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Eco-friendly Biosynthesis of Iron Oxide Nanoparticles using Lichen Ramalina fastigiata and its Biological Properties

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Abstract

Lichens are symbiotic organisms and enrich with more lichen compounds. Lichens are widely used as traditional foods, ethanomedicines, and holy sacrifice fires. In the present study, Collection of native species of high altitude lichen Ramalina fastigiata was collected. Fe_2O_3 NPs were synthesized using R. fastigiata lichen extract and characterized. FTIR and GC–MS analysis was performed to identify the bioactive compounds helpful in the synthesis of Fe_2O_3 NPs as capping and reducing agents. The antioxidant and anticandida activities were evaluated by in-vitro method. Results showed that the synthesized Fe_2O_3 NPs had a spherical shape and free radical scavenging activity for 2,2-diphenyl-1-picrylhydrzyl (DPPH) radicals with an IC50 value of 6.5±0.4µg/ml , 4.1 ± 0.2µg/ml ABTS and 18.8±0.05µg/ml H_2O_2 . The antibacterial activity of the synthesized Fe_2O_3 NPs against the test organisms were susceptible in the following order C. albicans > C. parapsilosis > C. tropicalis> C. krusei> C. glabrata. Thus Fe_2O_3 NPs synthesized from high altitude lichen extract demonstrated that it has strong antioxidant and anticandida property with more importance in the field of nanomedicine.

Keywords: Lichen, Nanoparticles, Antioxidant

1.Introduction

The symbiotic association between an algae and a fungus has resulted lichen. Approximately 17% are lichened, forming symbioses with green algae or blue-green algae. It is present in a wide range of habitats throughout the world and dominates 8% terrestrial ecosystems, globally 20,000 species of lichens have been reported [1]. They grow on rock, soil, wood and trees. Lichens have been used as bioindicator, medicinal values, bioremediation. Secondary metabolites of lichens are used in the pharmaceutical industry [2]. Recently, Nanotechnology has created a new revolution in the world, predominantly in the medical, agricultural, industrial sectors [3]. Silver NPs show unique reactivity and stability, recyclability in catalytic reactions [4] and showed inhibitory activity against human pathogens [5]. Among the metal nanoparticles, Iron (Fe_2O_3) nanoparticles have attracted considerable attention due to their applicability. In addition to their antibacterial activity which has been known since ancient times, various attributes of Fe_2O_3 nanoparticles have determined such as antifungal activity, anti-inflammatory effects, antiviral activity and anti-angiogenesis activity. Various studies demonstrated different species of lichens to fabricate unique NPs with different shapes, sizes, and physicochemical and biological activities. Studies stated that methanolic extracts of two lichen species, Xanthoria parietina and Flavopunctelia flaventior,

were shown to have the potential to reduce silver nitrate into Ag-NPs extracellular [6] and studies on Iron oxide Nanoparticles very rare in biological property.

Henceforth, due to multidrug resistance human microbes, antimicrobial agents are needed. Lichens are rich novel metabolites and need to explore in pharmaceutical research. Though exploration and screening of lichens for biological property and their nanoparticles synthesize research are limited reports today. In the present study the collection and identification of native lichen species in University campus and study the eco-friendly approach of Iron oxide nanoparticles by Ramalina fastigiata lichen extract and the evaluation their antioxidant and anti-candida activity

2. Materials and Methods

2.1 Survey and collection of lichen species

A brief survey of lichen distribution was done at different altitude covering various agro climatic zone of Kodaikanal. The study was conducted in the Attuvampatti (MTWU Campus). Different living and non-living substrata were collected along with lichen thallic to study the nature of attachment. During the collection of lichens were peeled off along with tree barks and small piece of rock substrates were collected carefully. The collected samples were cautiously packed in paper without disturbing the thallic and the substrata of the sample material and identification is specimens to Botanical Survey of India, Lucknow.

2.2 Preparation of sample and extract

The collected lichen sample of R. fastigiata was carefully segregated from other types of lichen. Then substrata from the species were removed gently without causing damage to the plant material. Lichen was thoroughly washed under tap water thrice to remove the contaminants present in the lichen. The lichen was then dried at 37℃ for 3-4 days for 4 to 5 hours. The dried lichen was powdered using blender and sieved. The powder was stored in airtight container at room temperature until extraction process. 10 g of lichen powder was taken and poured into a Soxhlet apparatus at 45 °C for 24 hours using ethyl acetate. After that, the solutions was collected and evaporated excess solvent in hot air oven. After evaporation, the dry extract of lichen was taken for further analysis.

