Chapter 46

Antimalarial Activity of Metallic Nanoparticles

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ABSTRACT

One of the most severe infectious diseases is malaria, which is characterized by high fever, severe anemia, and neurological sequelae including coma and stroke. *Plasmodium* species are the source of malaria, a parasitic disease that infects people when female *Anopheles* mosquitoes bite them. Metal NPs were primarily evaluated for their ability to kill bacteria and viruses. Nanoparticles can be made by different methods i.e. physical, chemical and biological methods. The most protective and environmental friendly method of nanoparticle synthesis is by using biological materials that is known as green synthesis. Diverse biological materials can be used for the production of nanoparticles i.e. leaves, seeds, flowers, stem, fruits etc. Different nanoparticles are synthesized by combining plant extract with mill molar and molar solutions of salts of different metals mixed in different ratios. The indication of nanoparticle formation was marked by a color change observed within 10-150 minutes. Confirmation of nanoparticle formation was achieved through ultraviolet spectroscopy, which revealed characteristics of plasmon vibrations. Characterization can be done by XRD, FTIR, SEMS and TEMS. Results of different articles showed that green-produced nanoparticles exhibited no cytotoxicity when applied to normal cells and affectively reduced malarial infection. So the green synthesized Nanoparticles can be the effectively reduced malarial infection further studies are required to enhance these findings.

KEYWORDS
Antimalarial activity, Characterization of nanoparticles, Cytotoxicity, Green synthesis, Metallic nanoparticles

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INTRODUCTION

One of the most severe infectious diseases is malaria, which is characterized by recurrent high fever, severe anemia, convulsions, and neurological sequelae including coma and stroke. An estimated 608,000 fatalities worldwide were attributed to malaria in 2022, with a mortality rate of 14·3 deaths per 100,000 people. According to the reported death rate, the following four nations accounted for 50% of all deaths: Nigeria (31%), the Democratic Republic of the Congo (12%), Niger (6%), and Tanzania (4%) (WHO, 2022).

Plasmodium protozoans are the source of malaria, a parasitic disease that infects people when female Anopheles mosquitoes bite those (White et al., 2014). Malaria badly affects economic growth and development in sub-Saharan Africa. Malaria remains a serious public health concern. In 2017, there were 219 million cases of malaria worldwide, and the disease was projected to have caused 435,000 fatalities. Ninety-two percent of these cases were from sub-Saharan Africa (Ta et al., 2014).

The world malaria report identified that the total cases reported in 2022 was expressively higher when compared to the pandemic situation in 2019. However, in nineteen year i.e. 2000 to 2019, there is a decrease in 10 million cases globally i.e. from 243 million to 233 million. But unfortunately, 11 million cases increased in 2020. No change was observed in 2021, however in 2022 there is an increase of 5 million cases in 2022, for a total of about 249 million cases (WHO, 2022).

There was also increase in a number of global malaria deaths in 2022 when compared to the death rate (864 000) in 2019. After that, there has been a decline in the Malaria deaths since 2000, 864 000 to 576,000. After the pandemic situation, the number of deaths increased from 55,000 in 2020, to 631,000 (WHO, 2022).

The most common parasite is *Plasmodium vivax*, but the most lethal is *Plasmodium falciparum*. Several species infect in humans, including *Plasmodium malariae, Plasmodium ovale, Plasmodium cynomolgi* and *Plasmodium knowlesi,* (White et al., 2014; WHO, 2018 and Ta et al., 2014). Despite substantial global initiatives to combat malaria, involving increased financing to support research and development, provision, and expedite its control (The 2020 malaria morbidity and mortality targets set by the Global Technical Strategy (GTS) are still far from being reached by adequate diagnosis, prevention measures, and effective treatment) (WHO, 2018).

