Review Article

Hygrophila schulli assisted green synthesis of silver and gold nanoparticles: A Review

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Received: 14 September 2023 Revised: 15 October 2023 Accepted: 21 October 2023

Abstract

Background: Number of attempts has been made for green synthesis of nanoparticles of different metals and metal oxides, revealing the significance of natural extracts in reducing metal source to the corresponding nanoparticles and applications in various scientific domains. Objective: The present article focus on applications of extracts of different organs of *Hygrophila schulli* in fabrication of nanoparticles of various metals like silver and gold. *H. schulli* is annual, belonging to family Acanthaceae. The plant has been reported to contain several phytochemicals like triterpnoids flavonoids, and hydrocarbons. In respective research attempts, metallic nanoparticles so synthesized were evaluated for one or more applications like anti-microbial or anticancer activity and/or photocatalytic activity. **Conclusions:** Mostly, polar extracts of *H. schulli* are used for green synthesize of nanoparticles. This indicates involvement of polar phytocompounds of *H. schulli* in reducing the metal source and stabilizing the nanoparticles. In conclusion, it could be noted that metal nanoparticles have better antimicrobial activity and photocatalytic potential over extract.

Keywords: *Hygrophila auriculata*, phytosynthesis, nanoparticles, biological activities

Introduction

Nanotechnology is an emerging science of developing the material having magnificent applications in various scientific domains. Among various nanomaterials, synthesis of nanoparticles (NPs) have been tried most of the times and evaluated for various applications like drug delivery (Lee et al. 2011), photo thermal therapy of cancer (Prashant and Ivan, 2007), photocatalysis, photodegradation of dyes (Thomas et al., 2015), chemical sensors and biosensors (Unser et al., 2015). These nanoparticles are of several types, Carbon-based NPs, metal NPs, ceramic NPs, Polymeric NPs, Lipid-based NPs. For synthesis of these NPs, approaches are there: Bottom-up approach and Top-down approach (Iravani, 2011). Considering the harmful effects of chemicals used in chemical methods on environment and requirement of high cost sophisticated machineries in other techniques, green approach has been developed which employs the natural extracts for reduction of metal ions from metal precursor, form NPs and stabilize them by capping (Ying et al. 2022).

Hygrophila schulli (Acanthaceae) is an annual, erect, thorny, semi-woody plant found along the banks of fresh water ditches or stagnant water canals and swampy land. Taxonomically it has been placed in Division, Tracheophyta; Class, Magnoliopsida; Order, Lamiales and Family, Acanthaceae. According to https://www.worldfloraonline.org/taxon/wfo-0000726761 searched on 12th August 2023, the plant *H. schulli* has several accepted synonyms like *Asteracantha auriculata, A. lindaviana, A. longifolia, A. macracantha, Bahel schulli, Barleria auriculata, B. cornigera, B. glabrata, B. hexacantha, B. longifolia, B. macracantha, B. spinosa, Hygrophila auriculata, H .lindaviana, H. longifolia, H.spinosa, Ruellia longifolia, Tenoria undulate*. It co-exists with marshy grasses and sedges (Nadkarni, 1978a). It is spiny but hispid herb which can reach upto maximum height of 1.5 m. It stems are unbranched, carry bears subquadrangular thickened nodes; leaves are subsessile with having oblong or linear lanceolate structure; spines

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DOI: https://doi.org/10.31024/apj.2023.8.5.1

are axillary, yellowish brown in colour with length of 2-3 cm; flowers are axillar, pale to purple-blue in colour; fruits are glabrous oblong capsules having 4 to 8 seeds (Bhogaonkar and Lande, 2011).

Pharmacological activities of *H. schulli*

Pharmacological activities of Leaves

In 1989, Fernando et al. preliminarily investigated the possible hypoglycaemic activity of *Asteracanthus longifolia*. Later, in 1991, they evaluated the effect of aqueous extract of leaves of *A. longifolia* on glucose tolerance in normal human and diabetic patients.

