EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES

http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 5
319-329
Year: 2018

BIOGENESIS OF GOLD NANOPARTICLES, ROLE OF FUNGAL ENDOPHYTES AND EVALUATION OF ANTICANCER ACTIVITY- A REVIEW

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Article Received on 17/03/2018 **Article Revised on 07/04/2018** **Article Accepted on 28/04/2018**

ABSTRACT
Due to the surge of interest in synthesizing nanoparticles in the recent couple of years using green routes, the field of nanotechnology has been advanced in the production of different metal nanoparticles which in turn have found innovative applications in various fields. These advances in turn provide an alternative for physicochemical methods of nanoparticles formation economically. Metals like gold and silver have been used for the biosynthesis of nanoparticles due to which they have attained a special focus. Gold nanoparticles have received tremendous importance in research because of their unique optoelectrical and photothermal properties. The scanty reports of nanomaterial synthesis from fungal endophytes have led to the development of 'myconanotechnology' as a new emerging domain of nanotechnology. This emerging field of nanoparticles synthesis using endophytic fungi is advantageous as fungi have the ability to produce large amount of nanoparticles by secreting large amount of enzyme proteins which can prevent the fast depletion of plant sources. Apart from this, fungus provides protection and survival condition to their host plants. Although many fungi have been reported as nano-biofactories of gold metal, relatively few reports are available about the synthesis of nanogold using fungal e; ndophytes. The present review is of a kind wherin it gives a vision of biogenic gold nano synthesis, unexplored endophyte-mediated gold nanomaterials and anticancer activity of green synthesized nanoparticles.

KEYWORDS: Fungal endophytes, Gold nanoparticles (AuNP's), Green synthesis, Anticancer activity.

1. INTRODUCTION
Nanotechnology has begun leaving adjusts of research facility and overcoming the new application to change our lives. Nanotechnology is science, engineering and technology led at the nanoscale which is in the range 1 to 100 nanometers. The concept of *nanotechnology* was given by Richard Feyman through his famous lecture entitled "There is a plenty of rooms at the bottom" at the American institute of Technology. The word *nanotechnology* was introduced by Prof. Norio Taniguchi of Tokyo Science University.

Then again biotechnology utilizes the learning and systems of science to control sub-atomic, hereditary and cell procedures to create items and benefits and is utilized as a part of differing fields from medicine to agriculture. Nanobiotechnology is thought to be one of a unique fusion of biotechnology and nanotechnology by which microtechnology can be merged to a molecular biological approach in genuine. Nanobiotechnology can assume a key part in creating and actualizing numerous valuable tools in investigation of life. Nanobiotechnology represents an economic alternative for chemical and physical techniques for nanoparticles formation. These methods of synthesis can be divided as intra cellular and extracellular (Ahmad *et al*., 2005). Among the variety of nanoparticles with their applications, metallic nanoparticles (especially gold and silver nanoparticles) are playing most prominent role in biology and medicine (Raghavendra *et al*., 2014; Siemieniec and Kruk, 2013).

With the rapid development of new chemical and physical methods, concern for environmental contaminations is regularly heightened as the said procedures involved in the synthesis of nanomaterials generates a large amount of hazardous by-products. Thus, there is an immediate need for 'green chemistry' that includes clean, nontoxic and environment-friendly methods of nanoparticle synthesis with precise control over the shape and size (Verma *et al*., 2011). The synthesis of nanoparticles with particular size, shape, composition and distinguished characteristics has overcome the extent of their applications in various fields including agriculture, cosmetics, textiles, food, medicine and environment (Oza *et al*., 2012). Advances in the field of nanotechnology have resulted in the production of different metal nanoparticles which have found innovative applications in various synthesis of noble metal nanoparticles using biological entities has great interest due to their physicochemical properties which are not observed either in individual molecules or in bulk metals. Nature has devised various processes for the synthesis of nano and micro scaled inorganic materials which have contributed to the development of relatively new and largely unexplored area of research based on biosynthesis of nanomaterials (Deendayal Mandal *et al*., 2006). Metal nanoparticles have been produced by different chemical methods which include the use of chemical reductants like hydrazine hydrate (Kajori and Padma, 2012). However biological methods, which make use of fungi, bacteria, algae and plants, are more eco-friendly and are being explored as alternatives to conventional techniques which inturn involve hazardous waste generation. Adopting green nanotechnology for this purpose will be a positive step towards reduction of global warming leading to sustainable development.

