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Ensino, pesquisa e inovação em botânica 2

Jesus Rodrigues Lemos
(Organizador)

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APRESENTAÇÃO

A obra “Ensino, Pesquisa e Inovação em Botânica 2”, seguindo o foco do primeiro volume, continua transitando por esferas que permitem a possibilidade de percepção do quão ampla e abrangente é esta grande área das Ciências Biológicas, esta, por sua vez, um grande campo do conhecimento.

Este segundo volume também traz a oportunidade ao leitor de enveredar por caminhos nos quais verificará uma amplitude de pensamento acerca do que pode ser explorado, e, ainda, provocando neste leitor o alargamento das suas perspectivas de realização de investigações envolvendo as plantas, organismos sobre os quais tem-se percebido e constatado, cada vez mais, sua fundamental importância na manutenção salutar da vida no planeta.

Somente por questões de uma fluência sequenciada deste título, os capítulos foram trazidos concebendo seus perfis principais dentro da proposta geral, assim, primeiramente são trazidos estudos com enfoque direcionados especificamente ao ensino formal e não-formal de Botânica, seguidos de pesquisa básica com subáreas mais tecnicistas, entretanto, todas desembocando em vieses nitidamente inovadores.

Assim, objetivamente, desejo aos leitores que aproveitem ao máximo das informações aqui contidas, reproduzindo-as, aplicando-as e expandido seus horizontes.

Jesus Rodrigues Lemos

SUMÁRIO

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CAPÍTULO 4

OPTIMIZATION OF MICROWAVE -ASSISTED EXTRACTION OF ANTI-CANCEROUS CONSTITUENTS OF TURMERIC *(CURCUMA LONGA L.)* BY RESPONSE SURFACE METHDOLOGY

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ABSTRACT: Turmeric (*Curcuma longa L.)* is a significant plant among cultivated spice crop in Pakistan. It belongs to a ginger family (Zingiberaceae).Turmeric has been used as anti-oxidant and anti-cancerous and ant-inflammatory properties containing herb all over the world. The most active constituents of turmeric are curcumin and its derivatives demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) collectively called curcuminoids. The present work reports on extraction of curcuminoids through Microwave-assisted technique optimized by Response Surface methodology (RSM).Three independent variables ethanol concentration (10-95%), extraction time (2-4minutes) , extraction temperature(250-400ºC) were selected. Total 27% yield of curcuminoids was calculated after crystallization of curcuminoids. The higher yield calculated at (95% ethanol), at time of (4minutes) and at (400ºC) temperature. The presence of curcuminoids was confirmed by UV-visible spectroscopy and curcumin showed maximum absorbance at 430nm to 465nm wavelength in ethanol solvent. Separation of curcuminoids was carried out by thin layer chromatography (TLC) analysis and results showed retention factor (RF) value at 0.78, 0.55, and 0.48 as curcumin, demethoxycurcumin, bis-demethoxycurcumin respectively. It is concluded that theoretical yield calculated by RSM was comparatively very close to experimental yield and Microwave-assisted extraction technique optimized by RSM was easiest and advanced way to obtain pure curcuminoids.

1 I INTRODUCTION

There is some truth to the old age that cancer is as old as the human race, but paleo

pathologic findings indicate that tumors existed in animals in prehistoric times, long before men appeared on Earth (Hajdu, 2011). The Egyptians attempted to treat tumors and cancers with cautery, knives, and salts, and introduced arsenic paste that remained in use as ‘‘Egyptian ointment’’ until the 19th century (Kim et al. 2014). The Sumerians, Chinese, Indians, Persians, and Hebrews of the same epoch were partial to herbal remedies such as tea, fruit juices, figs, and boiled cabbage, but in advanced cases, they did not hesitate to resort to solutions and pastes of iron, copper, sulfur, and mercury(Castiglioni, 2011). They believed that cancer was initiated by natural causes. They rationalized that excess or deprivation of blood, mucus, bile, and other body secretions, particularly at old age, may induce cancer. Remedies for cancer were compiled by Pliny the Roman (AD 23-79), in his Materia Medica (Jasvir et al. 2018. He recommended compound herbal and other remedies for internal use in advanced cancer before or after attempted surgery. His most highly praised prescription was a boiled mixture of ash of sea crabs, egg white, honey, and powdered feces of falcons (Talmadge and Fidler, 2010).Tumor is a mass composed of a group of abnormal cells. There are approximately 200 types of cancer (Jiang et al. 2013).