In 2006, Pawar et al. screened out the haematopoietic activity of *A. longifolia* on cyclophosphamide-induced bone marrow. They observed treatment with chloroform extract of *Asteracantha longifolia* leads to substantial improvement in erythrocyte and haemoglobin count on the 22nd day of administration of cyclophosphamide for 7 days. That also resulted in increase in bone marrow cellularity and number of α-esterase positive cells. Later in 2010, Pawar et al. reported the erythropoietic activity of *A. longifolia* leaves in rats. They first spectrophotometrically determined the iron content of *A. longifolia* leaves and then determined its use as erythropoietic agent in haloperidol induced iron deficiency anemic rat model. They noticed an administration of ethanolic extract of *A. longifolia* leaves leads to a significant increase in erythrocyte count, haemoglobin count, serum iron and serum protein etc. In 2014, Datta et al. performed comparative study of haematinic and iron utilization property of pre and flowering plant leaf extracts of *Asteracantha longifolia*.

Pharmacological activities of Roots

Shanmugasundaram and Venkataraman, 2006 examined the effectiveness of aqueous extract of *H. auriculata* roots (HAR) on carbon tetrachloride induced hepatotoxicity in mice. They found regeneration of hepatocytes, normalization of fatty changes and necrosis of the liver of mice of HAR treated group. HAR also exhibited significant in vitro antioxidant activity by inhibiting the oxidation of linoleic acid in both Ferric thiocynate (FTC) and Thiobarbituric acid (TBA) methods.

Pharmacological activities of seeds

In 1995, Handa and Singh, evaluated the hepatoprotective activity of *H. auriculata* against paracetamol and thioacetamide intoxication in rats. They tested methanolic extract of *H. auriculata* seeds by administering it to rats intoxicated with paracetamol and thioacetamide; and monitoring several enzymes like serum transaminases (SGOT and SGPT), alkaline phosphatase, sorbitol dehydrogenase, glutamate dehydrogenase and bilirubin in serum of rats.

Ahmed et al. 2001 studied the anti-tumor promoting activity of *A. longifolia* seeds in hepatocarcinogenesis in rats. They found that, treatment of rats with methanolic extract of *A. longifolia* seeds on alternate days, subsequent to carcinogen treatment, for 6 weeks reduced both the incidence of occurance and size-distribution of 2-acetylaminofluorene (2-AAF)- selected γ–glutamyl transpeptidase (γ-GT) –positive foci and tumor formation. This also exhibit dose-dependent suppression of ornithine decarboxylase (ODC) activity and thymidine incorporation into hepatic DNA.

Kannur et al. 2012 evaluated anti-inflammatory activity and found that *H. schulli* seed ethanolic extract decreases carrageenan-induced paw oedema in rats. They also evaluated antinociceptive activities using Eddy's hot plate test and tail immersion method; and noted the enhanced threshold capacity by inducing analgesia. In 2019, Ghosh and Mallick determined protective effect of *H. auriculata* seed ethanolic extract in cyproterone acetate (CPA)-induced sexual dysfunction in male albino rats. In this study, sexual functioning of male albino rats was disturbed by administration of anticancer drug, cyproterone acetate (CPA), which is used in treatment of prostate cancer. CPA-induced sexual dysfunction was determined as significant diminution in activities of superoxide dismutase, catalase, peroxidase and elevation of malondialdehyde and conjugated dienes levels. All these parameters were found came back to normal levels on treatment with *H. auriculata* seed ethanolic extract. In 2022, Islam et al. evaluated protective and antioxidant effects of *H. schulli* seeds. They found Ethyl acetate fraction (EAF) of crude methanolic extract (CME) had higher *in vitro* antioxidant activity. On *in vivo* antioxidant activity in cadmium intoxicated mice, the EAF showed a significant increase in serum catalase and SOD activity compared to the control group. EAF reduced the oxidation-based damage of nicked DNA damage RBCs. In this study, researchers confirmed that EAF could scavenge reactive oxygen species (ROS) and the extract can be used as a nutraceutical or functional food.