Most biogenic nanomaterials synthesized using diverse microbial flora are metallic nanomaterials such as silver and gold nanoparticles. Use of silver in therapeutics is well documented but the introduction of silver nanoparticles has resulted in expansion of its applications (Bankara *et al*., 2010). Silver nanoparticles are used as nanodevices, cosmetics, and biosensors, and also have been considered as potent antimicrobial agents against drug-resistant microbes (Ibrahim *et al*., 2015). Similarly, gold has been used for centuries in curing various ailments, and recently gold nanoparticles have demonstrated significant advances in medicine such as drug delivery, biocatalysts, and biolabeling (Baker *et al*., 2015). Among the biological entities, the use of microorganisms in the synthesis of nanoparticles has successfully competed with conventional methods in synthesizing size-dependent nanomaterials. Microorganisms can synthesize nanomaterials in aqueous solutions, and the nanomaterials can be easily separated, thus becoming ecofriendly and cost-effective (Hulkoti and Taranath, 2014). Microorganisms are an inexhaustible resource that can be preserved and reused, unlike plants, which can only be used once (Azmath *et al*., 2015). This causes an imbalance to plant diversity, especially among endangered species, and gives microorganisms an advantage over their plant counterparts (Azmath *et al*., 2015). Among the microbial community, "endophytes", have made a significant impact by secreting diverse secondary metabolites with numerous biological activities. Although there has been significant research on endophytes, involvement of endophytes in synthesizing nanomaterials is at an initial stage and it can generate major advances (Baker and Satish, 2015).

In view of all these, a brief survey of available literature for gold nanoparticles and their various biosynthesis processes, fungal endophytes in nanogold synthesis, anticancer activity of green synthesized gold nanoparticles and future aspects of synthesis are presented in this review.

1.1. Gold nanoparticles

Gold is one of the well known noble metals used in jewellery, dentistry and electronic industry. Gold is even used in few medications prepared for children to boost the immunity of the children because gold has tunable electrical and thermal conductivity. Gold in variety of forms has been used as medication throughout the history of civilization due to its safe affects. Gold and gold compounds have been used in treatment of rheumatic diseases and discoid lupus erythematosus (Parish 1999; Walter *et al*., 1991) and various inflammatory skin disorders such as pemphigus, urticaria and psoriasis (Thomas *et al*., 1993). Gold eyelid implants have been used in lagopthalmos patients and also for patients suffering from facial nerve palsy (Manhart *et al*., 2004).

Gold nanoparticles have been utilized for centuries by artists due to the vibrant colours produced by their interaction with visible light. More recently these unique optoelectronic properties have been looked and used in high technology applications. Forming gold into nanoparticles allows researchers to use gold in areas that are too small for bulk gold to reach and brings with it new potentialities. Gold nanoparticles (AuNP's) are even believed to display distinguished properties than that of bulk materials (Lavy *et al*., 2004; Nel *et al*., 2006; Uboldi *et* al., 2009). It was therefore natural for researchers to look to gold nanoparticles for medical applications rather than using other elements such as platinum which can be toxic in certain circumstances. In recent years, AuNPs have received huge importance in research because of their unique optical, electrical and photothermal properties (Dadras *et al*., 2009; Daniel and Astruc, 2004; Rosi and Mirkin, 2005). The review on the medical applications of gold compounds was first published by Fricker (1996). Since then, many reviews have been published till to date on gold, gold compounds, nanostructures and their importance in different fields. AuNP's have different uses or applications in nanotechnology as a platform for labelling of proteins and biomolecular detection. Many studies have reported successful synthesis of gold nanoparticles using microorganisms and biological systems. In the realm of nanoparticles, gold reigns supreme, stable and easy to handle. When compared to gold, silver has tarnished reputation, because it is more easily oxidized than gold and silver nanoparticles degrade far too easily for most uses.

1.2. Fungal Endophytes

Endophytes are life forms frequently fungi and bacteria that live between living plant cells. Endophytic fungi is an endosymbiont that live inside a plant for minimum part of its existence without causing apparent harm. These are moderately unexplored makers of metabolites valuable to pharmaceutical and agricultural ventures

(Petrini *et al*., 1992). Endophytic fungi are ubiquitos and have been found in all types of plants (Arnold *et al*., 2000). A few strains of a similar organism segregated from various parts of a similar host vary in their capacity to utilise diverse substances (Caroll and Petrini, 1983). So, endophytes can be isolated from different plants belongs to the different families and classes and can be grown under different ecological and geographical conditions (Petrini, 1986). The sort of association between an endophyte and plant is controlled by the genes of both organism and attuned by environment (Moricca and Ragazzi, 2008).

Fungal endophytes are separated into four classes based on host range, type of tissue(s) they colonize, colonization in planta, diversity in planta, transmission and their fitness benefits (Rodriguez *et al*., 2009).

Class 1 endophytes normally *Clavicipitaecous* endophytes are those, which involves a few number of phylogenetically related *Clavicipitaecous* species. They are fastidious in culture and limited to some cool and warm season grasses (Stone *et al*., 2004; Bischoff *et al*., 2004).

