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Blue Flower Extract: An Antioxidant-Rich Beverage, Mediated Bio-Synthesis of Metal, Metal Oxide Nanoparticles for Anti-Bacterial and Anti-Cancer Applications

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Abstract

This research emphasises the development of nanomedicine for cancer treatment through the exploration of the antibacterial and anticancer activities of different metallic nanoparticles (NPs) [Ag(0), Au(0) NPs] and metal oxides such as CuO, Fe_2O_3, ZnO, and NiO NPs. Through the green biosynthesis process, these NPs have been synthesised using an antioxidant-rich beverage, blue tea [blue flower, Clitoria Ternatea (CT), extract]. The surface functionalization with the high level of polyphenolic compounds, anthocyanin, catechin, etc., present in the blue flower extract, which resists the body from free-radical damage, has been confirmed through FTIR (Fourier Transform Infrared Spectroscopy) and EDX (Energy Dispersive X-ray Spectroscopy) analysis, which can enhance their stability and impart bio-compatibility, anti-bacterial, anti-microbial and anti-cancer activity. The antioxidant activities of both bare blue flower extract and CT-incorporated nanomaterials have been investigated using radical inhibition through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging assay, and the corresponding antioxidant abilities have been compared using median inhibition concentration (IC50) parameters. Furthermore, the anti-bacterial activity of the synthesised nanomaterials against various pathogenic organisms (gram-positive and gram-negative bacterial strains) has been evaluated by determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Finally, dose-dependent cytotoxicity analysis and anti-cancer activity in both in vitro and in vivo settings have established the anti-proliferative properties of the NPs against different cancer cell lines. In this chapter, it has been elaborated on how the antioxidant-incorporated nanomaterials can be used as potential antioxidants, antibacterials, and anticancer agents for commercial applications.

Keywords: *Anti-Cancer; Anti-Microbial; Apoptosis; Bio-Synthesis; Carcinogenesis; Clitoria-Ternatea; DPPH Free Radical; Nano-Materials*

Introduction

Blue butterfly-pea flower extract, commonly known as Blue-Tea, becomes a very popular beverage nowadays for daily intake. It is a caffeine-free herbal tea made from flower petals or whole flower extracts of Clitoria ternatea (butterfly-pea) plant and is high in antioxidants that resist the body from free-radical damage. Clitoria ternatea, also known as 'Aparajita' in Indian Ayurveda, and various parts of this plant, i.e., roots, leaves, flowers, seeds, etc., are important herbs in the Ayurvedic system with great medicinal values. These flower plants are mainly grown automatically in the tropical belt of India, Sri Lanka, Malaysia, Burma, and the Philippine Islands (Lakshan *et al.*, 2019). It has already been used since very old days as traditional ayurvedic-medicine as a memory enhancer, nootropic, anti-stress, anxiolytic, anti-depressant, anti-convulsant, tranquilizing and sedative agent for several neurological disorders. Such types of medicinal plants generally possess a wide range of chemical constituents (bio-active phytochemicals) such as flavonoids, polyphenols, terpenoids, alkaloids, quinines, tannins, etc. which exhibit high antioxidant, antimicrobial, antibacterial, antidiabetic, anti-inflammatory, anti-obesity, and anti-cancer actions and actually can decrease the risk of developing different chronic non-communicable diseases (Ullah *et al.*, 2020) and prevent different health issues and even the risk of cancer. The flower extract contains a significant amount of anthocyanin, a polyphenolic flavonoids compound, which is the main responsible for blue or purple colour and acts as a good anti-oxidant, playing a vital role in the prevention and management of a range of oxidative stress-related chronic diseases (Jeyaraj, Lim & Choo, 2022). The Blue-tea also contains catechin, particularly Epigallocatechin-3-gallate (EGCG), another important component of the human diet for daily intake with strong anti-cancer and anti-inflammatory behavior (Yoshizawa *et al.*, 1987). Koskei (2019) explored the effect of blue flower extract on human cancer cells, especially on breast cancer (J1NT1), cervical (HeLa), prostate (A2780), liver (HepG2), etc. (Koskei, 2019). After that, the blue flower extract becomes more attentive to modern anti-cancer research groups to implement as an anti-cancer agent to inhibit the growth of various cancerous cells and to reduce other chronic diseases, oxidation, DNA damage, cell cycle arrest, and low-grade inflammation.

Bio-synthesis of nanoparticles is a laboratory synthesis process where different biological resources, such as parts of plants (leaves, flowers, barks, seeds, etc.) and micro-organisms, have been used to prepare different nano-sized inorganic materials, especially metal and metal oxide nanomaterials (Vidana-Gamage, Lim & Choo, 2021). In recent years, this bio-synthesis process has been extensively studied for the development of more eco-friendly, non-toxic, most sustainable, cost-effective, and environmentally friendly ease of production, which can be extensively applicable in the biomedical field. In blue flower extract the antioxidant-rich phytochemicals act as good chelating/reducing agent where electrons have been transferred from anthocyanin to inorganic compounds, enabling the production of stable bulk metallic nano-materials with controlled sizes and shapes as well as capping/stabilizing agents, preventing the nano-particles from agglomerating with each other (Chatterjee *et al.*, 2022; Demirbas *et al.*, 2019). In addition, the bio-active phytochemical components have automatically been incorporated onto the surface of the nano-materials, i.e., surface functionalization, during bio-synthesis process. As a result, the potent antioxidant activity of the plant extract has been transmitted into the nano-particles, making them more bio-compatible and have been widely employed as more effective, cheaper, and lower-toxicity new therapeutic agents and nano-carriers for nano-delivery (Kumar *et al.*, 2020). So many in vitro and in vivo experiments have been carried out in the treatment or management of chronic diseases (Gonçalves *et al.*, 2022). It has been proved that antioxidant functionalised nano-materials exhibit higher free-radical scavenging activity (lower IC50 value) than bare antioxidant-containing natural extracts and have been widely used as potential antioxidants, anti-bacterial, and anti-cancer agent for commercial applications (Kumar *et al.*, 2020).