Pharmacological activities of whole plant

In 2014, Pareek and Barthakur, screened out the antibacterial and antifungal activities of petroleum ether, chloroform and methanol extracts of *H. auriculata* against bacteria *Salmonella typhi, V. cholera*, *Staphylococcus aureus*; and fungi *Aspergillus flavus, Cladosporium* and *Aspergillus niger*. Chloroform extract was found highly active against all the tested microbes.

Nair et al. 2015 evaluated the anticancer activity of

Asteracantha longifolia in 7,12-Dimethylbenz(a) anthracene-induced mammary gland carcinogenesis in Sprague Dawley rats. They found the decrease in tumor size on consecutive oral administration of methanolic extract of *A. longifolia.*

Traditional uses of *Hygrophila shulli*

According to Ayurvedic Pharmacopoeia of India, the plant *H. shulli* can be recommended for lithiasis (seeds) and gout (the whole plant and root). It can be used as diuretic, in treatment of urinary organs catarrh, and hepatic obstruction based oedema (Khare, 2007).

Recently, for the treatment of SARS-CoV-2, the Ministry of AYUSH, Government of India, has recommended a formulation composed of 15 plants, one of which is *Hygrophilla auriculata*, (used in 6.6%; Amanat and Krammer, 2020).

Phytochemicals present in *A. longifolia or Hygrophila spinosa*

So far, very few phytochemicals have been reported to be present in different parts of *A. longifolia or Hygrophila spinosa* (Fig. 1). Gupta et al. 1983 reported the isolation of lupeol, betulin and stigmasterol from *A. longifolia*. Then, Misra et al. 2000 isolated two compounds namely, 3-methylnonacosane and Lupeol acetate from aerial parts of *A. longifolia*, later in 2001, Misra et al. isolated 25-oxo-hentriacontanyl acetate and methyl 8-n-hexyltetracosanoate.

Taking the wide range of pharmacological activities, chemical constituents of *H. schulli* and applications of NPs into consideration, several attempts have been made for synthesis of different NPs using *H. schulli*. This summarizes the all these aspects of uses and phytochemicals of *H. schulli;* NPs synthesized using H. schulli and evaluation of their applications. As the plant, *Hygrophilla schulli* is known by several botanical names, the data collected to make this review was collected on searching with each of the synonyms given.

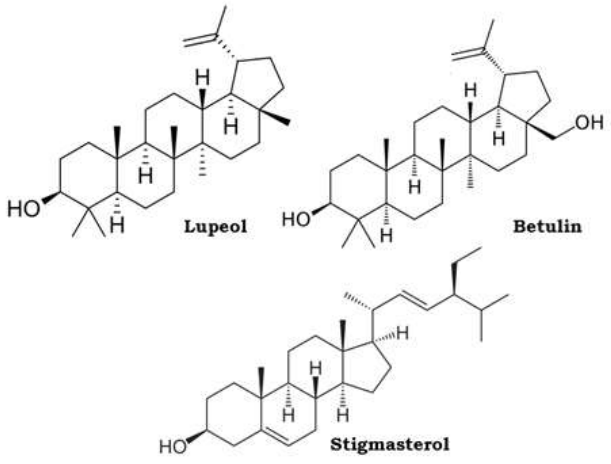

Figure 1: Phytochemicals present in *A. longifolia*

Green synthesis of metal nanoparticles using *Hygrophila*

First attempt of green synthesis of metal nanoparticles using *Hygrophila spinosa* was made by Ghosh et al. in 2017. They prepared aqueous extract of *H. spinosa* leaves using which they synthesized gold nanoparticles (Au NPs). Further, they characterized as Au NPs by techniques like UV-Visible spectroscopy (λ_{max} = 535 nm), DLS (dynamic light scattering) (size = 100–120 nm), zeta potential (–18.27 mV), TEM (transmission electron microscopy)(Size =15–20 nm) and fluorescence activity (quenching noted). Then, to evaluate hematological and antioxidant properties, iron deficiency anemia was developed in Swiss male albino mice by blood loss method. On treatment of these mice, an improvement was observed in hemoglobin percentage, total count of RBC, hematocrit value, serum iron concentration and decreased total iron binding capacity. Level of anti-oxidants like reduced glutathione, superoxide dismutase and catalase activity were found increased while lipid peroxidation was found decreased.