Resistance to Current Malarial Drugs

The malaria parasite has developed resistance to nearly all existing drugs, posing a significant obstacle to global malaria control efforts. Combination therapy of artemisinin-based (ACT), the most potent treatment option, which played a vital role in recent advancements in malaria control (White et al., 2014; WHO, 2018 and Ta et al., 2014). Strains of *P. falciparum* that are resistant to artemisinin have been documented from the Subregion of the Greater Mekong (GMS), Western Cambodia, and China's South (WHO, 2017; Dama et al., 2017 and Noedl et al., 2008). Treatment of malaria patients and efforts to control the disease in impacted areas have been severely hampered by the presence of artemisinin (ART) resistant parasites in addition to resistance to partner medication in ACT. The existence of ART-resistant parasites has been linked in multiple studies to increased rates of treatment adherence in patients with ACT (Fairhurst and dondrop, 2016; Tilley et al., 2016 and Woodrow and White, 2017). Kelch (K13) gene identified inside *P.falciparum* propeller domain, appears to be positive selection occurring on the artemisinin resistance pattern (Cheeseman, 2012, Miotto et al., 2013 and Takala-Harrison et al., 2013). This indicates that the specific genes responsible for delayed clearance or contributing to resistance to artemisinin has not been identified conclusively, however, 13 non-nonsynonymous variants have been identified as relevant markers (WHO, 2017; Dondorp et al.,2009; Witkowski et al.,2013, Ashley et al.,2014; Takala-Harrison et al.,2015; Ariey et al.,2014 and Straimer et al.,2015). The appearance or dissemination resistance to artemisinin from Asia to Africa mirrors previous occurrences with older anti-malarial medications such as Chloroquine and sulfadoxine-pyrimethamine (Trape, 2001; Trape et al., 2002 and Wongsrichanalai and Sibley, 2013), It would have a catastrophic impact on global malaria eradication initiatives. Despite widespread concern about the possible emergencies or dissemination of K13 mutations associated with artemisinin – resistance in Africa, the mutations identified thus for or infrequent and distinct from k13 polymorphism linked to diminished susceptibility in Asia (WHO, 2017; Djaman et al., 2017; Kamau et al., 2015; Murugan et al., 2017; Borrmann et al., 2013; Torrentino-Madamet et al., 2014; Cooper et al., 2015; Ouattara et al., 2015; Menard et al., 2016 and Taylor et al., 2015).

Alternative Source for the Treatment of Malaria by Nanoparticle

Therefore, provided the circumstances and the fact that many antimalarial treatments are supported by governments, non-profit organizations, and other entities, the ability of research and development to provide the next generation of anti-malarial drugs will determine the success of malaria control and global eradication (Tse et al., 2019). If the project fails, it may seriously impair the chances of successfully managing and eliminating malaria, particularly in Africa as outlined in the worldwide Technical Strategy 2016-2030 (WHO, 2022). Keeping in view of the above-mentioned problem in GMC nanotechnology seems to be the alternate treatment of malaria.

Nano products and metal nanoparticles offer significant utility and are inherently safe, finding a wide array of applications in renewable energies, biomedical devices, and health care catalysis, cosmetics, food, electronics, and environmental cleanup (Ealia and Saravanakumar, 2017; Gahlawat and Choudhury, 2019; Ahmed et al., 2016 and Khandel and Shahi, 2016).

Metallic Nanoparticles

In nanotechnology metallic nanoparticles have much important medical importance. Various approaches are available for creating new anti-malarial medication, with some originating from living organisms. The process of creating metal nanoparticles essentially included combining three components: the capping agent, the reducing agents, and the metal supply (usually noble metals like silver, gold, palladium, and titanium salt) (Ealia and Saravanakumar, 2017). Metal NPs were primarily evaluated for their ability to kill bacteria (Maiti et al., 2014; Patra and Baek, 2016 and Patra and Baek, 2017), fungi (Mallmann et al., 2015 and Arciniegas-Grijalba et al., 2017), and viruses (Narasimha, 2013 and Broglie et al., 2015). There is limited information available regarding the anti-plasmodial capabilities of metal nanoparticles (Dauda et al., 2017).

Methods for the Formulation of Metallic Nanoparticles

They can be prepared by using three methods, chemicals, physical, and biological. In nanotechnology metallic nanoparticles have much important medical importance. They can be prepared by using three methods, chemicals, physical, and Biological (Fig 1). Chemicals and physical methods pose challenges due to their expense, time intensiveness, and reliance on environmentally harmful reagents (Ealia and Saravanakumar, 2017 and Gahlawat and Choudhury, 2019).

