Abstract

Fresh or dried leaves of Moringa Oleifera (MO) are an excellent dietary supplement because they are rich in micronutrients. All parts of the tree are used in traditional medicine. In order to promote medicinal plants, a preliminary study using spectroscopic methods (UV-Vis - NIR absorption, transmission and fluorescence) was performed on extracts obtained from fresh leaves of Moringa Oleifera (MO) using different solvents. We also investigated the modification of the optical properties of the mixing of gold nanospheres with MO leaf extract and with a BSA biomolecule (Bovine Serum Albumin). Extractions from leaves contain chloroplasts which are natural bioactive chemicals that are a source of energy and contain carbon building blocks that have potential pharmaceutical uses. The change in the recorded UV–vis spectra would show that the interaction leads to the formation of nanoparticles-chloroplast complexes. The adsorption of gold nanospheres on chloroplasts would be a step toward developing bio-sensing and drug delivery.

Keywords: Biomedical, Gold Nanospheres, Moringa Oleifera, Plants, UV-Visible.

1. Introduction

The use of plants or vegetables as sources of drugs dates back to a long time ago. Moringa Oleifera (MO), often simply called Moringa, is a small tree species measuring up to 10 meters in height. It is native to northern India and is now naturalized in almost all tropical regions. It is resistant to drought, has a rapid growth cycle and is found in fields and gardens in West Africa and Asia. The MO plant, especially the leaves, is eaten in various forms and for many purposes[1]. The leaves are sometimes chewed fresh or crushed, or boiled into a vegetable sauce. Crushed or whole

dried leaves are also used in various beverages and in porridge or pasta, in alcohol, etc. for various potential applications. The Moringa tree is rich in photochemical compounds and has medicinal and nutritional properties (anti-inflammatory, etc.)[2-5]. Its main uses are shown in

Figure 1[1].

The mineralogical composition of plants is of a great interest because they are often consumed, even in traditional medicine, without special pre-treatment. It is necessary to control what patients ingest since the therapeutic effect of plant leaves depends on their organic and inorganic composition. To identify the different properties of leaves in their forms of consumption and the recommended forms, we carried out a spectroscopic study of MO leaves. The estimation of the leaves’ biochemical content or the simulation of their appearance is based on knowledge of their reflectance, transmission and absorption of light.

In the present work, we investigated the MO leaf extracts obtained with solvents such as acetone or Ethyl Alcohol 95°, or hot water. The study of the leaf extract is a prelude to our prospects of plant-based bio-synthesized nanoparticles[6-12] for antimicrobial applications[2-5]. We achieved the mixture of gold nanoparticles with an extraction of young MO leaves and an adsorption of the nanoparticles on the BSA biomolecule (Bovine Serum Albumin). The optical and electronic properties of gold nanoparticles are tunable by changing the size, shape, surface chemistry, or state of aggregation. More recently, optoelectronic unique properties of nanoparticles have been studied and used in high technology applications such as organic photovoltaics, sensory probes, therapeutic agents, drug administration in biological and medical applications, electronic devices and catalysis[13-15].


Figure 1. Uses of different parts of Moringa[1].


2. Material and methods

Fresh MO leaves were washed with distilled water before being crushed and were left in various solvents for 30 minutes before they were filtered and used. Some fresh and washed MO leaves were cut with scissors and introduced into hot water in a beaker. The solution was cooled and filtered before use.

Colloidal gold nanospheres were mixed with the extract of MO leaves at a volumetric ratio of 1:1 and were slowly stirred by hand for few minutes.

2.1. Reagents

Ethyl Alcohol 95°, acetone, distilled water and hot water were used. Our collaborators from ICEI (UBB - Cluj-Napoca, Romania) offered us the phosphate buffered saline (PBS), bovine serum albumin (BSA) and gold nanospheres.

2.2. UV-Vis-NIR spectrophotometry

The optical absorbance, fluorescence and transmittance spectra of the prepared solutions were recorded using a SM120 spectrometer from CVI Spectral and a SILVA NOVA spectrofluorimeter from StellarNet.

3. Results and discussion

3.1. Spectroscopic study of the Moringa leave extract

Nowadays, syntheses of nanoparticles based on medicinal plants were investigated\(^\text{[6-12]}\). The absorbance spectrum of the hot water filtrate has two peaks at 305 nm and 404 nm (Figure 2). The absorbance spectrum of the extract at room temperature with Ethyl Alcohol 95° or acetone showed two main peaks relating to chlorophyll a and b.

