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Antimicrobial and antioxidant potentials of biosynthesized colloidal zinc oxide nanoparticles for a fortified cold cream formulation: A potent nanocosmeceutical application

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ABSTRACT

Nanocosmeceuticals are promising applications of nanotechnology in personal care industries. Zinc oxide is an inorganic material that is non-toxic and skin compatible with self-cleansing and microbicidal properties. Herein, exploitation of colloidal zinc oxide nanoparticles (ZnONps) as potent biomaterial for a topical formulation of cosmetic and dermatological significance is employed. ZnONps were green synthesized using environmentally benign *Adhatoda vasica* leaf extract and characterized by UV–Vis absorption spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), dynamic light scattering (DLS), high-resolution transmission electron microscopy (HR-TEM) and energy-dispersive X-ray spectroscopy (EDX). The results reveal that the biosynthesized ZnONps exhibit an absorption peak at 352 nm. XRD and HR-TEM analyses confirm the hexagonal wurtzite structure of ZnONps with particle size of about 10 nm to 12 nm. Elemental analysis by EDX confirms the presence of zinc and oxygen. Zeta potential of −24.6 mV affirms the stability of nanoparticles. The antibacterial and antifungal activities of biosynthesized ZnONps exhibit mean zone of inhibition from 08.667 ± 0.282 to 21.666 ± 0.447 (mm) and 09.000 ± 0.177 to 19.000 ± 0.307 (mm) respectively, in a dose-dependent manner. The IC_{50} value exerted from the antioxidant activity of ZnONps is found to be 139.27 μg mL^{-1}. ZnONps infused cold cream formulation of microbicidal and antioxidant properties was further tested against clinical skin pathogens. The nano-based cold cream exhibited significant inhibitory action against *Candida* sp., which showed resistance against a commercial antifungal cream (2%). Therefore, this study demonstrates the exploitation of ZnONps as promising colloidal drug carriers in cosmeceuticals that can significantly alleviate human skin infections and oxidative stress induced cellular damage.

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1. Introduction

Cutaneous infections are one of the major concerns in pharmaceutical and cosmetic sectors. The global market involving technologies for the treatment of skin diseases reached $17.1 billion in 2015, and is expected to reach $20.4 billion by 2020 [1]. Among the major pharmaceutical companies, innovative therapies for certain skin diseases have renewed interest in the market. Novel dermatological and cosmetic formulations are employed with antibacterial and antifungal potentials to combat various skin conditions and infections [2]. These formulations comprise of fragrance, oils and fats that can undergo auto-oxidation and chemical degradation when exposed to air, thereby becoming odorless. Addition of antioxidants can preserve them, increase their shelf life and also protect the skin from cellular damages [3].

Formulations of biological origin are advantageous over synthetic formulations due to better patient tolerance, cost effectiveness and minimal adverse effects [4]. Several plants have been investigated for the treatment of various skin diseases ranging from itching to skin cancer [5]. The active phytoconstituents like alkaloids, flavonoids, tannins, saponins, glycosides, coumarins and lignins possess antibacterial, antifungal and antioxidant properties which are requisites for the treatment of skin diseases [6]. Therefore, cream, gel and soap formulations that contain these secondary metabolites of plants are effective in the treatment of various skin disorders caused by microbial infections [7].

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Chemical preservatives are used in cosmetics to increase their shelf life by avoiding microbial spoilage [8]. Compared to organic materials, inorganic powders like zinc oxide is stable and capable of withstanding harsh processes represent a promising alternative to chemical preservatives with microbicidal properties [9]. ZnO is also considered as one of the generally recognized as safe (GRAS) materials for humans [10]. It inhibits bacterial enzymes such as thiol peroxidases, glutathione reductases and dehydrogenases, owing to its pronounced antibacterial property [11]. The antifungal activity of ZnO occurs by the deformation of fungal hyphae and preventing the development of conidiophores leading to the death of fungal hyphae causing cellular destruction [12]. ZnO is used as a promising antioxidant in cosmeceuticals that is capable of penetrating the stratum corneum of skin and provides protection against reactive oxygen species (ROS) by inactivating the production of free radicals that damage the skin, and also help in promoting cellular repair and healing [13]. The efficacy majorly depends not only on the concentration of the active substance, but also on the size of the particles, their modification, and the degree of polydispersity [14].

Incorporation of nano-sized particles in cosmetic formulations can improve the stability of active constituents like vitamins, unsaturated fatty acids and antioxidants, thereby enhancing their therapeutic value [15]. Nanotechnology is a burgeoning field that has greater significance to revolutionize cosmetics and pharmaceuticals. Colloidal drug carriers like nanoparticles have tremendous scope in cosmetic and dermatological sectors due to their nanometric size and potential applications [16]. Modern cosmetic-oriented products consist of nano-sized particles, which are envisaged to replace conventional preservatives used in cosmetics [17]. They offer better skin penetration and UV protection, increased color and finish quality, long-lasting effects [18], and also eradicate or control the activity of various microorganisms [19].