Recently, in cancer research, nano-materials have been designed through bio-synthesis process in such a way that they can bind to specific sites on cancer cells or tumours to deliver drugs more effectively. after that the antioxidant functionalised nano-materials efficiently aggregate at the target location, releasing their surface-loaded anti-cancer agents to particular targeted sites during cancer therapy. Different metallic(0) nano-materials especially silver (0) and gold (0) nano-materials, iron oxide, and copper oxide nano-materials are the most popular and acceptable bio-synthesised and bio-compatible material in the medical sector (Fatimah *et al.*, 2020; George, Rajasekar & Rajeshkumar, 2021). Antioxidant-functionalised Au(0) NPs have been found to be excellent cancer preventives in both in vitro and in vivo settings, which could help to improve the effectiveness of cancer therapy. Also, Au(0) NPs possess superior photophysical and optical properties which make them interesting for cell imaging (Hosny *et al.*, 2022). Again, Silver possesses well-known potential to inhibit microorganisms, and its effect has been shown to further increase after transformation into nano-sized metallic Ag(0), becoming a popular anti-microbial, anti-fungal, anti-viral and anti-inflammation agent and having been extensively utilised in ointments/creams for burns and wounds to inhibit bacterial infections (Urnukhsaikhan *et al.*, 2021). Another research group has explored the potency of CuO and ZnO NPs for the treatment of bacterial infections and anti-cancer effects (Prabhu, Thangadurai & Bharathy, 2021).

With this evidence, this chapter focused on bio-synthesis of different metal and metal oxide nanomaterials using antioxidant-rich blue flower extract and assessed their anti-oxidant, cytotoxic activities. The antioxidant activities of both bare blue flower extract and CT-incorporated nano-materials have been investigated through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging assay, and the corresponding IC50 values have been determined. Furthermore, the bacterial activity of the synthesised nano-materials against various pathogenic organisms (gram-positive and gram-negative bacterial strains) has been compared and discussed. Finally, dose-dependent cytotoxicity analysis and anti-cancer activity in both in vitro and in vivo settings have been elaborated.

Nano-Scaled Metal, Metal Oxides

At first, the antioxidant-rich blue flower extracts have been prepared by following a standard methodology, starting from grinding about 25 g of fresh flower followed by maceration using 50 mL of water, followed by centrifugation at 600 rpm for 5min. The clear extracts have been collected into a sterilizsed bottle and preserved at 4°C until further use.

Silver (0) NPs has been synthesised by Neciosup-Puican *et al.* (2024) using antioxidant-rich blue flower extract, which acts as both a reducing and stabilizing agent for the reduction of silver ions. In this process, a 5 mM silver nitrate ($AgNO_3$) solution has been used as an optimisation condition at PH 10, with a reaction time of 30 min. Hosny *et al.* (2022) prepared Au(0) NPs by suspending varied volumes of 1 mM auric chloride ($HAuCl_4.3H_2O$) with 2 mL of blue flower extract. The reduction of Au^{3+} to Au(0) NPs has been performed by mixing varied volumes of 10 mM with 2 mL of blue flower extract, where the colour of the solution was changed from blue into light pink. Fatimah *et al.* (2020) used the ultrasound probe Delta DH68H (Taiwan) with a frequency of 40 kHz and power of 68 W to synthesize Gold(0) nanoparticles under blue flower extract (ultrasound-assisted method).

During CuO NPs preparation, 10 ml of the blue flower extract has been mixed with 100 ml of 1 mM $Cu(CH_3COO)_2$ solution, and the mixture has been heated at 80°C for 100 min. the colour of the solution changes to dark brown, which confirmed the formation of CuO NPs (George, Rajasekar & Rajeshkumar, 2021).

For the synthesis of iron oxide nanoparticles, 10 ml of 0.1 M $FeCl_3$ has been added dropwise to 100 ml of the blue flower extract under gentle but continuous agitation. Afterward, the pH of the solution has been adjusted to 8 by using 1 M NaOH. The change in the color of the resultant solution from violet to black indicated the successful formation of iron-oxide nanoparticles (Kachhawaha *et al.*, 2025).

In another study, ZnO and NiO NPs has been prepared through an eco-friendly biosynthesis process using aqueous flower extract of Clitoria ternatea under constant stirring at 60 °C for 2 h, followed by pH adjustment to 8 by using 1 M NaOH. The colour of the solution changed from violet to yellow or black, followed by precipitation (Prabhu, Thangadurai & Bharathy, 2021; Chatterjee *et al.*, 2022).

