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Antioxidant activity of silver nanoparticles synthesized by hydroalcoholic extract of Triphala

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Abstract

The aim of the present study was to investigate the in vitro antioxidant activity of hydroalcoholic extract of Triphala and its ecologically friendly synthesized silver nanoparticles. In the present study, we conducted an estimation of the extract using Hydrogen peroxide radical scavenging assay and superoxide radical scavenging assay to assess the antioxidant potential. The results were compared with the effect of standard ascorbic acid.

Primary phytochemical screening of the plant extract showed the presence of carbohydrates, alkaloids, glycosides, flavonoids, phenolics etc. The hydroalcoholic extract and its SNPs showed a concentration-dependent activity in both methods.

In the study, it was concluded that the hydroalcoholic extract of Triphala comprises pharmacologically important phytoconstituents like phenolic compounds and flavonoids which impart the potential of the extract as well as its SNPs in the modulation of oxidative stress owing to its strong antioxidant and free radical scavenging activities.

Keywords: Antioxidant activity; Hydrogen peroxide radical scavenging assay; Superoxide free radical scavenging assay; Silver nanoparticles

1. Introduction

Whenever a cell's internal environment is perturbed by infections, disease, toxins or nutritional imbalance, mitochondria diverts electron flow away from itself, forming reactive oxygen species (ROS) and reactive nitrogen species (RNS), superoxide radicals thus lowering oxygen consumption [1].

Increased oxidative stress at the cellular level can come about as a consequence of many factors, including exposure to alcohol, medications, trauma, colds, infections, poor diet, toxins, radiation, or strenuous physical activity [2]. The term "oxidative stress" has been used to define a state in which ROS and RNS reach excessive levels, either by excess production or insufficient removal. Being highly reactive molecules, the pathological consequence of ROS and RNS excess is damage to proteins, lipids and DNA [3]. The main diseases are diabetes, cancer, neurodegenerative diseases like Alzheimer's disease Parkinsons disease or ageing of organisms [4]. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules, thus protecting the cells. Antioxidants act by terminating the chain reaction of the formation of free radicals by removing free radical intermediates and inhibiting other oxidation reactions by being oxidized themselves [5].

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Plants are rich in phytoconstituents which exhibit pharmacological activities including antioxidant effect and can be used against many diseases [6]. Drug research attained a new horizon by associating nanotechnology with herbal as well as biotechnology. By incorporating herbal constituents in their nano form an improved pharmacokinetic and therapeutic profile can be effectively achieved.

In connection with the inflammation cascade, the antioxidants inhibit oxidation and protect the cells against free radicals, which are usually released by oxidative stress and play an important role in the pathogenesis of many diseases such as cancer, type 2 diabetes, obesity, AIDS, neurodegenerative as well as cardiovascular diseases [7]. In all such disorders, the antioxidants help in the reduction of damage to cells caused by the release of excess free radicals and oxidative stress as a result of the pro-oxidant and anti-oxidant homeostatic imbalance in the body. The pro-oxidant conditions dominate either due to increased generation of free radicals or due to their poor scavenging in the body which can be effectively controlled by antioxidants intake as ingredients in dietary supplements and are helps to maintain health and prevent oxidative stress-mediated diseases as well as delay ageing process [8].

Triphala is a well-known polyherbal medicine consisting of dried fruits of the three plant species *Phylanthus emblica* (Euphorbiaceae), *Terminalia bellerica* (Combretaceae), *and Terminalia chebula* (Combretaceae) in equal proportion. In ayurvedic medicine, triphala rasayana is classified as a *tridoshic rasayana* and it promotes long life and regeneration in patients of all constitutions and ages. It possesses antibacterial, anti-viral and anti-cancer properties. It is also effective in the treatment of acquired immune deficiency syndrome (AIDS) and to cure cataracts [9]. It is rich in polyphenols, flavonoids, vitamin C, gallic acid, chebulagic acid, chebulinic acid, anthraquinones, etc. by which it possesses both nutritional as well as health benefits such as demulcent or laxative, tonic, blood, and liver cleansing activities. So, it is also considered as one of the best and most valuable herbal preparations in the world [10].

2. Material and methods

2.1. Collection of plant material

The Triphala is composed of the three myrobalans, *Terminalia chebula*, *Terminalia bellerica* and *Phyllanthus Emblica* were collected from Kannur District in Kerala, India in October 2020 and were authenticated by Dr. P. Sreeja Msc, PhD, Assistant Professor and Senior Research Officer, P G Department of Botany and Research Centre, Sir Syed College, Thaliparamba, Kannur, Kerala. A voucher specimen No-99366 was deposited in the P G Department of Botany and Research Centre Herbarium, Sir Syed College, Thaliparamba, Kannur, Kerala for future reference. Dried fruits were ground to a coarse powder, passed through sieve no 24, stored in airtight container, and used for further extraction.