Green Synthesis as Cost – an Efficient and Environmentally Friendly Method

In response to this, innovative methods for synthesizing nanoparticles, known as Green synthesis, that use biological material. Green synthesis involves producing metal nanoparticles by harnessing the natural capping and reducing abilities of biomolecules derived from living things like microbes and plants**.** This approach is straightforward, cost–efficient, and environmentally friendly (Barabadi et al., 2019; Moher et al., 2009; Panneerselvam et al., 2011; Ponarulselvam et al., 2012 and Mishra et al., 2013). However extensive efforts are required to maintain the cultures of microorganisms so plant source is the best option for the formulation of Nanoparticles.

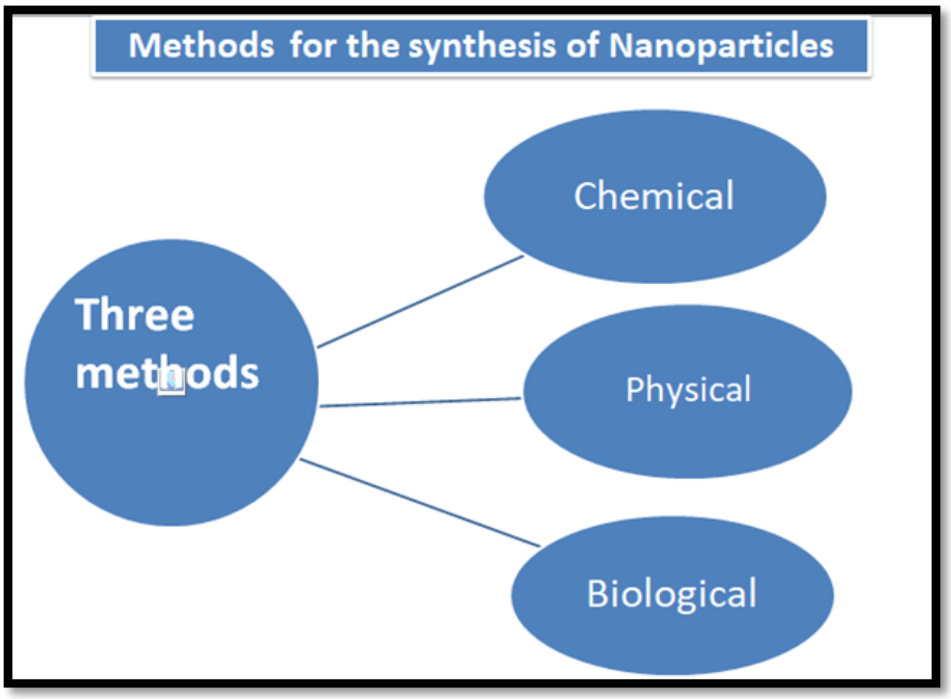

Fig. 1: Methods for the synthesis of metallic Nanoparticle

Different Biological Material that can be used for the Synthesis of Nanoparticles

Diverse biological materials can be used to produce nanoparticles i.e. leaves, seeds, flowers, stem, peels of fruits, fruits, etc. (Fig. 2). A summary of different plant materials used is given in Table 1. Leaves as the biological materials for the synthesis of metal NPs. Flowers, seeds, and barks were among the additional plant parts (Panneerselvam et al., 2015; Subramaniam et al., 2016 and Dutta et al., 2017).