Next attempt of green synthesis of metal nanoparticles was made by Bharathi et al. (2018). They used 1.5 ml aqueous extract of *Hygrophila auriculata* for phytosynthesis of silver nanoparticles (Ag NPs) using precursor, 30 ml of 1 mM aqueous silver nitrate. Material was separated from mixture by repeated centrifugation at 10,000 rpm for 20 min, followed by washing with sterile water and drying at 40 C. After characterization by UV, IR, XRD techniques, they screened Ag NPs for their antibacterial and antioxidant activities. Antibacterial activity was determined by agar well diffusion method against *S. aureus* (ATCC 6538), *B.cereus* (NCIM 2106), *P. aeruginosa* (ATCC 9027) and *E.coli* (ATCC 8739), and zone of inhibition (in mm) and minimum inhibitory con. (MIC) were determined. It was observed that Ag NPs inhibited the bacteria in dose-dependent manner. Anti-oxidant activity of Ag NPs was examined by three methods namely, DPPH radical scavenging activity, phospho molybdenum assay and reducing power assay. In all the three protocols, antioxidant activity of Ag NPs was found comparable with that of standard ascorbic acid.

In 2018, another attempt of green synthesis was made by Priya and Prakash, They prepared Ag NPs using aqueous extract of *Hygrophila auriculata* seeds. They stirred 50 ml of aqueous solution was prepared which consists of 1mM

silver nitrate with 5 ml of *H. auriculata* seed extract on a magnetic stirrer for 1 hr. Then, material was separated by centrifugation at 8000 rpm for 10 mins. For characterization of as synthesized Ag NPs was performed using advanced techniques like UV-Vis, FT-IR, Powder XRD, FESEM and EDX. Further, they evaluated the antibacterial activity against pathogens like *E. coli, P. aerugonisa, S. aureus*. Ag NPs showed higher antibacterial zone of inhibition than precursor silver nitrate.

Then, in 2019, Cittrarasu et al. synthesized Ag NPs using aqueous extract of *Barleria longiflora* leaves. About 20 gm of leave powder was boiled in 100 of distilled water for 15 min, filtered and filtrate was stored at 4 C. About 10 ml of this extract was mixed with 90 ml of 1 mM silver nitrate solution. This suspension was then stirred at 160 rpm on a magnetic stirrer until the colour changed from pale yellow to yellowish dark brown. Ag NPs so formed were then characterized by usual techniques like UV-visible spectroscopy (spectral properties), XRD (crystalline nature), FTIR (functional groups), and FESEM-EDX (surface architectures and the average size of the particles). Further, they tested Ag NPs (10 µg/ml) in volume of 50 , 75, and 100 µl for antibacterial activity against *Enterococcus sp., Streptococcus sp, Bacillus megaterium, P. putida, P. aeruginosa* and *S. aureus*, and the zone of inhibition (in mm) were noted. It was noted that, Ag NPs inhibited the growth of all microbes in dose-dependent manner. Higher the conc., higher the zone of inhibition. Later, they examined the photocatalytic activity for which 50 mg of the as-prepared Ag NPs (photocatalyst) was added into 100 ml solution (Methylene blue MB dye of 30 mg^{-1}) and stirred for 30 min to ensure the absorption–desorption equilibrium of dye molecules on the surface of the catalyst. Thereafter the suspension was placed under a high-pressure halogen lamp for irradiation under continuous stirring. An aliquot of 4ml of suspension was collected after every 15 min, and centrifuged to separate the suspended catalyst for UV-Visible absorption spectroscopic analysis. The final concentration of dye solution was calculated. It was resulted that, the absorbance of dye in visible region decreasing as the time passes (Highest at time 0 while lowest after 45 mins). This indicated the degradation of MB by light based catalysis using Ag NPs (Figure 2).