Class II endophytes comprise a diversity of species, all of which are members of the *Dikarya* (*Ascomycota* or *Basidiomycota*). They have ability to confer adaptation specific stress tolerance to hosts plants (Rodriguez *et al*., 2008).

Class III endophytes are differentiated on the basis of their occurrence and transmission. This includes vascular, nonvascular plants, woody and herbaceous angiosperm of Antarctic communities (Davis and Shaw, 2008; Higgins *et al*., 2007). They are especially known for their diversity within individual host tissues, plants and population.

Class IV endophytes have darkly melanized septa and are restricted to plant roots. They are generally *Ascomycetous* fungi which are conidial or sterile and that form melanized structures like inter and intracellular hyphae and also microsclerotia in the roots. This class of endophytes found in host plants like nonmycorrizal from Antarctic, alpine, sub alpine temperate zones and tropical ecosystem (Jumpponen, 2001).

Endophytic fungi have been considered as possible useful sources of natural products, which are able to produce substances of biotechnological importance and can also protect plants from insect attack and diseases (Borges *et al*., 2007; Strobel *et al*., 2004; Newman and Cragg, 2007). Some species of endophytic fungi have been identified as sources of anticancer, antidiabetic, insecticidal and immunosuppressive compounds (Strobel and Daisy, 2003). Some of them may produce secondary metabolites with potential for anticancer and antimicrobial property (Xu *et al*., 2008; Shu *et al*., 2005). *Fusarium oxysporum* has been *isolated* as an endophyte from medicinal plants with anticancer and antimicrobial activity (Xu *et al*., 2008). Other reports have also showed that certain endophytic fungi produced more metabolites similar to those produced by host plant with therapeutic function including alkaloids, steroids, terpenoids, flavanoids, quinines, peptides and phenolic acid (Shu *et al*., 2005; Strobel and Daisy, 2003).

Due to numerous activities exhibited by endophytes they can be exploited for nanoparticles synthesis using a simplified procedure as mentioned in the Flowchart (Figure 1).

Surface disinfection of different plant samples
↓ Leaves cut, surface sterilized with 0.01% mercuric chloride, washed thoroughly with sterile distilled water
Isolation of endophytic fungi from surface disinfected leaves on Potato dextrose agar media
↓ (at 25±2^0 C for 8 days)
Endophytic fungi then grown on Potato dextrose broth/Malt ,glucose, yeast extract, peptone media with agitation
↓ (at 25±2^0 C,200 rpm for 8 days)
Mycelia separated by centrifugation at 5000g at 20^0 C for 20 min
↓ Mycelia washed thrice with sterile distilled water
10 gm harvested wet fungal biomass taken
↓
1mM aqueous AuCl4 solution is added to fungal biomass
↓ Incubated at 25-27^0 C, 200rpm,maintained at dark and periodic sampling carried out
Colour of the mixture changes to pinkish ruby red colour
↓
Characterisation of Biosynthesized Gold nanoparticles

Scheme 1: Generalised flowchart for isolation of endophytic fungi (Shankar *et al*., 2003).

2. Synthesis of Gold Nanoparticles

Metallic gold nanoparticles have been examined for their use as tools for a new generation technological devices and many techniques are available now for producing nanoparticles. These techniques mainly fall into categories such as physical, chemical, and mechanical processes. The methods for making nanoparticles basically involve either a top down approach or a bottom up approach (Sapeur, 2008).

In top down synthesis nanoparticles are produced by size reduction from a suitable starting material (Meyers *et* al., 2006). Top down production methods introduce imperfections in the surface structure of the product which is a major limitation as the surface chemistry and other physical properties of nanoparticles are mainly dependent on the surface structure (Thakkar *et al*., 2010).