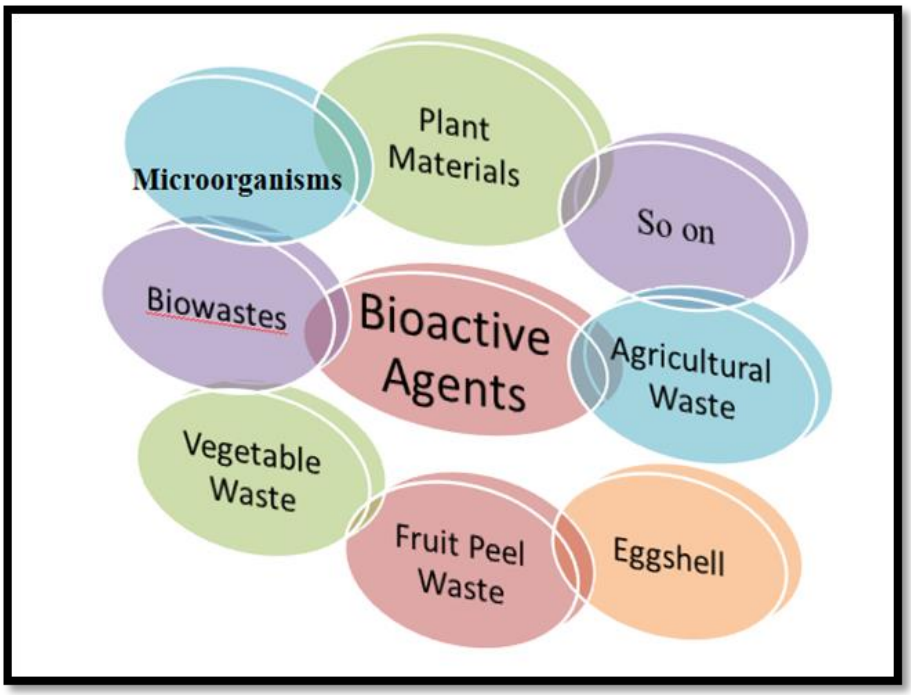

Fig. 2: Different biological materials used for the production of Nanoparticles

Table 1: Summary of different plant materials utilized in the production of Nanoparticles

Plant Source	References
Leaves	(Panneerselvam et al., 2011; Ponarulselvam et al., 2012; Mishra et al., 2013; Panneerselvam et al., 2015; Murugan et al., 2015; Subramaniam et al., 2016; Dutta et al., 2017; Sardana et al., 2018; Gandhi et al., 2018 and Rotimi et al., 2019
Flowers	(Subramaniam et al., 2016)
Seeds	(Murugan et al., 2016 and Rotimi et al., 2019)
Barks	(Dutta et al., 2017)

Preparation of Plant Extracts

The selected plant material is air-dried at room temperature. Subsequently, the dried plant material is finely ground using a mechanical blender. By using different quantities of plant material and deionized water extract can be made by simple method or by using Soxhlet extraction method by using Soxhlet Apparatus. By simple boiling and decantation method. Whatman no 1 filter paper is used to filter the resultant mixture. The filtrate obtained underwent lyophilization to produce a dry powder, which is stored in a covered centrifuge tube in the refrigerator at 4°C until needed for nanoparticle synthesis.

In the literature, the authors mostly used a simple method to create aqueous extracts, which needed to be combined with a material's predecessor to synthesize Nanoparticles. After thorough washing with double-distilled water and cutting, biological material (4-10g) was cooked. The mixture was then dripped out and filtered via Whatman N°1 filter paper utilizing water as the preferred extraction solvent.

Stock Solution of different Salts of different Metals

Millimolar and molar solutions of salts of different metals are prepared.

Synthesis of Nanoparticles

Different nanoparticles are synthesized by combining plant extract with mill molar and molar solutions of salts of different metals mixed in different ratios. Then the mixture underwent an incubation period with continuous stirring for different durations, in darkness to avoid photochemical reactions. The indication of nanoparticle formation was marked by a color change observed within 10-150 minutes following the mixing of the metal precursor and plant extracts.

After that mixture was centrifuged at different rpm for different time durations at room temperature. The resulting pellet is washed twice with double distilled water, air-dried, and utilized for subsequent assays (Panneerselvam et al.,2011; Ponarulselvam et al.,2012; Mishra et al.,2013; Panneerselvam et al.,2015; Rajakumar et al.,2015; Murugun et al.,2015; Subramaniam et al.,2016; Panneerselvam et al.,2016; Murugan et al.,2016; Dutta et al.,2017; Sardana et al.,2018; Gandhi et al.,2018; Rotimi et al.,2019; Karthik et al.,2013; Jaganathan et al.,2016; and Murugan et al.,2017).

Methods used for the Characterization of Nanoparticles

The research investigated the physical properties of metal nanoparticles across several dimensions: shape, size, and variability, as well as their chemical composition, in structural stability (refer to Table 4). Confirmation of nanoparticle formation was achieved through ultraviolet spectroscopy (UV -VIS), which revealed characteristics of plasmon vibrations. To determine the morphology and dimension of nanoparticles, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) were suggested. Elemental mapping based on atomic composition was made easier by CEM in conjunction with energy-dispersive X-ray spectroscopy (EDX, EDS), and crystallographic planes were exposed by TEM in conjunction with selected area electron diffraction (SAED). Using molecular vibrations, FTIR was utilized to investigate the interface between NPs and secondary metabolites. X-ray diffraction (XRD) offers information about crystallinity, nature, and additional aspects such as size and shape determination. Size distributions and terms of hydrodynamic radius were obtained by dynamic light scattering (DLS); some research concentrated on spectroscopy (AAS) (Subramaniam et al.,2016; Dutta et al.,2017; Gandhi et al.,2018 and Karthik et al.,2013) (Fig. 3).