Highly ionic nanoparticulate metal oxides such as zinc oxide nanoparticles (ZnONps) are promising biomaterials in nanomedicine that can be produced with larger surface areas and longer shelf life, and has synergistic effect with organic antimicrobial agents [17]. They are biocompatible to human cells [20], whereas, toxic towards a wide range of micro-organisms like bacteria [21] and fungi [22]. The antimicrobial activity of colloidal ZnONps has been assessed against human [23] and plant [24] pathogens. Antimicrobial properties of nanoscale ZnO particles have been the focus of various industrial applications as biocides coating in water treatment, paints and cosmetics [25]. The risk for humans from the usage of ZnONps in various sunscreens or cosmetics is considered negligible when used at concentrations upto 25% and *in vitro* genotoxic and photogenotoxic profiles of ZnONps results in no consequence to human health [20].

Eco-friendly synthesis of metal and metal oxide nanoparticles is preferred over chemical methods since it is a simple, non-toxic and cost-effective approach [26]. Colloidal ZnONps have been green synthesized using extracts of various parts of plants or trees like *Ruta graveolens* [27], *Camellia sinensis* [28], *Cassia fistula* [29], *Pithecellobium dulce* [19], *Lagenaria siceraria* [19], *Calotropis procera* [21], *Parthenium hysterophorus* [24] and *Rosa canina* [30].

Adhatoda vasica Nees (Acanthaceae), also known as Malabar nut tree is a native of South Asia which is being widely used in the Indian traditional medicine for the treatment of various respiratory disorders like asthma, chronic bronchitis and tuberculosis [31]. The leaves of *A.vasica* are also being used in Ayurvedic medicine exclusive for the treatment of various skin related diseases [32]. These medicinal qualities of *A. vasica* attribute to their active phyto-constituents like alkaloids, polysaccharides, vitamin C, polyphenols, proteins, glycosides, quinines, flavones, coumarins, triterpenes and essential oils. The principal constituents are quinazoline alkaloids and the chief alkaloid is vasicine [33]. Alkaloids have excellent antibacterial [34], antifungal [35] and antioxidant [36] properties. Such bioactive compounds act as reducing, capping and stabilizing agents in the synthesis of nanoparticles and also render biocompatible functionalities to the nanoparticles for various biomedical applications [26]. *A. vasica* is used in the synthesis of silver nanoparticles and assessed for antibacterial and antifungal [37], larvicidal [38] and electrochemical [39] activities. Herein, we report an eco-friendly synthesis of colloidal ZnONps by the biological reduction of Zn^{2+} ion using *A. vasica* aqueous leaf extract. Their physicochemical characterizations and evaluation of antibacterial, antifungal and antioxidant properties have been performed and exploited for a potent nano-based cosmeceutical application.

2. Materials and methods

2.1. Materials

Zinc acetate dihydrate [$Zn(CH_3COO)_2.2H_2O$] and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Hi-media laboratories, Mumbai, India and was used as received. All other reagents were of analytical grade. Ultrapure (Milli-Q) water was used throughout the experiments. Nutrient broth (NB), Mueller Hinton agar (MHA), Sabouraud dextrose broth (SDB) and sabouraud dextrose agar (SDA) used for the cultivation of bacteria and fungi were obtained from Himedia laboratories, Mumbai, India. Standard bacterial and fungal strains of clinical significance such as *Staphylococcus epidermidis* MTCC 435 (equivalent ATCC 155), *Escherichia coli* MTCC 443 (equivalent ATCC 25922), *Pseudomonas aeruginosa* MTCC 741 (equivalent ATCC 25668), *Aspergillus fumigatus* MTCC 6594, *Trichophyton rubrum* MTCC 296 and *Microsporum audouinii* MTCC 8197 were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. Clinical isolates *Staphylococcus* sp., *Streptococcus* sp., *Candida* sp. and *Fusarium* sp. were obtained from a local hospital.

2.2. Preparation of extract and phytochemical analyses

The fresh leaves of *Adhatoda vasica* were harvested in the month of September from Tiruchirappalli, Tamil Nadu, India, and were authenticated at the Department of Botany, St. Joseph's College of Arts and Science, Tiruchirappalli. The leaves were washed thoroughly twice and dried. 10 g of finely cut leaves were weighed and boiled with 100 mL of Milli-Q water at 100 °C for 10 min. The residues were removed by Whatman no.1 filter paper. The filtrate was cooled down and qualitatively analyzed for various phytochemical constituents by standard procedure [40]. The extract was further used for the synthesis of ZnONps.

2.3. Biosynthesis of colloidal ZnONps

ZnONps were synthesized by adding 1 mL of *A. vasica* aqueous leaf extract to $Zn(CH_3COO)_2.2H_2O$ solution (0.1 M, 50 mL) with rapid stirring for 10 min at room temperature. The pH was adjusted to 10 with NaOH and the mixture was continued to stir at 400 rpm for 1 h. The formation of ZnONps was observed visually by color change. The synthesized colloidal ZnONps were also subjected to phytochemical analyses as mentioned above to confirm the phytoconstituents that act as stabilizing and capping agents responsible for the reduction mechanism.

2.4. UV–Vis absorption spectroscopy

The colloidal ZnONps were characterized by UV–Vis absorption spectroscopy (Shimadzu/UV-2000, Japan) with wavelength range between 200 and 800 nm to confirm the reduction of Zn^{2+} ions. Then, the obtained nanoparticles were centrifuged thrice at 7500 rpm for 10 min at 4 °C by re-dispersing in Milli-Q water repeatedly to remove any uncoordinated biomoieties. This process of purification was performed to ensure better separation of ZnONps. The obtained pellet was air dried and used for further characterizations.

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