Evaluation Of Antioxidant and Antimutagenic Potential Of Biogenic Cu/Cr/Ni Trimetallic Oxide Nanoparticles

Thesis Submitted to

The Superior University Lahore

In Partial Fulfillment of the

Requirement for the Degree of

Master of Philosophy in Chemistry

By

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Roll No. SU92-MSCHW-F22-001

Session: 2022-2024

Faculty of Sciences

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DEDICATION

I would like to thank my parents for their love and support, who prayed for me at every turn, as well as my supervisor, who served as both my inspiration and my spirit, providing me with unwavering support and kind help without which this project would have remained an idle goal. Without them, this day would not have been possible.

ACKNOWLEDGEMENT

In the name of the Most Gracious and Merciful Allah. I am incredibly grateful to Almighty "Allah," who is the fount of all knowledge and wisdom bestowed upon humanity, for giving me the insight and foresight necessary to finish this project.

I want to sincerely thank to my supervisor Dr. Hina Zain and co-supervisor Dr. Muhammad Yasir for their insightful advice and upbeat approach to this research. She made sure that I had a conducive environment and advantageous circumstances during my study period, for which I am incredibly grateful. Without the assistance and knowledgeable direction of Prof. Dr. Muhammad Naveed Babur, Dr. Uqba Mahmood and Dr. Muhammad Mudassir Iqbal, this endeavor would not have been feasible.

Finally, I would want to express my sincere gratitude to my family. I could not have finished this attempt without their encouragement.

Hafiz Muhammad Saad Ali

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Abbreviations	Term
TMNPs	Trimetallic Nanoparticles
MRI	Magnetic Resonance Imaging
FRAP	Ferric ion Reducing Antioxidant Power
AAE	Ascorbic Acid Equivalents
FWHM	Full Width Half Maximum
DLS	Dynamic light Scattering

ABSTRACT

Nanotechnology encompasses the design, synthesis, characterization, and applications of particles in the nanometer scale range. Nanoparticles possess remarkable characteristics like enhanced reactivity associated with increased surface area, and their ability to interface with biological systems on the molecular level having flexible nature makes them very dynamic. Metallic nanoparticles possess remarkable properties like surface plasmon resonance, strong plasma absorption, and superparamagnetic behavior and pronounced Rayleigh scattering. In the current research project, trimetallic Cu/Cr/Ni oxide nanoparticles were synthesized following green synthesis method using orange peel aqueous extract. Trimetallic nanoparticles were ascertained by different analytical tools like UV/VIS spectroscopy, FTIR, XRD and SEM analysis. Strong UV/VIS bands at characteristic λmax indicated successful synthesis of Cu/Cr/Ni trimetallic oxide nanoparticles; absorption peaks in the range of 650-410 cm-1 in FTIR spectrum confirmed Cu/Cr/Ni oxides; characteristic bands in XRD for all three metals also indicated their successful synthesis; and finally SEM analysis showed the particles size of 12-15 nm with spherical morphology. Investigation about biological activities of such trimetallic nanoparticles (TMNPs) was carried out by Ames reverse mutation assay to examine mutagenic/antimutagenic potential while antioxidant activities were monitored by DPPH free radical scavenging and reducing power in terms of Fe3+ to Fe2+ reduction. Results of both assays indicated that such TMNPs exhibited high antioxidant potential (> 65% free radical scavenging and reducing power of 1.94 mg AAE/mL) and strong antimutagenic potential (> 85%). Thus, it is recommended such nanoparticles should be used in different medicinal formulations, neutraceuticals and fortified foods.

Keywords: Antimutagenic potential, Salmonella typhimurium TA98**,** DPPH free radical scavenging, anti-metastatic effects, Trimetallic nanoparticles, Antioxidant potential.

CHAPTER 1

1. INTRODUCTION

Nanotechnology, the field that explores the manipulation and engineering of matter at the nanoscale, has captivated the scientific community and the public imagination alike, promising revolutionary advancements across a wide range of disciplines. The development of nanoparticles, with their unique physicochemical properties, has opened up a world of creative possibilities, with applications spanning from drug delivery to materials science and beyond (1).

The health sector is considered as one of the most important places where nanoparticles are applied. Nanotechnology has great prospects for improving the diagnosis and treatment of diseases through new nano-therapeutics or diagnostic tools. Nanoparticles have been particularly effective in targeted drug delivery by delivering drugs to the target site precisely, thereby enhancing therapeutic efficacy and reducing off-target toxic effects (2). Nanoparticles have been further aided by their inherent properties for the creation of advanced imaging techniques, allowing for earlier and more accurate disease detection (3).

As the field of nanotechnology continues to evolve, researchers have made significant strides in understanding the potential risks and challenges associated with these novel materials. While the applications of nanoparticles are vast, concerns have been raised regarding their potential impact on the environment and human health (4). Ongoing research and rigorous safety assessments are crucial to ensure the deployment of nanotechnology in a wide range of industries and applications (5).

One of the key advantages of nanotechnology lies in the unique properties exhibited by nanoscale materials. Nanoscale matter can behave differently than the same bulk material, exhibiting altered melting points, color, strength, and chemical reactivity (6). These distinct characteristics stem from the high surface area-to-volume ratio of nanoparticles, which allows for enhanced interaction with their surrounding environment. This has enabled the development of a wide range of applications, including lightweight and strong materials for aerospace, water purification systems, high-density data storage, self-cleaning surfaces, and wearable health monitors (6),(7). Moreover, the control over matter on a nanometer scale has also unveiled new breakthroughs in medicine. Targeted drug delivery using nanoparticles has a huge receiving end in future for the precise and controlled release of therapeutic agents because they present immense advantages in comparison with traditional therapy. Moreover, diagnostic imaging using nanoparticles has allowed for diseases diagnosis at a very early stage resulting in improved patient outcomes based on timely interventions (8).As the field of nanotechnology continues to evolve, researchers are exploring new frontiers, including the integration of nanotechnology with other emerging technologies, such as biotechnology and renewable energy. The potential impact of this convergence is vast, with the promise of sustainable energy solutions, advanced biomaterials, and personalized medicine (9).

The intersection of nanotechnology and biomedicine has given rise to an exciting new field, known as nanomedicine. Nanoparticles, with their unique size and properties, have emerged as powerful tools for a wide range of biomedical applications, from diagnostics to therapeutics (10). One of the most promising aspects of nanoparticles in the biomedical realm is their ability to target and deliver therapeutic agents with enhanced precision and efficiency (11). Nanoparticles can be engineered to encapsulate and transport drugs, proteins, or genetic materials directly to the site of disease, improving the bioavailability and reducing off-target effects. This targeted drug delivery approach has shown particular promise in the treatment of cancer, where nanoparticles can selectively accumulate in tumor tissues and release cytotoxic payloads, while sparing healthy cells (3).

Nanoparticles have also been used for diagnostic imaging, in addition to evolution as a drug delivery tool. On account of their small sizes and tunable optical properties, nanoparticles are capable to interact with biological entities as anticipated, which thus laid the foundation for sensitive detection as well non-invasive imaging approaches. The advancements in magnetic resonance imaging (MRI) by means of the application of magnetic nanoparticles resulted in enhancement of the capacity for medical analysis, allowing earlier disease detection and more accurate monitoring of treatment response (11).

Moreover, nanoparticles have demonstrated the ability to modulate the immune system, opening up new avenues for vaccine development and immunotherapies. By leveraging the inherent properties of nanoparticles, such as their ability to mimic or interact with biological structures, researchers have designed innovative strategies to enhance the body's natural defenses against diseases (12).

The advancements in nanoparticle-based biomedical applications are not without challenges. Concerns regarding the potential toxicity and environmental impact of these materials must be addressed through rigorous safety assessments and responsible development. Nevertheless, the continued progress in this field holds great promise for transforming the way we diagnose, treat, and prevent a wide range of human diseases (13).The field of nanotechnology encompasses a diverse array of nanomaterials, each with its own unique properties and potential applications. These materials can be broadly classified into three main categories: inorganic nanoparticles, organic nanoparticles, and hybrid nanoparticles (14).

Mono-metallic nanoparticles have also been combined with other materials, such as polymers or biomolecules, to create hybrid nanostructures with enhanced functionalities (15), On the other hand, bimettalic nanoparticles, which incorporate two different metal elements, have shown improved catalytic, optical, and magnetic properties compared to their mono-metallic counterparts (16). Trimetallic nanoparticles, comprising three different metal elements, have further expanded the design space, enabling even more customizable properties for specific applications (17).

In recent years, the development of green synthesis approaches, which utilize natural reducing and capping agents from plant extracts or microbial sources, has gained traction. These eco-friendly methods offer a more sustainable alternative to traditional chemical synthesis while maintaining the desired nanoparticle characteristics (18). Understanding physicochemical properties and possible applications of nanoparticles needs their efficient characterization. The nanoparticles size, shape and composition are analyzed using techniques like transmission electron microscopy (TEM), X-ray diffraction (XRD) or dynamic light scattering (DLS) (19).

Carbon-based nanomaterials, including fullerenes, carbon nanotubes, and graphene, have also garnered significant attention in the field of nanomedicine. These materials offer unique structural and functional properties, such as high surface area, electrical conductivity, and mechanical strength, which can be leveraged for various biomedical applications (20).

Organic nanoparticles such as liposomes, polymeric nanoparticels and dendrimers are quite suitable materials for drug delivery system due to their biocompatibility and albeit biodegradable nature. For example, liposomes are effective mode of transporting different drugs to their target organs while reducing its side effects (21). Meanwhile, polymeric nanoparticles offers the flexibility to tune physicochemical properties and surface chemistry in order significantly enhance drug targeting ability, as well as long-term stability of drugs and bioavailability (22).

Hybrid nanoparticles, which combine multiple nanomaterials or integrate organic and inorganic components, have emerged as a versatile platform for biomedical applications. These hybrid systems can synergistically combine the unique properties of different nanomaterials, resulting in enhanced functionality and performance. For instance, hybrid nanoparticles composed of iron oxide nanoparticles and polymeric shells have been developed for simultaneous magnetic resonance imaging and drug delivery (23).

The diverse landscape of nanomaterials has greatly expanded the possibilities for nanomedicine, with each type of nanoparticle offering unique advantages and potential applications. As the field continues to evolve, the strategic design and integration of these nanomaterials are crucial for unlocking their full potential in areas such as targeted drug delivery, diagnostic imaging, and immuno-modulation (24),(26). However, the successful translation of nanomedicine from the laboratory to the clinic will require addressing challenges related to scalability, regulatory approval, and safety considerations (27).

Similarly, the synthesis of organic nanoparticles, such as liposomes and polymeric nanoparticles, often relies on self-assembly or emulsion-based techniques. These methods typically involve the encapsulation of bioactive compounds within the nanoparticle structure, allowing for the controlled release and targeted delivery of therapeutic agents (28). More recently, advanced techniques, such as microfluidics and microemulsion synthesis, have been developed to improve the control over the size, size distribution, and morphology of nanomaterials (29). These advanced synthesis methods not only allow for the production of highly uniform and reproducible nanoparticles but also enable the incorporation of multiple functionalities within a single nanoparticle platform (30).

The **top-down approach** for nanoparticle synthesis involves the breakdown of bulk materials into smaller nanoscale structures through physical and mechanical processes. This method typically employs techniques such as lithography, etching, and milling to produce nanoparticles with controlled size and shape (31). One of the key advantages of the top-down approach is the ability to precisely engineer the size and morphology of the resulting nanoparticles. However, this method is often limited by the need for specialized equipment, high energy consumption, and the potential for surface defects or impurities in the final nanoparticles (32).

In contrast, the bottom-up approach for nanoparticle synthesis involves the assembly of atoms or molecules into larger nanostructures through chemical and physical processes. This method typically employs techniques such as chemical reduction, sol-gel synthesis, and self-assembly to produce nanoparticles with a high degree of control over their size, shape, and composition (33). The bottom-up approach is often favored for its ability to produce highly uniform and monodisperse nanoparticles, as well as the potential for scalable production. However, this method can be more sensitive to reaction conditions and may require careful control over the synthesis parameters to achieve the desired nanoparticle properties (34).

Ultimately, the choice between top-down and bottom-up methods for nanoparticle synthesis depends on the specific application, the desired nanoparticle characteristics, and the available resources and expertise (31), (35), (36).

The unique properties and versatility of nanomaterials have enabled their widespread application in the field of medicine. Some of the key applications of nanomaterials in medicine include:

Drug Delivery: Nanomaterials, such as liposomes, polymeric nanoparticles, and metal nanoparticles, can be used as drug carriers for targeted and controlled delivery of therapeutics, improving the bio-distribution and pharmacokinetics of drugs (37), (38).

Tissue Engineering: Nanomaterials can be used to fabricate scaffolds and matrices that mimic the extracellular matrix, promoting cell adhesion, proliferation, and differentiation for tissue regeneration (39), (40).

Antimicrobial Applications: Certain nanomaterials, such as silver and copper nanoparticles, possess potent antimicrobial properties, making them useful for the development of infection-resistant medical devices and wound dressings (41), (42).

Cancer Treatment: Nanomaterials can be used as vehicles for the targeted delivery of chemotherapeutic agents, reducing the side effects of traditional cancer treatments and improving treatment efficacy (42), (43).

The use of nanoparticles in medicine has also raised concerns regarding their potential toxicity and environmental impact. Extensive research is ongoing to address these concerns and ensure the safe and responsible development of nanomedicines (31).

Nanomaterials exhibit unique physicochemical properties that distinguish them from their bulk counterparts. These properties include:

Small Size: Nanoparticles have a high surface-to-volume ratio, allowing for increased reactivity and interactions with biological systems (44).

Quantum Effects: At the nanoscale, materials can exhibit quantum effects, such as changes in optical, electrical, and magnetic properties (45).

Increased Reactivity: The high surface area of nanomaterials can lead to increased chemical reactivity, which can be both beneficial and potentially harmful (46).

Improved Biocompatibility: Certain nanomaterials, such as polymeric and ceramic nanoparticles, can be designed to be biocompatible and non-toxic, making them suitable for biomedical applications (47).

The specific properties of nanomaterials depend on their composition, size, shape, and surface properties. These properties can be tuned and engineered to optimize the performance of nanomaterials in various applications (26), (47), (48).

The electrical properties of nanomaterials are significantly different from their bulk counterparts due to the nanoscale dimensions. At the nanoscale, the ratio of surface atoms to interior atoms increases dramatically, leading to a higher surface-to-volume ratio. This increased surface area can enhance the electrical properties of nanomaterials (49), such as:

High Electrical Conductivity: Certain nanomaterials, such as carbon nanotubes and graphene, exhibit exceptional electrical conductivity due to their unique electronic structure (49).

Quantum Confinement: In semiconductor nanostructures, the movement of electrons is confined to the nanoscale dimensions, leading to the emergence of quantum effects, such as size-dependent energy levels and improved optical and electrical properties (50).

Enhanced Charge Transport: The high surface area and reduced dimensionality of nanomaterials can enhance charge transport and electron mobility, making them suitable for applications in electronics and energy storage (51).