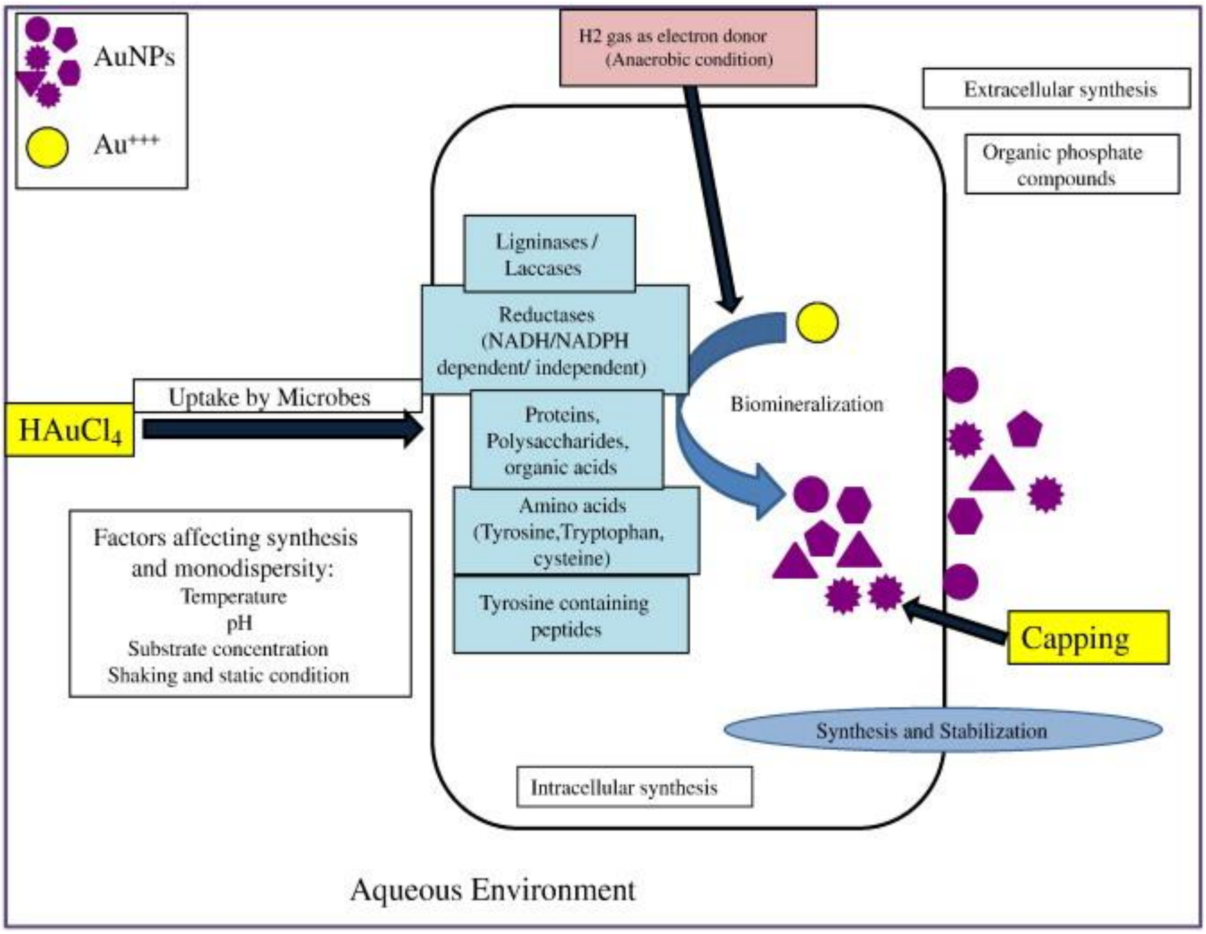
In bottom up synthesis the nanoparticles are built from smaller entities, like joining atoms, molecules and smaller particles (Mukherjee *et al*., 2001). The bottom up synthesis mostly relies on chemical and biological methods of production. Of the biological methods of synthesis, the methods based on using microbes have been widely reported (Dhillon *et al*., 2012; Mohanpuria *et al*., 2008). Microbial synthesis is readily scalable, environmentally friendly and compatible with the use of the product and can be explored for medical applications.

2.1. Biological synthesis of Gold Nanoparticles

The need for eco-friendly non-toxic methods for nanoparticles synthesis is developing interest in biological approaches which are free from the utilization of harmful chemicals as byproducts. An overview of biological synthesis of nanoparticles is focused below.

2.1.1. Plant mediated gold nanoparticles Synthesis:

Plants offer a best option for synthesis of nanoparticles as the protocols involving plant sources are free from toxic chemicals as the natural capping agents are readily supplied by the plants. Synthesis of gold and silver nanoparticles using Geranium leaf extracts have been reported by Shankar *et al*., 2003. Further, triangular gold nanoparticles and silver nanoparticles were also synthesized using Aloe Vera plant extracts by Singh *et al*., 2013. However, Singh *et al*., 2013. also reported the cost effective and environment friendly approach for green synthesis of AuNP's using chickpea leaf extract which act as a reducing agent as well as capping agent. Synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using neem (*Azadirachta indica*) leaf extract has been reported by Shankar *et al*., 2004.



Scheme 2: Biological synthesis of Gold Nanoparticles.

2.1.2. Algae mediated gold nanoparticles Synthesis: Algae are similar to yeast for biosynthesis of nanoparticles, but very few reports are available using algae as for bionano synthesis (Mukherjee *et al.*, 2008). The marine algae which has been used for the biosynthesis has produced highly stable extracellular gold nanoparticles in a comparatively short time period to other biosynthesizing processes (Mukherjee *et al.*, 2008).