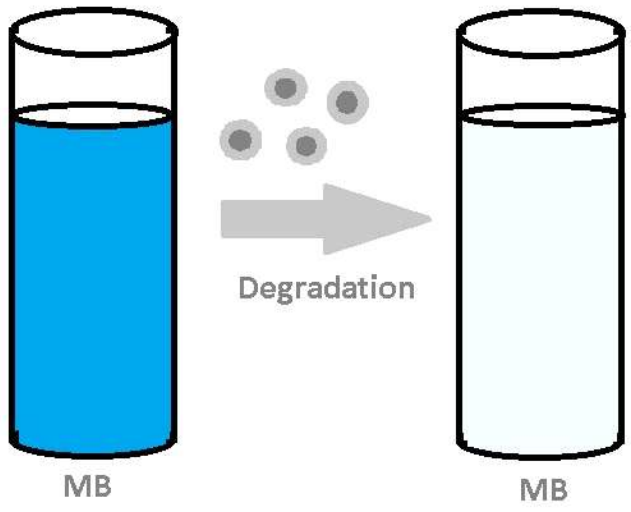

Figure 2: Photocatalytic degradation of dye, Methylene Blue

So far, it was clear that addition of aqueous extract of *H. auriculata* leaves to 1 mM silver nitrate solution converts silver ions to Ag NPs. This was again employed by Subhash et al. 2019 and prepared Ag NPs, characterized them and evaluated their larvicidal activity against larva of malarial causing mosquito, *Anopheles stephensi*. Initially, mosquitoes were fed on blood using a feeding unit fitted with Parafilm as membrane for 4 hrs. Then, larvicidal activity was evaluated as per WHO protocol. About 20 late III instar larvae were introduced into a 500 mL glass beaker containing 250 mL of dechlorinated water and the test concentrations of leaf extract or Ag NPs. For each concentration, five replicates were recorded at 24 h after exposure, during which no food was given to the larvae. Both extract and Ag NPs were found to exhibit mortality in larva, however, in terms of LC50 and LC90, it was significant for Ag NPs. LC50 (30.72 µg/mL) and LC90 (55.79 µg/mL) values of Ag NPs were almost 7 times lesser than those noted for extracts (LC50 = 230.27 µg/mL and LC90= 360.19 µg/mL). This may be because of small size of Ag NPs be able to move through cuticle of insects.

Later in 2019, Satpathy et al. optimized the process of green synthesis of Au NPs mediated by *Hygrophila spinosa* extract of and their biological applications. They used water soluble fraction of ethanolic extract of *H. spinosa.* First extract was undertaken for preliminary phytochemcial screening; determination of total phenolic and flavonoid content (TPC and TFC); GC/MS analysis and used for synthesis of Au NPs. Preliminary phytochemical screening showed the presence of primary metabolites like carbohydrates, proteins, amino acids, and secondary metabolites like flavonoids and tannins. Further, the total phenolic content (TPC) and total flavonoid content (TFC) of extract was found to be 21.33±2.37 mg gallic acid equivalent/gm dry weight and 33.22 ± 3.39 mg rutin equivalent/gm dry weight. GC/MS analysis revealed the presence of 12 different alkyl cycloheptasiloxane. For synthesis of Au NPs, different concentrations of tetrachloroaurate-(III) hydrate (1, 2, 4, 6 and 8 mM) and extract (10, 30, 50, 70 and 90% v/v) were tried. Other