Different Metals used for the Synthesis of Metallic Nanoparticles

Silver nitrate (AgNO3) served as a primary metal precursor for producing nanoparticles related to plants. Additionally, other noble metals such as gold, palladium, and titanium were utilized in nanoparticle synthesis (Rajakumar et al.,2015; Subramaniam et al.,2016; Dutta et al.,2017; Gandhi et al.,2018 and Karthik et al.,2013). The indication of nanoparticle formation was marked by a color change observed within 10-15 minutes following the mixing of the metal precursor and plant extracts characteristic and characterization of some metallic nanoparticles that were synthesized previously for antimalarial activity is summarized in Table 2 and Table 3.

Characterization Techniques used for Identification of Nanoparticles in Previous Studies

UV – VIS spectroscopy emerged as a pivotal method for examining nanoparticle behavior, including their formation, evolution, or aggregation.

The characteristics of plasmon vibration arise from the unrestricted movement of electrons on the surface of metallic materials. In the case of silver, these vibrations occur within the range of 400-450 nm (Panneerselvam et al.,2011; Ponarulselvam et al.,2012; Mishra et al., 2013; Panneerselvam et al., 2015; Murugan et al.,2015; Panneerselvam et al.,2016; Murugan et al.,2016; Dutta et al.,2017 and Sardana et al.,2018), while for gold, they occur around 540 and 560 nm (Rajakumar et al.,2015 and Karthik et al.,2013) and 8360 nm for titanium dioxide (Gandhi et al., 2018), most studies observed the formation of spherical- shaped Nanoparticles (Table 3). Globally, these nanoparticles ranged in size from 4 to 65 nm, and some research observed aggregation phenomena throughout production (Mishra et al., 2013 and Murugan et al., 2016). Additionally, certain research revealed the existence of other Bragg peaks (Ponarulselvam et al., 2012; Mishra et al., 2013; Panneerselvam et al., 2015 and Murugan et al., 2016). Signals related to oxygen or carbon atoms were detected using energy-dispersive X-ray analysis (Rajakumar et al., 2015 and Murugan et al., 2016), whole

additional Signals associated with chlorine were occasionally observed as well (Panneerselvam et al., 2016). The resultant nanoparticles' crystallographic planes and diffraction patterns were discovered by selected area electron diffraction (SAED) (Dutta et al., 2017).