2.4 Fourier Transforms Infrared Spectroscopy (FTIR) and GC-MS analysis

Fourier transform infrared spectroscopy (FITR) is a technique used to obtain functional groups present in the sample. The dried sample was once again finely powder in a mortar and pestle. Potassium Bromide (KBr) pellets were added to the sample and grained. The sample was analysed, using a Perkin Elmer instrument in the range of 4000 to 400cm^{-1}.Gas chromatography- mass spectrometry (GC-MS) is an analysis method that combines the features of gas –chromatography and mass spectrometry to identify different substances within a test sampling. Ethyl acetate extract of Ramalina sp. were analyzed using gas chromatography and mass spectrometry. So, filter the extract using whatman No1 filter paper and then 500µl of sample was used for the analysis.

2.5 Green synthesis of Fe_2O_3 nanoparticles using lichen

Ferric chloride ($FeCl_3$) was used as the precursor for the synthesis of the Fe_2O_3 nanoparticles. 0.1M ferric chloride was prepared and 50ml of aqueous solution of R. fastigiata was added drop wise (1:1 ratio)

and 1M of sodium hydroxide was added to reach pH 11. The solution was stirred using a magnetic stirrer until colour changes for the development of Fe_2O_3 NPs. The solution was centrifuged at 8000rpm for 20min and washed. The pellet was air dried and stored in a sterile vial. Green synthesized Fe_2O_3NPs were characterized using Ultraviolet-visible (UV vis) spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, X-ray Diffractometry (XRD), Scanning Electron Microscopy (SEM).

2.6 Antioxidant and Anticandida activity of Fe_2O_3NPs

Antioxidant activity of the Fe_2O_3NPs was determined by their ability to scavenge free radicals by in-vitro assays. The ability to scavenge DPPH free radicals was determined by 2 ml of this solution was added to 1 ml of dissolved Fe_2O_3NPs and incubated in the dark for 30 minutes and the absorbance was measure at 517 nm. The ABTS free radicals was determined by adding ABTS solution to different concentrations of Fe_2O_3NPs and the absorbance was measured at 734 nm. Moreover to study the ability of Fe_2O_3NPs in scavenging H_2O_2 free radicals, H2O2 was prepared in phosphate buffer and added to Fe_2O_3NPs and the absorbance was measured at 230 nm and Ascorbic acid was used as the standard [7].

Fe_2O_3NPs synthesized from R. fastigiata was tested for their anticandidal activity by well diffusion method. Fe_2O_3NPs were tested against C. albicans, C. parapsilosis, C. glabrata, C. tropicalis and C. krusei and Strains were purchased from MTCC. Using a cotton sterile swab each strain was swabbed on Potato dextrose agar plates and using gel puncture 6 mm wells were made on the agar plates. Different concentrations were taken 25µl, 50µl, 75µl and 100µl of Fe_2O_3NPs and added into the wells. The plates were incubated at 28℃ for 24 h and zone of inhibitions were measured [8].

3. Result and Discussion

3.1 Identification of Lichens

The collected lichen specimens were dried and identified by using standard manual (keys). The lichen species namely Ramalina fastigiata growing on barks of trees, were collected from the Mother Teresa Women‘s University Campus, Attuvampatty, Kodaikanal.

3.2Fourier Transform Infrared Spectroscopy (FTIR) and GCMS analysis

The FTIR analysis was used to analyse the functional group present in the lichen R. fastigiata demonstrates the presence of alcohol, alkane, nitro compounds, ether, alkyne, amine, carboxyl groups were present. GC-MS analysis Totally 5 compounds were identified in the ethyl acetate extracts of R. fastigiata and the identification is based on the peak area (%) and the retention time. The presence of three major compounds as 3-Methoxy-5-propylphenol,Furo[2,3-c]pyridine,2,3-dihydro-2,7-dimethyl,3.Alpha-Methylcholest-5-en-3-Beta.olNitrite with biological functions such as antioxidant and antimicrobial activities, anticancer activity.