2.2. Preparation of hydroalcoholic extract of Triphala (HAET)

HAET was prepared by the percolation method (about 48 h). The Triphala powder (200 g) was extracted with hydroalcoholic solution (96%, 500 mL) using a 2 L percolator. The extract was concentrated in a rotary evaporator to 50 mL, and then freeze-dried [11].

2.3. Primary phytochemical screening

For primary phytochemical screening, freshly prepared hydroalcoholic extract of Triphala was tested for the presence and absence of phytoconstituents such as carbohydrates, alkaloids, glycosides, tannins, flavonoids, phenolic compounds, steroids by using standard methods [12].

2.4. Green synthesis of Triphala-Silver Nanoparticles (TSNPs)

10.0 g of dried extract was suspended in 100 ml of 1mM silver nitrate (AgNO3) solution in 1:10 ratio (w/v). The mixture was centrifuged at 6000 rpm for 30 minutes, double filtered and the supernatant was used for further experiments. These nanoparticles are further characterized by UV-Visible spectroscopy and Transmission electron microscopy (TEM) [13].

2.5. Determination of antioxidant activity of Triphala extract and TSNPs

2.5.1. Hydrogen peroxide radical scavenging assay:

A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of extract and its SNPs (125 -2000 µg/ml) were prepared from stock concentration of 10 mg/ml and added to H_2O_2 solution (0.6 ml). A control without the test compound but an equivalent amount of distilled water was taken. Optical density was read at 230 nm after 10 minutes [14].

$$\% \text{ Inhibition} = \frac{\text{opticaldensityofcontrol} - \text{opticaldensityoftest}}{\text{opticaldensityofcontrol}} \times 100$$

2.5.2. Superoxide free radical scavenging assay:

Different concentrations of samples (125-2000 µg/ml) from stock solution of 10 mg/ml, 0.05 ml of Riboflavin solution (0.12 mM), 0.2 ml of EDTA solution [0.1M], and 0.1 ml NBT (Nitro-blue tetrazolium) solution [1.5 Mm] are mixed in two test tubes. The reaction mixtures were diluted up to 2.64 ml with phosphate buffer [0.067 M]. Control without the test compound but with an equivalent amount of distilled water was taken. The absorbance of the solution was measured twice after illumination for 5 minutes and 30 minutes in fluorescent at 560 nm on a UV-Visible spectrophotometer [15,16].

$$\% \text{ Inhibition} = \frac{opticaldensityofcontrol - opticaldensityoftest}{opticaldensityofcontrol} \times 100$$

2.6. Statistical analysis

Data were expressed as mean ± standard deviation for separate groups for determinations in triplicates. IC_{50} values were calculated via non-linear regression analysis (sigmoidal fitting with variable slope) using GraphPad Prism v. 5.0(GraphPad Software Inc., USA).

3. Results and discussion

3.1. Preliminary phytochemical screening

Preliminary phytochemical screening of the leaf extract showed the presence of alkaloids, glycosides, carbohydrates, flavonoids, phenolic compounds, proteins, and steroids.

3.2. *In vitro* antioxidant activity of silver nanoparticles by hydrogen peroxide assay method

Table 1 *In vitro* antioxidant activity of silver nanoparticles by hydrogen peroxide assay method

Groups	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
Ascorbic acid	25	21.37	79.63
	50	38.65	
	100	56.13	
	250	79.19	
	500	93.10	
HAET	25	17.23	168.94
	50	29.54	
	100	39.41	
	250	55.91	
	500	69.24	
TSNPs	25	19.25	96.41
	50	31.02	
	100	52.14	
	250	68.47	
	500	83.37	

Antioxidant potential of the HAET and TSNPs was evaluated using Hydrogen peroxide assay method. The extract exhibited antioxidant activity in a dose dependent manner. The hydroalcoholic extract showed a maximum inhibition of 55.91% while the standard ascorbic acid exhibited maximum inhibition of 79.19% at a concentration of 250 µg/ml.