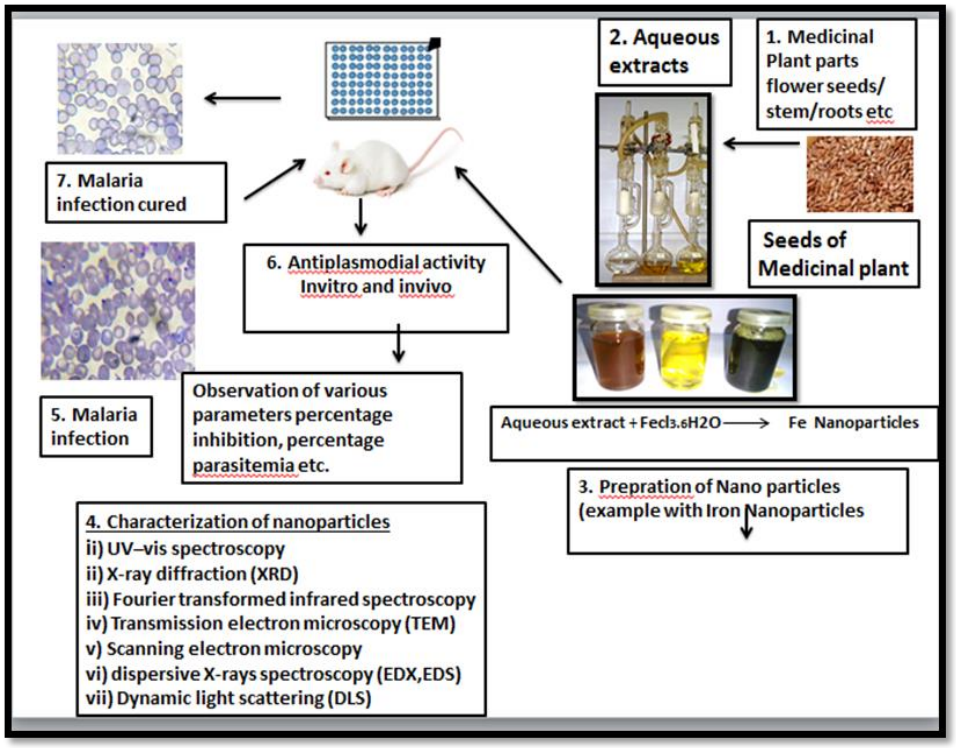

Fig. 3: Schematic diagram for synthesis, characterization, *In vivo* and *In vitro* antiplasmodial activity of metallic nanoparticles

Power X-ray diffraction is a noteworthy characterization method in solid-state chemistry (Das et al., 2014), used to evaluate nanoparticles generated from biological extracts like plants or earthworms and identify the crystalline phases. The anti-plasmodial properties were more extensively evaluated with Nano silver (57%) and Nano gold (29%). The analysis was made easier by contrasting the acquired patterns with those in the Giant Committee on Powder Diffraction Standards (JSPDS) database, which was originally known as the International Center for Diffraction Data (ICDD). While biosynthesis typically yields pure palladium, gold, or titanium dioxide NPs, the case differs for silver, where the outcomes may include Nano silver, silver chloride, Nano crystallites, or their mixture (Ahmed et al.,2016; Khandel and shahi, 2016 and Broglie et al.,2015).

Table 2: Summary of Physio-chemical characterization of NPs reported in the literature

Physio-chemical characteristic	Characterization method used	References
Plasmon resonance	UV-Vis	(Panneerselvam et al., 2011; Ponarulselvam et al., 2012; Panneerselvam et al., 2015; Rajakumar et al., 2015; Murugan et al., 2015; Subramaniam et al., 2016; Panneerselvam et al., 2016; Murugan et al., 2016; Dutta et al., 2017; Sardana et al., 2018; Gandhi et al., 2018; Rotimi et al., 2019; Karthik et al., 2013; Jaganathan et al., 2016; Murugan et al., 2017)
Shape, size and Size distribution	FESEM/SEM, EDX, EDAX	(Panneerselvam et al., 2011; Ponarulselvam et al., 2012; Panneerselvam et al., 2015; Rajakumar et al., 2015; Murugan et al., 2015; Subramaniam et al., 2016; Panneerselvam et al., 2016; Murugan et al., 2016; Rotimi et al., 2019; Karthik et al., 2013; Jaganathan et al., 2016 and Murugan et al., 2017)
	HRTEM/TEM, SAED	(Mishra et al., 2013; Rajakumar et al., 2015; Dutta et al., 2017; Sardana et al., 2018; Gandhi et al., 2018; Rotimi et al., 2019; Karthik et al., 2013 and Murugan et al., 2017)
Silver content	AAS	(Mishra et al., 2013)
Interface NPs-metabolites	FTIR	(Mishra et al., 2013; Rajakumar et al.,2015; Murugan et al., 2015; Subramaniam et al., 2016; Murugan et al.,2016; Dutta et al., 2017; Gandhi et al., 2018; Rotimi et al., 2019; Jaganathan et al., 2016 and Murugan et al., 2017)
Size distribution	DLS	(Mishra et al., 2013 and Gandhi et al., 2018)
Crystallinity of the structure	XRD	(Panneerselvam et al.,2011; Mishra et al., 2013;Panneerselvam et al., 2015; Rajakumar et al., 2015; Murugan et al., 2015; Subramaniam et al., 2016; Panneerselvam et al., 2016; Murugan et al., 2016; Dutta et al., 2017 and Karthik et al., 2013)
Stability	Zeta potential	(Panneerselvam et al., 2011; Rajakumar et al., 2015; Subramaniam et al., 2016 and Murugan et al., 2016)

Table 3: Summary of some characteristics of metallic NPs reported in the literature