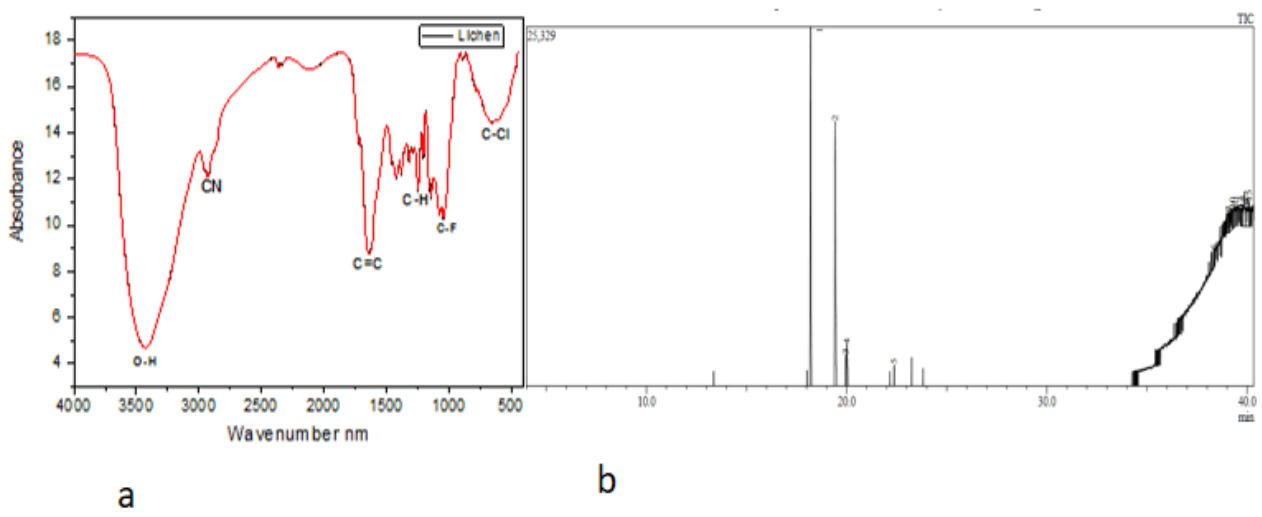

Figure 1: FT IR analysis and GC MS analysis of R. fastigiata

3.3 Characterization of Iron Oxide Nanoparticles

Fe_2O_3NPs were rapidly synthesized using R. fastigiata extracts which was evident from the visible colour change from pale yellow to brown with the increase in time. The UV–Visible spectra of the synthesized Fe_2O_3NPs from the lichen extracts showed surface plasmon bands in the range of.200-350nm. UV- Visible spectrum was carried out to confirm the synthesis of Iron oxide nanoparticle where a peak was attained in the wavelength of 219nm (Fig. 2). The lichen metabolites necessity to lead the fabrication of NPs from the lichen extracts [9]. The FT-IR spectrum (Fig. 2b) revealed the functional groups of the Fe_2O_3NPs synthesized using R. fastigiata showed peaks at 3432cm^{-1},2123cm^{-1},1604cm^{-1},1384cm^{-1},1074$cm^{-1}$and 617cm^{-1}which corresponded to hydroxyl, alkyne,alkanes,nitro compounds, ether and alkyl halide respectively. The presence of amide and nitro groups depict that the proteins play a major role in capping the nanoparticles and preliminarily would have aided in effective reduction of the particles [10]. The SEM with energy-dispersive X-ray spectroscopy (EDX) was studied. The SEM micrograph showed that the synthesized Iron oxide NPs has spherical morphology and the composition of Iron oxide was found to be Iron and oxides with higher amount in the composition were evaluated using the EDX graph and similar results was showed by Dheekshana et al. [11] which showed spherical shaped Fe_2O_3 NPs.

