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GREEN TEA DERIVED SILVER NANOPARTICLES BOOSTED THE ANTIOXIDANT POTENTIAL OF GREEN TEA

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Abstract

In order to enhance the antioxidant potential of green tea, phyto-mediated synthesis of silver nanoparticle approach was used. The synthesized green tea silver nanoparticles (GTAgNPs) were characterized using spectrophotometric techniques (UV-Visible and FTIR). GTAgNPs showed characteristic peak nearly at 410nm and the obtained FTIR spectra confirmed that polyphenolic compounds of green tea were responsible for the biogenic synthesis of silver nanoparticles. Antioxidant activity of resultant nano-formulation was assessed using DPPH radical scavenging assay. Green tea derived silver nanoparticles (GTAgNPs) exhibited higher scavenging activity than green tea.
Keywords: Green tea (GT), Nanoparticles (NPs), Silver nanoparticles (AgNPs), Green tea derived silver nanoparticles (GTAgNPs), Antioxidant.

Introduction

Green tea is acclaimed to be one of the healthiest drinks worldwide. It is initially originated in China but in recent years it gained popularity in other countries also. It is generally consumed in the form of aqueous infusion. It is minimally processed form of tea obtained from pan fried or steamed leaves of plant *Camellia sinensis* which belongs to flowering plant family *Theaceae* (Wang *et al.,* 2011). It also possesses a unique composition of phytochemicals which are highly water soluble and responsible for the varied types of biological activities. Tea leaves are composed of various types of polyphenols which includes flavonols, theanine, caffeine, leucoanthocyanins and phenolic acids. About 60-80% of polyphenols in green tea are catechins (mainly (+)-catechin, (–)-epicatechin gallate, (+)-gallocatechin, (–)-epigallocatechin, (–)-epicatechin, (–)-epigallocatechin gallate and (+)-gallocatechin gallate) (Puligundla *et al.,* 2017; Sharangi, 2009). Various studies concluded that these non-nutritive bioactive components of green tea are responsible for its health nurturing properties such as anti-oxidant, anti-diabetic, anti-oncogenic, anti-angiogenic, anti-obesity, anti-allergic and anti-microbial (Sharangi, 2009; Oh *et al.*, 2013; Giri *et al.,* 2020). Due to its various nutraceutical applications, it is extensively studied for its health promoting potential. Polyphenolic compounds of green tea are potent in scavenging free radicals and have much higher antioxidant activity than many herbal infusions and dietary supplements (Costa *et al.,* 2012; Farooq and Sehgal, 2019). However, some clinical studies reported that to attain health benefits high amount of green tea (nearly 5-10 cups daily) consumption is required (Voung *et al.,* 2011). The outcomes of clinical trials summarized that green tea has mild to moderate effects in preventing type II diabetes, cancer, brain stroke and cardiovascular associated diseases (Choan *et al.,* 2005). Poor solubility, decreased bioavailability and poor stability of bio-active phytochemicals inside our body are the major obstacles in the therapeutic potential of green tea. Various methods are applied to solve this problem, out of all; one method is the usage of nanoparticles. The synthesis of nanoparticles by bio-reduction process is the novel approach to enhance the therapeutic efficacy of plant based bio-active compounds (Puligundla *et al.,* 2017; Kaur *et al.*, 2018).