Results and Discussion

The bio-synthesis of gold and silver nanoparticles [Au(0) and Ag(0) NPs] has been carried out through the reduction of aqueous gold metal ions, gold(III) chloride salt ($HAuCl_4.3H_2O$)

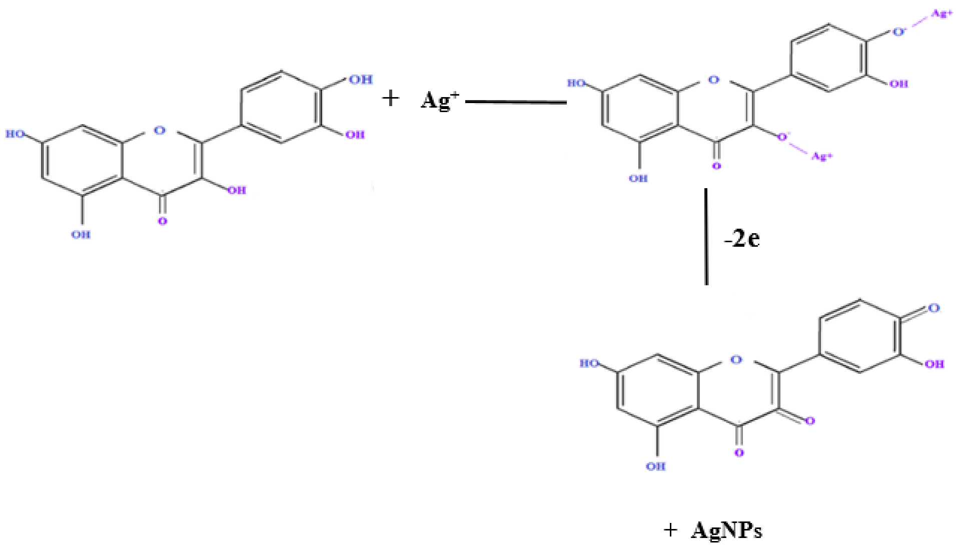

Figure 1: Proposed Mechanism of Bio-Synthesis Reduction of Metal Ion in Presence of Anthocyanin Flavonoids

and silver nitrate ($AgNO_3$), respectively, by antioxidant-rich phytochemicals that are present in blue flower extracts. During this reaction, the metal ions are converted from their mono/di/trivalent state to a zero-valent state followed by nucleation and growth to form metal nanoparticles (Figure 1). Numerous research groups have demonstrated that the presence of functional groups, such as hydroxyl and carboxyl, in a plant's phytochemicals (anthocyanin) primarily acts as reducing, capping, and stabilising agents (Chatterjee *et al.*, 2022). Similarly, when those antioxidant-rich blue flower extracts have been added to the aqueous metal salt solution, followed by pH adjustment to an alkaline medium using NaOH or NH_4OH, the colour of the solution changes from light blue to dark, indicating the formation of metal oxide nano-particles. The pH of the reaction medium is one of the most important factors for nano-material synthesis, which has generally been adjusted to alkaline medium.

During bio-synthesis process, the bioactive phytochemical components, anthocyanin, have automatically been incorporated onto the surface of the nano-materials. Several research groups have confirmed the presence of anthocyanin functional groups on the surface of nano-materials through FTIR analysis (Figure 2a), where the main peak at 3387 cm^{-1} is present in the FTIR spectrum of synthesised Ag(0) NPs corresponding to the involvement of anthocyanin's hydroxyl groups (free O-H stretching band). The involvement of methyl and carbonyl groups of anthocyanin has also been located at 2931 and 1603 cm^{-1}, respectively.

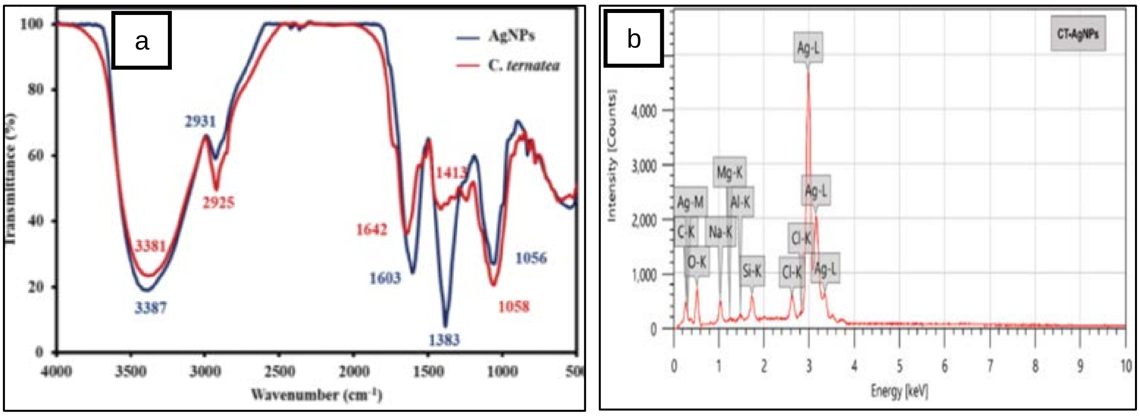

Figure 2 (a): IR Spectra of CT-Ag NPs and Blue Flower Extract; (b) EDX Analysis of CT-AgNPs (Chatterjee et al., 2022; Singh et al., 2025)

Very recently, Singh *et al.* (2025) has verified the presence of bio-active compounds on the surface of the synthesised NPs through EDX analysis (Figure 2b), where some data for carbon (21.63%) and oxygen (4.54%) have been visualised in addition to predominant silver (66.49%) elemental composition.