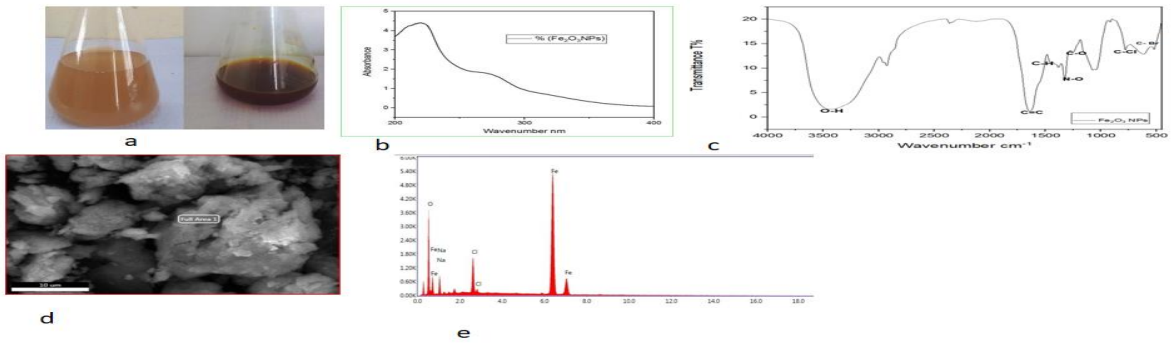

Figure 2: Nanosynthesis of R. fastigiata .a) Colour change b) UV spectrum c)FT IR analysis d) SEM analysis e) EDX

3.4 Antioxidant activity

Antioxidant activity was studied using in-vitro methods for the ability of the synthesized Fe_2O_3NPs using lichen extract. The results showed that Fe_2O_3NPs exhibited free radical scavenging property against DPPH, ABTS and H_2O_2 free radicals. Free radical scavenging activity for DPPH radicals with an IC50 value of 6.5±0.4µg/ml. ABTS free radicals was scavenged for the with the IC50 value of 4.1 ± 0.2µg/ml. Fe_2O_3NPs effectively inhibited H_2O_2 free radicals with an IC50 value of 18.8±0.05µg/ml. The rate of inhibition 90% was observed for DPPH and H_2O_2 free radicals at a concentration 50µg/ml. This showed that the antioxidant activity of the Fe_2O_3NPs was concentration dependent and at a higher concentration of the Fe_2O_3NPs efficient scavenging activity was obtained. Goga et al.[12] stated that lichen are rich in bioactive compounds especially phenols and flavonoids that are incredible antioxidants and aid in the reduction and capping of NPs.

3.5 Anticandida activity of R. fastigiata Fe_2O_3NPs

The inhibitory activity of selected candida strains were tested using R. fastigiata Fe_2O_3NPs. The results showed that R. fastigiata Fe_2O_3NPs were effective in suppressing the growth of Candida species harmful yeast pathogens in well diffusion method. At 100µg/ml the anticandida activity was observed in the order of susceptibility as follows C. albicans > C. parapsilosis > C. tropicalis> C. krusei> C. glabrata respectively. C. albicans was highly vulnerable to Fe_2O_3NPs at a concentration of 100µg/ml. The anticandida activity was concentration dependent, at higher concentration of Fe_2O_3NPs the zone of inhibition increased 19.3±1mm. The ability of Lichen-based nanoparticles have to destroy cell membranes, oxidize the cellular components and produce hydroxyl free. Similar studies was reported using plant extract Allium species, Allium ampeloprasum and Furcraea foetida [13]. Only a few studies on the antifungal effect of Fe_2O_3NPs have been published as the topic has received only marginal attention [14].

Table1: Anticandidal activity of R. fastigiata Fe_2O_3NPs

Organisms	Zone of Inhibition (mm)			
	25µl	50µl	75µl	100 µl
C. albicans	5.3 ± 1.2	11.2 ± 2.1	14.2 ± 1.5	19.3 ± 1.1
C. parapsilosis	3.2± 0.2	8.3 ± 0.3	10.4 ± 3.1	16.1 ± 1.2
C. tropicalis	2.1± 0.2	6.3 ± 0.3	8.4 ± 5.1	13.3 ± 1.3
C. krusei	2.4 ±1.3	4.5 ± 2.1	6.1 ± 3.2	11.3 ± 3.1
C. glabrata	2.0 ± 1.2	3.9 ± 1.2	4.1 ± 1.4	7.1 ± 1.4

4. Conclusion

The present study showed an eco-friendly, non-toxic, reasonable and superficial approach to synthesize Fe_2O_3NPs using R. fastigiata extract induced by high altitude lichen species. FTIR and GCMS analysis shown the role of lichen compounds as strong reducing and capping agents in the biosynthesize process. The study stated that lichen Fe_2O_3NPs more effective in antioxidant property that was evaluated using in-vitro assays and the anticandida activity. Fe_2O_3NPs synthesized from lichen extract was safe and can be used in modern drug development.

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