Nanoparticles mean particles having size measurements on nanometer scale (1 nm-100 nm) and the synthesis of these nanostructures comes under the branch of nanotechnology. Today is the era of nanotechnology as these nanostructures are gaining popularity in every field of science that is from material science to biology, medicines, electronics, cosmetics and drug delivery (Sudha *et al.,* 2017). Metallic nanoparticles can be synthesized by chemical, physical and biological methods but various chemical and physical methods require high energy consumption, toxic waste products and are also not ecofriendly. Biological method includes the exploitation of microorganisms, fungus, natural enzymes, compounds and plant extracts for the bio-reduction process of metal precursors (Prabhakar *et al.,* 2011a; Prabhakar *et al.,* 2011b). This method have various advantages such as, ecofriendly method, non-toxic waste products, economically friendly and also enhance the therapeutic aspect of bioactive components of plant extracts (Moldovan *et al.,* 2016). Inorganic metallic nanoparticles are highly exploited in cellular drug deliveries due to their richness in functionality, high compatibility, selectivity and specificity and increased bioavailability. Among all metallic NPs, silver nanoparticles are extensively studied due to its unique and varied types of biological activities such as anti-microbial, anti-angiogenic, anti-viral, anti-inflammatory and anti-cancer (Selvan *et al.*, 2018; Al-sheddi *et al.,* 2018). There are many studies in which different plant extracts are used for the synthesis of AgNPs such as *Azadirachta indica* (Ronavari *et al.*, 2017; Kaur *et al.,* 2018), *Ocimum sanctum* (Ahmed *et al.*, 2010), *Curcuma longa*, *Allium cepa* (Selvan *et al.*, 2018), Green tea (Rolim *et al.*, 2019), *Pongamia, pinnata* (Priya *et al.,* 2016), *Cinnamomum camphora* (Sun *et al.,* 2014), *Asphodelus aestivus* (Falal *et al.,* 2017), *Prosopis farcta* (Salari *et al.*, 2018), *Lippia nodiflora* (Sudha *et al.,* 2017), *Achillea millefolium* (Yousaf *et al.*, 2020) and many more, all these studies concluded increased biological activities of plant extracts. Present study was designed to synthesize silver nanoparticles through phyto-reduction process employing green tea aqueous extract and the evaluation of antioxidant activities of green tea and green tea synthesized silver nanoparticles (GTAgNPs) (Nandi *et al.*, 2020).

Material and Methods

Plant extract preparation

Freshly prepared green tea was purchased in the pack of 250gm from CSIR-IHBT, Palampur, India. 2% (w/v) infusion of green tea was prepared by steeping it for 5 min. in hot double distilled water (90-95°C). Green tea extract then filtered with the help of Whatmann filter paper and then centrifuged for 20min. at 6000rpm. The supernatant was used for the synthesis of silver nanoparticles and for determination of antioxidant assay. Rest of the extract was stored at 4°C for further use.

Green synthesis of silver nanoparticles

Silver nanoparticles were synthesized by using silver nitrate solution through bio-reduction method. The aqueous solution of $AgNO_3$ (0.5mM) was prepared in a conical flask and covered with aluminum foil. To 99ml of $AgNO_3$ solution, 1ml of green tea extract was added drop wise with constant stirring (pH = 9, 80-85°C). The mixture was stirred continuously for 5 minutes till brown color appeared. The resultant solution was then cooled at room temperature and stored at 25°C for further use (Masooleh *et al.,* 2018).

Characterization of silver nanoparticles

UV-Visible Spectroscopy

Formation of nanoparticles is indicated by the color change in the solution of plant extract and also determined by the UV-Visible spectra. UV-Visible spectra help us in determining the synthesis and size of nanoparticles up to a certain extent. Measurements were taken at room temperature by using Shimadzu's UV-Visible 1800 series spectrophotometer, after the dilution of synthesized solution containing nanoparticles between the range from 300-500nm. Quartz cuvette was used to run the samples and double distilled water was used as blank. (Kumar, 2020, Kumari, 2019)

FTIR (Fourier Transform Infra-red Spectroscopy)

FTIR spectroscopy was used to determine the functional groups which correspond to the coated phytochemicals on the surface of nanoparticles. Spectra was obtained in the mid infra-red range from 4000 cm^{-1}- 400 cm^{-1} at room temperature.

Antioxidant assay

DPPH Assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl) was used to evaluate the antioxidant activity of green tea infusion and green tea synthesized silver nanoparticles (GTAgNPs) in the solution form. DPPH is stable free radical compound having dark blue coloured methanolic solution. These free radicals accept hydrogen from the antioxidant compound and get neutralized. For the experiment, DPPH (11mg) was dissolved in 50ml of methanol (100%). This stock solution was then diluted to set the absorbance at 520nm between the ranges of 0.8-1. Different concentrations of green tea or GTAgNPs were added to 2ml of DPPH solution in separate test tubes. The reaction mixture was incubated at 37°C for 45 minutes in water bath. After incubation, absorbance was taken at 520nm through SHIMADZU UV-Visible Spectrophotometer (Mensor *et al.,* 2001; Vyas *et al.*, 2017; Vyas, 2019).

