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Published in:
Talanta Open

DOI:
10.1016/j.talo.2025.100500

Published: 01/12/2025

Document Version
Publisher's PDF, also known as Version of record

Talanta Open 12 (2025) 100500

Contents lists available at ScienceDirect

Talanta Open

journal homepage: www.sciencedirect.com/journal/talanta-open

Intrinsic fluorescence in *Moringa Oleifera* seed extract

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ARTICLE INFO

Keywords:
Autofluorescence
Moringa oleifera
Amino acids

ABSTRACT

Autofluorescence, an intrinsic property of biological molecules that emit light upon excitation, is gaining significance as a noninvasive technique for studying plant-derived bioactive compounds. This phenomenon enables detection of biomolecules such as chlorophyll, lignin, flavonoids, and alkaloids without requiring external dyes or destructive extraction processes. Intrinsic fluorescence can also aid in detecting and identifying biomolecules that typically require extensive sample preparation (molecule labelling with external fluorophores), eliminating the need for external probes. The present study aims to report the intrinsic autofluorescence in the *Moringa Oleifera* plant, highlighting its potential to enhance noninvasive bioactive compound identification and quality assessment in plant-based products.

1. Introduction

Autofluorescence, the natural emission of light by biological molecules upon excitation, has recently emerged as a useful plant-based pharmaceutical characterization tool. It can sometimes interfere with conventional fluorescence labeling techniques, but it can offer a noninvasive, label-free method for analyzing plant tissues and identifying bioactive compounds. Various plant biomolecules, including chlorophyll, lignin, phenols, flavins, quinones, alkaloids, and terpenoids, alongside proteins and polypeptides, can contribute to autofluorescence, emitting light in distinct spectral regions. This enable spectral imaging and compound differentiation based on unique fluorescence signatures [21].

Moringa Oleifera, which is widely recognized for its reported nutritional and medicinal properties, contains a diverse array of bioactive compounds that can contribute to fluorescence [20]. Polyphenols and flavonoids in Moringa leaves and seeds, isothiocyanates and glucosinolates [25], as well as proteins, peptides, and phytosterols [17] in its seeds, all exhibit varying degrees of fluorescence. Additionally, these compounds may contribute to Moringa's reported health benefits—such as antioxidant, anti-inflammatory, and neuroprotective effects [8–10].

Analysis of autofluorescence at multiple excitation wavelengths, generates color-coded fluorescence images that can map bioactive compounds across different plant tissues [16]. This technique enhances spectral separation, allowing for more precise identification of phytochemicals while minimizing the need for destructive extraction methods. In pharmaceutical research and quality control, fluorescence imaging can be used to monitor the composition of Moringa-derived supplements. Autofluorescence-based detection methods may thus offer new insights into the bioavailability and metabolic pathways of Moringa compounds in potential medical, cosmetic or food applications [6].

Plant autofluorescence remains under explored in *Moringa Oleifera*. A deeper understanding of these properties could lead to improved methods for characterizing its bioactive compounds.

2. Methodology

2.1. Preparation of Moringa Oleifera (MO) seed extract

The extraction of *Moringa Oleifera* (MO) seed components was carried out using *Moringa Oleifera* seed powder from Indian origin and it involved two-step procedure. In the first step, MO seed powder (50 % w/v) was mixed with Milli-Q water in a centrifuge tube and stirred at 80 rpm for 24 h using an IKA Trayster digital stirrer (IKA Werke GmbH & Co. KG, Staufen, Germany). The mixture was then filtered through a 0.2 mm Nalgene filtration unit (Thermo Scientific) connected to a Membran vacuum pump (Vacuubrand GmbH +CO KG, Wertheim, Germany).

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https://doi.org/10.1016/j.talo.2025.100500
Received 25 March 2025; Received in revised form 2 June 2025; Accepted 9 June 2025
Available online 9 June 2025

After filtration, the pH of the filtrate was adjusted to 9 using a Metrohm pH meter (Mettler Toledo, USA). The samples were stored at −25 °C overnight in a Liebherr refrigerator (Liebherr, Bulle, Switzerland), after which they were freeze-dried for five days using a Labconco Freezone dryer (Labconco, Missouri, USA). The freeze-dried samples were later re-dispersed in buffer at pH 9 for further analysis.