2.1.3. Bacteria mediated gold Nanoparticles Synthesis: Bacteria have been most extensively researched for synthesis of nanoparticles because of their fastidious growth and the ease for genetic manipulation. Bacteria have also been used to synthesize gold nanoparticles (Mourato *et al.*, 2011). The bacteriogenic gold nanoparticles have been used in many applications (Liangwei *et al.*, 2007).

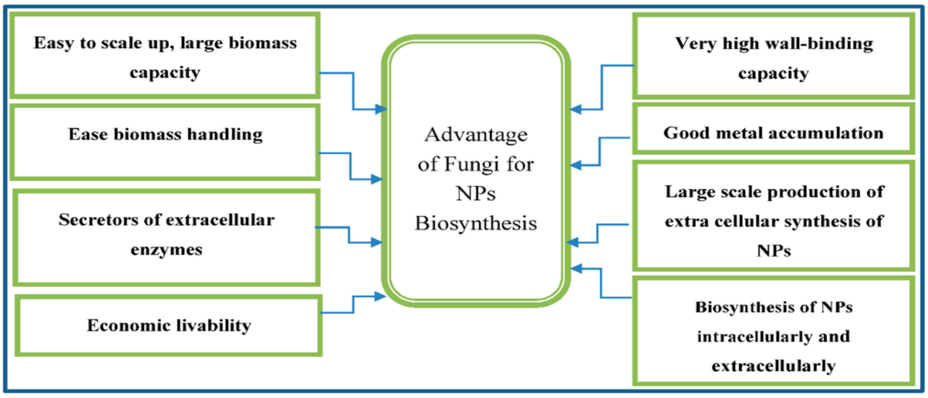
2.1.4. Actinomycetes mediated gold Nanoparticles Synthesis: Most of the actinomycetes especially the thermophilic actinomycetes, *Thermomonospora* sp. when exposed to gold ions got reduced extracellularly (Rai *et al.*, 2009). These micro-organisms have developed numerous special adaptations to survive in such extreme habitats.

2.1.5. Yeast mediated gold Nanoparticle Synthesis: The biosynthesis of AgNPs and AuNPs by using an extremophilic yeast strain isolated from acid mine drainage has been reported. The synthesized AgNP's with diameter 20nm and AuNP's with diameter 20 to 100 nm were well dispersed and capped by proteins which were secreted by yeast (Konishi *et al.*, 2004).

2.1.6. Virus mediated gold Nanoparticles Synthesis: Biological synthesis of nanoparticles have also been extended to biological particles like viruses. Cowpea chlorotic mottle virus and cowpea mosaic virus have been utilized for the mineralization of inorganic materials (Gade *et al.*, 2008).

2.1.7. Fungi mediated gold Nanoparticles Synthesis: The fungi occupies the center of the stage in the studies of biosynthesis of nanoparticles because of its metal tolerance and bioprecipitation**.** Fungi are efficient secretors of extra cellular enzymes and it can easily obtain large scale production of enzymes. Further advantages of using fungal mediated synthesis of gold nanoparticles include economic viability and ease in handling biomass.The fungi are one of the good biological agents in the synthesis of metal nanoparticles. Indeed, fungi are regarded as more advantageous for AuNP's biosynthesis compared to other microorganisms with regard to the following reasons.

- Fungi **produce large amounts of extracellular enzymes** which catalyse the heavy metal ions and produce nanoparticles. Due to which fungi can produce Nanoparticles at faster rate in comparison with chemical synthesis (Saha *et al.*, 2010)
- Fungi are **easy to isolate and subculture** as they have simple nutritional requirements. Serial dilutions, plating and hyphal extraction are the simple methods required to isolate fungi. Fungi are totipotent in nature and therefore hyphae or spores can be used to grow fungus and can be sub-cultured to obtain pure isolate. (Saha *et al.*, 2010)
- Fungi can **produce nanoparticles extracellularly** which is suitable for easier downstream processing and handling of biomass. Extracellular synthesis of AgNPs by *Aspergillus* sp. has been reported (Shankar *et al.*, 2003)
- Some of the additional advantages are that Fungi are able to **sustain under high agitation and flow pressure** as compared to bacteria and plants (Ahmad *et al.*, 2005).



Scheme 3: Fungal advantages for Nanoparticles synthesis.

Ahmad and coworkers (2005) reported both extra as well as intracellular synthesis of AuNP's by fungus *Trichothecium* sp. which resulted in the rapid extracellular formation of AuNP's of spherical rod-like and triangular nanoparticles in stagnant condition whereas reaction of the biomass under shaking conditions resulted in intracellular growth of the AuNP's.