% scavenging was calculated by the formula:

$$\%\ \text{DPPH scavenging activity} = \left[\frac{\text{Absorbance of control}\ \ \text{Absorbance of test}}{\text{Absorbance of control}} \times 100\right]$$

Result and Discussion

UV-Visible Spectroscopy

Formation of silver nanoparticles was indicated firstly by the change in the colour of the resultant solution containing mixture of silver nitrate and green tea extract. When green tea extract was added drop wise in silver nitrate solution, there was change in colour from colourless to yellow and with continuous stirring (5min.) yellow colour changed to brown. The appearance of dark brown colour is the characteristic feature of silver nanoparticles and this is due the unique optical activity exhibited by the silver nanoparticles named as LSPR (Localized Surface Plasmon Resonance). LSPR exhibited by the metallic silver nanoparticles electrons after interacting with the light of particular wavelength. The colour of the silver nanoparticles solution depends upon the size of NPs and pH of the solution (Masooleh *et al.*, 2018, Katoch, P. 2019).

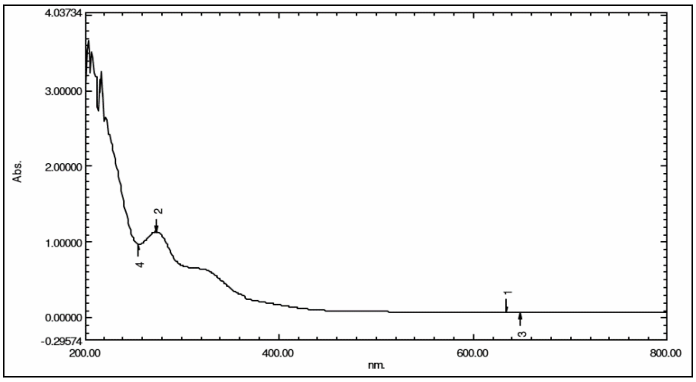

Fig. 1: UV-Visible spectra of green tea aqueous extract.

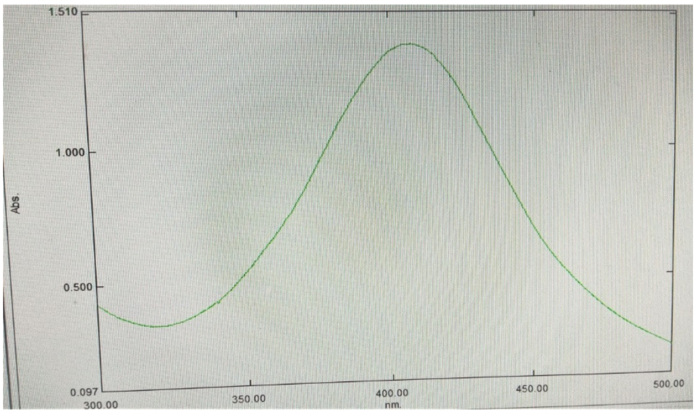

Fig. 2: UV-Visible spectra of green synthesized silver nanoparticles (GTAgNPs)

The UV-Visible spectra of green tea infusion illustrated characteristic peaks between the range of 260-350nm wavelength and sharp peaks at lower wavelength (Fig. 1). These peaks mainly correspond to absorbance of flavonoids and other polyphenolic compounds present in green tea extract. The UV-Visible spectra of green tea synthesized silver nanoparticles showed emergence of broad peak centred near 410nm in the range of 370nm- 500nm (Fig. 2). This region corresponds to the absorption region of silver nanoparticles (AgNPs). An earlier study demonstrated formation of spherical shape and colloidal nature of silver nanoparticles at similar absorbance and range (Rolim *et al.*, 2019).

FTIR Spectroscopy

FTIR technique was used to recognize the functional groups that are responsible for the bio-reduction of metal precursor and coated residues of reducing agent present on the surface of synthesized silver nanoparticles (Fig. 3). FTIR spectra of GTAgNPs revealed characteristic peaks at 3261.74cm^{-1} and 1645.33cm^{-1}. These peaks were due to the functional groups of phytochemicals present in green tea which get attached on the surface of AgNPs. The peak at 3261.74cm^{-1} corresponds to the stretching vibration of O-H bond present in the alcohol and phenol groups whereas 1645.33cm^{-1} peak represents the bending vibration of N-H bond present in the proteins of plant extract (Masooleh *et al.,* 2018). Our results are in agreement with earlier studies that polyphenolic content present in green tea is majorly responsible for the bio-reduction of silver nitrate to form silver nanoparticles (Sun *et al.,* 2014; Onitsuka *et al.*, 2019; Vilchis-Nestor *et al.,* 2008; Mehsooleh *et al.*, 2018).