Adjusting the pH of *Moringa Oleifera* seed particles from their original acidic pH (~3) to an alkaline pH (~9) enhances their functional performance. At alkaline pH, increased protein solubility, reduced particle aggregation, and improved surface charge stability are observed, leading to better dispersion and bioavailability. This pH shift also activates phenolic and carboxyl groups, enhancing reactivity for applications such as coagulation, biosorption, and antioxidant activity. In contrast, acidic conditions promote aggregation and limit functionality, making pH 9 more favorable for diverse applications involving *Moringa Oleifera* [22].

2.2. Confocal microscopy

MO seed extract at pH 9 was observed by Zeiss® LSM 710 confocal laser scanning microscopy (Jena, Germany) equipped with a Zeiss AXIO Examiner Z1 inverted microscope with 63 × 1.4 DIC water immersion lens (Zeiss Plan-apochromat). Samples were auto-fluorescent and excited at a wavelength of 488 nm, and the emission range was from 499 to 629 nm. Images were acquired using Zeiss Zen software and analyzed using Fiji-ImageJ software. The histogram obtained represents emission spectra.

2.3. Amino acid profiling

The analysis was performed as triple determination; 30 mg of solid sample is weighed in a 4 mL glass vial using the appropriate vials for hydrolysis. The weight was recorded to four decimal digits. 1 ml of 6 M HCl was added, covered with nitrogen, seal, and hydrolyzed in a heating cabinet. Samples are heated at 110 °C for 18 h, then cooled before filtration. pH was adjusted by adding 1.5 ml of 0.2 M KOH and 1.6 ml buffer and transfer 1 ml for Agilent HPLC 1100 instrument (Santa Clara, CA, USA) coupled with an Agilent ion trap mass spectrometer. Amino acids were identified based on retention time and mass spectra from external standards. Calibration curves were prepared and measured by HPLC-MS.

Samples and standards were analysed using a mobile phase consisting of 10 mM Ammoniumformiate and acetonitrile(A) and 10 mM Ammoniumformate in water (W); flow rate is 0.5mL/min and injection volume is 1 mL at different gradients.

3. Result and discussion

3.1. Confocal imaging

Fig. 1 illustrates the natural fluorescence exhibited by *Moringa Oleifera* (MO) seed extract, demonstrating that it contains compounds capable of emitting light without the need for external fluorophores or dyes. The fluorescence is bright enough to stand out from the background and spans a broad range of wavelengths. The emission appears in a particulate or aggregated pattern, suggesting the presence of fluorescent biomolecules that may be forming clusters. The intensity and distribution of fluorescence provide insight into how the extract disperses in the solvent. It must be noted that the buffer used as the solvent does not exhibit any fluorescence under the same imaging conditions, confirming that the observed emission originates solely from the MO seed extract.

The dominant green fluorescence hints at the presence of naturally occurring fluorophores such as flavonoids and phenolic compounds. Flavonoids like quercetin and kaempferol are known to fluoresce when excited in the visible range, though their emission characteristics can shift depending on factors such as solvent polarity and pH. Additionally, if residual chlorophyll is present, it may contribute weak fluorescence in the green region. Given that these compounds are well-documented constituents of MO, they likely play a role in the extract's intrinsic fluorescence [18].

Previous studies have reported fluorescence in Moringa leaf extracts, with one study identifying a broad emission peak around 530 nm. This blue-green fluorescence was attributed to aromatic compounds such as polyphenols, coenzymes, and alkaloids [7]. Additionally, other research indicates that fluorophores like lignin and flavonoids can be excited at 488 nm, emitting within the 500–700 nm range. Similarly, tannins exhibit fluorescence when excited at 488 nm, with emission spanning 490–700 nm, while coumarins fluoresce between 500 and 600 nm when excited in the 400–500 nm range [3]. Another key class of natural fluorophores includes cofactors, with NADH (reduced β-nicotinamide adenine dinucleotide) being one of the most prominent contributors to biological fluorescence [15].

Broadband emission has been observed across nearly all amino acid and protein samples. The persistence of broad emission spectra confirms the presence of multiple fluorophores. However, this broad emission may limit the specificity needed for precise fluorophore identification