Nanomaterials also exhibit unique magnetic properties that differ significantly from their bulk counterparts. Some of the key magnetic properties of nanomaterials include:

Superparamagnetic: At the nanoscale, certain materials can exhibit superparamagnetic behavior, where individual nanoparticles behave as single magnetic domains and can be easily magnetized and demagnetized by an external magnetic field (8).

Enhanced Magnetic Susceptibility: The high surface-to-volume ratio of nanomaterials can lead to an increase in their magnetic susceptibility, making them more responsive to external magnetic fields (52).

Spin-Dependent Effects: In some nanomaterials, the spin of electrons can play a significant role in their magnetic properties, leading to phenomena such as giant magnetoresistance and spin-dependent tunneling (53).

The optical properties of nanomaterials are also significantly different from their bulk counterparts. Some of the key optical properties of nanomaterials include:

Surface Plasmon Resonance: Certain metallic nanoparticles, such as gold and silver, can exhibit strong light-matter interactions due to the collective oscillation of their conduction electrons, known as surface plasmon resonance (54).

Quantum Confinement: In semiconductor nanostructures, the confinement of electrons and holes to the nanoscale dimensions can lead to size-dependent optical properties, such as tunable absorption and emission wavelengths (55).

Improved Light Absorption and Scattering: The high surface-to-volume ratio of nanomaterials can enhance their ability to absorb and scatter light, making them useful for applications in solar cells, photo catalysis, and optical sensing (56).

These optical properties of nanomaterials have found applications in areas such as photovoltaics, optical sensing, bio imaging, and nano photonics.

The mechanical properties of nanomaterials can also differ significantly from their bulk counterparts due to their small size and high surface-to-volume ratio. Some of the key mechanical properties of nanomaterials include:

Enhanced Strength and Toughness: Certain nanomaterials, such as carbon nanotubes and graphene, exhibit exceptional mechanical strength and toughness, making them suitable for applications in structural materials and composite reinforcement (57).

Improved Hardness and Wear Resistance: Nanostructured materials, such as nanocomposites and nanoceramics, can exhibit increased hardness and wear resistance compared to their bulk counterparts, making them useful for wear-resistant coatings and cutting tools (58).

Size-Dependent Mechanical Behavior: The mechanical properties of nanomaterials can be strongly influenced by their size, shape, and surface characteristics, leading to size-dependent phenomena such as the "smaller is stronger" effect (59).

These unique mechanical properties of nanomaterials have enabled their use in a variety of applications, including structural materials, coatings, and microelectromechanical systems (60).

Nanomaterials possess a high surface-to-volume ratio due to their small size, which can significantly enhance their catalytic properties. Some key aspects of the high surface area and catalytic properties of nanomaterials include:

Increased Surface Area: The small size of nanomaterials leads to a dramatic increase in their surface area, with a corresponding increase in the number of active sites available for catalytic reactions (46).

Improved Catalytic Activity: The high surface area and unique atomic arrangement of nanomaterials can enhance their catalytic activity, leading to improved efficiency and selectivity in various chemical processes (61).

Tunable Catalytic Properties: The size, shape, and composition of nanomaterials can be tailored to optimize their catalytic properties for specific applications, such as in energy conversion, environmental remediation, and chemical synthesis (62).

CHAPTER 2

2. LITERATURE REVIEW

The interesting and fundamentally distinct physical, chemical and optical properties of metallic nanoparticles in comparison to their corresponding bulk materials have provoked enormous attention among the scientific community because they open a new approach through which relation between photons and electron excitation sates become possible. These properties are due to the high surface-to-volume ratio and quantum-size effects exhibited by nanostructured materials. Metallic nanoparticles can be prepared using numerous chemical and physical approaches allowing perfect regulation of the size, shape as well as composition of engineered particles (4).

Monometallic nanoparticles from silver, gold and copper (among the most studied elements) have played some important role in several areas of research, as catalysis, electronics or biomedicine (63). As expected silver nanoparticles have shown immense antimicrobial activity which has been recognized due to their capacity of destroying the bacterial cell membranes, hindering cellular respiration and producing reactive oxygen species (64), (65). Moreover, bimetallic nanoparticles composed of two separate metallic elements have been found to be much higher in catalytic activity, optical efficacy and antimicrobial performance than their monometallic counterparts (17), (63).

Considerable effort has been made to develop novel synthesis methods for the preparation of metallic nanoparticles. Several processes have been reported in the literature for the preparation of metallic nanoparticles and some are chemical reduction, thermal decomposition and hydrothermal methods (66). On the other hand, size, shape and composition of nanoparticles strongly depend on various reaction condition such as selecting synthetic method, reducing agents, capping agents, and reaction temperature, and composition of the resulting nanoparticles (63), (66).

Biological methods along with the traditional chemical and physical have shown great potential in synthesizing metal nanoparticles. These "green" synthetic methods use nontoxic reducing and capping agents that come from sources such as plant extracts, microorganisms, or enzymes to synthesize nanoparticles more robustly while avoiding environmental damage (66).

The structural, composition and properties of metallic nanoparticles influences largely to the kind of applications that these materials can bring. Metal nanoparticles can be synthesized with various forms, and played significant roles for the development of many properties; there are several analytical techniques as Transmission Electron Microscopy (TEM), X-ray diffraction (XRD) and Dynamic Light Scattering (DLS) to determine the particle size and distribution size. Due to the remarkable properties of metallic nanoparticles in different applications, such as sensors, catalysts, antibacterial coatings and biomedical imaging (63), (67).

Metallic nanoparticles have been one of the most widely studied applications for antimicrobial purposes. Taking silver nanoparticles as an example, they have long shown exceptional antibacterial and antiviral activities to a large extent; this also attracts huge attention in the development of medical implant devices, wound dressings and water purifying systems (65), (68).Gold nanoparticles have been observed for its remarkable capacity of capturing hydrogen peroxide and it can be utilized in cancer cell therapy. With improved research in the field of newer synthesis methods, especially attempts at green synthesis processes may increase the possibility of more sustainable and eco-friendly metallic nanoparticles in the future (68), (69).

Various techniques for trimetallic nanoparticles synthesis have been introduced such as co-reduction method is used in which all three metal precursors are simultaneously reduced to produce an alloyed nanoparticle (70). This method provides control over the composition and stoichiometry of the nanoparticles, yet can be difficult to accomplish with a homogeneous distribution of all three metals (16). One strategy is based on successive reduction, also called sequential reduction method in which the precursors of each three metals were reduced separately by changing reducing agents or potential to reduce metal ions. This approach can produce either core-shell or phase-segregated nanoparticle morphologies depending on the reactivity and solubility of the metal components (63), (71).

A similar approach using galvanic replacement reactions has been used in the synthesis of trimetallic nanoparticles where a sacrificial metal template is employed as an alternative to drive the reduction and deposition mechanism for all three metallic precursors. The disadvantage of this process is that similar to the other methods; however, nanoparticles produced by it can be hollow or porous nanoparticle structures which in some applications like catalysis and drug delivery considered as a great advantage (71).

Various other methods have also been used in the synthesis of trimetallic nanoparticles, where metal precursors are mixed and reacted at high temperature and pressure (solvothermal or hydrothermal methods). Taking into consideration, the above described methods of achieving ternary structure, which also dictate reaction conditions and precursors composition is very essential with regards to both structural, chemical and functional properties of a resulting trimetallic nanoparticles. Careful selection and optimization of the synthetic route facilitate property tuning, allowing trimetallic nanoparticles to be optimized or adjusted according to their intended application (16).

In this method, metal precursors are deposited on a porous support material (e.g. activated carbon or silica) and the subsequent reduction-thermal treatment leads to trimetallic nanoparticles formation in CNTs-based composites (15). Owing to their diverse physical, chemical and biological properties metallic nanoparticles such as monometallic, bimetallic or trimetallic formulations have become very useful for various antimicrobial applications. A plausible synthesis route to obtain antimicrobial trimetallic nanoparticles could be the impregnation method, where these materials are anchored in a porous material like activated carbon or silica (72).

This methodology allows a high degree of precision in tuning the composition, dimensions and spatial arrangement of the trimetallic nanoparticles (TNPs), as well as optimal interactions with support materials. Utilization of the support material chosen can likely play an important role to control and retain antimicrobial potentiality as well as performance concerning trims nanoparticles (73). As example, supporting on activated carbon can improve sequestration of microorganisms and release metal ions; in turn silica support increases nanoparticles stability as well as their dispersion (74), (75).

The processes in the impregnation method are generally as follows:

1) Support material preparation,

2) Impregnation of the metal precursors on to a support.

3) Ammonia reduction and partial calcinations to form trimetallic nanoparticles.

The synthesis is optimized to enhance the composition, size and dispersion of trimetallic nanoparticles for their optimal antimicrobial activity. Ag-Cu-Zn and Ag-Pd-Pt trimetallic nanoparticles synthesized by the impregnation method have shown a higher antimicrobial activity than their corresponding monometallic/bimetallic forms in recent studies (15), (76), (77). The enhanced antimicrobial effect of trimetallic nanoparticles are related to synergistic effect of three different metals, making it more potent and broad-spectrum capability (78).In addition, the incorporation of trimetallic NPs in polymer matrices i.e. chitosan and polyvinyl alcohol can enhance their antimicrobial activity as well as stabilization and hence they could be potential application for wide range of applications like medical devices, textiles (79).

Apart from impregnation method, galvanic replacement reactions have been used as an alternative to produce trimetallic nanoparticles with more bactericides activity (16). The galvanic replacement method uses a sacrificial metal template spontaneously react with other metal precursors in the redox process, promoting hollow and core-shell nanomaterials formation. Such a strategy enables the accurate regulation of composition, morphology and surface feature of trimetallic nanoparticles to fulfill desired antimicrobial performance (80). In one of the study, Ag-Cu-Zn trimetallic nanoparticles were prepared by galvanic replacement method which exhibited excellent antifungal and antibacterial properties than typical monometallic and bimetallic particles (72).Functionalization of the support by polymers or biomolecules can also improve antimicrobial and biocompatibility properties on their external surface due to galvanic replacement in trimetallic nanoparticles (15).

The synthesis of trimetallic nanoparticles (TMNPs) by using the impregnation and galvanic replacement methods gives a new step in their potential use as potent broad-spectrum antimicrobial materials. By tuning the composition, size and distribution of these trimetallic nanoparticles, also considering their interactions with support materials (e.g. mesoporous silicas) as well polymer matrices in form of thin films, it is possible to establish a new generation antimicrobial agents showing excellent activity across diverse microorganisms for several applications including medical devices, textiles and water purification systems and many others (81).

Impregnation method produced trimetallic nanoparticles exhibiting a notable enhancement in antimicrobial activities over their monometallic and bimetallic counterparts due to ligand effect and the interaction of three metallic components, after optimizing synthesis conditions followed by other steps involving charge affinity difference (72). The galvanic replacement reaction has been used as well to synthesize trimetallic nanoparticles with increased antimicrobial properties. During galvanic replacement, the spontaneous redox reaction takes place between a sacrificial metal template and other metal precursors to obtain hollow, porous or core- shell type structures. Here, this enables precise control over the full chemical composition and morphology of such as-synthesized trimetallic nanoparticles to optimize their antimicrobial performance (16).

These compounds constitute excellent antimicrobial agents and have biocompatibility, making them attractive new biomaterials. The introduction of these functional elements to the surface can be achieved by employing the galvanic replacement method in addition to provide an additional functionality needed for this class of materials (74). This work shows that the synthesis of trimetallic nanoparticles using both impregnation and galvanic replacement methods may provide an excellent way to enhance both antimicrobial efficacy and versatility for metal-based nanomaterials. This will enable a new generation of powerful and broad-spectrum antimicrobial agents with diverse applications in medical devices, industry and food manufacturing units (82).

Nanoparticles may be monometallic or multi-metallic in nature (63). Monometallic nanoparticles are elements that contain only a single metal having unique physical and chemical appearances. There is more than one synthetic method to generate these nanoparticles, although chemical methods remain the most common. Monometallic nanoparticles, specifically those of silver (Ag), gold (Au) and copper have been shown to possess an extraordinary antibacterial efficacy in numerous studies against diverse bacteria like Gram-positive, Gram-negative, and viruses as well (63), (65).

Bimetallic nanoparticles are meanwhile, unlike monometallic nanoparticles that consists a composite of two different metallic elements. Due to their unique properties, bimetallic nanoparticles are more competent than monometal in catalysis (65), (83). Bimetallic nanoparticles can be synthesized by many chemical methods which enable tuning the size, shape and composition of yielded nanoparticles (63).

During the last decade, new synthesis methods have been developed for the growth of bimetallic nanoparticles. Metallic nanoparticles are prepared using chemical reduction, thermal decomposition and hydrothermal methods mostly. The possible pathways of synthesis and different reaction conditions, like the reducing agent, capping agent or reaction temperature have a major influence on their size, shape as well as chemical composition (16).

Biological methods have appeared to be a new promising alternative method for the synthesis of metallic nanoparticles in addition to chemical and physical approaches broadly applied. These “green” methods of synthesizing nanoparticles implement natural reducing and capping agents i.e. using plant extracts, microorganisms or enzymes for nanoparticle production leading to greener eco-friendlier alternatives (65).

Trimetallic nanoparticles combine three distinct metals and, although monometallic and bimetallic particles are more frequent, the synthesis of trimetallic NPs has also been pursued. Additionally, these metallic nanoparticles exhibit specific properties such as catalytic activity, optical characteristics and antimicrobial efficacy that can be further boosted through the inclusion of a third metal (65). The types of trimetallic nanoparticle are the same as those in bimetallic nanoparticles, including mixed alloyed particles and core shell alloyed or separate core-shell-shell structures (83).

The extent of mixing and atomic ordering in trimetallic nanoparticles can depend on parameters such as the strength of bonds among the three metals, surface energy, atomic size or electronic/magnetic effects (84). Similarly, this tri- metallic nanoparticles synthesis can be done via various approaches such as core-shell/hollow/core-alloy type structures based on compositions and desired application or properties (16).

Synthesis of trimetallic nanoparticles is a rapidly evolving field, and ongoing research is focused on developing new synthesis methods and exploring the potential applications of these more complex metallic nanomaterials (85). One of the potential methods is green synthesis which includes using biological agents like plant extracts or microorganisms to synthesize trimetallic nanoparticles, as they are considered more sustainable and eco-friendly ways for metal nanoparticle capping (86).

The synthesis of non-hierarchical trimetallic nanoparticles can be achieved by chemical methods including co-reduction, sequential reduction and thermal decomposition or biological approaches using plant extracts or microbial enzymes as reducing and capping agents (40).

Aside from the fact that tri-metallic nanoparticles present additional possible applications as a different species of metal to create an alloy, where multi-faceted catalytic and antibacterial properties are acelerated due to synergistic effects between three distinct metals (16). Metal doping such as incorporating a third metal with the nanoparticles can alter their electronic structure, improve catalytic activity on its surface, or change its reactivity against deactivation. The optical properties of trimetallic nanoparticles are also tunable by controlling the synthesis to regulate their plasmonic resonance and photoluminescence which depend on composition, size as well including internal architecture (87).