Characterisation of the Green Synthesised Nano-Materials

During the biosynthesis process, the colour change was obtained from light blue to dark brown (Figure 3) for Ag(0) NPs and from light blue to very intense pink for AuNPs, which are the primary indicator of the formation of corresponding nano-materials. UV-visible spectrophotometer has primarily been used to monitor the formation of the nano-materials by recording the spectrum of the reaction mixture from 200-900 nm, and the product formation was further confirmed by the development of an absorption peak at ~400 nm (Figure 3a) for Ag(0) NPs, ~530 nm (Figure 3b) for Au(0) NPs, which is associated with the surface plasmon resonance (SPR) band, as metals have free electrons and exhibits excitation of longitudinal plasmon vibrations and formation of quasi-linear superstructures of nanoparticles (Neciosup-Puican *et al.*, 2024).

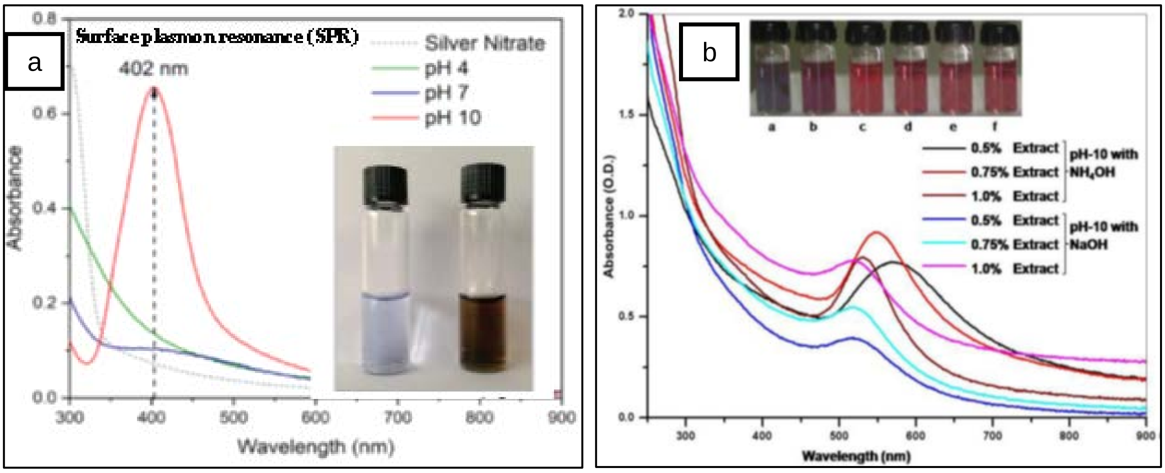

Figure 3: UV-Vis Spectra of (a) Ag(0) NPs; and (b) Au (0) NPs at Different pH Medium, Inset: Corresponding Photography of Reaction System (Neciosup-Puican et al., 2024; Fatimah et al., 2020)

Again, the pH of the reaction medium is one of the most important factors for nano-material synthesis, which has generally been adjusted by 0.1 M NaOH or NH_4OH to obtain different pH-containing solutions having acidic, neutral and alkaline medium. But only in alkaline medium, maximum metal, metal oxide nano-particle formation has occurred, and corresponding dark colouration and UV-visible spectra have been developed. Chatterjee *et al.* (2022) have investigated the synthesis of Ag(0) NPs at different PH medium and obtained brown-coloured NPs of 15 to 50 nm at only PH 10 medium (with a UV absorbance peak at 402 nm) (Figure 3a). Spherical gold nanoparticles (7-29 nm) synthesised using flower extract of Clitoria ternatea (CT) have been reported by Neciosup-Puican *et al.* (2024). Such flower extract has been further reported to be an effective bio-reductant and stabilizing agent for Au (0) NPs. The size of the synthesised Au (0) NPs has been found to decrease (56.5 ± 13.6 to 24.7 ± 8.2) with increasing extract concentration (0.5%-1.0%) in an alkaline medium. The reaction time has also been optimised through the development of UV-vis spectral bands at different times ranging from 0 to 90 minutes and reveals that the synthesis of Au (0) NPs with an average particle size of 5.5 ± 2.7 requires 20 minutes to complete. In the case of metal oxide synthesis, various phytochemicals present in blue flower extract have also been shown to play a significant role in the bio-reduction of metal ions into metal oxides.

The crystalline structure, purity, and average size have generally been determined by the powder X-ray diffraction (XRD) pattern, where sharp peaks corresponding to specific crystal planes confirm the presence of the metal and metal oxide nanoparticles and their crystalline nature. Pure maghemite (Fe_2O_3) (JCPDS ID. 39 1346) phase of iron oxide with one major orientation along the (311) plane (Figure 4) has been successfully synthesised by Dutta *et al.* (2012). In transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM), the surface modification has also been visualised which confirms the formation of hetero-nanostructure with an average size of about 40 nm.