Bio reduction of aqueous gold ions was also carried out using fungus *Verticillium* sp. (Beveridge *et al.*, 1997) led to the formation of nanoparticles with fairly well defined sizes. Finally it was concluded that surface trapping of chloroaurate ions in fungal cells was due to electrostatic interaction with positively charged groups of enzymes that are present in the mycelia cell wall.

3. Fungal endophytes as a source of gold nanoparticles

Endophytic fungi have characteristics of both plants and fungi. The interface between endophytic systems and nanomaterials is a relatively new and unexplored area and may open avenues in the future to push the frontier forward in coming decades and so as plants. There are a few already reported literatures which have proven that endophytes and their hosts can easily synthesize nanoparticles.

Shankar *et al*., 2003. synthesized gold nanoparticles extra-cellularly using geranium leaves and its endophytic fungus. Sterilized geranium leaves and an endophytic fungus were separately exposed to aqueous chloroaurate ions. Rapid reduction of the metal ions was observed resulting in the formation of stable gold nanoparticles of variable size in both the cases. In the case of gold nanoparticles synthesized using geranium leaves, the reducing and capping agents appeared to be terpenoids while they were identified to be polypeptides/enzymes in the *Colletotrichum* sp. case. The biogenic gold nanoparticles synthesized using the fungus were essentially spherical in shape while the particles grown using the leaves exhibited a variety of shapes including rods, flat sheets and triangles. This report confirmed the monodispersity of nanoparticles with endophytic fungus as compared to its host.

Verma *et al*., 2010 reported the endophytic fungus *Aspergillus clavatus* can be isolated from sterilized stem tissues of *Azadirachta indica*. When the biomass of fungus was challenged with 1 mM HAuCl4 aqueous solution, results showed that triangular gold nanoparticles were formed along with some spherical as well as hexagonal morphology. It was also observed that the synthesis of gold nanotriangles was extracellular and showed a high aspect ratio. The study reported served as a unique single-step green protocol for the generation and stabilization of nontoxic gold nanotriangles, exploitable in a myriad of diagnostic and therapeutic applications.

Vallinachiyar *et al*., 2015 reported the use of less explored endophytic fungi were in the synthesis of gold nanoparticles. Extracellular gold nanoparticle formation was observed by the change in colour of the solution to dark pink. This was followed by characterizing the nanoparticles using various instrumental analyses like UV-Vis spectroscopy, TEM and FTIR. The obtained gold nanoparticles were found to be spherical with slight aggregation and their size was found to be in the range of 15 - 35 nm. Their biocompatibility was been assessed using cytotoxicity assay and the results showed that these biogenic gold nanoparticles do not induce significant cytotoxicity in normal and cancer cell lines.

Alappat *et al*., 2012 reported biological synthesis of gold nanoparticles using the various endophytic fungi, from *Bauhinia variegate* and the leaf and flower extracts of *Bauhinia variegate Linn*. was been demonstrated and it was concluded that the biosynthesis route was more advantageous than the chemical synthesis due to the highly controlled reproducible synthesis, generation of water-soluble and biocompatible particles. Since the production didn't involve any chemicals such as toxic surfactants or organic solvents, the process was found to be 100% ecofriendly and the nanoparticles toxicity was eliminated. Furthermore, the low cost of the method as well as its simplicity and efficiency offered an alternative to chemical synthetic methods of gold nanoparticles.

Manjunath *et al*., 2017 has reported green synthesis of Gold nanoparticles from *Penicillium citrinum* isolated from brown algae. The synthesised AuNPs showed significant antioxidant potential compared to the standard. Recently Manjunath *et al*., 2017 again have optimized a method for biogenic synthesis of gold nanoparticles (AuNPs) from *Cladosporium cladosporioides*, an endophytic fungus of the seaweed, *Sargassum wightii* to understand the mechanism of the gold nanoparticle synthesis using the fungal extract. The study showed the involvement of NADPH-dependent reductase and phenolic compounds in the bioreduction of the gold metal salts to nanoparticles. The AuNPs showed significant antioxidant as well as the antimicrobial activity. Hence, this study showed a great potential for the development of a cost effective antimicrobial treatment utilizing biogenic gold nanoparticles.

Table 1: Gold Nanoparticles Synthesized by different Endophytic Fungi.