Moreover, trimetallic nanoparticles have displayed increased antimicrobial capacity against a wide diversity of microorganisms viz., bacteria, fungi and viruses which are good contenders for their usefulness in biomedical areas (87). The versatility of trimetallic nanoparticles, coupled with their amenability to the development through composition and structure manipulation renders them increasingly useful in diverse applications such as catalysis, sensing, optoelectronics, biomedicine and others (88).

The properties and applications of the trimetallic nanoparticles can be greatly influenced by its morphology and structure. For example, bimetallic nanoplates/nanosheets have been paid more attention because the properties of 2D materials are predominantly composition, structure and morphology-dependent (83). These two-dimensional trimetallic nanostructures usually possess unique structure and superior catalytic, optical, magnetic properties over bulk/spherical counterparts that lead to attractive applications in the fields of catalysis, sensing, biomedicine (16). Chemical synthesis strategies for the preparation of trimetallic nanoplates and nanosheets, include seeded epitaxial growth, co-reduction, solvothermal reactions, galvanic replacement and electrode deposition (83).

Properties of such trimetallic nanostructures are found to be quite different depending on the specific synthetic approach and reaction conditions used. For instance, trimetallic nanoplates Pt-Pd-Ag and Au-Pd-Pt exhibited enhanced catalytic activity and selectivity compared to their monometallic or bimetallic counterparts. In particular, the current ability to control trimetallic nanoparticles shapes and structures dictates their designed synthesis with most beneficial morphologies for targeted technological applications (89).

The catalytic activity and selectivity of trimetallic nanoparticles are closely related to their chemical composition. Changing the metal component proportions offer control over the electronic, catalytic, optical and magnetic properties of these nanoparticles for specific applications. As an example, in bimetallic nanoparticles the addition of a

third metal improved catalytic performance as it could tune not only electronic structure but also surface reactivity thereby enhancing selectivity and activity in catalysis reactions (90).Analogously, composition of all three metal components can be used as a means to tune the optical characteristics: plasmonic resonance and photoluminescence (87). Additionally, the synergistic action of these three metals provides trimetallic nanoparticles with broad-spectrum antimicrobial activity in respect to both Gram-negative and Gram -positive bacterial strains that surpasses mono- or bimetallic nanomaterials. In general, the chemical composition of trimetallic nanoparticles is an important design strategy to improve their performance in different applications (91).

Completely new properties and performance of trimetallic nanoparticles, may result from the selection or oxidation states for these individual metal components. As example, the catalytic activity of trimetallic nanoparticles can be altered due to different relative oxidation states caused by their influence in electronic structure and reactivity on the surface (85). Among them, trapping and release of charge carriers at their surfaces may cause changes in the optical properties like plasmonic resonance (phenomena associated with collective electron oscillations) and luminescence on solid state that are manifested as photoluminescent behaviour for these trimetallic nanoparticles (55).

In addition, the oxidation state of metals used in trimetallic nanoparticles may affect their antimicrobial activity as different states display distinct levels of reactivity and can lead to varied efficiency for specific species ranging from bacteria to fungi (92). Thus, characterizing the control and manipulation of metal oxidation state in trimetallic nanoparticles needs to be properly understood for their design and optimization at a nanoscale level (93).

There are many ways to synthesize trimetallic nanoparticles like co-reduction, galvanic replacement or solvothermal synthesis (91). The selection of a specific synthetic method and the kind of reaction environments will determine all factors in turn such as shape, composition or oxidation status at surface level that collectively influence desirable properties for targeted applications (94).

The trimetallic nanoparticles surface morphology could also have a considerable effect on their features and functioning in various application performances (74). A good example is the catalytic activity of trimetallic nanoparticles, which can be greatly influenced by their surface structure as exposed facets and atomic coordination at surfaces play key roles on adsorption and activation of reactant molecules (16).

Also, the external morphology is obligatory to expose a remarkable behavior of both plasmonic resonance and photoluminescence since it determines how they can alter localization and enhancement of electromagnetic field due to those excellent morpho-specific properties (88). In addition, the surface morphology of trimetallic NPs plays an important role in their antimicrobial activity; attributes such as irregularities and roughness at surfaces can impact interactions between nanoparticles and microbial cells along with metal ions and reactive oxygen species release into growth medium (95). In conclusion, the surface morphology of trimetallic nanoparticles is one key design parameter that can be tuned for different conductivities in various technological applications (92).

Trimetallic nanoparticles have attracted potential applications due to their effective catalysis, sensing and biomedical activity (96). The composition, oxidation state and surface morphology of these nanoparticles can be controlled to modulate the properties as well as performance (97). In conclusion, continued research and development is allowing trimetallic nanoparticles to be versatile entities in several fields of study that can serve as improved technological solutions (98).

Trimetallic nanoparticles are also deposited or inserted on a similar support material or substrate which can significantly affect their efficiency and performance (16), (99). Support material will provide a considerable stability to the nanoparticles and prevent agglomeration along with restricting its interaction with the surrounding environment. For instance, enhancement of catalytic activity might be achieved by supporting trimetallic nanoparticles onto high surface area materials (e.g. oxides or porous carbon based supports) promoting both the number and accessibility of active sites for reactant molecules adsorption/activation (85).

Dielectric properties of support media, however, play a crucial role in determining the optical characteristics of trimetallic nanoparticles; they can largely determine localization and enhancement values for electromagnetic fields around these nanoparticles. Moreover, the support material can alter the release and diffusion of metal ions or reactive oxygen species, hence influence antimicrobial property and also affect interaction between nanoparticles with target microorganisms (100). Therefore, the support material choice is a critical design factor that can be used to design trimetallic nanoparticle performance of products for their end applications in different fields viz., catalysis; sensing and biomedicine (77).These studies combined with the flexibility to find and tune ideal support-materials, allow designing the nanoparticle-support interactions governing trimetallic NPs for generating highly efficient and versatile systems that that can address a wide range of technological challenges (101).

A number of metallic (monometallic and bimetallic compositions) nanoparticles have been shown to possess equally potent antimicrobial activities against an array of microorganisms including Gram-positive, Gram-negative bacteria, fungi as well as viruses (65). The ways in which metallic nanoparticles exert their antimicrobial effects are varied, also free radical generation through oxidative stress, loss of genetic material or proteins function interference with cellular respiration, depending on many issues such as specific physical and biological parameters (63).

When compared with monometallic nanoparticles, bimetallic systems are highly efficient in killing a wide variety of microorganisms by the combined action of each metal constituents. The mix of metals that have some distinctive attributes in redox potentials and ion release profiles, as well as antimicrobial mechanisms can be more effective for the robust and broad-spectrum anti-microbial activity (102). To illustrate further, silver-gold bimetallic nanoparticles have been reported to exhibit higher antibacterial and antiviral activity than those of the former alone; with the preponderant effect exercised via gold accounts for its stabilizing behavior inducing synergistic actions in comparison (63).

Additionally, copper-zinc bimetallic nanoparticles have good antibacterial and antifungal activities which can be reasoned by the synergy of Cu ions with reactive oxygen species formation (63). Recently, the development of trimetallic nanoparticles for antimicrobial application is being explored to improve their efficacy and broad spectrum activity against a larger domain of microbes when compared with mono- or bimetallic NPs (95).

Apart from as antimicrobials, metallic nanoparticles are also catalyzed in different industrial processes. Metallic nanoparticles are in principle promising catalytic materials due to their unique chemical and physical properties that include large surface-to-volume ratios, tunable compositions and increased reactivity (103).

One of the main features of metallic nanoparticles in catalysis is their great potential for facile dispersion and support on several substrates like carbon, metal oxides or polymers (16). This was enabling the effective accessibility of catalytically active surface area and enhancement in catalytic performance. In addition, the introduction of more than one metal constituent in bimetallic or trimetallic nanoparticles may induce synergistic effects which enhance both catalysis and selectivity when compared to their monometallic analogues (83), (85). For instance, Pt-Pd bimetallic nanoparticles have attracted a lot of interest due to their excellent catalytic performance in many reactions such us the oxygen reduction reaction, hydrogenation and fuel cell applications. In the same way, Pt-Pd-Ru trimetallic nanoparticles have shown good catalytic performance and form a great synergistic material in methanol oxidation reaction which is essentially important for direct methanol fuel cells (104).

Metallic nanoparticles are also important applications in the Fischer-Tropsch synthesis of hydrocarbons from synthesis gas (a mixture of carbon monoxide and hydrogen). In this way, nanometallic based iron, cobalt or ruthenium catalysts convert synthesis gas to long-chain hydrocarbons (55). The Fischer Tropsch synthesis with metallic nanoparticles presents some advantages which are:

1- Better catalytic performance and selectivity owing to their large surface-to-volume ratios, as well unique structures of low dimensions.

2- Elevated thermal stability and deactivation resistance, especially for Fischer-

Tropsch process that usually involves high-pressure high-temperature environment.

3- Alter the noble metal composition in combination or else change their particles structures for tuning relates to modulation of product distribution/prerequisite which may result with adjustable end products (105).

In addition, the recycling and recovery of valuable metal catalysts that are used in Fischer-Tropsch process like platinum group metals and rhenium is necessary for economic and environment friendly technology (106). Current researches concentrate on synthesizing a novel type of metallic nanoparticle catalysts and their superior performance can be attributed to promising catalytic activity, selectivity or stability in the Fischer-Tropsch synthesis as well reprocessing technologies for regenerating/collecting and recycling the used catalysts (105).

Metallic nanoparticles also have an important application in the catalytic reforming of methane, one of its most significant applications being for hydrogen and synthesis gas production. This method involves the use of metallic nickel, cobalt or ruthenium nanoparticles as active catalysts during methane conversion into hydrogen and carbon monoxide. Therefore, such nanoparticles based process for methane reforming offers many benefits:

1- Enhanced activity and selectivity as compared with the conventional supported catalysts due to their high surface: volume ratios, unique structural properties.

2- Increased resistance to coke formation, a typical problem encountered in methane reforming operations; and

3- The possibility of tuning the product distribution by varying composition and structure in metallic nanoparticles (107).

So far, there have been some research works on enhancing the catalytic performance and stability of multimetallic NP catalysts consisting of nickel, cobalt, and copper.

Improving activity, selectivity and resistance to deactivation for metallic nanoparticle catalysts is a fundamental goal of current research in the field of methane reforming. The integration of these catalysts in viable reactors and optimization processes is crucial to the large scale application of methane reforming technology for hydrogen or syngas production (108).

One of the key applications in which metal nanoparticles are used for direct synthesis of hydrogen peroxide (H_2O_2) from suitable precursors, namely hydrogen and oxygen. The hydrogen peroxide is a chemical used in many applications, and among other sectors to pharmaceuticals, personal care or water treatment. One such alternative is the direct synthesis of hydrogen peroxide, H_2O_2, from its elements - oxygen and hydrogen, which causes less harm to the environment compared with traditional anthraquinone method. In fact, metal nanoparticles such as palladium gold or platinum, have demonstrated a good capacity to catalyze the direct synthesis of hydrogen peroxide with high selectivity.

The high surface-to-volume ratios and tunable compositions of these nanoparticles enabled a selective catalytic formation of hydrogen peroxide with suppression on undesired over-hydrogenation or oxidation reactions. In addition, a combination of the supported metallic nanoparticle catalyst could be used for extra stabilization and recyclability-assessment strategies to make direct hydrogen peroxide synthesis more established in continuance. Briefly, catalysis with metal nanoparticles has become a revolutionary method for the improvement of catalytic functionalities which makes use for various applications ranging from chemical transformations to environmental remediation as well as energy conversion (109), (110).

The success of progression in catalytic uses for metallic nanoparticles has no doubt been heavily linked to the capability to both design and synthesize these particles with requisite compositions, structures and morphologies. Current research indicates that attention is increasingly turning towards the development of highly active and environmentally benign metallic nanoparticle as catalysts due to their superior efficacy, and green benefits in most areas where energy production or conversion, chemical synthesis, pollution prevention/reduction processes are needed (110).

The selective hydrogenation of alkynes to alkenes using metallic nanoparticles is an important industrial process, being involved in the synthesis of different fine chemicals and pharmaceuticals. Selective hydrogenation of this kind has traditionally been conducted with noble metal catalysts, e.g. platinum or palladium. Nevertheless, the high price and limited supply of these noble metals has motivated a considerable interest in exploring non-noble metal catalysts, such as cobalt-based alternatives (111).

Recently, several studies have shown that metallic catalysts based on palladium nanoparticles can deliver better catalytic activity for the selective hydrogenation of alkynes to alkenes. Maybe this case the increased reactivity and selectivity of these nanoparticle catalysts arises from their high surface-to-volume ratios that give a rise to available active sites as well tuning adsorption/reaction kinetics through control blueprints for size, composition &surface structure. In addition, the utilization of Pd based nanoparticle catalysts supported on different oxide support can further enhance its stability and recycle ability making it more competent for industrial applications (112).

Future advancements in this area are directed towards developing increasingly selective, as well as more stable metallic nanoparticle catalysts for the hydrogenation of a variety of alkyne substrates and exploring new reaction conditions or catalytic support material to further improve the overall catalysis performance and sustainability. The selective hydrogenation of alkynes to alkenes with metallic nanoparticle catalysts is a relevant example in which the distinctive properties provided by these nanomaterials have been recently exploited for tackling key challenges within organic synthesis and fine chemistry (112), (113).

Apart from traditional metal catalysts, the catalytic potential of graphene-based materials in selective hydrogenation has also been investigated. The graphene-based carbocatalysts have several advantages over the traditional metal catalysts due to their abundance and low cost, which can also be tuned through functionalization (introduction of certain groups) or by controlling processing conditions leading to defects in structure (114).

Herein, using the selective hydrogenation of acetylene in an ethylene-rich stream as a model reaction, we demonstrate that such catalytic activity can be achieved by carbocatalysts based on graphene. This absent in metal additives high selectivity achieved by graphene-based carbocatalysts is a further advance as it suggests the possibility to create novel, we could say more sustainable and also cheaper catalytic systems for different hydrogenation reactions (112).The predictability of the localized charge-rich region and defects on the carbocatalytic activity has made it an attractive research topic for graphene-based catalysts for hydrogenation because these composites can trap atomic hydrogen through Van der Waals electronic interactions that are far superior to interactions observed between metals and hydrogen. Further studies in this direction are focused on elucidating the critical factors capable regulating selective manufacture of graphene-based carbocatalysts interwoven with a tunable nano-materials which ultimately lead towards optimization of catalysis as rational design or engineering improves prospects. The rise of carbocatalysts based on graphene and metallic nanoparticles as a possible substitutes for metal catalysts is a testament to the increasing role played by nanomaterials in selective hydrogenation and catalysis (115), (116).