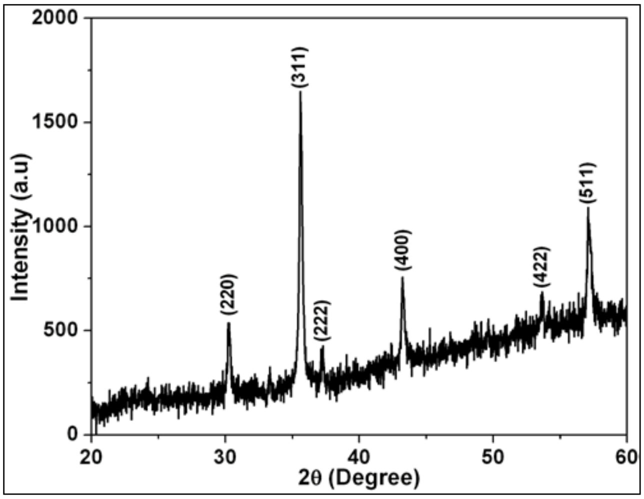

Figure 4: XRD Pattern of the Fe_2O_3 Nanoparticles (Dutta et al., 2012)

Determination of the Antioxidant Activity

Numerous studies showed that the foods or beverages rich in antioxidant components anthocyanins and anthocyanidins mainly possess the ability to act as free radical scavengers against harmful oxidants such as reactive oxygen (ROS) and nitrogen species (RNS) generated inside biological systems during electron transfer reactions by losing or accepting electrons. When the antioxidant functionalised nano-materials has been synthesised through the biosynthesis process, it exhibits superior free-radical scavenging activity compared to bare antioxidant-containing natural extract. From the chemical point of view, the compounds containing dibenzopyran or pyrone ring structure (Figure 5) and so many conjugated double bonds and phenolic hydroxyl groups can accommodate the unpaired electrons through extensively delocalizing over the conjugated system or Hydrogen Atom Transfer (HAT) or Single Electron Transfer (SET) mechanism and effectively scavenge peroxyl (ROO.), alkoxyl (RO.), OH., O_2^-, NO. and other nitrogen or sulphur-containing free radicals.

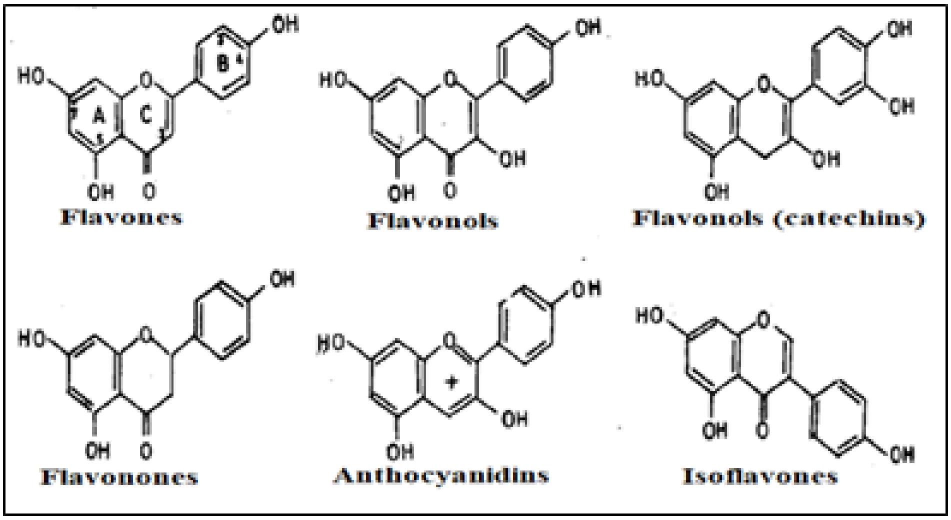

Figure 5: Chemical Structure of Different Natural Flavonoids Compounds

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging assay has been generally used in antioxidant research to determine the antioxidant properties of both tea extract and synthesised nano-materials. DPPH is commercially available as a stable free radical, an organic nitrogen free radical, representing any kind of free radical within the body, which is used to test antioxidant capabilities. A compound is considered a good antioxidant if it can readily donate an electron to the electron-deficient DPPH radicals, thereby terminating the chain reaction of electron transfer between molecules. This action helps prevent oxidative damage to healthy cells and vital biomolecules such as DNA, proteins, and cellular membranes.

During radical scavenging experiments, when the antioxidant anthocyanine, EGCG molecules react with DPPH free radicals, electron transfer occurs, the antioxidant molecules are oxidised and the DPPH is reduced. The investigation has generally been carried out spectrophotometrically because DPPH has an absorption band at 515 nm which disappears gradually upon reduction by an antioxidant compound. Lower absorbance of the reaction mixture indicates higher DPPH free radical scavenging activity (Fang *et al.*, 2017; Baygar & Ugur, 2017). Ascorbic acid has been used as a reference antioxidant because it can react with DPPH, quickly reaching a steady state immediately. Free radical scavenging activity has generally been expressed as the % of inhibition; the higher the percentage inhibition, the more antioxidant the particular compound is. The percentage of inhibition has been calculated by the following equation:

% DPPH scavenging effect = [(ODcontrol – ODsample) / ODcontrol] × 100,

where ‘control’ has been prepared without a tested antioxidant tea sample or nano-material sample. A strong antioxidant, ascorbic acid, has been used as a reference sample.

