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GREEN SYNTHESIS AND CHARATERIZATIONS OF THE IRON NANOPARTICLES (INPS) FROM ALLIUM SATIVUM (GARLIC) STEM EXTRACTION

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Abstract

The main objective of the research was to explore the phytochemical composition of Allium Sativum (garlic) and prepare nanoparticles by extracting it by using its polar. A phytochemical screening test was carried out to identify the polar extraction of garlic. The results of the screening test indicating the presence of proteins, flavonoids, phenols and tannin compounds in the extract. Later on, iron nanoparticles (INPs) were also produced with the help of the extract. The produced INPs were analyzed by UV-visible spectroscopy and FTIR (Fourier-transform infrared spectroscopy). Analysis of the UV-visible and FTIR determined that the iron was present in the polar extract. The iron nanoparticles synthesized had a peculiar peak of

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289 nm. Furthermore, the credit of synthesis of iron nanoparticles was attributed to the active phytochemical compounds in the extract.

Graphical Abstract: Allium Sativum (Garlic) extract for synthesis of the iron nanoparticles

INTRODUCTION

Nanotechnology is a trans-disciplinary field that deals with manipulation and control of matter at a nanoscale, has received considerable attention and recognition as a result of its ability to transform many industries (Deshmukh and Hussain, 2024). Nanotechnology is a revolution in a discipline with enormous potential of advancement in many sectors. This opens remarkable opportunities to create materials with tailor-made properties by properly managing and controlling matter at the nanoscale which is generally between 1 and 100 nanometers (Roco, 2011). Nanotechnology involves the production, definition, manipulation, and use of materials, structures and devices at the nanoscale (Roco, 2011), (Whitesides, 2003). On a nanoscale, materials exhibit exceptional properties regarding their physical, chemical and biological properties compared to their larger-scale equivalents (Ferrari, 2004). The underlying origins of these properties are the enhanced surface area-volume ratio, quantum confinement effects and the changed electronic,

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optical, magnetic and catalytic properties (Whitesides, 2003), (Ferrari, 2004). Nonanomaterials feature unique characteristics, which have led to their ubiquitous application in different industries. Their ability to manipulate and form structures and devices with remarkable accuracy at a nano level has brought new opportunities to many areas like electronics, medicine, energy, and environmental de-contamination (Ojha, 2004). Nanomaterials have been found useful in medicine in targeted drug delivery, imaging, and diagnostics (Peer et al., 2007), (Shi et al., 2017). Moreover, nanotechnology has contributed greatly in energy generation and storage, environmental cleanup, and in the field of aerospace (Chhowalla et al., 2013), (Kumar, et al., 2013), (Díaz-Marcos et al., 2025). Because better nanofabrication techniques have been developed, both top-down and bottom-up, nanotechnology has advanced substantially. The methods have been instrumental in enhancing the development of nanotechnology (Whitesides, 2003), (Kumar et al., 2011). The methods have been used to tightly regulate the size, shape, composition, and assembling of nanomaterials, which make it possible to design materials that are specific to application (Roco, 2011), (Whitesides, 2003).

Nanoparticles can be classified into two broad categories; Organic NPs and Inorganic NPs, Organic nanoparticles are made of carbon-based compounds and are frequently highly biocompatible and versatile. They are usually applied in many other processes like drug delivery, imaging, sensing and bioengineering. Polymeric nanoparticles are one of the brightest examples of the organic nanoparticles, formed out of synthetic or natural polymers. These nanoparticles are controllable (size, shape, surface properties), and desired functionalities are attained (Khan et al., 2019). Polymeric nanoparticles have the benefits of controlled drug release, stability and the capability to encapsulate hydrophobic and hydrophilic compounds.

Inorganic nanoparticles are non-carbon based materials and they also have a great variety of compounds such as metals, metal oxides, semiconductors. These nanoparticles have special physical and chemical characteristics, which enable their use in electronics applications, catalysis, energy storage and environmental remediation. As an example, metal nanoparticles, e.g. gold or silver nanoparticles, are plasmonic and find application in bio sensing and surface-enhanced Raman spectroscopy (Chen et al., 2018). Quantum dots are semiconductor nanoparticles with an optical property that is dependent on size and have been used in imaging, solar cells, and light-emitting devices.