Sl. No	Host Name	Endophytic fungi	Size	Activity	Reference
1.	*Pelargonium graveolens* plant	*Colletotrichum* sp.	20-40 nm	--	Shankar *et al*., 2003
2.	*Bauhinia variegate* plant	*Pencillium citrinum*	--		Alappat *et al*., 2012
3.	*Garcinia xanthochyumus* and *Aravae lanata* plant	--	16-35 nm	Cytotoxic activity	Vallinachiar *et al*., 2015
4.	*Azadirichta indica* plant	*A. clavatus*	20-35 nm	--	Verma *et al*., 2010
5.	Marine algae	*C. cladosporiodes*	<100 nm	Antioxidant and Antimicrobial activity	Manjunath *et al*., 2017
6.	Brown algae	*P. citrinum*	--	Antioxidant activity	Manjunath *et al*., 2017

-- Not reported

4. Gold nanoparticles as Anticancer agents

Nanotechnology, a multidisciplinary research field involving different fields of science has great potential for early detection, accurate diagnosis, and personalized treatment of cancer (Cai and Chen, 2007). Cancer nanotechnology is an interdisciplinary area with potential applications in fighting cancer. The continued development of cancer nanotechnology holds the promise for personalized oncology in which genetic and protein biomarkers can be used to diagnose and treat cancer based on the molecular profile of each individual patient.

Gold nanoparticle is unique in a sense because of its intriguing optical properties which can be exploited for both imaging and therapeutic applications. Gold nanoparticles have been investigated in diverse areas such as in vitro assays, in vitro and in vivo imaging, cancer therapy, and drug delivery. In order to be useful for cancer treatment, the AuNPs must be non cytotoxic for normal cells. Many studies have been carried out to study the anticancer effects of green gold nanoparticles.

Michalet *et al*., 2005. reports showed that cell and phantom imaging using gold nanoparticle serves as a proof-of-principle for their potential applications in live animals or cancer patients.

Dhar *et al*., 2010. has reported the cellular uptake studies and cytotoxic effect of biosynthesized gold nanoparticles in human glioma cell line LN-229 and human glioma stem cell line HNGC-2. The gold nanoparticles showed greater cytoxicity by killing the glioma cell lines and the glioma stem cell lines also.

Audrey and didier, 2012. reports indicated that some AuNP's are toxic at a concentration of 100 mM for cancer cells, but not for immune cells. The positively charged AuNP ligands are usually toxic at concentrations weaker than those at which negatively charged ligands would be cytotoxic.

Lokina and narayanan, 2013 studies shows that cytotoxicity on hela cancer cell of gold nanoparticles synthesized from grape fruit extract was very inevitable results with addition to antimicrobial activity.

A recent study conducted by Balasubramani *et al*., 2015 reported that AuNPs synthesized from *A. leptopus* exhibit good anticancer activity against MCF-7 breast cancer cells at 257.8 μg/ml.

Green-synthesized AuNPs from *Gymnema sylvestre* leaf extracts were also investigated by Arunachalam *et al*., 2014. for their anticancer effects against hepatocellular carcinoma (HepG2) cells. The study inferred that these AuNP's exerted significant cytotoxic effects against HepG2 cancer cells at a maximal concentration of 250 μg/mL.

Another study demonstrated by Abel *et al*., 2016. showed the cytotoxic efficacy of *Cassia tora* against colon cancer cell lines. The study revealed that the activity of *C. tora* at three different doses (25, 50, and 75 μg/mL) was dose-dependent and the 75 μg/mL dose showed the highest activity against the colon cancer cell lines.

Deshpande *et al*., 2011. reported the Biofunctionalized gold and silver nanoparticles synthesized using different plant extracts of guava and clove and studied its invitro anti-cancer efficacy against four different cancer cell lines. This study suggested that flavonoids functionalized gold nanoparticles synthesized using aqueous clove buds extract were more potential than guava leaf extract for their anti-cancerous activities. The XTT assay inferred that the functionalized irregular shaped gold nanoparticles synthesized with aqueous clove bud extract showed a satisfactory anti-cancer effect on all the cell lines. The silver nanoparticles synthesized using same extracts were devoid of anti-cancer activity. The XTT assay thus revealed dose-dependent cytotoxicity to cancer cell lines. The study also revealed that the free radicals generated by gold nanoparticles were the reason for anti-cancer effect.

Rajeshkumar. 2016. studied the Anticancer activity of extracellularly synthesized gold nanoparticles by MTT assay against Hep-G2 and lung cancer cell (A549) lines. The study showed increased anticancer activity at an increased concentration of gold nanoparticles. This was found to be an easy and eco-friendly biosynthesis approach. Thus the gold nanoparticles synthesized were more efficient in the biomedical applications in cancer treatment for their high anticancer activity.

Another study by Anand *et al*, 2015. reported that AuNPs synthesized from *Moringa oleifera* flower aqueous extract- showed anticancer activity against A549 lung cancer cells. A dose of 50 μg/mL AuNPs showed potential activity against this lung cancer cell line.

Ravi *et al*., 2013. studied anticancer activity of gold nanoparticles which were synthesized using aqueous flower extract of *C. guianensis*. From this study they inferred that the pharmacological properties of *C. guianensis* provide the anticancer activity of newly formed gold nanoparticles. Interestingly it was also demonstrated that the functionalization of gold nanoparticles as anticancer nanomaterial has been achieved without doping of molecules.

