoparticles, a faint sheen that caught the light like morning dew. This coating likely came from phytochemicals—flavonoids, proteins, and sugars— in the plant extract used to create it, a bit like the thin golden sheen that appears when the liquid finally dries. These organic molecules cling to the nanoparticle's surface, building a thin layer that wraps it like a snug, protective shell. The coating keeps the nanoparticles steady, prevents them from sticking into messy clumps, and helps them mix easily with biological systems (Sangeetha et al., 2011). In several spots on the SEM images, you could see moderate clumping—small particles huddled together, like sand grains clinging after a splash of water. It's no shock—green- synthesized nanoparticles skip synthetic surfactants and rely on plant-based stabilizers, but those natural coatings can't always keep the particles from bunching up, like fine sand left behind when a wave pulls back. Even so, the clusters looked pale and loosely pressed together, like dusted flour, so they didn't disturb how smoothly the nanoparticles spread or how well they performed. This finding suggests that although a few particles stuck together—like tiny grains of sand—they each still held onto enough of their own

function to act on their own (Agarwal et al., 2017).
Beyond their shape, the SEM micrographs showed nanoparticles glowing with even brightness and crisp contrast, a sign that electrons were interacting uniformly and the composition stayed consistent throughout. These even scattering patterns show that the nanoparticles are made mostly of zinc-based material, with no large impurities—like a smooth field untouched by stray stones. Combining SEM results with techniques like XRD or FTIR gives a clear, well-balanced picture of purity and successful synthesis, confirming the material's quality (Iravani, 2011). The SEM images clearly show that the zinc nanoparticles made with plant extract are neatly rounded, nanosized, and wrapped in a thin organic layer that's characteristic of green nanomaterials. These traits show how well plant extracts drive nanoparticle synthesis—clean, affordable, and gentle on living systems, like a reaction blooming quietly in green solution. The SEM results sharpen our view of the nanoparticles' structure, confirming their promise for a wide range of scientific and industrial uses—for instance, they reflect light like fine silver dust under the lens.
The morphology of the surface resulting after gold plating is an extremely disordered granular structure of clustered particles packed together tightly covering the region scanned by SEM (Fig5). The texture is similar to that of biosynthesized or plant-mediated zinc nanoparticles in which organic residues tend to be left on the surface. The gold coating has not only increased the conductivity of the sample but also sharpened the boundaries of the particles making the agglomeration patterns more pronounced.On higher magnifications, particles are seen to have a rough uneven structure with the presence of several micro-crevices and fractured regions. The presence of such cracks shows that the dried nanoparticles are brittle and the sample is sensitive to high-vacuum conditions in SEM. The existence of the above- mentioned voids can also be a result of disintegrated bio-organic capping agents in the course of the drying or sputter-coating process. The presence of zinc can also be evidenced by the elemental map overlay of C + O + Na + Zn that is provided, which evidently shows that the zinc is balanced over the chosen area rather well. The Zn (L) clusters are marked in yellow and carbon and oxygen are in the background as a result of the plant extraction products or the beginning product. The scattered distribution of Zn signals leads to assume efficacious development of zinc-based nanoparticles as opposed to big crystalline fragments.The separate carbon map (C K) displays a slight but general distribution over the whole field. This indicates the phytochemicals of organic nature which stabilize the nanoparticles particularly where plant extracts like fenugreek, pumpkin or even shillajat are utilized in the manufacturing. These organic residues also serve as the basis of capping of the nanoparticles as well as avoiding over-aggregation during synthesis.
The oxygen elemental map (O K) can be observed all over the surface of the particles, which proves the possibility of the zinc oxide (ZnO) formation instead of pure metallic zinc. Oxygen Zinc association In green synthesis pathways, oxidation may readily take place because of aqueous and plant rich environment. The red areas (OH) can as well be the traces of oxidized organic substances, which are inherent to the web of the nanoparticles.The sodium mapping (Na K) shows an irregular but distinct presence. This component is usually acquired through the aqueous extraction medium or salts that are inherent in plant tissues. Though sodium does not appear in the structure of the nanoparticle as such, its identification indicates that small amounts of trace minerals in the extract are adsorbed to the synthesized particles.A dedicated zinc map (Zn L) shows high and concentrated spots of zinc that are mainly concentrated in dense clusters. The yellow color is an indication that there was intense Zn impurity, proving that the synthesis of nanoparticles was successful to form zinc-rich areas. The spatial homogeneity of the diverse clusters also supports the way the elemental map is a result of carbon (blue), oxygen (red), sodium (green), and zinc (yellow). The multicoloured areas are bright and indicate the co-existence of organic matrix components binding with zinc which is a distinctive characteristic of plant-derived nanoparticle synthesis.The EDS spectrum is another level of assurance of such. These high-energy peaks surrounding the low-energy area are all indicative of carbon, oxygen, and sodium which would be anticipated of bio-organic coats. Nevertheless, the sharp zinc peaks at about 1 keV and again around 8 10 keV are a strong indication of the presence of zinc in its metallic or oxide state in its significant amount in the region studied. These high heights indicate that zinc is not the trace element but the prevailing element of the sample. These clearly defined peaks would guarantee high reliability of the elemental composition.The total counts indicated on the spectrum provide an indication on the fact that the signal of zinc has high magnitude in comparison to the background noise. This increased signal strength indicates that the nanoparticles have a high amount of zinc loaded, which is indicative of successful biosynthesis devoid of undesired elements.The peaks of zinc distribution throughout the spectrum should also be considered indicative of purity in the synthesized nanoparticles. The areas marked in blue square in the SEM image are the carefully selected mapping area based on the central and the most morphologically dense area in the sample synthesis process was clean and governed by plant metabolites and precursors of zinc. This has guaranteed the lack of biasness in the elemental readings since they are a clear representation of the actual composition and structure of the synthesized Zn nanoparticles: it is clear that the nanoparticles are made up of zinc oxide to a considerable degree but meanwhile surrounded and stabilized by natural organic molecules. The presence of sodium and carbon also confirms the presence of stabilizing factors of plant origin, whereas the zinc maps prove the successful formation and stabilization of zinc nanoparticles.As a whole, the SEM - EDS analysis is a strong confirmation of the success of the formation and stabilization of zinc nanoparticles. The homogeneous distribution of Zn, discrete Zn peaks, and organic capping markers are all indicators that the produced nanoparticles have the desired composition, structural integrity and surface chemistry that could be utilized into other applications (biological or material based).