To sum up, the potential of nanotechnology is an exciting prospect of scientific and technological advances due to its capability of manipulating and engineering materials on a nano scale. Due to its special characteristics and innumerable uses, nanomaterials are an important future research and development field.

Human beings have, over the years, depended on Nature to support their fundamental needs to manufacture foodstuffs, shelters, apparel, means of transportation, fertilizers, flavor as well as fragrances and, last but not least, medicines. Plants have been the foundation of advanced traditional medicine systems that have existed over thousands of years and are still able to bring new solutions to mankind. Whereas certain of the medicinal actions of the plants have since been established to be erroneous, the medicinal plant therapy is founded on the empirical data collected by hundreds and thousands of years (Gurib-Fakim, 2006). Herbal drugs are resorted to both in the treatment of infectious and chronic diseases. In addition, plants and microbes are themselves stabilizing agents because they contain capping and stabilizing agents (Andleeb, 2020).

Allium Sativum was also known as a food and spice used in various countries thousands of years ago. Most cultures have also applied it in a variety of medicinal purposes. Nevertheless, large-scale scientific research began only a few years ago. Nowadays it is one of the most studied herbal remedies and the highest selling. Most commonly used orally, topically, garlic has been used to treat hypercholesterolemia and prevent arteriosclerosis, prevent or treat infections and to maintain general health. The most studied attribute of it is the reduction in the total serum cholesterol levels (Pittler & Ernst, 2015).

Garlic (Allium Sativum) is thought to have been domesticated in Central Asia, occupying the area between modern day Iran and Kazakhstan. It got to other places of the world by way of trade and exploration. Garlic is a highly grown and used crop globally, with China, Pakistan, India, South Korea, Russia, and the United States being among the top producers and consumers of the crop (El-Saadony et al., 2024). China is the world leader in production of dry bulbs of garlic with an annual output of approximately 21 million tons (more than 81% of the global output). Iran, with 54.247 tons of dry bulbs produced per year, is the 25 th most prolific garlic producer in the world (FAO, 2013). Garlic traditionally occupies a niche in the Iranian traditional agricultural complex due to the culture, its medicinal benefits, and and acclimatization to the climatic conditions of Iran (Ammarellou, 2022).

Garlic (Allium Sativum) is a vegetable crop of the family Allicaceae and one that is shallow rooted. Allium is an early crop which originated in central Asia and is spread across the northern hemisphere in the temperate, warm temperate, and boreal regions.

There are 7 known genus Allium species of which out of 750, the 7 species are grown in several parts of the globe. Garlic is a genus Allium, one of the earliest vegetables grown, and the second most commonly grown Allium, after onion (Allium Cepa) (Ammarellou, 2022).

TABLE 1 - TAXONOMICAL POSITION OF ALLIUM SATIVUM

KINGDOM	PLANTAE
Phylum	Tracheophyta
Class	Liliopsida
Order	Asparagales
Family	Alliaceae
Genus	Allium
Specie	Sativum

MATERIALS AND METHODS

Green Synthesis of Metal Nanoparticles

Green synthesis involves the synthesis of nanoparticles with the use of plant extract. Nanoparticles may be prepared through a number of biological procedures. A number of different pathways have been established to synthesize nanoparticles of salts of the respective metals biologically or biogenically. Nanoparticles have been produced using microorganisms, entire plants, tissue and fruits, plant extracts and marine algae. The biogenic synthesis is not only effective as it causes less harm to the environment than certain other physicochemical methods of production, but it is also applicable to mass-produce large amounts of nanoparticles that are contamination-free and have a defined size and morphology (Altammar, 2023)

1.3.1 Green Synthesis of $FeCl_3$ Nanoparticles

Green synthesis of iron chloride nanoparticles involves the use of environmentally friendly methods and natural sources to obtain these nanoparticles. Here's a general approach for the green synthesis of iron chloride nanoparticles:

Materials

i. Iron chloride precursor (such as $FeCl_2$ or $FeCl_3$)
ii. Reducing agents (such as plant extracts or biological molecules)
iii. Stabilizing agent (such as plant extracts or natural polymers)
iv. Solvent (such as water or ethanol)

The plant of Allium sativum was collected from Various regions (Manki, Sheikh Jana, and Tordher) of district Swabi-Kpk, Pakistan. The plant was first dried and then grinded to powdered form, after that 50g of grinded plant was mixed with 500ml of Methanol and covered with Aluminum foil for one week. After one-week solution containing methanol and plant was filtered with the help of filter paper and then for evaporating methanol the filtrate was placed in water bath for three days, three days past methanol was evaporated and extract was collected.