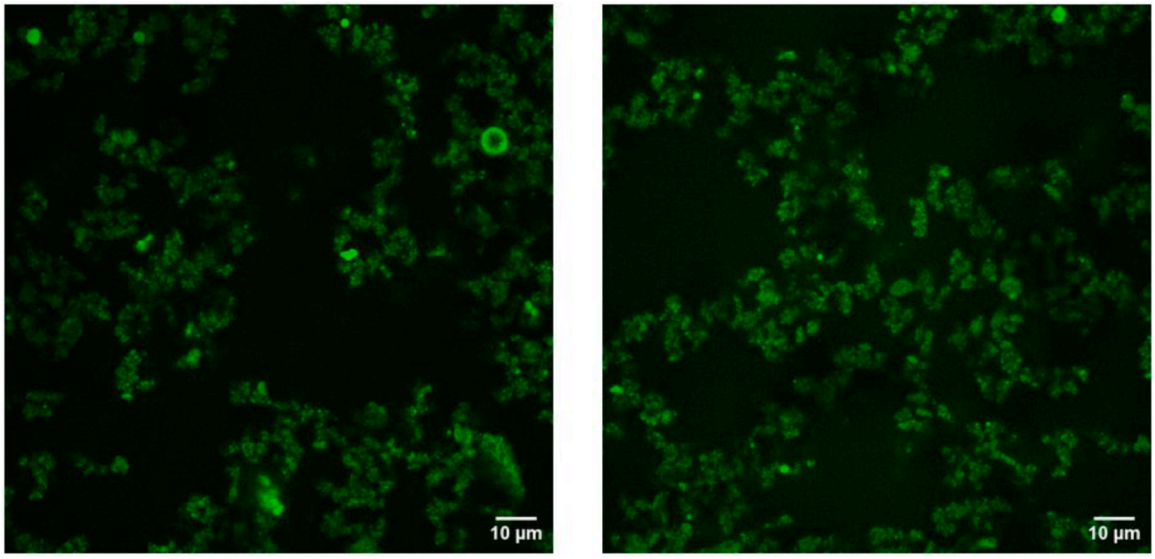

Fig. 1. Confocal images of *Moringa Oleifera* seed extract.

[23]. Emission in this region could also be dependent on various other fluorophores, such as Quinine, Suberin, Flavonoids (emitting in 400–600 nm), Ferulate (450–650 nm), Kaempherol (500–800 nm), Stilbenes (490–700 nm), Riboflavins (480/540 nm) which are present in plants. The blue-green fluorescence (450–600 nm) is mainly derived from aromatic compounds in the leaf epidermis or seeds, such as polyphenols, coenzymes, and alkaloids, with specific examples including flavonoids (e.g., quercetin and rutin) and phenolic acids [6,7].

The most relevant fluorescence features identified in this study were emissions in the ranges of 480–640 nm as seen in Fig. 2. These bands were primarily associated with amino acids, phenolic compounds, and oxidation products, as well as pigments that contribute to the fluorescence signature of natural extracts. This broad spectral response underscores the complexity of MO seed extract fluorescence and highlights the need for further investigation into the specific compounds responsible [19].

The emission intensity is not a reliable parameter for identification, as it fluctuates within the sample. Factors such as excitation power, background fluorescence, and sample concentration can influence intensity, making it unsuitable for quantitative identification [23].

3.2. Amino acid profile

In addition to plant-based fluorophores, aromatic amino acids—namely tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phe)—are known to contribute to the intrinsic luminescence of proteins and peptides. However, the exact mechanisms underlying their fluorescence, particularly their contribution to unusual blue emission, remain unresolved [5].

Amino acid profiling was made using mass spectrophotometer as described above in the methodology section, on MO seed extract at pH 9 along with original MO seed powder, shown in Table 1. The values are given in mg/g of the sample, with a comparison to FAO/WHO reference values for essential amino acids. These data are relevant for the intrinsic fluorescence of MO seed extract, as several amino acids especially aromatic ones contribute to fluorescence. Among the aromatic amino acids that could be fluorescence contributors, tryptophan was not detected, while the phenylalanine and tyrosine were present and increased with pH change. This can contribute to the overall emission profile.

Other non-aromatic amino acids such as histidine, proline and glycine also increase with pH and though its not a fluorophore in itself, it can interact with other compounds which can influence fluorescence indirectly [24]. Even if free aromatic acids do not fluoresce at this excitation, their interaction with flavins, phenolics can contribute to the intrinsic green fluorescence. Free amino groups can react with phenolic compounds to form Schiff bases or quinonoid structures, these compounds exhibit redshift fluorescence, emitting in green region [4]. The interaction of amino acids with sugars can lead to AGE (Advanced Glycation End) products, which exhibit green-yellow fluorescence especially in protein rich plants [1]. Phenylalanine can form complexes with polyphenols, which may shift their fluorescence to longer wavelengths. Tyrosine when complexed with flavins or phenolics can participate in electron transfer and fluoresce at higher wavelengths [13]. Cysteine forms disulfide bonds and interacts with quinonoid structures, which may enhance fluorescence [11]. Histidine and lysine contain amine groups that can form Schiff bases with phenolics or flavins [2]. Arginine has a highly reactive guanidine group which can bind to flavins to enhance fluorescence [12]. These interactions may lead to a red-shift fluorescence, explaining why green fluorescence is observed. Also at higher pH, amino acids can alter the electron exchange of flavins and polyphenols, possibly shifting their fluorescence to longer wavelengths, which could be the case in this extract at pH 9. Endogenous fluorophores, such as carbonyls, also serve as the main molecular components responsible for fluorescence in amino acids. These carbonyl-based emitters can generate bright, well-defined spots with high emission counts [23].