By producing hydrogen gas, the dehydrogenations process raises profit efficiency to a significant degree. In addition, metallic nanoparticles exhibited excellent catalytic characteristic in dehydrogenation reactions that own important applications for the synthesis of many advanced fine chemicals and fuels. One of the factors by which metallic nanoparticles exert high catalytic activity on hydrogen release is that they have a large surface, in comparison to other types solid legal catalysts and this allow direct interaction with the ionization materials, as well their tune-ability properties allows them function for different purposes based on how they were synthesized hence allowing it to be tailored towards significant dehydrogenation products while avoiding formation of undesirable polymorph process. For instance, platinum-based nanoparticle (NP) catalysts have been studied for dehydrogenation of alcohols to aldehydes and ketones, which is a fundamental reaction in the preparation of a wide variety of organic compounds (115).

The capability to design and synthetize metal nanoparticles with tailored compositions, structures, and morphologies have also supported the evolution of dehydrogenation catalysts selective toward a wide array of substrates. Further research in this area aims to address the development of even more effective metallic nanoparticle catalysts for dehydrogenation reactions that are both economically and environmentally sustainable, as well as the identification novel reaction pathways and optimization of catalytic performance by deriving active sites through specific procedures (116).

In addition to the use of metallic nanoparticles in catalytic CO oxidation, there has also been considerable interest in these materials for hydrogen generation via various reactions like water-gas shift and steam reforming. These reactions are essential for making clean and renewable hydrogen, which can be used in fuel cells and as a precursor to various chemicals. Over the past few years, significant efforts have been made to develop metal nanoparticle catalysts for these hydrogen production routes with an overall goal of improving their activity selectivity and stability (117). For instance, the use of nickel-based nanoparticles have been studied in water-gas shift reaction due to their high activity and selectivity towards hydrogen production (110), (118).

Incorporation of additional metal or metal oxide components, e.g. iron and ceria, can further improve both the catalytic reactivity and sintering resistance for these nickel-based nanoparticle catalysts as well. Currently knowledge on the composition, structure and morphology of metallic nanoparticle catalysts for enhanced production is been continuously addressed in an effort termed to further improve hydrogen generation due to its ability as illustrated up top, targeting higher yields without forgetting limitations associated with deactivation of a metal nanoparticles based feature or sustainable action (119).

Metallic nanoparticles, with their high surface energy and tunable composition/structure have displayed extraordinary properties in versatile catalytic applications. Metallic nanoparticles hold the promise of tackling some major catalysis challenges ranging from alkyne selective hydrogenation to water-gas shift and steam reforming for H2 production. As the research in this area continues to grow, it is anticipated that metallic nanoparticle catalysts with yet greater efficiency and sustainability will have profound implications on the future of catalysis as well as

general production chemical industry for essential chemicals/fuels (120).

The dehydrogenation of formic acid to generate hydrogen and carbon dioxide is one the potential applications for metallic nanoparticles in catalysis. Formic acid is considered as a highly regarded medium in storing and carrying hydrogen possessing sufficient storage density of H_2, low toxicity with easy production, transport and handling. Several recent studies have shown that metallic nanoparticles (particularly, platinum, palladium and ruthenium) can catalyze the dehydrogenation of formic acid to produce H_2 with excellent activity and selectivity (114).

With the large surface area as well as tunable properties of these metallic nanoparticles, it is possible to design catalyst with desired performance in terms of both activity and selectivity while also addressing issues related to stability and reusability. For an instance, the idea of being used as stable supports other than using metal oxides or reaching to bimetallic nanoparticles has apparently contributed a lot in highlights on strategies for enhancing catalytic performance and durability.

Future studies in this field will further improve the economic and sustainable strategies for formic acid dehydrogenation, as well as find out about the reaction mechanisms that dictate catalytic behavior (121).Dehydrogenation of alkanes to form alkene is another fundamental application point of metallic nanoparticles in catalysis, the intermediates are very important chemicals for diverse applications. Dehydrogenation onto metallic platinum, palladium or rhodium nanoparticles has provided excellent selectivities and catalytic activity for these reactions (114).

The hight surface area and tunable traits of these metallic nanoparticles can tailor catalyst development to maximize catalytic performance while also target challenges in the stability and coke formation. For instance, strategies such as bimetallic nanoparticles or the promotion with metal oxide supports have been employed to improve over catalytic activity and selectivity towards a specific product from pyrolysis while also trying to impart resilience against deactivation. Future research in this field should pay more attention to the design of catalysts not only for alkane dehydrogenation but also other types of catalytic reactions, with specified focus on

using recyclable and more sustainable Ag-based metallic nanoparticles as a substitute material. Another perspective can further develop an understanding concerning the underlying mechanistic pathway (including coke formation) that responsible for both high-performance materials interactions between various components when exposed to reactive conditions (122).

A promising hydrogen storage material, ammonia borane can be dehydrogenated over metallic nanoparticles; a recent application of such type that is rapidly emerging in catalysis. Ammonia borane can emit hydrogen via a catalytic dehydrogenation process, and metallic nanoparticles such as cobalt, nickel, or ruthenium-based ones display notable activity/selectivity toward the reaction (120).

Using these metallic nanoparticles, both the catalyst design can be optimized to improve catalytic performance (by tuning its high surface area) and challenges associated with stability of a catalyst and kinetics for hydrogen release will also be addressed. One example is the employment of bimetallic nanoparticles or metal oxide support as a means to in enhancing overall catalytic activity, selectivity and deactivation resistance. Work in this direction is further being carried to develop highly effective and sustainable bimetallic nanoparticle catalysts for dehydrogenation of ammonia borane while its specific reaction mechanisms, principles that determine catalytic performance are also under extensive investigation (121).

The distinct features of metallic nanoparticles including high specific surface area, ability to tune composition and structure which make them more important in catalytic proceedings with superior activities. Metallic nanoparticles have shown the promise of being able to tackle significant problems in catalysis ranging from selective hydrogenation of alkynes to production of hydrogen via water-gas shift and methane steam reforming reactions (114). In addition, research in this area will advance further using metallic nanoparticle catalysts that are more efficient and sustainable than those currently available on a large scale; the effects of these advancements may significantly affect future catalysis of various chemicals, fuels (121).Metallic nanoparticles also find heavy usage in catalytic oxidation reactions, particularly for the oxidation of CO and the epoxidation of propylene. These

trimetallic nanoparticles-having three distinct metallic components in the nanoparticle concerto show a higher catalytic activity and selectivity for these reaction as compared to their monometallic or bimetallic counterparts (123).

The interaction effects between the various metallic components in these trimetallic nanoparticles are responsible for modified catalytic activity, selectivity and stability. For instance, the use of a third metal may have an influence on how it will adsorb/activate reactant molecules or could make more stabilised surface structures that are resistant to deactivation (120).Future challenges in the field relate to unravelling the mechanism which is responsible for defining and enhancing into catalysis operation of trimetallic nanoparticles, as well as targeted synthesis with nature inspired methods towards a broader than ever range of oxidative transformation (109).

The tremendous properties such as high surface area, tunable composition and structure renders the metallic nanoparticles to be more crucial for various catalytic applications. Metallic nanoparticles have shown to possess excellent efficacies in the solution of catalytic challenges, ranging from selective hydrogenation of alkynes over production of hydrogen via water-gas shift and steam reforming reactions (SRM), as well as oxidation processes such carbon monoxide and propylene. As research in this area progresses further, more advanced metallic nanoparticle catalysts that are even practical and sustainable will undoubtedly play a vital role toward the future of catalysis for important chemical and fuel synthesis (122).

In addition, catalytic performance of metallic nanoparticles for the selective hydrogenation of alkynes to alkenes has been reported, and many precious metal nanoparticles have shown superior catalytic activity for this reaction (e.g., palladium NPs, rhodium NPSs or ruthenium NPs). Due to the high surface area and special electronic properties of metallic nanoparticles, they have higher adsorption capacity for reactant molecules, which can contribute to improving its catalytic performance. Palladium nanoparticles are frequently used as catalysts for the selective hydrogenation of acetylene to ethylene, an important reaction in the petrochemical industry. The incorporation of Au and/or Ag enhances the catalytic activity and selectivity of these nanoparticle catalysts even more profoundly by synergistic effects (124).

In addition, metallic nanoparticles, in particular platinum or palladium-containing catalysts with high activity as well as selectivity to methanol formation have shown promising activities for methane partial oxidation (119). The high surface area and special electronic properties of metallic nanoparticles make them a good adsorption/activation site for reactant molecules, thus boosting the catalytic activity. Platinum nanoparticles supported on different substrates, for instance, have been widely investigated as a catalyst to oxidize methane into methanol. Synergistic effects are also achieved with the addition of a 2nd and/or 3rd metal component, such as gold or rhodium so that catalytic performance can be further enhanced using these nanoparticle catalysts (107).Current research in the field is aimed at elucidating the fundamental mechanisms that control how catalytically active metallic nanoparticles promote methane-to-methanol oxidation, and designing catalysts with even higher activity for this critical reaction (103).

Metallic nanoparticles have further demonstrated their role in a spectrum of electrochemical reactions including the transformation of carbon dioxide to methane or methanol by (107). These distinctive features of nanoparticle such as high surface area and modifiable electronic structure can promote the adsorption and activation processes for reactants, thereby improving catalytic performance (125). Using the electrochemical reduction of carbon dioxide, one study found that a large variety of transition metal surfaces (gold, silver, zinc etc) are able to produce CH4 or methanol. Although the selectivity for such products is usually low, we note that all of these metals can catalyze these reactions (107). Future research directions in this domain concentrate on enhancing the catalytic efficiency of metallic nanoparticles to its maximum level regarding electrocatalysis applications, accompanied by an insight into their refined mechanist reactivity (126).

Precious-metal nanoparticles such as platinum and palladium members have also shown excellent catalytic activity with high selectivity in the isomerization of alcohols, which is crucial for fuel cell applications. Platinum nanoparticles supported

on carbon materials such as for example graphitic structures have attracted much attention with respect to their use in the electrochemical oxidation of methanol and ethanol. Second or third metal components, such as ruthenium (Ru) and tin (Sn), can be used to enhance the catalytic activity/selectivity of these nanoparticle catalysts by introducing a synergistic effect with CO (127).

Ongoing research in this area is focused on understanding the underlying mechanisms that govern the enhanced catalytic performance of metallic nanoparticles for the electrochemical oxidation of alcohols. Developing even more efficient and sustainable catalysts for this important reaction is also under consideration. Metallic nanoparticles, such as those composed of platinum and its alloys, have similarly shown remarkable catalytic activity and selectivity for the oxygen reduction reaction. This reaction is a critical procedure for both fuel cells and metal-air batteries. For example, palladium-based electrocatalysts with little or no platinum have been studied for their effectiveness in catalyzing the oxygen reduction reaction in alkaline media. The high surface area and distinctive electronical property of nanoparticle catalysts can not only enhance the absorption and activity of oxygen but also improve the catalytic efficiency. Ongoing research in this field is aimed at understanding the particular mechanisms that enable nanoparticles’ catalytic performance to be boosted for the oxygen reduction reaction. Furthermore, even more, efficient and sustainable catalysts are utilized in this important reaction. Potential applications In addition to the well-studied bimetallic and trimetallic nanoparticle catalysts, more complex, multicomponent metallic nanoparticles have been examined for a variety of catalytic applications. These higher-order metallic nanoparticles, which include four or more distinct metallic components, may offer even more opportunities for tuning and optimizing catalytic performance via synergistic effects between the metal constituents.

For example, a new research study shows the increased catalytic activity for fuel cell applications by using tetrametallic nanoparticles made of platinum (Pt), palladium (Pd), nickel (Ni) and copper (Cu). The additional metal parts may develop leading electronic and structural features for more favorable deactivation resistance than simple bimetallic or trimetallic nanoparticle catalysts (128).

Ongoing researches in this field are particularly directed toward extending applications of these third-order metallic nanoparticle catalysts for developing versatile and sustainable catalytic systems suitable to a large number of electrochemical and non-electrochemical processes (103).

In addition to their catalytic function, trimetallic nanoparticles also can be utilized as prospects for biomedical applications such as antimicrobial agents and cancer therapeutics (16). Trimetallic nanoparticles show synergistic effects by three different metal components that can exhibit stronger antimicrobial activity, i.e., a more significant inhibition of bacterial growth and biofilm formation than monometallic or bimetallic counterparts (74).

Specifically, trimetallic nanoparticles were found to be more potent in killing cancer cells and may have greater capability for tumor cell penetration due to their capacity for various cytotoxicity mechanisms including anti-angiogenic/anti-metastatic effects (129). Research into this field is ongoing to define the basic mechanism of action by which trimetallic nanoparticles mediate their biological effects and improve its design as well as synthesis for better therapeutic performance (130), (131).

Further, not only they possess antimicrobial and anti-cancer properties, trimetallic nanoparticles have also demonstrated excellent antioxidant activity that might be useful in numerous biomedical applications (132). It means that trimetallic nanoparticles show unique electronic and structural properties due to synergetic interactions between three different metal components leading them to be promising candidates with high antioxidant activity by scavenging of ROS in oxidative stress systems (75).Such antioxidant power could be of particular interest for therapeutic applications, to potentially help preventing the damage in healthy tissues from oxidative stress commonly promoted during disease treatment like cancer, inflammation and neurodegenerative disorders (133). Future in this area will look into the mechanistic and still unknown mechanism of trimetallic nanoparticles as an antioxidants leads to a better understanding even for their much wider role i.e., from drug delivery systems, scaffolds (tissue engineering) or diagnostic platforms (129).

CHAPTER 3

3. METHODOLOGY

The research work carried out in this project was conducted in different institutions. Synthesis of tri-metallic nanoparticles was carried out at Analytical Chemistry Lab., the University of Lahore, Lahore. Characterization of thus synthesized trimetallic nanoparticles was performed from LUMS University, Lahore; while evaluation of antioxidant and antimutagenic potential was carried out at Biochemistry Lab., University of Agriculture, Faisalabad. The detailed description of all such experimental is given in quite detail:

3.1. Chemicals/ Reagents

All the chemical used in the research were of analytical grade. The detail is as follows:

1,1-diphenyl-2-picrylhydrazyl	DPPH	(Sigma-Aldrich)
Chromium Chloride Hexahydrate	$CrCl_3.6H_2O$	(Sigma-Aldrich)
Copper Sulfate Pentahydrate	$CuSO_4.5H_2O$	(Sigma-Aldrich)
Davis-Mingioli Salt		(Sigma-Aldrich)
D-Biotin	$C_{10}H_{16}N_2O_3S$	(Merck)
Deionized water	Dist. H_2O	(Sigma-Aldrich)
D-Glucose	$C_6H_{12}O_6$	(Sigma)
Dimethyl Sulfoxide	DMSO	(Sigma-Aldrich)
Ethanol	C_2H_5OH	(Merck)
L-Histidine	$C_6H_9N_3O_2$	(Sigma)
Methanol	CH_3OH	(Sigma)
Nickel Chloride Hexahydrate	$NiCl_2.6H_2O$	(Sigma-Aldrich)
Sodium hydroxide	NaOH	(Merck)

3.2. Apparatus/Glassware

- Aluminum foil
- Centrifuge tubes
- Conical flask
- Drop pipette

- Falcon tubes
- Filter paper
- Glass beakers
- Measuring cylinder
- Petri dishes
- Stirring rod
- Test tubes

3.3. Instruments

Centrifuge machine	(H-200NR; Kokusan, Tokyo, Japan)
Hot plate with Magnetic stirrer	(MH-6D)
Oven	(SLFHP522-H)
pH meter	(HI2202-edge-blu)
Refrigerator	(HR1-140S)
Thermometer	(Ambala 14355768830)
Weighing balance	(FA2204)

3.4. Growth Media

Nutrient agar medium	(Oxoid)
Potato dextrose agar	(Oxoid)

3.5. Bacterial Strains

Salmonella typhimurium TA98	(MolTox)
Salmonella typhimurium TA100	(MolTox)

3.6. Collection and Extraction Of Orange Peels

Orange (Citrus sinensis) waste peels were collected from different fresh juice corners of Lahore. Then, such peels were given pretreatment like washing, shade drying, and grinding to get fine powder which were preserved in air tight container. Then powdered peels were extracted with distilled water in the temperature range 60 to 70°C and the filtrate was preserved as aqueous extract at 4°C (134).