The consumption of herbal medicines is increasing steadily throughout the world as an alternative treatment for a number of health problems including heart diseases, diabetes, high blood pressure and even certain types of cancer (Jasvir et al. 2018). Herbs and spices can modify micro biota which can stimulate growth within organisms that protect against cancer as well as within microorganisms that may serve to increase cancer risk (Chirstine et al. 2008)**.** Culinary herbs and spices generally serve as antioxidants but may also serve as pro-oxidants at higher exposures. Inflammation, tumergenesis, and carcinogen bio activation influence cancer risk and tumor behavior, but interventions which inhibit these processes can contribute to cancer(Schmidt and Weber, 2006).

Turmeric (*Curcuma longa* L.) is a spice that has received much interest from both the medical/scientific worlds as well as from the culinary world. Turmeric is a rhizomatous herbaceous perennial plant (*Curcuma longa L.*) of the ginger family zingiberaceae(Priyadarsini, 2014). Turmeric is known to possess antibacterial, anticancer, antifungal, Antioxidant and anti –inflammatory properties (Susan et al. 2017). The major component, curcumin, is a polyphenolic compound classified as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration(An et al. 2011). Curcumin is available in various formulations: capsules, tablets, ointments, energy drinks, soaps and cosmetics (Gupta et al. 2013). Curcumin is the main component of turmeric; it is also known as diferuloylmethane and is a yellow-orange crystalline solid (Zhu et al. 2017).

Recently it has been shown that phenolic compounds have an inhibitory effect on cancer and its ability to metastasize (Zhang et al. 2013). Curcuminoids, the principal pigment and bioactive compounds in turmeric, are composed of curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl-1,6-heptadiene-3,5-dione) and its derivatives desmethoxycurcumin (DMC) and bisdesmethoxycurcumin (BDMC), which have been widely studied for antioxidant,

anticancer, ant mutagenic, and antibacterial functions(Hu et al. 2016). Curcuminoids are used in cosmetics as ingredients in skincare products due to their antioxidant, anti-inflammatory, and anti-aging activities (Arct et al. 2014). Curcuminoids are yellow pigments whose main compounds are curcumin (70–75%), desmethoxycurcumin (10–20%) and bisdesmethoxycurcumin (5–10%) and represent 2–9% of the active components in turmeric (Sahne et al. 2017).

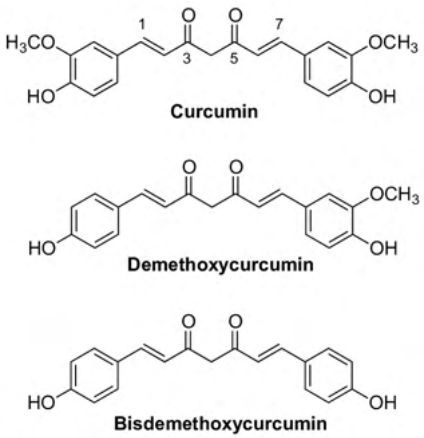


Fig: 1.1 Chemical structures of Curcuminoids.

In this research we are using microwave-assisted extraction technique optimized by response surface methodology. In the present time, the application of computer software technology in the research process has brought about remarkable results, gradually replacing traditional methods. One of the methods used to optimize the conditions that affect the extraction of plants is the response surface methodology. Response surface methodology (RSM) is a method used to simultaneously optimize two or more factors affecting the experiment process (Chandrap et al. 2018). Response Surface Method used to find the interaction of different affecting factors. Response surface methodology explores the relationship between different explanatory variables or different response variables. This method was first introduced by George E. P. Box and K. B. Wilson in 1951.Experiments is conducted at experimental designs and output is recorded (Chelladurai et al. 2021).

RSM is also capable of designing experiments. The designed experiments will consists of 12 or 20 experiments for optimization of 2 and 3 factors affecting the extraction process, respectively. In addition, RSM also facilitates the experiment detection and manipulation. Finally, with the simultaneous optimization of multiple factors, RSM results in the equations for prediction, and 3D models that describe the interaction of conditions on the response factor (Bahl et al. 2018).In the current stage, green technology has always been the subject of many studies Desai et al. 2014).A particular attraction in this field is microwave extraction because of its ability to transfer energy to the material, its environmental-friendliness and

most importantly, the ability to improve the quality(Abdurrahman et al. 2012) .