Preparation of Stock and Salt Solution: In green synthesis of Fe-NPs using methanolic extract of *Allium sativum,* two types of solutions were prepared (i) Stock solution and (ii) Salt solution. For preparing stock solution 2g (weighed via digital balance) of extract was dissolved in 200ml of distilled water and stirred for 30mins so that extract gets dissolved completely. After this it was analyzed via UV Visible spectroscopy for checking concentration, the solution was concentrated so we diluted the solution by adding distilled water.

Similarly, 1mmol salt solution was prepared by dissolving 0.0162g of $FeCl_3$ in 100ml of distilled water.

Chemicals Used: Following chemicals utilized in research work conducted on "Green synthesis of Iron Chloride nanoparticles". These chemicals were chosen based on their specific roles in the experimental procedures and their compatibility with the desired outcomes. Methanol, Chloroform, Sodium hydroxide, Sulphuric acid, Hydrochloric acid, Copper Sulphate, $FeCl_3$, Distilled water and Detergents were used for cleaning purposes.

Apparatus Used in Experiments: The success of any scientific experiment heavily relies on the careful selection and utilization of appropriate apparatus. Plant stem was crushed using grinder. A double spectrophotometer (Spectro Lab, Model-UV 2601) equipped with quartz cuvettes having a path length of 1.0 cm was employed to measure UV-Vis spectra. Precise weights were determined using an analytical balance (QHAUS). Water bath was used for evaporating methanol from extract. The magnetic stirrer (Model DLAB, MS-H-S) was employed to mix the substances.

Phytochemical Analysis of Allium Sativum Stem Extract

Phytochemical analysis is a scientific research that has been concentrating on the identification, isolation, and characterization of bioactive compounds in plants. The colors, flavors and aromas of different plant species are due to these compounds which are called phytochemicals. Phytochemical research is very essential in investigating the therapeutical, nutritional, and pharmaceutical promise of plants and in comprehending its biological functions. The fractions of the Allium Sativum stem were all tested. Because of the existence of various phytochemical compounds, such as phenols, tannins, flavonoids, terpenoids, proteins and saponins. General findings revealed the existence of various compounds in all the fractions and lack thereof.

Phenols: Allium sativum stem extract was transferred into a 1M $FeCl_3$ solution and allowed to mix a few minutes, appearance of dark blue, i.e. presence of phenols.

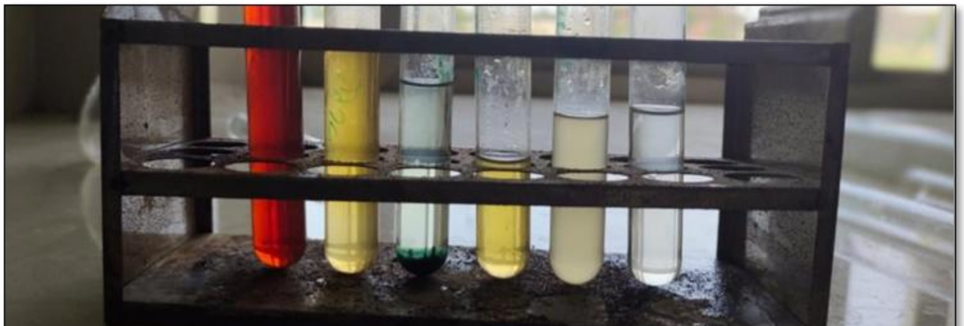

FIGURE 1: PHYTOCHEMICAL TESTING

Flavonoids: Plant extracts were dissolved in sodium hydroxide solution and then few drops of hydrochloric acid were added. A yellowish color solution turned colorless will indicate the presence of flavonoids.

Terpenoids: Salkowski test were performed for checking presence of terpenoids in extract. 2ml chloroform and 3ml H2SO4 were added to extract solution, appearance of reddish-brown color will indicate the presence of terpenoids.

Proteins: 2ml of NaOH were added to extract solution and then few drops of 1M $CuSO_4$ were added to it, and waited for 5 to 6 min, appearance of bluish color will indicate the presence of proteins.

Saponins: 5ml of distilled water were added to extract solution after 15 to 20 min appearance of white foam will indicate the presence of saponins if present.