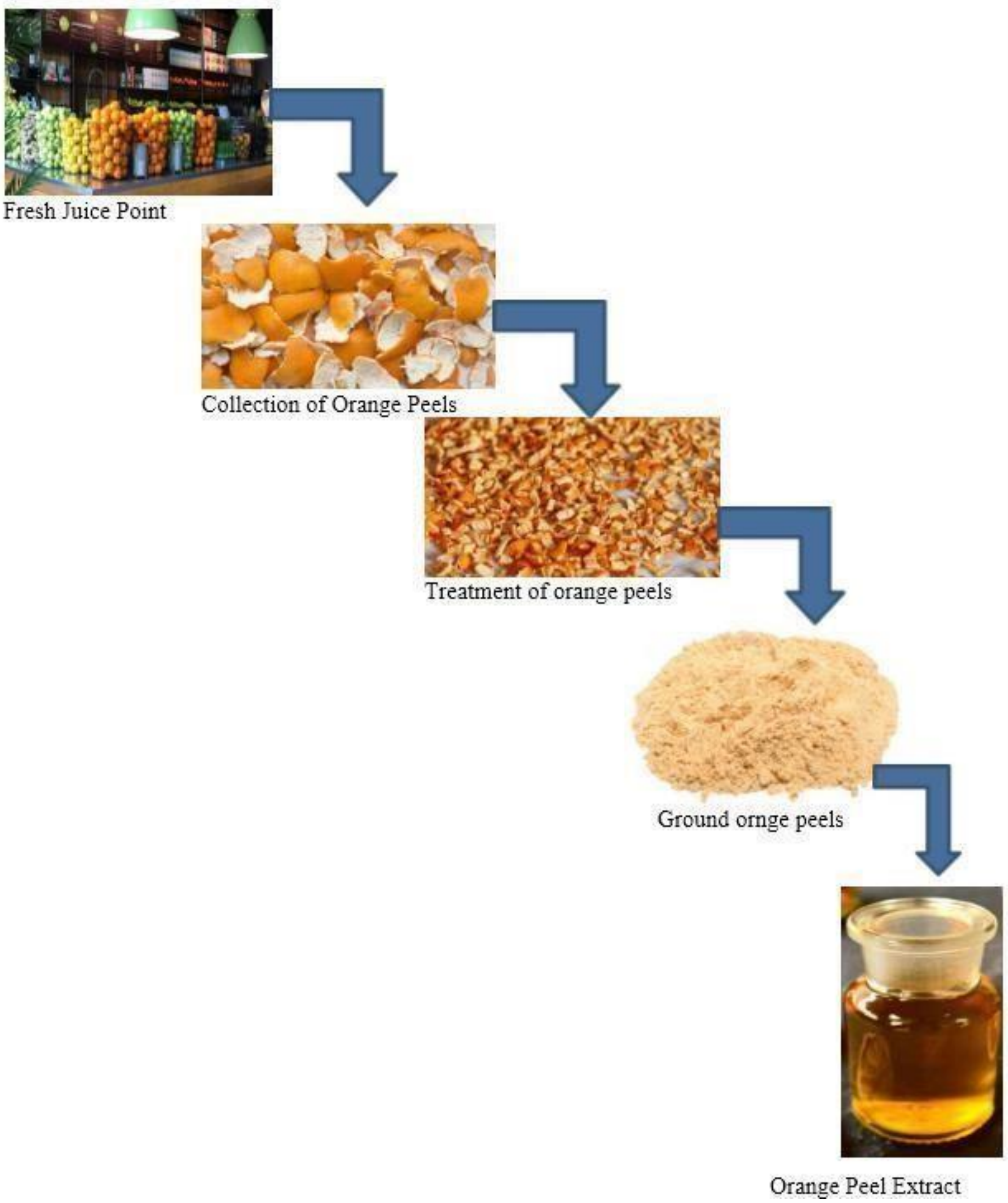


Figure 3.1. Schematics of orange peel collection, treatment and extraction

3.7. Green Synthesis of Trimetallic NPS

Cu/Cr/Ni trimetallic oxide nanoparticles were prepared by following already described methods of (135). Typically, it involves the application of plant extract containing numerous phytochemicals which serve as reducing agents to convert precursor salts of all three metals into their nanoparticles. Solutions of each precursor salt (NiCl2.6H2O (0.5M); CrCl3.6H2O (0.5M) and CuSO4.5H2O (0.5M)) were mixed in a beaker and stirred at 80oC with continuous addition of orange peel extract drop wise. pH of the reaction mixture was maintained in the range of 9 to 10. After completion of the reaction, the solution was cooled down to ambient temperature; and trimetallic NPs thus synthesized were obtained by centrifugation for 15min at 3000 rpm. Such trimetallic oxide NPs were then washed several times with ethanol to remove unwanted impurities which may come from plant extracts and other chemical reagents. The sedimenting trimetallic oxide NPs were preserved at 40˚C in vacuum oven to maintain their stability and to avoid any condensation or agglomeration (136), (137).

3.8. Characterization of Tmnps

3.8.1. Ultraviolet-Visible Spectroscopy (UV-Vis)

To determine the optical characteristics of trimetallic nanoparticles, a straightforward method of analysis known as UV-Visible spectroscopy using UV-Visible spectrophotometer (BioMate 160 UV/Vis, ThermoFisher Scietific, US) was used. The findings of UV/VIS spectrophotometer are presented in the form of spectra, which illustrate the highest absorption as peaks. Before beginning the actual analysis of the samples, an appropriate blank sample has to be performed in order to reduce the error value (139).

3.8.2. Scanning Electron Microscope (SEM)

Trimetallic nanoparticles which were prepared in this project were also investigated under scanning electron microscope (Nova 450 NanoSEM) to evaluate the textural and structural informations (140).

3.8.3. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis is very crucial for examining different functional groups of multiple phytochemicals present in plant extracts and responsible for bio-reduction of precursor metal ions to generate metallic nanoparticles. FTIR spectrometer (Nicolet iS5, Thermo Fisher Scientific, US) provided both the transmission and absorption spectra of the material in the infra-red regions of electromagnetic spectrum. The transmission of infrared light at a certain frequency may be used to study the vibration of chemical bonds that are present in a sample. The absorbed infrared radiation is then used in the process of determining the functional groups present in a test sample (141).

3.8.4. Powder X-ray Diffraction (XRD)

The study of X-ray diffraction is predicated on the constructive interaction of monochromatic X-rays with the crystalline sample. Size of nano-crystallites and degree of crystallinity was determined by Philips diffractometer system (λ=1.54060 nm, 40 Kv and 30mA) with 2°/min scanning rate, in the range of 20–80°. The unique diffraction peaks of X-ray properties formed during examination reveals information about the crystalline sample.

3.9. Bioactivities

3.9.1. Antioxidant Activity

Antioxidants are chemical compounds that help cells avoid damage caused by reactive oxygen species (ROS) and free radicals. Food and beverages with antioxidant activity is one of the most fascinating topics to come from science at some point. These antioxidants serving to protect the body from vital damage caused by free radicals, including: cardiovascular disease development; reduced premature aging and heart disease structure; anemia formation stimulated acts on essential lymphocytes meanwhile mediates various cancer types as well as inflammation (144). The antioxidant activity of the TMNPs was determined by means of free radical scavenging test using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Methanolic solution of DPPH (4 mL) were added to each test tube containing methanolic solution of plant extract (0.4mL) and methanolic mixture of TMNPs (5mg/mL; 0.4mL) and allowed to incubate at 370C for half an hour. All the test tubes were properly covered with aluminum foil to prevent the solution from light. The absorbance was measured at 517nm, against blank (pure methanol) using a UV spectrophotometer (IRMECO 5000) after 30 min. Same experiment was performed with different dilutions (conc. ranging from 5000-156.25 µg/mL) of trimetallic nanoparticles.

The antioxidant activity was determined as scavenging percentage using the following formula.

$$\% \text{ Scavenging activity} = [1- \frac{\text{Absorbance sample}]}{\text{Absorbance control}} \times 100$$

IC50 which is defined as the concentration of TMNPs required to neutralize 50% radicals, was also calculated and expressed in µg/mL. A higher IC50 value represents lower antioxidant activity and vice versa (145).

3.9.2. Reducing Power

Ferric ion Reducing Antioxidant Power (FRAP) was carried out following the procedure of (146) with slight changes. In brief, methanolic solution of TMNPs (5 mg/mL) was mixed with 2.5 of potassium ferricyanide and phosphate buffer (2.5 mL, pH 6.6). To this, 2.5 mL of a 10% solution of trichloroaceticacid was added and centrifuged (H-200NR; Kokusan, Tokyo, Japan) at 200 rpm for 10 min. The supernatant was diluted with deionized water (1:1) and 0.1% FeCl3 solution (0.5 mL) was added, then absorbance was read at 700 nm (IRMECO 5000). Reducing power of nanoparticles was expressed as mg of ascorbic acid equivalents (AAE)/g. A calibration curve was obtained following the same procedure with ascorbic acid.

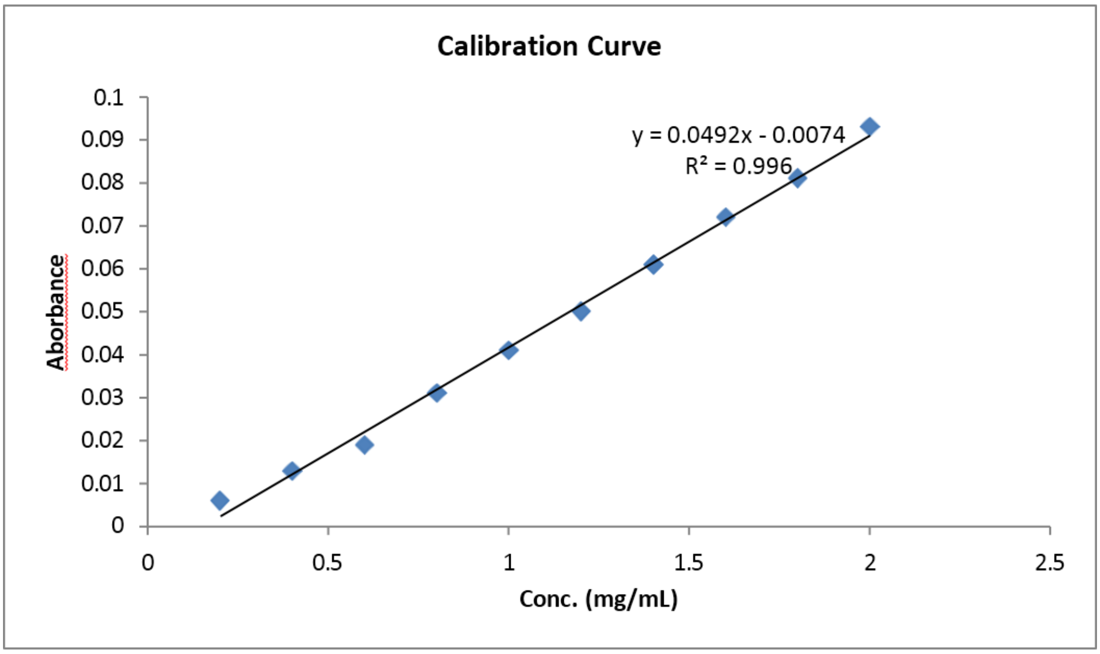


Figure 3.2. Reducing Power calibration curve using ascorbic acid as positive standard

3.9.3. Antimutagenic activity

The mutagenic and antimutagenic potential was examined by modified Ames bacterial reverse-mutation assay, carried out in liquid culture. A mixture of different components in fixed concentration as: bromocresol purple (2.38 mL), D-biotin (1.19 mL), D-glucose (4.75 mL), L-histidine (0.06 mL) and Mingioli salt (21.62 mL), was labeled as ‘reagent mixture’.

Aqueous solution of TMNPs was mixed with reagent mixture different beakers at the amounts as shown in Table 3.1. Two bacterial strains, S. typhimurium TA98 and S. typhimurium TA100 were incubated in nutrient broth at 37oC for overnight. The content of each beaker was transferred into a Eliza plate and each plate was packed in air-tight wrap to avoid evaporation for 4 days. Purple coloration in ‘blank’ plate indicated the sterility of experiment. Any yellow, off white or even turbid well was considered as positive mutant while purple as negative mutant. Numbers of all positive wells were scored visibly. The ‘background’ plate (no TMNPs and no standard mutagen added) exhibited spontaneous mutation. The NPs were considered toxic if all wells in the test plate showed purple coloration. For NPs to be regarded as mutagenic, the number of positive wells had to be more than twice the number of positive wells in the ‘background’ plate (spontaneous mutation) (165). The %age mutagenecity was obtained by the following formula:

$$\text{\%age Mutagenicity} = \left[\frac{(\text{number of mutants on plate4} - \text{spontaneous mutatants})}{(\text{number of mutants on plate3} - \text{spontaneous mutatants})}\right] \text{x } 100$$

The antimutagenic effect was measured by the following formula:

$$\text{Antimutagenecity (\%age)} = \left[1 - \frac{(\text{number of mutants on plate5} - \text{spontaneous mutatants})}{(\text{number of mutants on plate3} - \text{spontaneous mutatants})}\right] \text{x } 100$$

The antimutagenic effect was considered as ‘strong’, ‘moderate’ or ‘weak’ if when %age inhibition of mutagenecity was more than 40%, 25 – 40% or less than 25%, respectively (166).

Table 3.1. Ames reverse mutation test set-up.

Plate No.	Treatment	Volume added (mL)				
		Standard Mutagen	**TMNPs solution**	**Reagent mixture**	**Deionized Water**	***Salmonella* test strain**
1	Blank	___	___	2.5	17.5	___
2	Background	___	___	2.5	17.5	0.005
3	Standard Mutagens	0.1	___	2.5	17.4	0.005
4	Samples for Mutagenic Test	___	0.005	2.5	17.5	0.005
5	Samples for Antimutagenic Test	0.1	0.005	2.5	17.4	0.005

3.10. Statistical Analysis

Each experiment was presented as average of three parallel experiments and presented as mean ± S.D (167).