In the case of hot water, the heat may have dissolved some chloroplasts. The observed peak would be the presence of other chloroplasts having withstood the heat or simply that of other conformations resulting from transformation under the effect of temperature of chloroplasts\(^\text{[16]}\).

The fluorescence spectra of the Moringa green leaf extract are presented in Figure 3. The fluorescence spectra of the filtrate from hot water, under an excitation at 390 nm, has a broad peak centered around 530 nm. The extraction with acetone has a fluorescence spectrum with three peaks at 545, 685 and 736 nm, respectively. On this spectrum, there are two main areas of fluorescence bounded by: 450-600 nm and 650-775 nm. For these areas, it would be the blue-green fluorescence and red fluorescence and infrared fluorescence, respectively. The red and near-infrared fluorescence generally emanates from chloroplasts, sources of leaf chlorophyll. As for the blue-green fluorescence, it would come from the aromatic

![Figure 2. Absorbance of Moringa green leaf extract](image)

![Figure 3. Fluorescence spectra of Moringa green leaf extract](image)

compounds that are found in the leaf’s epidermis (polyphenols, coenzymes, alkaloids)\cite{cerovic1999ultraviolet}. The ratio of chlorophyll/polyphenols is a determining factor for agronomic forecasts through to prognosis on the need for nitrogen fertilization\cite{cartelat2005optically}. The chlorophyll fluorescence measurement gives the stress disturbing of the plants’ photosynthetic activity\cite{kalaji2010use}.

3.2. Study of complexes based on gold nanoparticles

We characterized the nanospheres obtained from ICEI Cluj-Napoca (Romania) for bio-sensing. Figure 4 shows the transmittance of gold nanoparticles (gold nanospheres, GNPs) and that of a biomolecule BSA (bovine serum albumin). It shows an absorption between 450 and 600 nm for gold nanoparticles and before 300 nm (about 280 nm)\cite{dzagli2010study} for the BSA. The SM 120 spectrometer used this time has its range set between 290 and 1100nm.

It is noted in Figure 4 that in the near infrared where the sample is not absorbent the transmission the coefficient is close to 100%, while transmission falls to about 10% or less and in the UV - visible spectrum where the sample’s absorbance is strong.

Figure 5 presents spectra obtained also by the SM120 spectrometer. These are absorbance spectra of gold nanospheres and of the mixture of nanoparticles and extract of young Moringa leaves and adsorption of nanoparticles on biomolecules BSA, respectively. The absorbance spectrum of gold nanospheres admits a peak at 517 nm. With the addition of the biomolecule BSA,

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Transmittance spectra of nanoparticles and complex GNP @ BSA}
\end{figure}

\footnotesize


a first peak forms around 523 nm due to the complexes obtained based on the biomolecule BSA, but over time, a blue shift is observed with the formation of a new peak between 380 and 450 nm during its evolution. These observations confirm that an interaction is performed between the BSA and GNPs. One can see on Figure 5 that the absorbance spectrum’s peaks of the obtained complex become broader and move towards the longer wavelengths. The moving of the peaks to longer wavelength indicates the formation of gold nanoparticle aggregates\textsuperscript{[15, 21]}. The absorbance spectrum of the direct mixture of gold nanospheres and the leaf extract admits a peak around 657 nm and two secondary peaks at 560 and 602 nm. The absorbance spectrum of the gold nanospheres’ mixture and the biomolecule BSA after 15 minutes, and the extract of the leaf admit a peak around 663 nm. The evolution of this complex is provided by the changes induced on the spectra. This result confirms the detection of biomolecules, which is of paramount importance for the discovery and development of biomolecular mechanisms and medical diagnostics.

**Conclusion**

During this exercise, we investigated the MO leaves’ extract and demonstrated the interaction of GNPs with biomolecules and MO leaf chloroplasts by UV-VIS spectroscopy, fluorescence and transmittance. This contributes to the study of the extract of MO leaves and demonstrates the feasibility of using nanoparticles to characterize therapeutic solutions. Future research will investigate the synthesis of Ag nanoparticles from the leaves of medicinal plants for biomedical applications.