DISCUSSION

It was evident in this study that through these green and natural way employing Fenugreek seed and leaf extracts, zinc oxide nanoparticles could be successfully prepared. The process utilizes materials of plant origin instead of relying on detrimental chemicals and costly laboratory reagents that are non-toxic, inexpensive, and eco-friendly. This renders the synthesis procedure very appealing to large-scale applications in the future. Fenugreek is a famous medicinal plant that consists of numerous useful natural elements like flavonoid, alkaloid, phenolic, proteins, saponins, and carbohydrates. These compounds are significant in the process of forming the nanoparticles since they aid in the conversion of zinc salts into zinc oxide particles and also in the protection of the particles against aggregation. To put it in straightforward words, the plant extract is at once a reducing agent and a stabilizing agent. The obtained characterization results highly favor the achievement of successful formation of zinc oxide nanoparticles. FTIR analysis revealed the existence of various functional groups including hydroxyl, amine and carbonyl groupings that are common amino groups in plant metabolites. These groups are thought to take an active role in the reduction as well as the stabilization process. Zn -O bonds were detected, proving that ZnO nanoparticles were obtained successfully. Probably, the natural molecules of fenugreek did not break off due to the presence of the nanoparticles on the surface they were bound to, forming a protective layer. This coating may enhance the stability, dispersibility, and the compatibility in the biological systems. The morphological technique like SEM helps in learning about the surface appearance, the shape, and the approximate size of the nanoparticles. The nanoparticles produced through green synthesis have a tendency to assume a spherical, rod-like, or hexagonal shape depending on the conditions during preparation and extract components. The significance of EDX analysis is also that it confirms the presence of the main elements zinc and oxygen in the sample. Zinc oxide particles are nanoscaffolds, which have unique properties that are not observed when using bulk particles. The surface area of smaller particles is many times larger, and this raises chemical reactivity and enhances the performance in most applications. Due to this fact, zinc oxide nanoparticles are heavily researched in terms of antimicrobial, antioxidant, catalytic, anti-inflammatory, and UV-protective applications. They can be applied in medicine, cosmetics, agriculture, water purification, food packaging and environmental protection. In the production of such nanoparticles with the help of fenugreek extract, they can obtain some extra biological advantages as a result of the medicinal constituents of the plant. Fenugreek finds extensive application in ancient medicine, traditionally to treat digestion, immunity, strength, metabolism, and reproduction. Consequently, the complex of zinc oxide nanoparticles with bioactive compounds obtained in fenugreek can offer an even better therapeutic resource. Zinc in itself is a mandatory mineral that is needed in numerous biological functions such as enzyme activity, immune response, and hormonal equilibrium as well as reproductive functions. It is significant in the production of sperms and the usual male fertility. Fenugreek is a traditionally vitality and reproductive-supporting herb. It implies that the zinc oxide nanoparticle dispersed via of fenugreek could lead to the successful applications in the fertility-related and nutritional studies. But prior to such use, more extensive biological tests are required. The behavior of nanoparticles can vary with consideration of their size, concentration, and exposure pathway. As such, lab assessment along with animal trials is most critical. In general, this research demonstrates the latest approach to the scientific study which refers to the use of nanotechnology along with herb resources. It shows how a simple and sustainable strategy can be used to make useful nanoparticles out of an ordinary medicinal plant such as fenugreek. These nanoparticles may find application in various healthcare and industrial sectors with supplementary optimization, safety testing and application-based research.