CHAPTER 4

4. RESULTS

The current research work involves the synthesis of Cu/Cr/Ni trimetallic oxide NPs and their chemical by stat-of-the-art techniques including UV/VIS spectroscopy, FTIR, XRD and SEM analysis; and finally the evaluation of antioxidant potential and antimutagenic potential. The detailed description of all results of this project is given as follows:

4.1. Characterization of Trimetallic Nanoparticles (TMNPS)

4.1.1. UV/VIS Spectroscopy

Results of UV-Vis spectroscopy have been presented in the figure (4.1) which exhibited three sharp bands with λmax at 261.04 nm, 426.98 nm and 574.31 nm. Peak at 261 nm showed the presence of Ni and NiO-NPs, peak centering 426.98 nm represented Cr- NPs while that at 574.31 nm showed the presence of Cu and CuO-NPs (135), (147), (148).

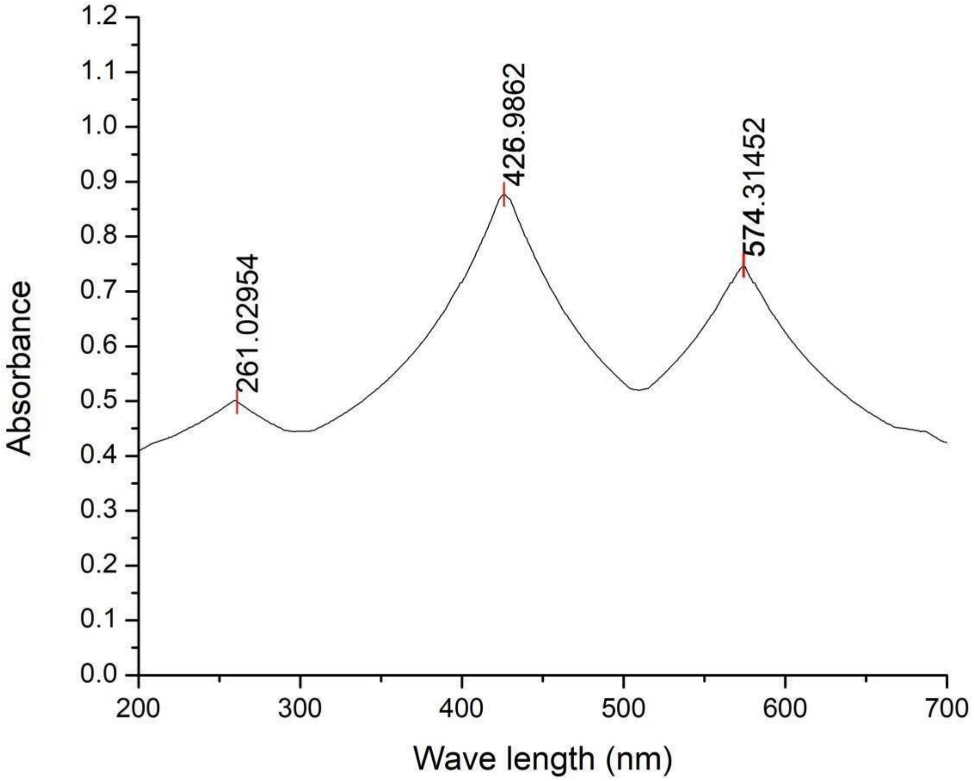
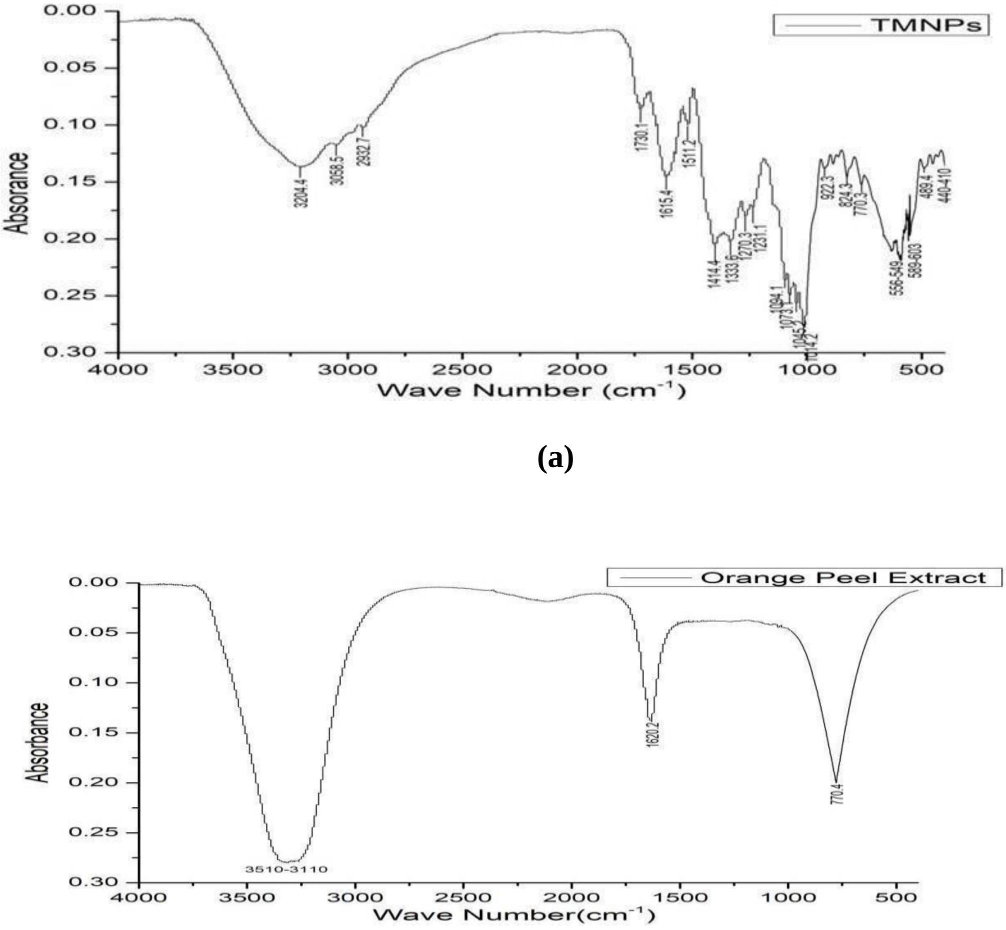


Figure 4.1. UV Spectra of Cu/Cr/Ni trimetallic nanoparticles

4.1.2. Fourier Transform Infra-Red Spectroscopy (FTIR)

FTIR spectrum of biosynthesized trimetallic nanoparticles has been presented in figure (4.2a). A broad peak at 3204.4 cm-1 corresponds to O–H stretching vibrations (149); a shoulder peak at 3058.5 cm-1 also indicate the phenolic components involved in green synthesis of TMNPs (146) while other shoulder peak at 2932.7 cm-1 also a representation of hydrogen bonded phenolic functional groups (147). The complete FTIR profile has been presented in table (4.1) showing different peaks of FTIR spectrum of trimetallic oxide nanoparticles. These peaks demonstrate the successful synthesis of Cu/Cr/Ni trimetallic oxide by phytochemicals present in plant extract. Numerous FTIR peaks indicate the existence of different plant secondary metabolites serving as bio- reductants and capping agents to avoid agglomeration.



(a)

(b)

Figure 4.2 (a) FTIR spectra (a) Green synthesized TMNPs (b) Orange peels extract

Table 4.1. FTIR peaks with their characteristic functional groups for trimrtallic oxide nanoparticles.

Sr. No	Peak Position (cm^{-1})	Functional group	Vibrational mode	References
1	3204.4	O–H of phenolics	Stretching	(149)
2	3058.5	O-H due to the entrapped moisture or any phenolic components	Stretching	(146)
3	2932.7	H-bonded alcohol or phenol	Stretching	(147)
4	1730.1	C=O	Stretching	(150)
5	1615.4	Aliphatic C–H	Stretching	(151)
6	1511.2	C=C of aromatic rings	Stretching	(150)
7	1414.4	C-O of aromatic rings	Bending	(150)
8	1333.6	C-H	Stretching	(152)
9	1270.3	Hydroxyl at aromatic rings	Stretching	(150)
10	1231.1	C-H Aliphatic	Stretching	(150)
11	1094.1	C-H Aliphatic	Bending	(150)
12	1073.1			
13	1045.2			
14	1014.2			
15	922.3	Chromyl (Cr=O)	Stretching	(153)
16	824.3	Cr-O	Stretching	(151)
17	770.3	C-H Aliphatic	Out of plane bending	(150)
18	589-603	Cu-O; Cr-O	Stretching	(147),(153)
19	556-549	Cu-O	Stretching	(152)
20	489.4	Ni–O	Stretching	(151)
21	440-410	Trimetallic (Cu/Cr/Ni) oxide NPs	Stretching	(154)

4.1.3. X-Ray Diffraction Analysis

XRD spectrum figure (4.3) of trimetallic nanoparticles shows major peak at 36.64˚ that corresponds to Cu and CuO and Cr2O3 NPs (154,155) while other major peak at 43.49 ˚ represents Cr NPs and that at 63.19˚ stands for Ni and NiO NPs (154).

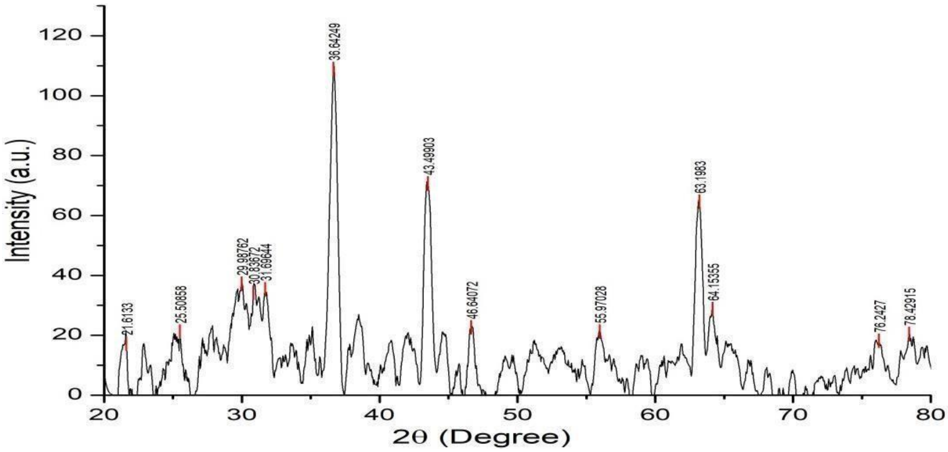


Figure 4.3. X-ray diffraction pattern of Cu/Cr/Ni oxide trimetallic NPs

Other major peaks around 40-50o and 50-60 o are characteristics of oxides of all three metals (135). while all miner peaks indicate that little amount plant extract exist on the surface of nanoparticles serve as capping agent to avoid agglomeration.

Table 4.2. Calculations for size of Cu/Cr/Ni oxide trimetallic NPs.

Peak Position (2θ)	**FWHM**	**Crystallite Size D (nm)**	**Average D (nm)**
21.4464	0.34883	23.17886	3.06003
25.22518	0.73379	11.09409	
27.80524	1.08569	7.538097	
29.85903	1.38002	5.957761	
31.03629	0.60065	13.72654	
31.75444	0.59657	13.84478	
34.98821	0.55791	14.92991	
36.66019	0.60639	13.80122	
38.41441	0.79518	10.57946	
43.48922	0.55391	15.44091	
56.00381	0.59324	15.16692	
63.15763	0.54216	17.2	
64.15091	0.6966	13.45888	
76.16067	0.75023	13.45256	
78.75668	1.57379	6.530458	

4.1.4. Scanning Electron Microscopy (SEM)

Morphological characterization of TMNPs was carried out by scanning electron microscopy. Fig (4.4) presents photograph of SEM results to analyze particle shape and size of trimetallic oxide NPs synthesized by green synthesis using orange peel extract. The image shows the spherical morphology for TMNPs and size was found to be 12-15 nm calculated from SEM image using Image J software (156).

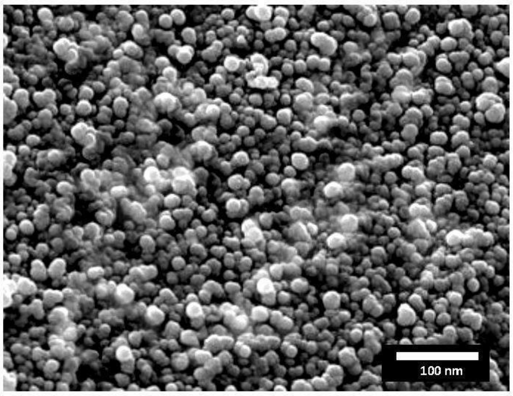


Figure 4.4. SEM image of Cu/Cr/Ni trimetallic oxide nanoparticles.

4.2. Bioactivities of Trimetallic Oxide NPs

4.2.1. Mutagenic screening and antimutagenic potential of Cu/Cr/Ni oxide NPs

Table (4.3) presents the mutagenic behavior of green synthesized trimetallic oxide nanoparticles. All wells in ‘Blank’ plate appeared purple figure (4.5a) showing the sterility of the experiment. Figure (4.5b) represents the ‘Background’ plates showing the spontaneous mutation for bacterial strain TA 98. Figure (4.5c) represents the ‘Background’ plates showing the spontaneous mutation bacterial strain TA 100. The effect of standard mutagens i.e. K2Cr2O7 for TA98 has been presented in figure (4.5d). The effect of standard mutagens i.e. NaN3 for TA 100 has been presented in figure (4.5e), showing the effectiveness of such toxic compounds as standard mutagens. Figure (4.5f) showing the mutagenic effect of TMNPs on TA 98. Figure (4.5g) showing the mutagenic effect of TMNPs on TA 100, mutagenic behavior of TMNPs indicating their non-mutagenic behavior towards both strains and figure (4.5h) showed mutagenic behavior of nanoparticles was compared with H_2O_2 on TA 98 and figure (4.5i) on TA 100 serving as positive control for mutagenicity test. TMNPs exhibited only 15-16% mutagenicity for both bacterial strains and regarded as non-mutagens while H_2O_2 presented 70-71% mutagenicity for both selected bacterial strains as shown in table (4.3).

Figure (4.5j) showing the antimutagenic effect of TMNPs on TA 98 and figure (4.5k) showing the antimutagenic effect of TMNPs on TA 100 presented the antimutagenic potential of TMNPs and positive standard (ascorbic acid), respectively for both selected bacterial strains. Figure (4.5l) showing the antimutagenic effect of positive control (ascorbic acid) TA 98 and figure (4.5m) showing the antimutagenic effect of positive control (ascorbic acid) TA 100.

Trimetallic oxide nanoparticles exhibited more than 85% antimutagenicity for TA98 and more than 92% for TA100 which are quite comparable to the positive control, ascorbic acid as presented in table (4.3).

Table 4.3. Percentage mutagenicity/antimutagenecity of TMNPs in comparison to positive standards.