Kamalapriya. 2015. studied the anticancer activity of Gold Nanoparticles with MCF 7 breast cancer cell lines with reference to standard anticancer drugs such as Letroz and Tamoxifen. The results revealed good anticancer activity of gold nanoparticles in par with the mentioned drugs. The study also showed increased anticancer activity at an increased concentration of gold nanoparticles. The study mentioned yielded reliable results in favour of gold nanoparticles.

The invitro anticancer activity of gold nanoparticles (AuNP) synthesized using leaves extract of *Bauhinia tomentosa* by Mukundan *et al.,* 2017. was confirmed by MTT assay laryngeal HEp-2 carcinoma cells cell lines. The study showed IC50 values of extract at 53.125 µg/mL and AuNP's at 34.375 µg/mL. The AuNP's inhibited the proliferation of HEp-2 cells in a dose and time dependent way. The IC50 value of AuNP showed that the concentration required to inhibit 50% of HEp-2 cells was less than that of *B. tomentosa* leaves extract. The green synthesized gold nanoparticles were predicted to be potential agent in cancer therapy in the current study.

Parida. 2011 demonstrated the general toxicity of gold nanoparticles synthesized from *Allium cepa* L plant extract with MCF-7 cancer cell line for in vitro anticancer activity. AuNP's cell viability test showed the cytotoxic activity on MCF-7 cancer cell line which was in dose dependent manner. This inferred that high concentration of AuNP's would reflect low cell viability. Hence AuNP's effectively inhibits the growth of MCF-7 cancer cell line.

Preetam *et al*., 2016. synthesized Biologically stabilized gold nanoparticles from the flower aqueous extract of *T. divaricata*. The gold nanoparticles synthesized demonstrated potent anticancer activity against MCF-7 cell line. Their results concluded that the antioxidant molecule present in *T. divaricata* may be responsible for both reduction and capping of gold nanoparticles which possess potential applications in medicine and pharmaceutical fields.

Nanoparticles are conjugated with antibodies against exclusive cancer cell surface receptors and are used to specifically bind with cancerous cells. The functionalized nanoparticles are used for targeted entry into cells. Phthalocyanine-stabilized gold nanoparticles have been shown to be a potential delivery vehicle for photodynamic therapy (Nowack *et al.*, 2011). Different nanoparticles have also applied as targeted drug delivery and biomarkers agents for diagnosis and medical treatment of cancer. Gold nanoparticles functionalized with targeted specific biomolecules can effectively destroy cancer cells or bacteria **(**Wang *et al*., 2010).

5. CONCLUSION AND FUTURE OUTLOOK

The Biosynthesis methods are always cheap, reliable and nontoxic over the physical and chemical methods as it does not use any toxic chemicals and they also exert an anticancer effect. Since fungal endophytes occupying the unusual habitat have capacity to survive under stress conditions and thus must have set of enzymes and metabolites not found in their wild-type counterparts. For this reason, fungal endophytes could be a better candidate for synthesizing nanomaterials. Fungal endophytes also acts as a potential source of natural products for the search of new and biologically active compounds. They are the unexplored area of research. There are very few reports available on biosynthesis of gold nanoparticles from endophytic fungus isolated from different medicinal plants, hence there is need to explore wide range of endophytic fungi which are potent producers of AuNp's.

Future prospect of endophytes-mediated nanoparticle synthesis involves further exploration of endophytic fungi for gold nanoparticles synthesis, an elaboration of laboratory synthesis to industrial scale, elucidation of fungal enzymes involved in the synthesis of nanoparticles and their biological activities. The endophytic fungal based nanoparticles can have huge commercial applications in medicine, health care and new drug discoveries in the coming years.

ACKNOWLEDGEMENTS

Authors are pleased to thank Department of Biotechnology, Teresian College(affiliated to University of Mysore) for providing facilities.

Ethical issues

The authors declare no ethical issues.

Conflict of interest

The authors report no competing interest.

Review highlights
What is current knowledge? Nanotechnology is the study of extremely small things which can be extended to other science fields such as biology, chemistry, physics, energy science and engineering. Nanoparticles being the building blocks of nanotechnology and gainging the interest in young researchers are being synthesized by emerging Biosynthesis methods. Gold nanoparticles are been synthesized by different organisms like fungi,bacteria and plants. Fungi carrying the centre of stage in Nananoparticle synthesis due to its increased reduction capacity,ease in handling etc. **What is new here?** Fungal endophytes are found to be unexplored systems for gold nanoparticles synthesis. Fungal endophytes must have set of enzymes and metabolites not found in their wild-type counterparts responsible for gold salt reduction. Eco-friendly methods provide a safe way to extend nanoparticles applications in medical field.

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