CONCLUSION

Conclusion Finally, the current research was able to prove the idea that the green synthesis of zinc oxide nanoparticles with the use of fenugreek seed and leaf extracts is a simple, cost-effective, and environmentally-friendly approach. Plant extracts eliminate the application of the toxic reducing chemicals and contributes to a safer overall process by humans and the environment. This is among the key benefits of green nanotechnology. Fenugreek has a high source of natural compounds which have served to transform zinc ions to zinc oxide nanoparticles and also stabilise the resulting particles. This dual action leads to making the plant extract an effective natural source in making nanoparticle. The successful synthesis of nanoparticles was proven using different characterization techniques. The presence of plant-based functional groups in reaction was revealed by FTIR analysis. The existence of Zn -O bonds was an indication that zinc oxide particles have been formed. Additional methods like the SEM, EDX, DLS and zeta potential are good methods to understand the size, shape, purity and stability of the suspension. These findings indicate that nanoparticles synthesized can be used in the future with regards to science and industry. They are small in size with a high level of surface area; this makes them more active and effective compared to much larger conventional materials. Zinc oxide nanoparticles already have a great variety of positive traits, including antibacterial, antioxidant prospects, photocatalytic, wound healing, and UV protecting. These particles can also bear the added value of herbal medicinal compounds when produced through the use of fenugreek extract. Fenugreek is known to be useful both as food and as medicine. It has long been associated with the need to enhance digestion, blood sugar control, immunity, increase levels of energy and boost reproductive health. Thus, the zinc oxide nanoparticles in combination with the fenugreek phytochemicals can unveil great opportunities in the field of medicine and health sciences. The article also promotes the concept of a sustainable scientific development. Green synthesis technologies decrease the amount of waste, decrease the cost of production and minimize pollution. This renders them feasible in the event of large-scale production in the future. Meanwhile, further studies are required. Before these nanoparticles are commercially uptaken or used in the clinical field, scientists have to consider greatly pertinent issues such as toxicity, dose-response, chronic effects as well as the interaction between these nanoparticles and other biological interactions. There is also need to standardize the processes of preparation in order to obtain uniform particle quality. Future studies can be directed towards the examination of antimicrobial properties, antioxidant action, fertility advantage, drug delivery prospects, and farmers. Competition of nanoparticles produced out of fenugreek seeds and leaves can also be used to determine the preferred source material. Performance could be even enhanced with surface modification and formulation research. Overall, the mediation of ZnO nanoparticles with fenugreek is one of the most promising combinations of ancient herbal knowledge with modern nanoscience. They provide a natural, safe and novel pathway in the synthesis of multi functional nano particles, which could find application in health, agricultural, cosmetic, and environmental applications. The paper offers a sound-based research and practical implementation in green nanotechnology in the future.

Contribution Statement with Credit Authorship

Ms. Alshifa Memon *(Research scholar) has contributed to study conception and design, material preparation, data collection and compilation of experimental data. Dr. Priyanka Singh* (as supervisor) has guided her for designing of experiment, analysis of data and drafting of manuscripts. *Dr. Yoge*sh *Kumar Singh* has helped the candidate for therapeutic efficacy of plant extract and nanoparticle. *All authors have read and approved the final manuscript.*

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