RESULTS AND DISCUSSION

3.1 Phytochemical Screening of Allium Sativum: Result of phytochemical screening test of aqueous Stem extract of Allium sativum is given in table 2. Aqueous extract showed the presence of Proteins, flavonoids, phenols and tannins, while tests for saponins and terpenoids give negative results.

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TABLE 2: PHYTOCHEMICAL ANALYSIS OF ALLIUM SATIVUM

Phytochemicals	Aqueous extract
Saponins	_
Proteins	+
Flavonoids	+
Phenols	+
Terpenoids	_
Tannins	+

Green Synthesis of $FeCl_3$ Nanoparticles: To prepare Iron nanoparticles via green synthesis using Allium sativum stem extract, the Iron salt solution and extract solution were mixed in different ratios (1:1, 1:2, 1:4, 1:6, 1:8, 1:10, 1:12, 1:14, 1:16 1:18 and 1:20) in different vials and kept on stirring for 1 hour. The color in reaction mixture in each vial changed till 1:14. The color produced in these ratios was best and beyond these ratios, there was no prominent color changed. It was noted that the best nanoparticles were synthesized in 1:14 which was confirmed by UV-Visible spectrophotometer. The UV Vis spectra showed characteristic peak for iron NPs at 289 nm which indicates the formation of nanoparticles.

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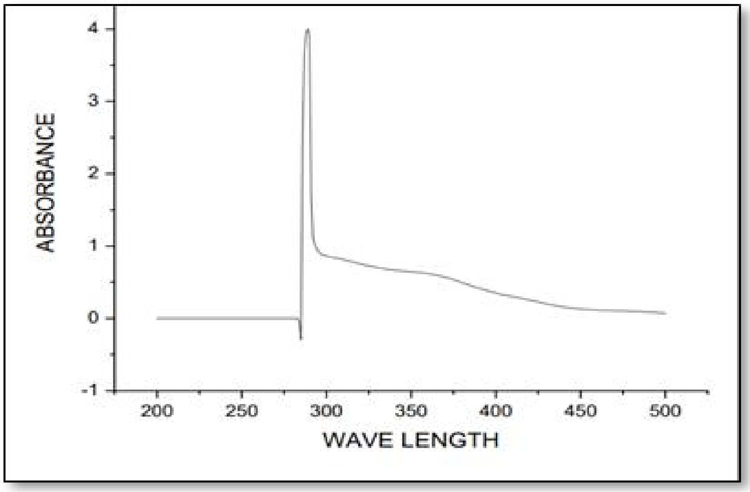

FIGURE 3: UV-VISIBLE SPECTRA OF IRON NPS

Spectroscopic Analysis of Nanoparticles

UV-Visible spectroscopy is the first indication in the formation of nanoparticles, and we also know that most of the nanoparticles give λmax in the range of 280-550 mm. In the above figure λmax given in range 289 nm of iron which indicates the formation of nanoparticles. Green synthesis of silver nanoparticles using plant extract of *Allium Sativum* as stabilized reducing agent was carried out with positive result.

Optimized Ratio of Nanoparticles: UV spectra were taken for different ratios. Below peaks in the graph show different ratios of Iron salt $FeCl_3$ vs plant. The best Peak among (1:10,1:12,1:14,1:16 and 1:18) is the one which have the sharpest peaks and ranged in 280-350nm. Results showed the formation of iron NPs at peak of 289nm for 1:14. The synthesis of $FeCl_3$ NPs was easily determined by UV-Visible spectrophotometer. The spectra were taken for the stock solution as well as when the color changed due to addition of metallic salt. The spectra were compared to each other for the conformation of nanoparticles.

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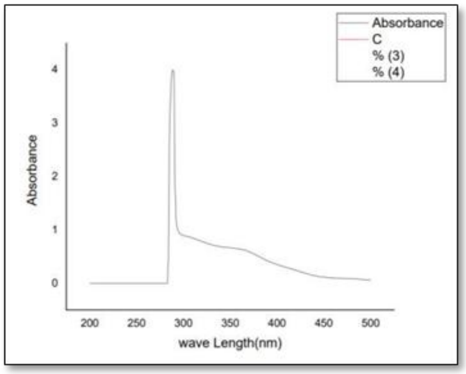