		TA 98			**TA 100**		
	Samples	**Mutants**	**%age Mutagenicity/ Antimutagenicity**	**Effect**	**Revertants**	**%age Mutagenicity/ Antimutagenicity**	**Effect**
	Background	8	---	---	12	---	---
	$K_2Cr_2O_7$	92	---	---	---	---	---
	NaN_3	---	---	---	90	---	---
Mutagenic Potential	TMNPs	22	16.7±0.33	Non-Mutagenic	24	15.4±0.21	Non-Mutagenic
	Positive Control (H_2O_2)	68	71.4±0.45	Mutagenic	67	70.5±0.81	Mutagenic
Antimutagenic Potential	TMNPs	20	85.71±0.56	Strong Antimutagen	18	92.3±0.74	Strong Antimutagen

	Positive Control (Ascorbic acid)	19	86.9±0.71	Strong Antimutagen	18	92.3±0.88	Strong Antimutagen

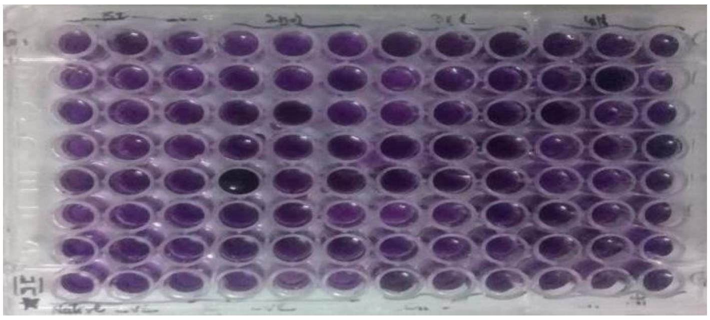
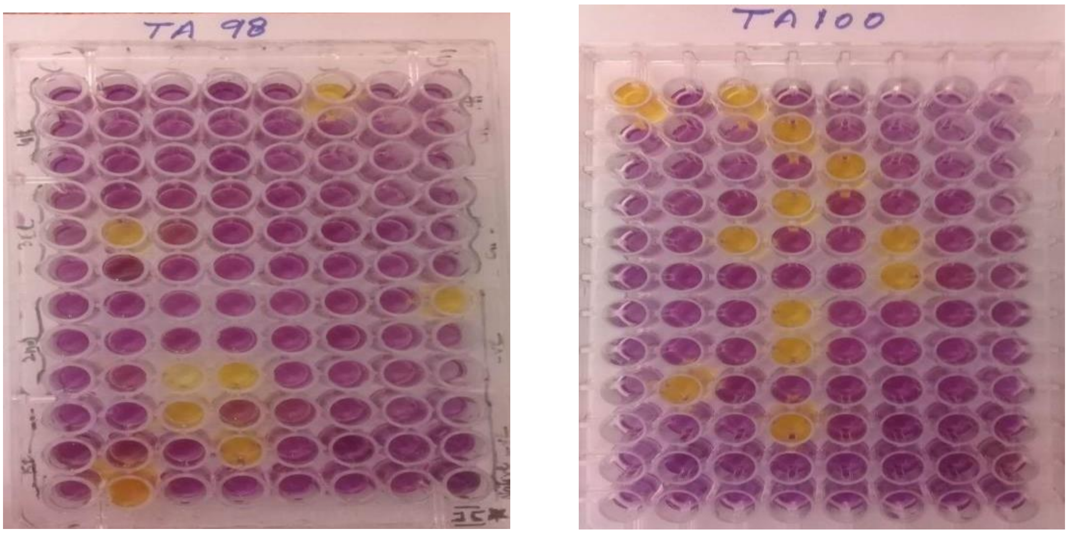
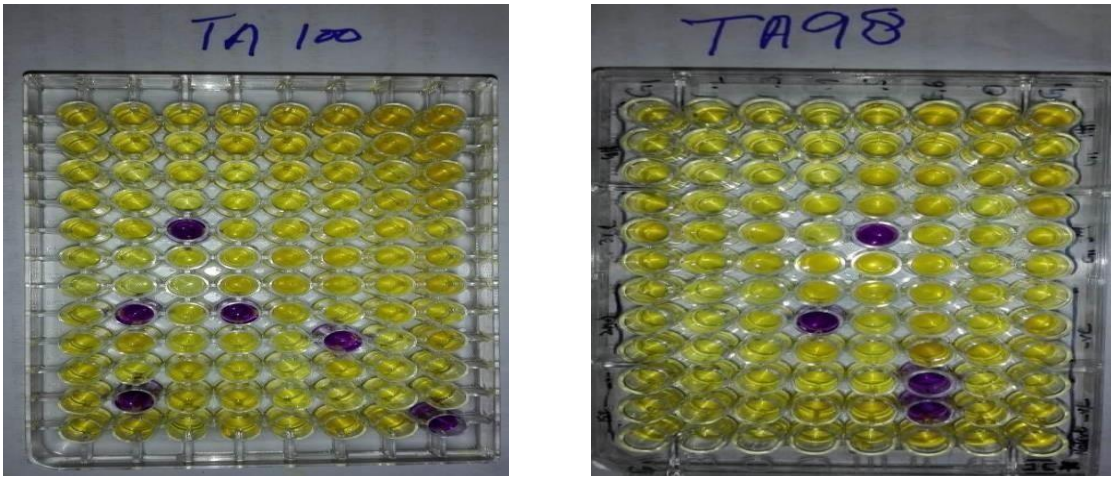


Figure 4.5 (a) The 'Blank' plate with no bacterial strain and no nanoparticle sample.



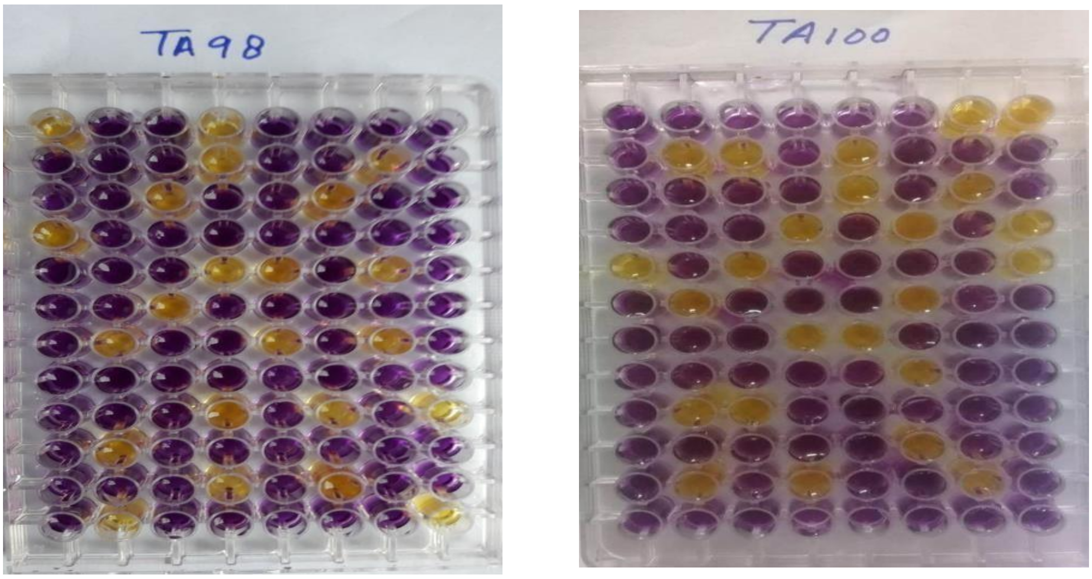
(b) (c)

Figure 4.5: The 'Background' plates showing spontaneous mutation (b) For bacterial strain TA 98 (c) For bacterial strain TA 100.



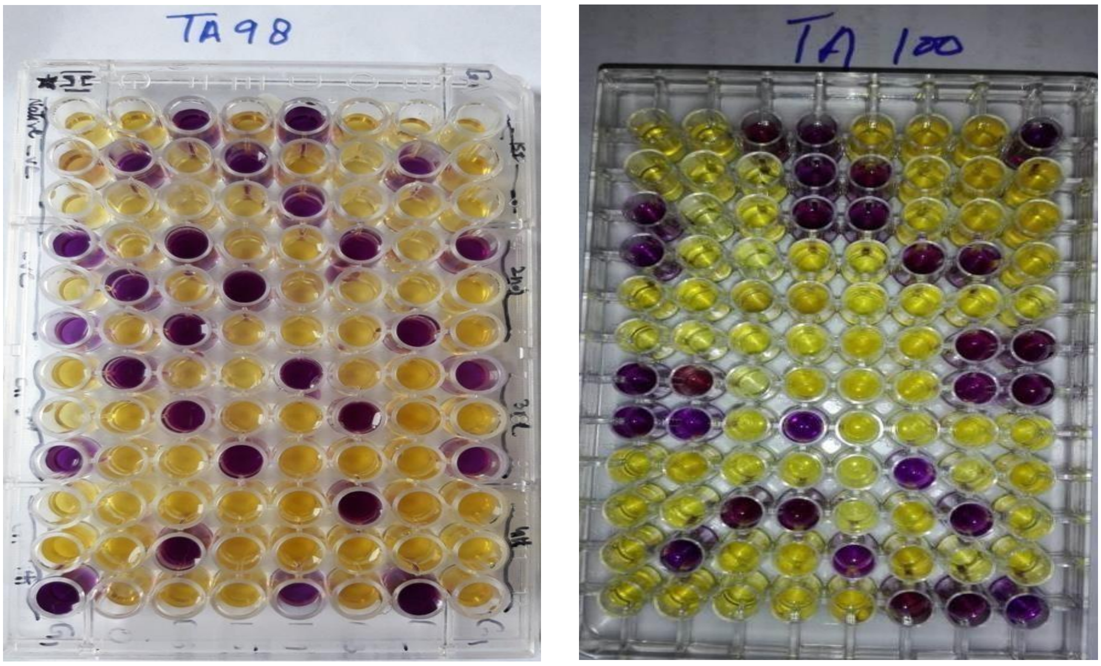
(d) (e)

Figure 4.5: The effect of standard mutagens (d) On TA 98 ($K_2Cr_2O_7$) (e) On TA 100 (NaN3).



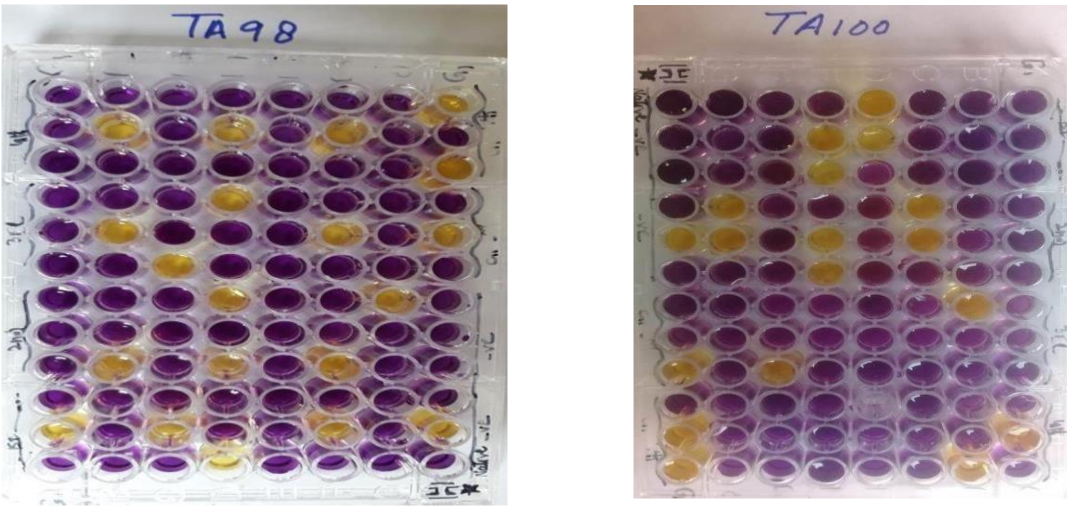
(f) (g)

Figure 4.5: The mutagenic effect of TMNPs (f) On TA 98. (g) On TA 100



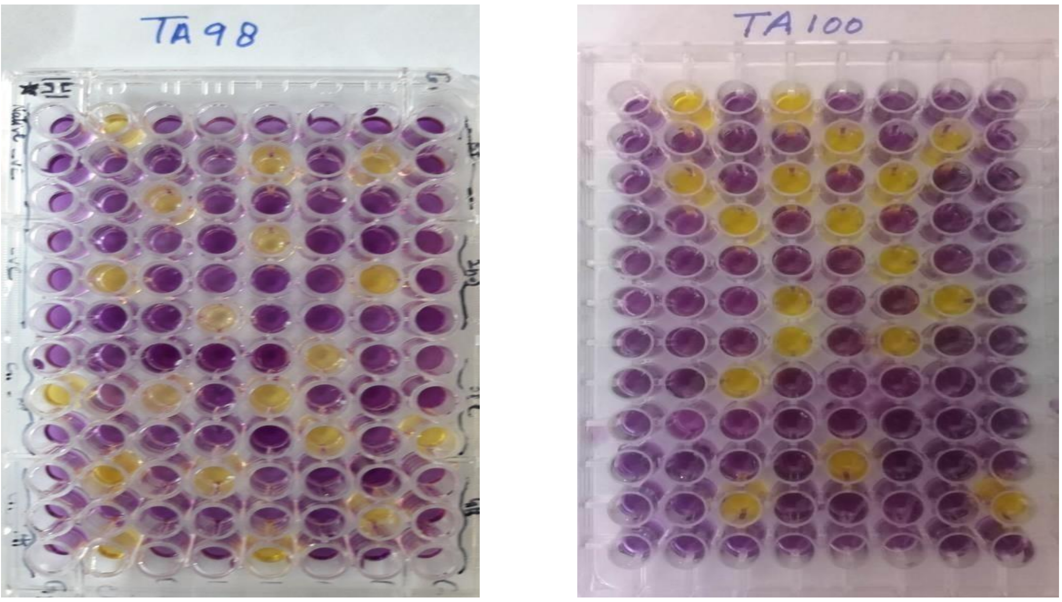
(h) (i)

Figure 4.5: The mutagenic effect of positive control (H_2O_2) (h) On TA 98 (i) On TA 100



(j) **(k)**

Figure 4.5: The antimutagenic effect of TMNPs (j) On TA 98 (k) On TA 100



(l) (m)

Figure 4.5 : The antimutagenic effect of positive control (ascorbic acid) (l) On TA 98 (m) On TA 100

4.2.2. DPPH free radical scavenging

The results of antiradical activity for TMNPs have been presented in table (4.4) and figure (4.6). It has been found that as the concentration of nanoparticles was increased, scavenging of DPPH free radical was also increased which clearly demonstrate that TMNPs were completely involved in neutralization of DPPH. Maximum value of %age scavenging (65.94%) was achieved at 5 mg/mL with IC50 value of 2.867 mg/mL. Such %age scavenging (65.94%) was found to be superior to that of Cu-NPs (62%), (152).