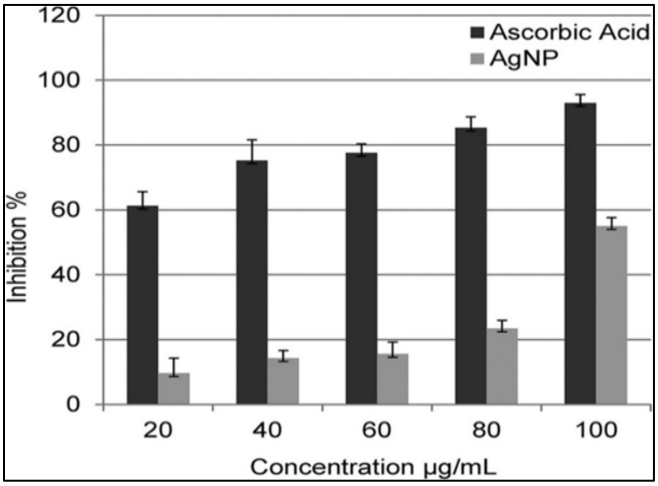

Figure 6: Antioxidant Activity of Bio-synthesised Ag NPs and Reference Ascorbic Acid (Baygar & Ugur, 2017)

During the experiment, when the tested antioxidant sample has added to Control DPPH solution, the absorbance value, i.e., ODcontrol, gradually decreased owing to the inhibition effect of the antioxidant sample, making it decompose some harmful free DPPH radicals, and hence the amount of total DPPH radicals decreased. Baygar and Ugur (2017) have reported that various concentrations of 20, 40, 60, 80 and 100 μg/ml of biosynthesised AgNPs exhibited 9.66%, 14.27%, 15.59%, 23.46% and 54.99% free radical scavenging capability, respectively (Figure 6). When compared with standard ascorbic acid, the antioxidant activity of the biosynthesised NPs was found to increase gradually with the increase in the treatment dose (dose-dependent matter).

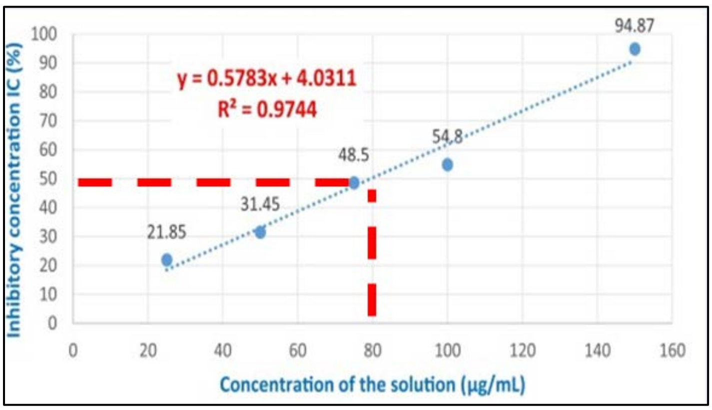

Figure 7: Calibration Curve for Determination of IC50 Value (Fatimah et al., 2020)

Furthermore, Median inhibition concentration (IC50) calculations are widely used to determine the concentration of antioxidant molecules that can do 50% inhibition, i.e., how much of an antioxidant molecule or drug is needed to inhibit a biological process by half

(50%). High IC50 values mean that a relatively high amount (concentration) of a drug or compound is needed to inhibit 50% of a biological process; that compound is less potent and requires a larger amount to achieve the desired effect, whereas the lower IC50 value in microg/ml is more acceptable to us, and the compound is more potent because a small amount of the compound can produce significant inhibitory effects. The IC50 calculation has been carried out graphically using a calibration curve in the linear range by plotting the antioxidant concentration vs the corresponding scavenging effect.

From the experimental data (Figure 7), the more preferable low IC50 value in the range of 50-200 µg/ml of Ag(0) NPs has been determined through constructing calibration curves which exhibit increasing % DPPH radical scavenging ability, but a concentration of 140 µg/ml of Ag(0) NPs exhibits the highest antioxidant activity (~80%). Furthermore, the IC50 value of Ag NPs is around 80.37 µg/ml, comparable with the IC50 value of standard ascorbic acid, 44.10 µg/ml (Fatimah *et al.*, 2020). Kumar *et al.* (2020) have reported that Ag(0) NPs synthesised using tea extract show a significantly lower IC50 value, i.e., 55.86 µg/ml, compared to tea extract alone (IC50 1920 µg/ml), indicating its strong anti-oxidant potential relevant to that of standard ascorbic acid.

Anti-bacterial Activity

Nano-materials especially metal-based ones, can inhibit bacterial growth through various mechanisms such as bacterial cell-membrane damage, production of reactive oxygen species (ROS) inside the bacterial cell or interference with bacterial metabolism and DNA replication. When high levels of polyphenolic compounds, anthocyanins, catechins, etc., have been functionalised on the surface of the NPs, the anti-microbial and anti-inflammatory effect has been increasing much more. Silver possesses famous potential to inhibit micro-organisms and their effect has been shown to further increase after transformation into nano-sized material. The Ag(0) and Au(0) NPs are also well-known anti-bacterial agents because they can interact very easily with bacterial cell walls through attraction between the microbial cell wall's negative charge and NPs' positive charge (owing to possessing negative zeta potential). After interaction, the permeability function of the bacteria cell membrane changes, and hence bacterial integrity has been disrupted and caused cell death (Yamanaka, Hara & Kudo, 2005). Again, after interaction of the NPs with bacterial cells, the thiol group in the electron transport chain enzyme has been disrupted because of the affinity of Ag(0) NPs to Sulphur and Phosphorus elements, which are abundantly found in bacterial cell wall, main responsible for anti-bacterial properties of the NPs. The capability of NPs to enter and accumulate inside the wall of a bacterial cell increases with decreasing the size of the NPs.