However, to improve the yield in the extraction of turmeric extract not only does the choice of extraction method matter, but it is also necessary to consider the optimization of the influencing conditions through response surface methodology (Bach et al. 2017). Microwave extraction and response surface methodology are used to extract turmeric extract and optimize the factors that influence the extraction process. (Tran et al. 2019). The requirement for the advancement of high performance in the extraction method is essential(Barzegar et al. 2019).

The aims and objectives of the present study were as follow; Optimization of extraction process by response surface methodology (RSM). To find a maximum yield of turmeric extract by Microwave-Assisted Extraction Technique and to characterize the Anti-Cancerous compounds of turmeric by different analytical techniques.

2 | MATERIALS AND METHODS

Turmeric (*Curcuma longa* L.) plant is used in this research. It belongs to (zingiberaceae) family. The main compound (Curcumin) present in turmeric is an important bio-active Constituent of turmeric. Yellow color of turmeric is due to this curcumin compound. There are lots of benefits we obtain from turmeric. It is used as spice, food coloring, and dyes. It has anti-cancer, anti-oxidant, anti-inflammatory effects that help in cure of cancer, inflammation, oxidative stress, joints pain and other diseases. The main focus of this research was to highlight the anti-cancer compounds of turmeric. For this purpose different techniques were used for the optimization of extraction process of turmeric and characterization of its anti-cancerous compounds were used.

Following techniques were used: Response Surface Methodology (RSM) for the theoretical prediction of the values, Microwave Assisted Extraction Technique (MAE)

1. Filtration
2. Ultra-Violet Visible Spectroscopy
3. Thin Layer Chromatography (TLC)

2.1 Response Surface Methodology (RSM)

Response surface methodology (RSM) was introduced by Wilson and Box. Response surface methodology is a collection of mathematical and statistical techniques based on a polynomial equation to the experimental data. It is an optimization tool that can identify interrelationship between variables as being adopted by experiment in food and herbal plants extraction. In this research Response surface methodology was applied for the optimization of extraction conditions. It is performed by using design expert software 8.0.7.1.

In Response surface methodology different steps were followed as

1. Central composite design (CCD)

- Analysis, Fit summary, ANOVA, Model graphs , Predicted vs. actual yield
- 3D Graphs

2. Final equation

2.2 Central Composite Design (CCD)

Before applying the Response Surface methodology (RSM), it is first necessary to choose an experimental design that will define which experiments should be carried out in the experimental region being studied. RSM helps to determine the best experimental design in order to identify the relationship between variables. Three independent variables Extraction time (X1), Extraction temperature (X2), Ethanol concentration (X3) were selected. After selecting variables in design expert it provided us 17 run at different ethanol concentration, extraction time, extraction temperature. CCD Model is presented in Table 1.

Factors	**Code**	**Levels**	
		Low level	High level
Ethanol concentration (%)	X1	10	95
Extraction time (Minutes)	X2	2	4
Extraction temperature (ºC)	X3	250	400

Table1: 3.3.1 Central composite Design (CCD) for the extraction of Turmeric (*Curcuma longa L.)*

2.3 Microwave Assisted Extraction

2.3.1 Principal of working

Microwave-assisted extraction (MAE) is an automated green extraction technique that offers many advantages such as the reduction of the extraction time and solvent consumption. MAE largely complies with the minimum criteria required for modern sample preparation techniques, and provides a very attractive alternative to conventional approaches for the extraction of organic compounds from a wide variety of matrices. MAE is a conventional technique for the extraction of active components from medicinal plants, using microwave energy to heat solvents containing samples, thereby partitioning analytes from a sample matrix into the solvent. The main advantage of MAE is its ability to rapidly heat the sample solvent mixture, resulting in its wide applicability for the rapid extraction of analytes, including thermally unstable substances. MAE can reduce the extraction time to < 20 min and solvent consumption below 20 ml. Furthermore, the recoveries obtained are usually higher than the conventional methods.

3 | EXTRACTION EXPERIMENTS

Fifteen experiments were performed by ultrasonic extraction method given by design Expert software

Runs	Ethanol Concentration (%)	Extraction Time (Min)	Extraction temperature	Solvent to drug ratio(g/ml)	Solution
1	10.00	2.00	250.00	1:10	20ml
2	10.00	2.00	400.00	1:10	20ml
3	52.50	1.32	325.00	1:10	20ml
4	52.50	4.68	325.00	1:10	20ml
5	52.50	3.00	325.00	1:10	20ml
6	95.00	2.00	250.00	1:10	20ml
7	95.00	4.00	250.00	1:10	20ml
8	52.50	3.00	325.00	1:10	20ml
10	95.00	2.00	400.00	1:10	20ml
11	10.00	4.00	250.00	1:10	20ml
12	52.50	3.00	198.87	1:10	20ml
13	10.00	4.00	400.00	1:10	20ml
14	52.50	3.00	325.00	1:10	20ml
15	52.50	3.00	451.13	1:10	20ml
17	95.00	4.00	400.0	1:10	20ml

Table1: 3.3.1 Central composite Design (CCD) for the extraction of Turmeric (*Curcuma longa L.).*

YIELD ESTIMATION

After cyrstallization of curcuminoids yield was calculted by dividing total dry weight of total extract by total weight of dry powder.