Characteristics	Detail	References
Metal source	AgNO3	(Panneerselvam et al.,2011; Ponarulselvam et al.,2012; Mishra et al.,2013; Panneerselvam et al.,2015; Murugan et al.,2015; Panneerselvam et al.,2016; Murugan et al.,2016; Dutta et al.,2017; Sardana et al.,2018; Karthik et al.,2013 and Murugan et al.,2017)
	HAuCl4	(Subramaniam et al.,2016; Dutta et al.,2017; Rotimi et al.,2019 and Karthik et al.,2013)
	Pd (OAc)2	(Rajakumar et al.,2015)
	TiCl4	(Gandhi et al.,2018)
Production time	< 30 min	(Panneerselvam et al.,2015; Sardana et al.,2018; Gandhi et al.,2018 and Karthik et al.,2013)
	>30 min	(Panneerselvam et al.,2011; Mishra et al.,2013; Rajakumar et al.,2015; Subramaniam et al.,2016; Panneerselvam et al.,2016 and Murugan et al.,2016)
Shape	Spherical or mainly Spherical	(Panneerselvam et al.,2011; Ponarulselvam et al.,2012; Mishra et al.,2013; Panneerselvam et al.,2015; Rajakumar et al.,2015; Panneerselvam et al.,2016; Murugan et al.,2016; Dutta et al.,2017; Sardana et al.,2018; Gandhi et al.,2018 and Jaganathan et al.,2016)
	Other shapes (cubical, polygonal, triangular, oval, ellipsoid, rectangular)	(Panneerselvam et al.,2015; Rajakumar et al.,2015; Murugan et al.,2015; Subramaniam et al.,2016; Murugan et al.,2016; Dutta et al.,2017; Sardana et al.,2018; Gandhi et al.,2018 and Karthik et al.,2013)
NPs aggregation Phenomenon	Yes	(Mishra et al.,2013 and Murugan et al.,2016)

FTIR analysis was conducted to examine biomolecule extracts at the interfaces of silver, gold, palladium, and titanium metal oxides. Using this technique, molecular vibrations within or on the surface of artificial nanoparticles can be observed. The results of spectroscopic research showed that the absorption frequencies closely matched the known molecular properties. Alcohols are present, i.e. when O-H (stretch, H-bonded) is present at 3200-3600 and C-O (stretch) is present at 1050-1150. Alkanes show C-H (bending) at 1350-1480 and C-H (stretch) at 2860-3000. Comparably, C=C (stretch) is found in alkanes at 1620-1680 and N-H (stretch) and N-H (bending) are found in amines at 83300-3500 and 1600, respectively, along with C-N (stretch) at 0180-1360. Carbonyls show C=O (stretch) at 1670-1820, whereas aromatics show C-H (stretch) at 3000-3100 and C=C (stretch) at 1400-1600 (Hanson, 2013).

Anti-plasmodial Activity of Nanoparticles

The experiments primarily focused on in vitro setups, although a few also involved in vivo studies (Rajakumar et al.,2015 and Gandhi et al., 2018), or a combination of both (Murugan et al.,2016 and Murugan et al.,2017). Chloroquine was used as a positive control in most of these studies to evaluate exposure to different laboratory strains of Plasmodium falciparum, including INDO (CQ-resistant), 3D7 (CQ-sensitive), FcB1/Colombia (CQ-sensitive), and Dd2 (CQ-sensitive) (Table 3). Control groups included distilled water, uninfected or maybe infected erythrocytes, or culture medium. P. falciparum field strains were collected by some research from patients in healthcare facilities (Panneerselvam et al.,2016; Murugan et al.,2016 and Dutta et al., 2017). P. berghei served as the model organism and studies involving animal models to evaluate malarial susceptibility and NPs were given intraperitoneally (Karthik et al., 2013) or orally (Murugan et al., 2016). To assess the anti-plasmodial activity of nanoparticles, the 50% inhibitory concentration (IC50) and the percentage of suppressed parasite development were used as endpoints. Panneerselvam et al., (2015), Observed a decrease in parasite growth rate ranging from 26% to 83% at doses of 25µg/mL and 100µg/mL, respectively. Murugan et al. (2016) Reported reduced action against Plasmodial ranging from 6.4% to 42.8%. Results based on IC50 varied among studies but usually fell into one of three groups: (I) The results showed that nanoparticles outperformed the positive control (Murugan et al., 2015; Subramaniam et al., 2016; Panneerselvam et al., 2016 and Murugan et al., 2016), (II) that they outperformed plant extracts (Mishra et al., 2013; Rajakumar et al., 2015; Murugan et al., 2016 and Dutta et al., 2017), and (III) that they underperformed both the positive control (Chloroquine) and the plant extract (Subramaniam et al., 2016; Panneerselvam et al., 2016; Murugan et al., 2016 and Dutta et al., 2017). Using chloroquine as a reference, metal nanoparticles were found to be more effective in 9 out of 11 investigations (Mishra et al., 2013; Rajakumar et al., 2015; Murugan et al., 2015; Subramaniam et al., 2016; Panneerselvam et al., 2016; Murugan et al., 2016; Jaganathan et al., 2016 and Murugan et al., 2017). For example, Gajanathan et al., observed that their NPs had IC50 values of 49.3µg/mL and 55.5µg/mL against the chloroquine-sensitive-strain of P.falciparum 3D7 and the chloroquine-resistant strain of INDO, respectively, in comparison to 81.5µg/mL and 86.5µg/mL, respectively, of chloroquine (Jaganathan et al.,2016). According to Murugan et al., (2016) nanoparticles had IC50 values of 63.18µg/mL and 69.24µg/mL against 3D7 and INDO strains, respectively, whereas extracts had IC50 values of 82.41µg/mL and 86.12 µg/ml. This suggests that nanoparticles have greater anti-plasmodial activity