The synthesised NPs have been evaluated for anti-bacterial capabilities towards gram-positive and gram-negative bacterial stains through calculation of the inhibition zones and Minimum Inhibitory Concentration (MIC) in µg/ml of the nano-antibiotics i.e., quantitative measurement of the lowest concentration of the NPs that inhibits the growth of a given strain of bacteria. Different bacterial stains show different diameters of inhibition zone (DIZ) in mm units (Figure 8) in the disc diffusion assay experiment. But Minimum inhibitory concentration (MIC) and Minimal Bactericidal Concentration (MBC) have generally been determined with different bacteria in µg/ml units during anti-bacterial activity testing (Wintachai *et al.*, 2019).

In the case of CT-Ag(0) NPs, the MIC has been evaluated as 0.5 mM (0.05 mg/mL) and the corresponding MBC, the lowest concentration, as 4.5 mM, which kills 99% of the initial bacterial population against *E. Coli* bacteria (Wintachai *et al.*, 2019). Again, Antioxidant functionalised Gold(0) NPs have been found to be excellent anti-bacterial agents, and using a special ultra-sound assisted synthesis method, smaller and more uniform Au(0) particles illustrated enhanced anti-bacterial activity through providing more surface contact with micro-organisms. S. aureus bacteria have been found to be most resistant to Au(0) NPs with a minimum inhibition zone of ~20 mm (Figure 8b) (Fatimah *et al.*, 2020).

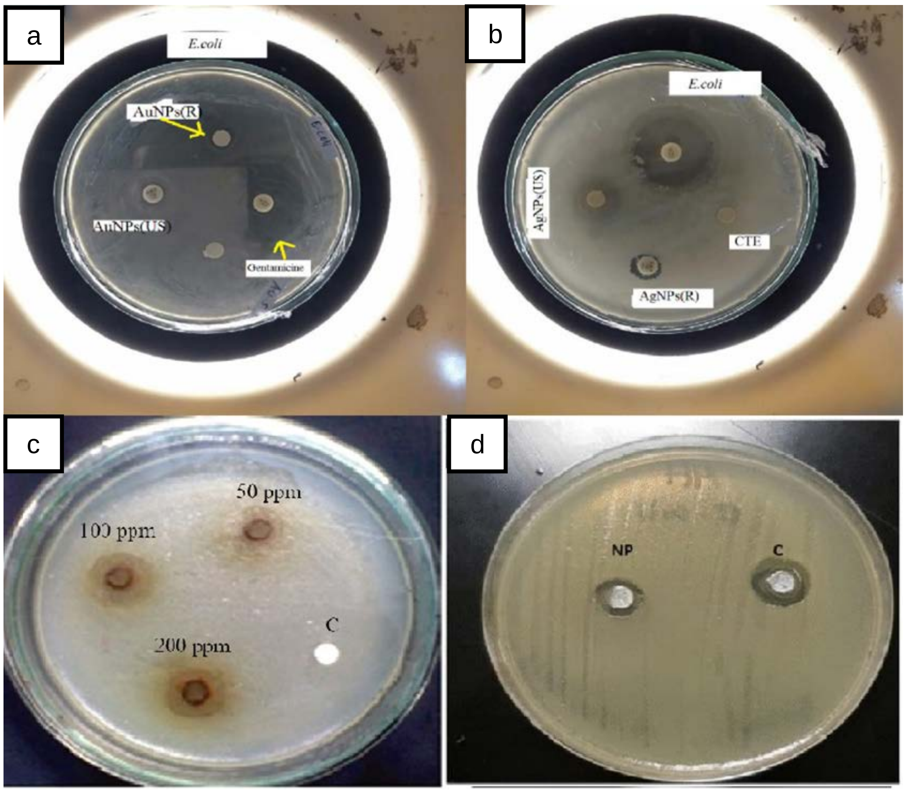

Figure 8: Photography of Disc-Diffusion Assay of (a) Au(0) NPs; (b) Ag(0) NPs; (c) Fe2O3 NPs; and (d) ZnO NPs Against E.coli Bacteria (Wintachai et al., 2019; Fatimah et al., 2020; Naiel et al., 2022)

Among the metal oxide NPs, ZnO and TiO_2 NPs are very popular for their characteristic ultraviolet ray scattering properties and wound-healing applications. Naiel *et al.* (2022) have reported that phyto-synthesised ZnO NPs exhibit potent anti-microbial, anti-fungal activity against gram-negative bacteria *E. Coli*. The anti-bacterial capabilities of those ZnO NPs have been investigated against various pathogenic organisms with different concentrations of ZnO NPs using the agar well diffusion method, which exhibits different sizes of the inhibition zone based on the concentration of the NPs. In another study, bio-synthesised ZnO NPs exhibits anti-bacterial activity against both Escherichia Coli and Staphylococcus aureus bacteria after 24 hours incubation with an 11 mm and 10 mm diameter bacterial growth inhibition zone, respectively (Figure 8d) (Naiel *et al.*, 2022). Again, Fe_2O_3 NPs showed a higher zone of inhibition of E. Coli in the range of 18-26 mm (Figure 8c) (at a concentration of 10 mg/ml). Using the very popular Fenton mechanism, iron can easily generate ROS inside the bacterial cell; that ROS could completely inhibit the function of protein and mitochondria and cause DNA damage and cell lysis.