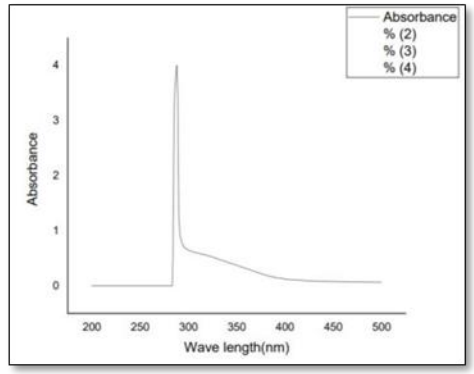

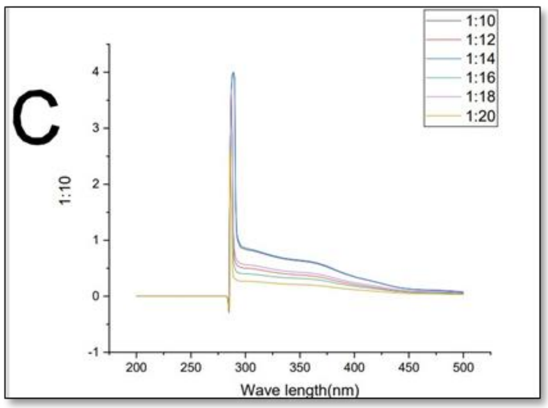

FIG 4: DIFFERENT UV-VISIBLE SPECTRA OF IRON NPS

Characterization via FTIR: To confirm the existence of Fe_3O_4 nanoparticles in plant Allium sativum extract, FTIR spectroscopy were performed, results have been shown in below figure. The formation of Iron nanoparticles was confirmed by FTIR spectroscopy.

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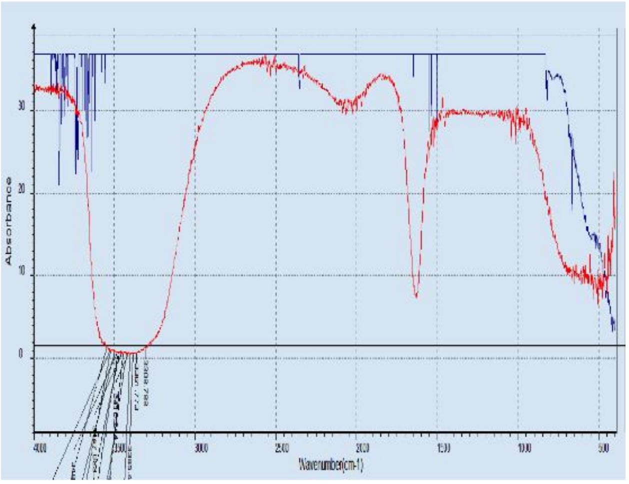
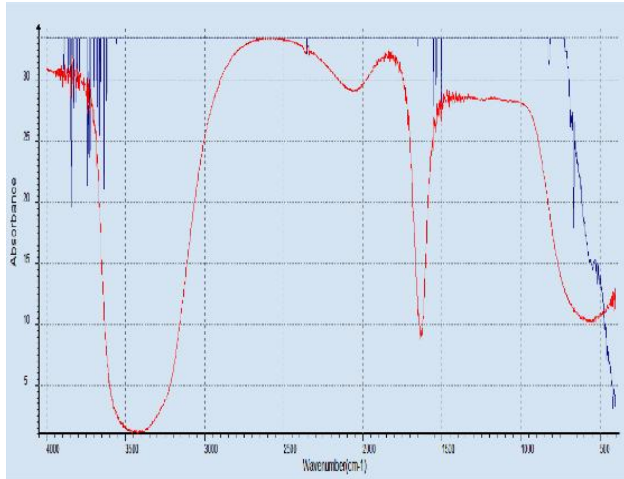

FIG 4,5: FTIR RESULT

Comparison of FTIR spectra of Allium sativum extract and prepared Fe-NPs demonstrated peak broadening in certain region, many differences were found in the fingerprint region of both extract and Fe-NPs spectra, so extract is involved in reduction of Iron Fe^{+3} ions from its higher oxidation state to lower oxidation state Fe.