Yields of extracts obtained were calculated as follows :

$$\text{Yield (\%)} = \frac{\text{Total weight of recoverd extract}}{\text{Total weight of dry powder}} \times 100$$

4 I ANALYTICAL TECHNIQUES (FOR CHARCATERIZATION OF SAMPLES)

Results of Extraction of Curcuminoids

We performed fifteen experiments given by deign expert software. During first experiment ethanol concentration was 10% and extraction time 2 minutes and temperature was 250ºC.So total curcuminoids yield we calculated from this extracted solution was 5.810mg/2g.WE see that due to lower concentration of ethanol and lower temperature curcuminoids contents lead to less in yield. After this second experiment was carried out and we used 10% ethanol concentration and extraction time was 2 minutes and extraction temperature was 400ºC so yield we calculated was 6.621mg/2g.Agter third experiment was carried out and we used 52% ethanol concentration and extraction time 1.32 minutes and extraction temperature was 325ºC and calculated yield was 9.10 higher than above mentioned yield because of the higher concentration of ethanol and temperature than these experiments. After this during 4th experiment we used again 52% ethanol concentration extraction time was 4.68 and extraction temperature was 325 and we calculated the yield of 14.910mg/g due to the higher concentration of curcuminoids. During the fifth experiments we used 52% ethanol concentration and extraction time was 3 minutes and extraction temperature was 325. The calculated yield was 13.4mg due to the higher content of curcuminoids in the solution. During the 6th experiment we used 95% ethanol concentration and extraction time was 2 minutes and extraction temperature was 250ºC and calculated yield was 15.601mg due to the higher content of curcuminoids.

Fig:4.2.1 Extracted samplesafter Micro-wave assistedextraction.

TOTAL YIELD

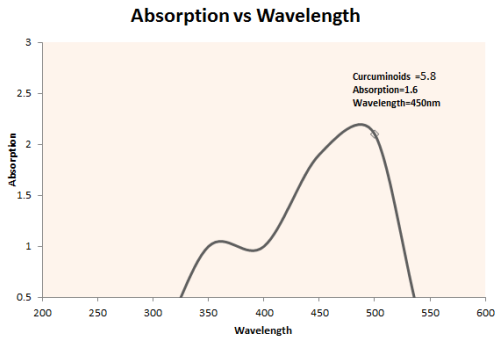
Total yeild weas estimated as follows :

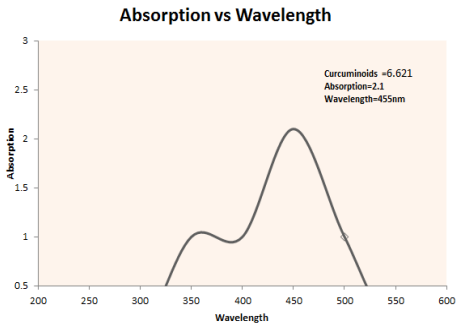
$$\text{Yeild (\%)} = \frac{\text{Total weight of recoverd extract}}{\text{Total weight of dry powder}} \times 100$$

$$= \frac{182.0561mg}{30g} \times 100$$

Total yield = 27% total number of curcuminoids

5 I UV-VISIBLE SPECTROSCOPY RESULTS





These graphical explanation of uv results shows that higher number of curcuminoids containing solution shows peak near to the standard peak of curcumin which is 423nm.So when we see the grapp Figure.(i) it shows peak at 455nm and it solution contains 5.810 number of curcuminoids content.In the next figure.(ii) it shows 465nm peak very farther away from the standard peak of curcumin due to the less amount of curcuminoids.Furthermore solution containing 9.160 curcuminoids yiled shows peak at 445nm and absorbance at 1.7.After this solution containing 14.910mg curcuminoids yield shows peak at 440nm and absorbance at 2.4.Next solution shows wavelength peak at 445nm and absorbance at 2.3.Furthermore solution containing 15.6mg curcumin yield shows peak at 435nm which is slightly closer to standard peak of curcumin.Solution containing 17.051 and 17.8 and 17.8 shows most closer peaks to the standard peak of curcumin so these are the best peaks we see.Solution containing 10.0 , 4.8, 6.625mg yield of curcuminoids shows lowest peak and farther away from standard peak because of the lower concentration of curcuminoids.So according to the results we conclude that solution containg higher content of curcuminoids shows good peaks near 430-435 nm and solution containing lower numbe rof curcuminoids shows lowest peak at 44—465nm.