Anti-cancer Activity

In the case of cancer cells, the good antioxidant can oxidize itself and attack cysteine residues of proteins in cancerous cells, inhibit their growth, and induce apoptosis (programmed cell death). In this way, strong antioxidant-containing materials can inhibit the development of various cancer cells and metabolic pathways that enhance apoptosis, suppress cell proliferation, and inhibit angiogenesis, resulting in cancer cell growth and carcinogenesis. With the help of nano-biotechnology, engineered NPs have been synthesised in such a way that highly porous morphology and a high surface-to-volume ratio of the NPs can enhance the anti-cancer effects. Again, when a high level of polyphenolic compounds, anthocyanins, catechins, etc., has been functionalised on the surface of the NPs, the anti-tumour and anti-cancer effects have been increasing much more, which could help to improve the effectiveness of cancer therapy, cancer cell imaging and prevention methods. Actually, anthocyanin and catechin (EGCG) from tea extract possess high levels of cancer prevention action; they have been widely used in the manufacture of medicine capsules and are prescribed to cancer patients undergoing treatment with radiotherapy. Again, tea catechin (EGCG) shows additional health benefits as a cancer preventive and can-do anti-tumour activity through inhibition of metallo-proteinase activity, inducing the formation of ROS in the mitochondria of cancer cell lines. According to Hsu *et al.* (2012), anthocyanin in blue tea plays a role in cancer prevention through suppressing cell proliferation of COLO 320 DM (IC50 = 64.9 µg/ml) and HT-29 (IC50 = 55.2 µg/ml) by blocking cell cycle progression at the G0/G1 phase inducing apoptotic cell death.

Ag(0) and Au(0) NPs have already been widely used for cancer cell imaging owing to possessing superior photophysical and optical properties. Antioxidant-functionalised gold (0) NPs have been found to be excellent cancer preventives, suppress cell proliferation, have minimum toxicity and can be used to deliver drugs to cancer cells. Again, iron oxide (Fe_2O_3) NPs have already been investigated in cancer research due to holding unique magnetic properties which enable various applications like magnetic targeting and drug delivery, cancer hyperthermia, and magnetic resonance imaging (MRI). The Fe_2O_3 has been designed and utilised to target specific cancer cells, and their magnetic properties allow for precise control of therapeutic agents using an external magnetic field.

The anti-cancer activity of bio synthesised NPs has been investigated by Ansar *et al.* (2020) through the reduction of 3-(4,5-dimethyl thiazole-2-yl)2,5-diphenyl Tetrazolium bromide dye (MTT), and that MTT assay has been generally utilised to explore cytotoxic effects and apoptosis. It has been demonstrated that CT-Ag(0) NPs are potent anti-cancer agents, which exhibits cytotoxic activity against MCF-7 breast cancer cell lines with a very low IC50 value of 17 µg/ml and also exhibit cell death at higher concentrations of nanoparticles i.e., cytotoxicity increases proportionately with increasing concentrations of Ag(0) NPs with a maximum effect at 100 µg/ml with an IC 50 of 55 µg/ml (Ansar *et al.*, 2020). In another study revealed that CT-Ag(0) NPs are able to kill almost all lung cancer cells (A549 and L-132) at 30 µg/ml concentration. Among the metal oxide NPs, ZnO NPs have been investigated for their anti-skin cancer and cytotoxic effect against cancer cell lines, where dose-dependent cytotoxicity analysis reveals that higher concentrations of 500 or 250 µg/ml ZnO can significantly reduce cell viability at 22% and 38%, respectively and the IC50 of skin cancer has been reported as 409.7 µg/ml (Naiel *et al.*, 2022).

Conclusion

The proposed nano-scaled materials have been successfully produced through a green bio-synthesis route using antioxidant-rich blue Aparajita flower aqueous extract. Using flower extract as reducing and stabilizing agents for synthesis offers advantages, such as the fact that flower extract is readily available, sustainable, eco-friendly, cost-effective, and non-hazardous. The synthesised nano-materials has been widely explored as efficient anti-bacterial and anti-cancer agents owing to their possessing superior anti-oxidant activity. They exhibit different anti-oxidant efficiencies based on different size, shape and morphology. The anti-oxidant performances have also been improved by tuning the size and morphology through the ultrasound-assisted synthesis way. The prepared NPs exhibited efficient antimicrobial activities against both Gram-positive and Gram-negative bacteria. Moreover, a low amount of NPs can produce significant inhibitory effects against the development of cancer cells. Therefore, the antioxidant-incorporated nano-materials can be used as potential anti-oxidant, anti-bacterial, anti-cancer agent for commercial applications.

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