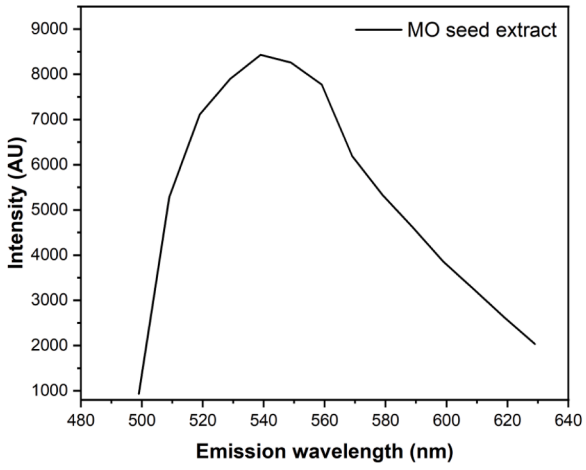

Fig. 2. Excitation-dependent emission spectra of *Moringa Oleifera* seed extract. AU: arbitrary unit from the Zeiss® LSM 710 confocal laser scanning microscope.

Table 1
Amino acid composition of *Moringa Oleifera* seed and extract at pH 9 obtained from mass spectrophotometry.

AMINO ACIDS (mg/g sample)	Original seeds ± Standard deviation	MO extract at pH 9 ± Standard deviation	FAO/ WHO ref [14]
Essential amino acids			
Lysine	3.4 ± 0.1	2.9 ± 0.2	4.2
Threonine	5.7 ± 0.4	9.3 ± 0.3	2.8
Valine	9.3 ± 0.4	16.3 ± 0.5	4.2
Methionine	2.4 ± 0.3	6.6 ± 0.4	2.2
Isoleucine	5.8 ± 0.2	11.1 ± 0.2	4.2
Leucine	10.8 ± 0.4	19.8 ± 0.2	4.2
Phenylalanine	6.7 ± 0.2	10.9 ± 0.2	2.8
Tryptophan	ND	ND	ND
Conditionally essential amino acids			
Proline	14.7 ± 0.4	27.3 ± 0.5	
Glycine	13.1 ± 0.3	24.5 ± 1.1	
Arginine	32.2 ± 2.3	86.3 ± 2.3	
Cysteine	4.2 ± 0.7	17.4 ± 1.5	
Tyrosine	3.7 ± 0.3	5.3 ± 1.0	
Histidine	3.0 ± 0.3	6.0 ± 0.7	2.1
Non-essential amino acids			
Alanine	9.9 ± 0.3	18.2 ± 0.2	
Aspartic acid	14.8 ± 0.1	19.5 ± 0.8	
Serine	9.5 ± 0.4	16.4 ± 0.6	
Glutamic acid	51.9 ± 1.5	110.1 ± 3.1	

4. Conclusion

Moringa Oleifera (MO) seed extract shows intrinsic fluorescence that could be influenced by tyrosine and phenylalanine, along with naturally occurring fluorophores such as flavonoids and phenolics. The significant increase in amino acid concentration at pH 9 suggests that the extraction process enhances the availability of these biomolecules, which may contribute to the broad fluorescence emission observed. Further research is needed to precisely identify the fluorophores responsible for MO extract fluorescence, for enabling its potential use in future applications.

CRediT authorship contribution statement

Namrah Azmi: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Sachin Rathod:**

Writing – review & editing, Data curation. **Bruno Telli Ceccato:** Writing – review & editing, Data curation. **Rasmus Kranold Mikkelsen:** Writing – review & editing, Methodology, Formal analysis. **Jon Otto Fossum:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

Acknowledgment: This work received funding from Norwegian University of Science and Technology (NTNU). The authors would also like to thank Inge Houlberg Holmberg for performing the amino acid composition analysis at DTU.

Data availability

Data will be made available on request.

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