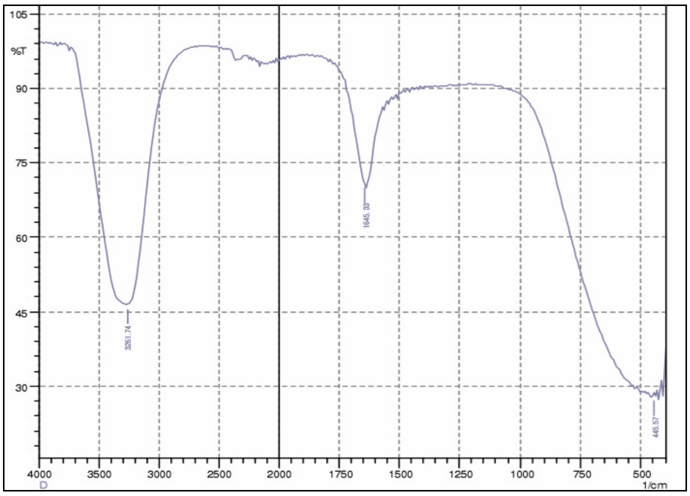

Fig. 3: FTIR spectra of green synthesized silver nanoparticles by using green tea extract.

Antioxidant activity evaluation of GTAgNPs

Free radicals are usually generated inside our body during various metabolic reactions and these free radicals are the reason for many health ailments such as aging, cancer, diabetes, cardiovascular problems, neurodegenerative diseases and many more (Selvan *et al.,* 2018). It is the need of hour to develop new formulations that can show higher antioxidant potential than crude extracts. The green synthesis of nanoparticles employing plant extracts with well-known antioxidant properties can be one of the ways to obtain a nano formulation in solution form with enhanced radical scavenging ability as compared to the plant extract used for its synthesis. DPPH assay is the most frequently used antioxidant assay to evaluate the antioxidant activity. Antioxidant activity of plant extract is evaluated by the ability of donating hydrogen to scavenge the free radicals of DPPH (Falal *et al.,* 2017, Chauhan, S. *et al* 2017). GTAgNPs showed higher scavenging activity than green tea at all concentrations (Fig. 4). The lower is the EC_{50} (Effective concentration to scavenge 50% of DPPH radicals) of the tested drug or extract higher is the antioxidant capacity. GTAgNPs exhibited lower EC_{50} (25.3µg/ml) in comparison to green tea (37µg/ml). The increment in scavenging activity may be due to the increased surface area for the interaction between polyphenols and DPPH free radicals in case of GTAgNPs in solution form. The coating of polyphenolic residues over the surface of silver nanoparticles may increase the interaction and ability of polyphenols present in green tea for donating hydrogen to free radicals.

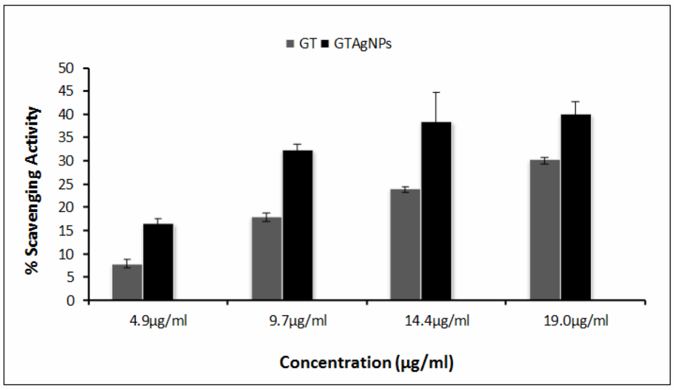

Fig. 4: DPPH scavenging activity of green tea (GT) and green tea derived silver nanoparticles (GTAgNPs). Data represented as MEAN ± S.D (n=3).

Conclusion

Green tea derived silver nanoparticles can be a better option than green tea as far as antioxidant property is concerned. The future applications of these nanoparticles can be in prevention and therapy of oxidative stress related diseases, food industry and cosmetics.

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