than plant extracts. The current investigation assessed the antimalarial activity of Silver NPs produced from crude extracts of the marine seaweed S. tenerrimum (Ag-ST), in vivo as well as in vitro studies. With IC_{50} values of 7.71 ± 0.39 µg/mL and 23.93 ± 2.27 µg/mL against Plasmodium falciparum and Plasmodium berghei, respectively, the result demonstrated that Ag-ST NPs had excellent anti-plasmodial action (Veeragoni et al., 2023).

Using an IC_{50} value of 3.41 µg/mL, it was discovered that the produced ZnO NPs exhibited good anti-plasmodial properties. According to the results of this study, ZnO NPs showed excellent anti-plasmodial activity. These findings may be applied to future in vivo investigations to develop anti-plasmodial drugs (Najoom et al., 2021).

Cytotoxicity of Nanoparticles

Out of 17 studies, 7 conducted cytotoxicity assessments of the synthesized nanoparticles (Mishra et al., 2013; Rajakumar et al., 2015 and Karthik et al., 2013). Among the seven investigations, four concluded that the nanoparticles had little to no negative effect on the cell lines that were investigated (Mishra et al.,2013; Panneerselvam et al., 2015; Dutta et al., 2017 and Gandhi et al., 2018). The remaining three studies, on the other hand, documented severe side effects that included apoptosis (Jaganathan et al., 2016), necrosis, cytopathic effects (Rajakumar et al., 2015), behavioral changes, physical appearance changes, and mortality of laboratory animals (Karthik et al., 2013). PBMCs are readily available, round-nucleated cells extracted from buffy coats or blood. Systemic toxicity and medication resistance are the two main side effects of chemotherapy medications. Because human PBMCs are susceptible to novel medications and toxins, they are frequently utilized in cytotoxicity testing (Bendale et al., 2017). Using the MTT test, the cytotoxic effect of Iron oxide nanoparticles on PBMCs was thus assessed. We observed that the cell viability of PBMCs treated with varying concentrations of FeO NPs (5,10,15,20, and 25 µg/mL) did not significantly change. Conversely, it was shown that as the concentration of FeO NPs was raised, after PBMCs were treated with them, the cell viability decreased. The cell viability percentage in relation to various concentrations (Figure 4). FeO nanoparticles were successful in achieving 78% viability on PBMCs at 25 µg/mL. According to ISO 10993-5:2009, If cell viability exceeds 70%, Iron Oxide nanoparticle activity is considered non-toxic (López-Badillo et al., 2021). Therefore, it is possible to classify the produced FeO NPs as non-toxic. Our results are corroborated by other investigations that found that green-produced nanoparticles exhibited no cytotoxicity when applied to normal cells (Zangeneh et al., 2020). FeO NPs' lack of toxicity gives them greater stability for use in applications related to drugs and other medicines.

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