CONCLUSION

In the current investigation, the stems of Allium sativum were examined using phytochemical screening before extracting them in larger quantities to identify various classes of bioactive compounds. The screening results indicated the presence of proteins, flavonoids, phenols, tannins and saponins in the stem extract. Previous research has reported that Allium sativum exhibits insecticidal and antimicrobial activity, as well as a significant analgesic effect. To further enhance these activities, the researchers subjected the stem extract of Allium sativum to treatment with iron tri chloride, resulting in the synthesis of iron nanoparticles (Fe-NPs). The active phytochemicals present in the extract played a pivotal role in the synthesis of these iron nanoparticles. The Fe-NPs demonstrated a characteristic peak at 289 nm. The synthesis of these nanoparticles holds potential for enhancing the reported activities of Allium sativum, and their production can be achieved through a straightforward and cost-effective method.

REFRENCES

Altammar, K. A. (2023). A review on nanoparticles: characteristics, synthesis, applications, and challenges. *Frontiers in microbiology*, *14*, 1155622.

Ammarellou, A., Yousefi, A. R., Heydari, M., Uberti, D., & Mastinu, A. (2022). Biochemical and botanical aspects of Allium sativum L. sowing. *BioTech*, *11*(2), 16.

Andleeb, S., Tariq, F., Muneer, A., Nazir, T., Shahid, B., Latif, Z., ... & Al Farraj, D. A. (2020). In vitro bactericidal, antidiabetic, cytotoxic, anticoagulant, and hemolytic effect of green-synthesized silver nanoparticles using Allium sativum clove extract incubated at various temperatures. *Green Processing and Synthesis*, *9*(1), 538-553.

Chen, Y., Fan, Z., Zhang, Z., Niu, W., Li, C., Yang, N., ... & Zhang, H. (2018). Two-dimensional metal nanomaterials: synthesis, properties, and applications. *Chemical reviews*, *118*(13), 6409-6455.

Chhowalla, M., Shin, H. S., Eda, G., Li, L. J., Loh, K. P., & Zhang, H. (2013). The chemistry of two-dimensional layered transition metal dichalcogenide nanosheets. *Nature chemistry*, *5*(4), 263-275.

Deshmukh, K., & Hussain, C. M. (Eds.). (2024). Functionalized Magnetic Nanosystems for Diagnostic Tools and Devices: *Current and Emerging Research Trends*.

Díaz-Marcos, J., & Mendoza González, J. (2025). Shaping the future of nanotechnology: a perspective on education, dissemination and ethics. *Frontiers in Nanotechnology*, *7*, 1524578.

El-Saadony, M. T., Saad, A. M., Korma, S. A., Salem, H. M., Abd El-Mageed, T. A., Alkafaas, S. S., ... & Ibrahim, S. A. (2024). Garlic bioactive substances and their therapeutic applications for improving human health: a comprehensive review. *Frontiers in immunology*, *15*, 1277074.

Ferrari, M. (2005). Cancer nanotechnology: opportunities and challenges. *Nature reviews cancer*, *5*(3), 161-171.

Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular aspects of Medicine*, *27*(1), 1-93.

Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. *Arabian journal of chemistry*, *12*(7), 908-931.

Kumar, V., Kumari, A., Guleria, P., & Yadav, S. K. (2011). Evaluating the toxicity of selected types of nanochemicals. *Reviews of environmental contamination and toxicology*, 39-121.

Ojha, A. K. (2004, April). Nano-electronics and nano-computing: status, prospects, and challenges. In *Region 5 Conference: Annual Technical and Leadership Workshop, 2004* (pp. 85-91). IEEE.

Peer, D., Karp, J. M., Hong, S., Farokhzad, O. C., Margalit, R., & Langer, R. (2007). Nat. Nanotechnol. *Nat. Nanotechnol*, *2*, 751.

Pittler, M. H., & Ernst, E. (2007). Clinical effectiveness of garlic (Allium sativum). *Molecular nutrition & food research*, *51*(11), 1382-1385.

Roco, M. C. (2011). The long view of nanotechnology development: the National Nanotechnology Initiative at 10 years. *Journal of nanoparticle research*, *13*(2), 427-445.

Shi, J., Kantoff, P. W., Wooster, R., & Farokhzad, O. C. (2017). Cancer nanomedicine: progress, challenges and opportunities. *Nature reviews cancer*, *17*(1), 20-37.

Pakistan Journal of Medical & Cardiological Review
https://pakjmcr.com/index.php/1/about
Online ISSN Print ISSN
3007-2387 3007-2379

Whitesides, G. M. (2003). The'right'size in nanobiotechnology. *Nature biotechnology*, *21*(10), 1161-1165.