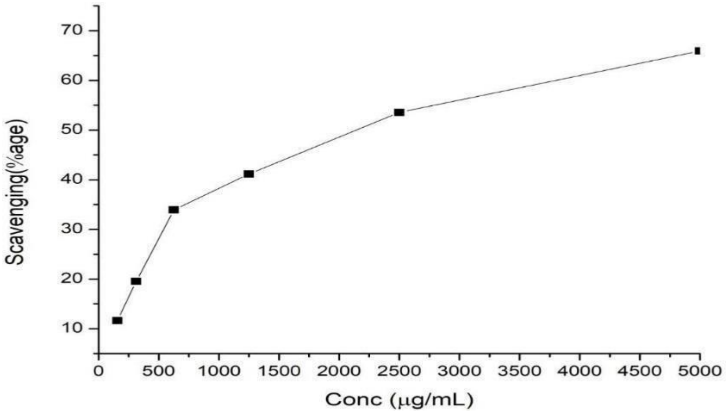


Figure 4.6. Effect of concentration of TMNPs on percentage scavenging of DPPH.

Table 4.4. DPPH scavenging potential (%) and IC50 of trimetallic oxide nanoparticles

Concentration of TMNPs (µg/mL)	Absorbance of sample (A_s)	Absorbance of Control (A_b)	% Scavenging	IC_{50} (µg/mL)
156.25	0.856	0.969	11.66	2867.92
312.5	0.78		19.50	
625	0.64		33.95	
1250	0.57		41.18	
2500	0.45		53.56	
5000	0.33		65.94	

4.2.3. FRAP

The reductive capability of nano-materials was shown as mg Ascorbic Acid Equivalents (AAE)/mL solution of sample. The result of this assay showed reducing power of green synthesized TMNPs was 1.94±0.012 mg AAE/mL at 2.5 mg/mL concentration of TMNPs.

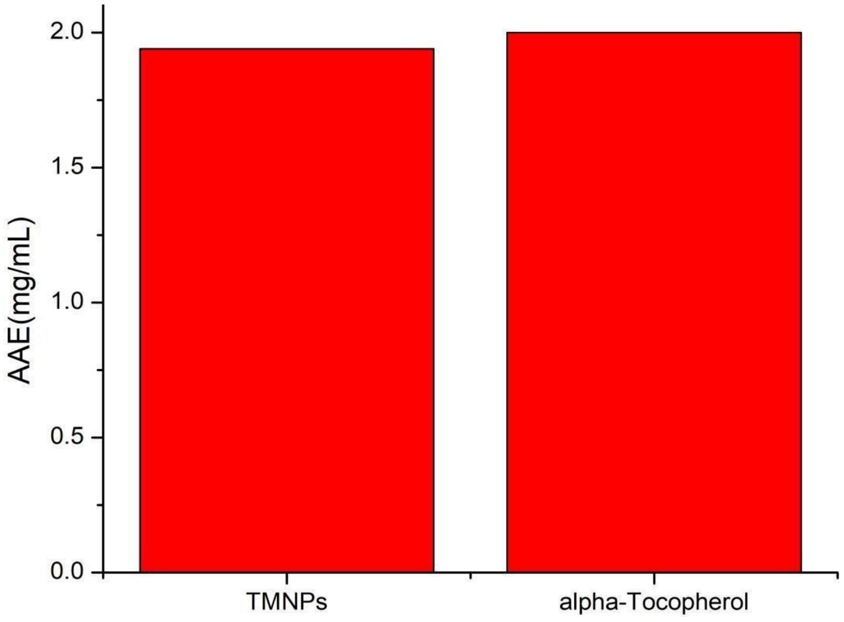


Figure 4.7. Reducing power of TMNPs in comparison to α-Tocopherol expressed in mg AAE/mL

CHAPTER 5

5. DISCUSSION

The current research project was planned to synthesize novel trimetallic nanoparticles of copper, chromium and examined their antioxidant and antimutagenic potential. The research plan involved three stages. Firstly, the synthesis of Cu/Cr/Ni trimetallic NPs by following green synthesis using orange peel extract as bio-reducant; secondly, the chemical characterization of thus synthesized TMNPs by stat-of-the-art techniques including UV/VIS spectroscopy, FTIR, XRD and SEM analysis; and finally the evaluation of antioxidant potential by DPPH scavenging activity and antimutagenic potential by Ames reverse mutation assay using two bacterial strains i.e. Salmonella typhimurium TA98 and TA100.

Precursor metal salts solution exhibited a change in color on treatment with aqueous extract of orange peels. Such color change is associated with a phenomenon known as surface plasmon resonance, in which free electrons at the surface of metal nanoparticles show resonance with electromagnetic radiations of specific wavelength range. Literature showed that electromagnetic radiations of 250-280 nm causes resonance in the Ni-O nanoparticles, those of 410-440 nm produce resonance in Cr-O nanoparticles while Cu-O NPs exhibited resonance by electromagnetic radiations of 550-580 nm region. The results of UV-Vis spectroscopy have been presented in the figure (4.1) which exhibited three sharp bands with λmax at 261.04 nm, 426.98 nm and 574.31 nm. Peak at 261 nm showed the presence of Ni and NiO-NPs, peak centering 426.98 nm represented Cr-NPs while that at 574.31 nm showed the presence of Cu and CuO-NPs. (135) also biosnthesized Cu/Cr/Ni trimetallic nanoparticles using Coriander sativum extract as bio-reducant and evaluated their antimicrobial potential. UV/VIS characterization produced very similar results by presenting λmax values at 261 nm, 426 nm and 574 nm for Ni, Cr and Cu nanoparticles, respectively.

Green synthesis of trimetallic nano-maerial was ascertained by FTIR analyses which were conducted in the range of 4000-400 cm-1 using Thermo Scientific equipment i.e. Nicolet 6700 FTIR spectrometer. Absorbance peaks at variable frequencies represent different functional groups associated with multiple phytochemicals which serve as reducing and capping agents for trimetallic nano-crystallites; also those related to trimetallic oxides. FTIR spectrum of biosynthesized trimetallic nanoparticles has been presented in figure (4.2a). A broad peak at 3204.4 cm-1 corresponds to O–H stretching vibrations (149) that is the crucial component of different phenolic compounds present in plant extract; a shoulder peak at 3058.5 cm-1 also indicate the phenolic components involved in green synthesis of TMNPs (146) while other shoulder peak at 2932.7 cm-1 also a representation of hydrogen bonded phenolic functional groups (147).

A small peak at 1730.1 cm-1 stands for C=O stretching vibrations of carbonyl groups of multiple phytochemicals including phenolic acids, flavonoids, tannins and others (150). A sharp peak at 1615.4 cm-1 indicates C-H stretching peak of aliphatic components of multiple plant secondary metabolites (151). A small peak at 1511.2 cm-1 stands for C=C vibrations of benzene rings representing the aromatic portion of different plant natural products involved in green synthesis of nanoparticles (150). Peak at 1414.4 cm-1 represents C-O bending vibration of aromatic rings (150) while that at 1333.6 cm-1 stands for C- H bending vibrations of aliphatic components of various natural products (152). Peaks at 1270.3 cm-1 indicates hydroxyl at aromatic rings. Peaks at 1231.1 cm-1 specifies C-H stretching vibrations while peaks at 1094.1, 1073.1, 1045.2 and 1014.2 cm-1 represent C-H stretching vibrations (150). Fine peaks ranging from 922.3 cm-1 to 824.3 cm-1 shows the presence of chromium in trimetallic nanoparticles by representing chromyl (Cr=O) and Cr-O vibrations (151),(153) while fine peaks around 770 cm-1 correspond to aliphatic C-H out of plane bending vibrations (150). Peaks around 589-603 cm-1 indicate the oxides of Cu and Cr (147),(153) while peaks around 550 cm-1 ascertain the presence Cu-O nanoparticles (152) and peak at 489.4 cm-1 represents Ni-O stretching vibration (151) while fine peaks around 420 cm-1 shows the indication of metallic oxides of all three metals i.e. Cu/Cr/Ni (154). Most of these peaks are associated with TMNPs as orange peel extract only exhibited few peaks in FTIR spectrum. Orange peel extract showed three peaks figure (4.2b), one broad band around 3510-3110 cm-1 that corresponds to O-H stretching vibrations of multiple phenolic bioactive phytochemicals. Peak around 1620.2 cm-1 stands for aliphatic C-H stretching vibrations while that at 770.4 cm-1 represents the C-H bending vibrations (157).

The crystallinity and size of nanoparticles was calculated from XRD peaks by using Debye Scherrer's formula (158).

$$D = \frac{K\lambda}{\beta\cos\theta}$$

Where D is the size of nanocrystallite in nanometer unit, λ is wavelength of X-ray radiations which were used as light source and it is kept constant during complete analysis, only changes from instrument to instrument. Value of λ is taken in nanometer unit, which was 0.15406Å (0.15406 nm) in our study. K is another constant known as Debye-Scherrer’s constant whose value depends upon geometry of compound and its value is 0.9. β is FWHM (full width half maximum) of XRD peaks which was calculated from XRD plot using Origin software. θ is the diffraction angle of X-rays representing the spacing in the crystal lattice. All the results of calculations from origin software derived from XRD spectrum are shown in table (4.2). The size of trimetallic crystallites was calculated to be 13.06 nm. XRD spectrum figure (4.3) of trimetallic nanoparticles shows major at 36.64° that corresponds to Cu and CuO and Cr2O3 NPs (154),(155) while other major peak at 43.49 ° represents Cr NPs and that at 63.19° stands for Ni and NiO NPs (154).

Other major peaks around 40-50o and 50-60 o are characteristics of oxides of all three metals (135). while all miner peaks indicate that little amount plant extract exist on the surface of nanoparticles serve as capping agent to avoid agglomeration.

Morphological characterization of TMNPs was carried out by scanning electron microscopy. Fig. (4.4) presents photograph of SEM results to analyze particle shape

and size of trimetallic oxide NPs synthesized by green synthesis using orange peel extract. The image shows the spherical morphology for TMNPs and size was found to be 12-15 nm calculated from SEM image using ImageJ software (156), (159) also achieved spherical morphology of Ag/Au/Pt trimetallic nanoparticles to investigate their electrocatalytic activity for methanol oxidation.

Mutations range from chromosomal alternations involving millions of nucleotides to a change in a single base pair. Different types of chromosomal alternations in almost all sorts of cancers and tumors are reported. Various kinds of tumors additionally establish due to distinct chromosomal modifications. Some tumors are also formed through a series of mutations in these genes (160), (161).

Table (4.3) presents the mutagenic behavior of green synthesized trimetallic oxide nanoparticles. All wells in 'Blank' plate appeared purple figure (4.5a) showing the sterility of the experiment. Figure (4.5b) (4.5c) represents the 'Background' plates showing the spontaneous mutation for both bacterial strains. The effect of standard mutagens i.e. K2Cr2O7 for TA98 and NaN3 for TA 100 has been presented in figure (4.5d) (4.5e) showing the effectiveness of such toxic compounds as standard mutagens. Figure (4.5f) (4.5g) showed mutagenic behavior of TMNPs indicating their non-mutagenic behavior towards both strains. Figure (4.5h) (4.5i) shows mutagenic behavior of nanoparticles was compared with H_2O_2 serving as positive control for mutagenicity test. TMNPs exhibited only 15-16% mutagenicity for both bacterial strains and regarded as non-mutagens while H_2O_2 presented 70-71% mutagenicity for both selected bacterial strains as shown in table (4.3).

Figure (4.5l) and (4.5m) presented the antimutagenic potential of TMNPs and positive standard (ascorbic acid), respectively for both selected bacterial strains. Trimetallic oxide nanoparticles exhibited more than 85% antimutagenicity for TA98 and more than 92% for TA100 which are quite comparable to the positive control, ascorbic acid as presented in table (4.3). Such results are in close agreement to those reported for Ag NPs (168) against similar bacterial strains (TA98 and TA100).

The ability of trimetallic nanoparticles to scavenge free radicals was tested in this assay. DPPH is a dark pink colored free radical. It is then converted to a non-radical molecule diphenylpicrylhydrazine with the help of some reducing species and appears as yellow. This neutralization of free radical is effected by the receipt of a proton or electron from different bioactive compounds existing in plant extracts or nano-mterials (162). The results of antiradical activity for TMNPs have been presented in table (4.4) and figure (4.6). It has been found that as the concentration of nanoparticles was increased, scavenging of DPPH free radical was also increased which clearly demonstrate that TMNPs were completely involved in neutralization of DPPH. Maximum value of %age scavenging (65.94%) was achieved at 5 mg/mL with IC50 value of 2.867 mg/mL. Such %age scavenging (65.94%) was found to be superior to that of Cu-NPs (62%) as reported by (152). Identical results for DPPH scavenging potential (63% at conc. 3mg/mL) were presented by (163) for

CuO/ZnO/NiO nanocomposites.

FRAP (Ferric reducing antioxidant potential) is one of the key determinants in search to analyze any material for its ability as an antioxident. The antioxidant potential of nano-materials is commonly evaluated on the extent to which they reduce Fe3+ to Fe2+. Nanoparticles were here employed as the reducing agents i.e. antioxidant in this assay which precipitates Fe3+ ions into Ferrous ferricyanide complex, a prussian blue coloration that was spectrophotometrically monitored at 700 nm wavelength (164). The reductive capability of nano-materials was shown as mg Ascorbic Acid Equivalents (AAE)/mL solution of sample. The result of this assay showed reducing power of green synthesized TMNPs was 1.94±0.012 mg AAE/mL at 2.5 mg/mL concentration of TMNPs. Such results of Cu/Cr/Ni oxide trimetallic NPs was found superior to those of individual mono-metallic NPs as reported by (146) for Cr-NPs (27 µg AAE/mL), (156) for CuO-NPs (50 µg AAE/mL) and (149) for NiO-NPs (529 µg FSE/mL). Such results of antioxidant potential in terms of reducing power are quite comparable to a standard antioxidant compound i.e. α-tocopherol as shown in figure (4.7).

CHAPTER 6

6. CONCLUSION

The current study was aimed to design Cu/Cr/Ni trimetallic oxide nanoparticles through biogenic method using orange peel extract. Phytochemicals present in plant extract served as reducing agents during synthesis of metallic nanoparticles, also functioned as capping agents for individual nanoparticles help to avoid condensation and agglomeration. Successful biosynthesis of nanoparticles was ascertained by different analytical characterization techniques including FTIR, UV-VIS, SEM and XRD analysis. Results of all such instrumental techniques confirmed the successful synthesis of Cu/Cr/Ni trimetallic oxide NPs. After effective biosynthesis process, TMNPs were evaluated for their bioactivities like antioxidant and antimutagenic potential by already reported state-of-the-art in vitro bioassays. Results showed their high antioxidant potential by presenting high percentage scavenging to DPPH free radicals; and high Fe3+ to Fe2+ reducing potential. Results of Ames reverse mutation assay indicated their non-mutagenic behavior by presenting very small percentage of mutation in the selected bacterial strains; however, results of antimutagenic assay exhibited high percentage of antimutagenic potential against selected standard mutagens rendering TMNPs as strong antimutagens. Thus, it is concluded that such biosynthesized trimetallic oxide NPs can be strongly recommended to be used for treatment of multiple cancers and tumors in combination with already available medicines.

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