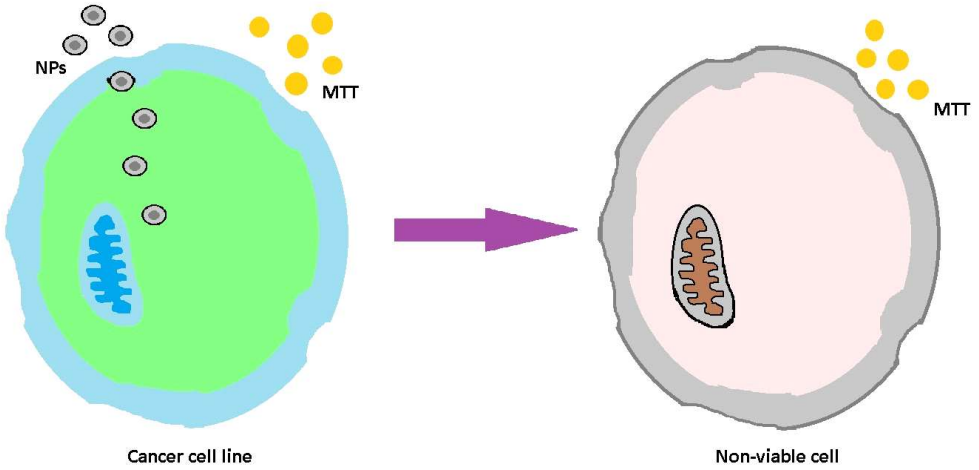

Figure 3: MTT assay in cancer cell lines. Due to cell death, no conversion of MTT to other compounds like formazan is observed

variables were temperatures (room temperature, 40, 60, 80 and 100°C), time duration (15, 30, 45 and 60 min) and pH (2, 4, 6, 8 and 12). Each sample of Au NPs was characterized by UV-vis spectroscopy, Particle Size/Zeta Potential analyzer, X-ray diffraction, transmission electron microscopy, field emission gun scanning electron microscopy, and FTIR spectroscopy. It was found that, the optimized synthesis of Au NPs, about 1 mM tetrachloroaurate-(III) hydrate need to be mixed with 10 % v/v of *H. spinosa* at 80°C for 45 mins at pH 2. Further, they evaluated Au NPs for total antioxidant capacity (TAC) and in-vitro anticancer activity. TAC was represented as copper reducing equivalent (CRE) showed that activity of AuNPs was dose dependent, but the potency is less than the extract. *In vitro* anticancer activity was performed using dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay on cell lines MCF-7 and MDA-MB-231 (breast cancer), SKOV-3 (ovarian cancer) NCI/ADR (multi-drug resistant) and U-87 (glioblastoma, brain cancer); and percent cell viability was determined for all test cell lines (Fig. 3). It was observed that cell viability decreases as con. of Au NPs increases from 12.5 to 200 µg/ml. Highest activity was observed for U-87 (lowest cell viability, 27.89%).

Now, latest in 2021, Nayak et al. used hydroalcoholic extract of *Hygrophila auriculata* to synthesize Ag NPs. They added 15 ml of extract slowly to 100 ml of 2 mM silver nitrate solution in triple distilled water with continuous stirring at 60°C. After 4 hrs, colour of solution changed from colorless to yellow to brown. That reaction mixture was centrifuged at 6000 rpm for 15 min. to get pellet which was rinsed thrice with triple distilled water and dried at 80°C. The resultant material was characterized by UV spectroscopy, Particle size analysis, zeta potential determination, FTIR Resonance, X-Ray Diffraction, Scanning Electron Microscopy and Transmission Electron Microscopy. Surface Plasmon Resonance phenomenon was observed at 422 nm. Average particle diameter was 313.8 nm and Polydispersity index was 0.481. Zeta potential was found to be -10.8mV. XRD pattern showed four intense peaks characteristic for Ag NPs were observed at 38.08°, 46.19°, 64.5° and 77.4° corresponding to the (111), (200), (220) and (311) planes respectively. TEM and SEM imaging showed that particles were spherical in shape, with size of 100 -140 nm.

Conclusion

From this review, it can be concluded that, the plant *Hygrophila schulli* has several pharmacological activities. Polar extracts of its leaves and seeds are useful not only in reduction of precursor of metal to corresponding metal nanoparticles, but also in stabilizing as synthesized nanoparticles by capping around them. The detail phytochemical prospection of plant has not been reported so far. Hence, further research works pertaining to advanced phytochemical analysis of different parts of this plant; their use in green synthesis of metal oxide nanoparticles like zinc oxide, copper oxide, titanium dioxide nanoparticles and evaluation of their use in different scientific domains could be undertaken.

Acknowledgment

We, the authors of this review article are thankful to Dr. Sanjay B. Chordiya, Chairman, Suryadatta Group of Institute, Bavdhan for encouraging us and providing internet and computer facilities to carry out this review work.

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