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SOBRE O ORGANIZADOR

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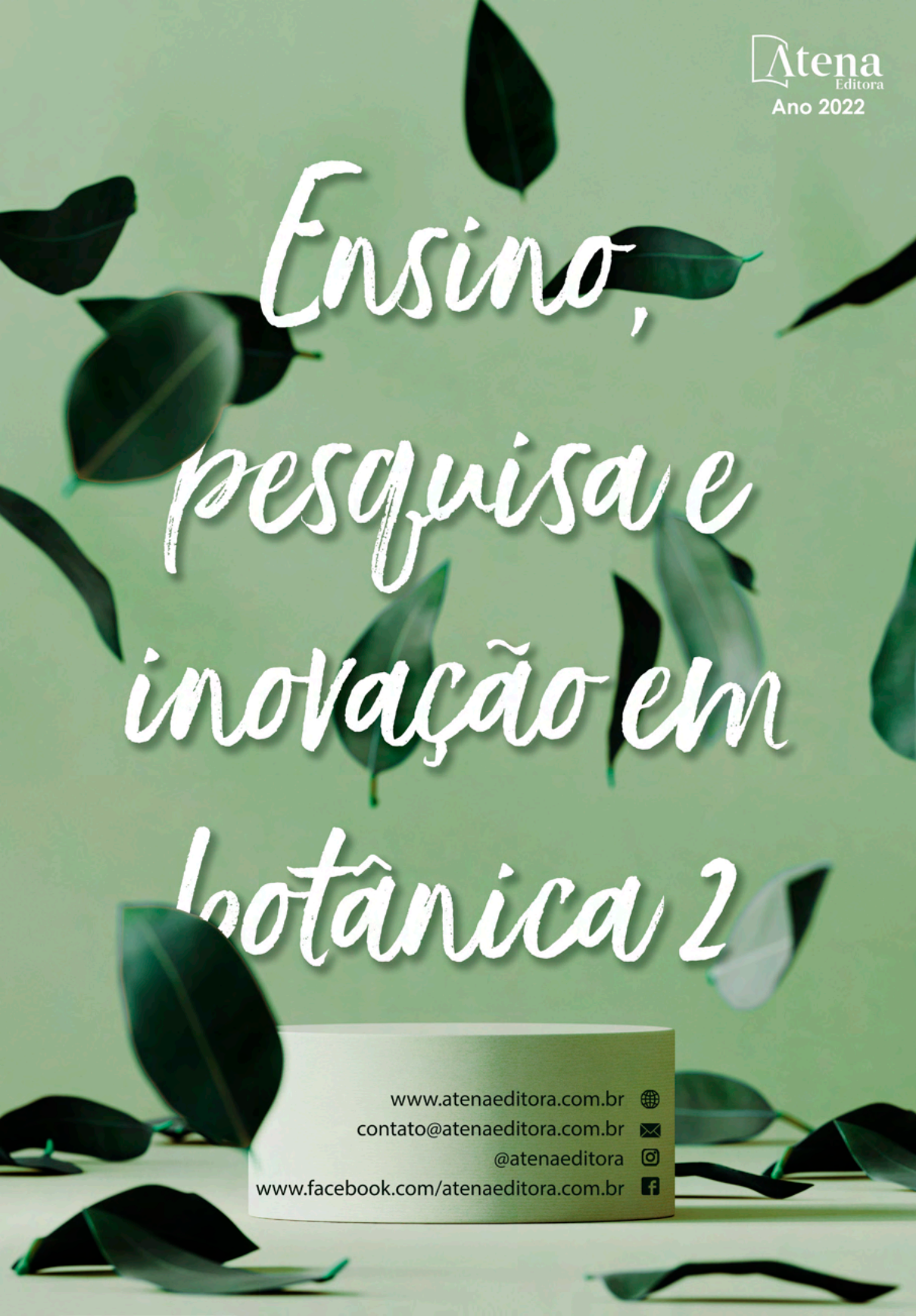
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