The silver nanoparticles showed 68.47% inhibition at 250 µg/ml. The IC_{50} values of the standard ascorbic acid was found to be 79.63 µg/ml, the hydroalcoholic extract showed 68.94 µg/ml and the silver nanoparticles was found to be 96.41 µg/ml. Hydrogen peroxide assay is often used to evaluate the ability of natural antioxidants to donate electron. Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of certain plant extracts which are again enhanced with nanoparticles. (Table 1 and Figure 1)

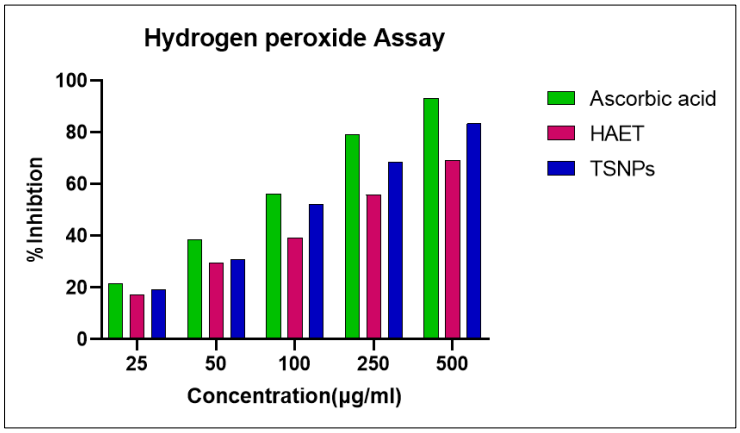

Figure 1 *In vitro* antioxidant activity of silver nanoparticles by hydrogen peroxide assay method

3.3. *In vitro* antioxidant activity of silver nanoparticles by superoxide radical scavenging assay method

Antioxidant potential of the HAET and TSNPs were evaluated using superoxide radical scavenging assay method. The extract and its SNPs exhibited antioxidant activity in a dose dependent manner. The hydroalcoholic extract showed maximum inhibition of 59.03% while the standard ascorbic acid exhibited maximum inhibition of 83.21% at a concentration of 250 µg/ml. The silver nanoparticles showed 64.71% inhibition at 250 µg/ml. The IC_{50} values of the standard ascorbic acid were found to be 67.17 µg/ml, the hydroalcoholic extract showed 95.97 µg/ml and the silver nanoparticles were found to be 82.22 µg/ml. (Table 2 and Figure 2)

Table 2 *In vitro* antioxidant activity of silver nanoparticles by superoxide radical scavenging assay method

Groups	Concentration (µg/ml)	% Inhibition	IC50 (µg/ml)
Ascorbic acid	25	16.08	67.17
	50	34.72	
	100	69.03	
	250	83.21	
	500	91.62	
HAET	25	12.72	95.97
	50	29.97	
	100	50.70	
	250	59.03	
	500	72.23	
TSNPs	25	13.98	82.22
	50	32.87	
	100	56.31	
	250	64.71	
	500	76.40	

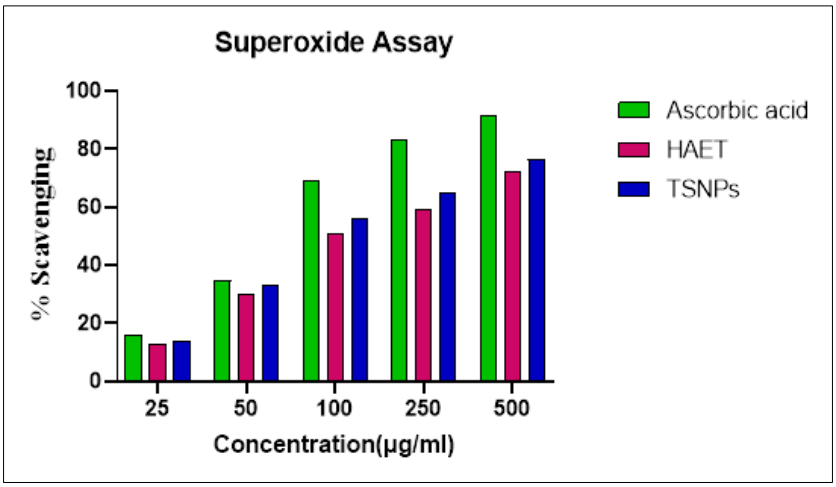

Figure 2 *In vitro* antioxidant activity of silver nanoparticles by superoxide radical scavenging assay method

4. Conclusion

In the study, all the results suggested that the hydroalcoholic extract of Triphala and its silver nanoparticles exhibit a significant antioxidant potential which was proved by the techniques such as hydrogen peroxide as well as superoxide radical scavenging assays. The antioxidant properties elicited by the extract may be due to the presence of flavonoids and phenolic compounds extensively present in it, moreover, the potential can be enhanced by administering it in nanoparticle form. It was evident that the Triphala extract and its SNPs were potent sources of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. They may result in a new antioxidant drug with reduced particle size, minimum toxicity and potential for synergism with currently existing antioxidants in the market.

Further studies are needed for the isolation and identification of individual component in the extract and its SNPs and *in vivo* studies should be conducted for understanding their molecular mechanism as well as pharmacokinetic profiling as an antioxidant prior to clinical use.

Compliance with ethical standards

Disclosure of conflict of interest

No potential conflict of interest was reported by the authors.

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