v. 2, n. 11, 2022

Journal of
Agricultural
Sciences
Research

FRAGMENTATION EFFECT OF LEAVES AND SEEDS GREEN FROM BLACK PEPPER (*Piper nigrum*) ON CONTENT AND YIELD OF ESSENTIAL OIL

Eduardo da Silva Bandiera
Universidade do Estado de Mato Grosso, Campus Universitário Professor Eugênio Carlos Stieler, Curso de Ciências Biológicas, Tangará da Serra - MT
http://lattes.cnpq.br/8308839445833882
https://orcid.org/0000-0003-1333-7131

Diones Krinski
Universidade do Estado de Mato Grosso, Campus Universitário Professor Eugênio Carlos Stieler, Curso de Ciências Biológicas, Tangará da Serra - MT
https://lattes.cnpq.br/9473229586446780
https://orcid.org/0000-0002-4719-5314

Abstract: The Piperaceae family comprises 5 known genera (*Macropiper*, *Zippelia*, *Manekia*, *Peperomia* and *Piper*). The species *Piper nigrum* L. is known as black pepper and in the Brazilian market it is widely used in the sausage, perfumery and pharmaceutical industries. In addition to industrial, pharmaceutical and food use, the essential oil (EO) of *P. nigrum* stands out due to its biological properties. Therefore, this work aimed to extract and quantify EO from green leaves and seeds from black pepper and compare the content and yield of extractions using different processing of these plant parts (whole and crushed). For this, green leaves and green seeds of *P. nigrum* were collected in the municipality of Tangará da Serra/MT for hydrodistillation of their EO using Clevenger apparatus for 4 h. As a result, it was found that the values of content and average yields extracted from seeds were almost five times higher when whole seeds were used. The amount of EO extracted from the leaves did not show significant differences between the extractions performed with whole and crushed leaves.
Keywords: Black pepper. Piperaceae. Secondary metabolite. Mato Grosso. Tangara da Serra.

INTRODUCTION

The Piperaceae family comprises 5 known genera (*Macropiper, Zippelia, Manekia, Peperomia* and *Piper*) with approximately 3,700 species distributed around the world, occurring mainly throughout the tropical region, usually in shady places (Brito and Pereira, 2019). In Brazil, the Piperaceae family is represented by the genera *Manekia*, *Peperomia* and *Piper*, the latter two having the largest number of known species (RITO et al., 2021).

In Brazil, the genus *Piper* L. has approximately 291 species (MELO; ALVES, 2019) (Figure 1). In this genus, the species *Piper nigrum* L. is popularly known as black pepper or black pepper, it stands out commercially in the domestic market, and can be used in the sausage, perfumery and pharmaceutical industries. According to Machado et al. (2021) black pepper is an indispensable ingredient in the preparation of various dishes of animal and vegetable origin.

Piper nigrum is classified as a perennial plant (EMBRAPA, 2004; GARCIA et al., 2000; PISSINATE, 2006; VELOSO et al., 1995) with a smooth, round, knotty and branched stem, whose leaves are entire, laminated, oval, with acute apex with 7 main veins. Its flowers are small, white and arranged in spikes, while its fruits are globular, red when ripe, and when they dry, they present a thick, rough and dark surface (GARCIA et al., 2000; PISSINATE, 2006).

This species is among the only spices in which its fruits can be marketed in four different versions of grains (black - grains mostly mature, threshed from the ears and dried in the sun or in dryers; white - grains threshed, dried and husked; green - still immature threshed grains; and red - ripened and threshed grains), although the most common form of marketing is black (EMBRAPA, 2004; PISSINATE, 2006; CARNEVALLIA; ARAÚJO, 2013).

Brazil stands out commercially with this species, as it is the first western country to produce black pepper on a large scale, with record productions each year, with the state of Espírito Santo at the top of the ranking, surpassing the state of Pará with production. above 60 tons since 2018 (MACHADO et al., 2021). In addition to the well-known industrial, pharmaceutical and food use for the species, the essential oil (EO) use of *P. nigrum* has also been highlighted in agricultural research due to its antimicrobial, insecticidal, nematicide and acaricide activity (RODRIGUES; OLIVEIRA, 2021), in addition to being used as an active agent to prolong the longevity of foods (DE MELO et al., 2021).

Due to these characteristics, several studies have been carried out with EO extracted from its seeds, which have a high content of piperine, an alkaloid characterized by the intense odor characteristic of this species (CARDOSO et al., 2005). Piperine has been the most abundant compound found in the EO of black peppercorns, which makes this chemical constituent important for the trade and study of the species (COSTA et al., 2021).

However, there are still few studies that evaluate the content and yield of EO from *P. nigrum* leaves, probably because its fruits have greater economic appeal and, consequently, greater scientific interest, which ends up focusing studies on this plant part, quite different from other studies with EOs have already been carried out on most species of the genus *Piper*, which generally use mainly leaves to obtain this raw material and consequent research (KRINSKI; FOERSTER, 2016; SANINI et al., 2017; KRINSKI et al., 2018ab; DE SOUZA et al., 2020).

Considering this, the development of research and methods for obtaining of *P. nigrum* OEs that generate results and advantages such as low costs and simplicity in extraction, aiming to generate efficient protocols for the production of the essential oil, in addition to its optimization, are necessary (TRAN et al., 2020).

Thus, the present work aimed to extract the OEs from the leaves and green seeds of black pepper and compare the content and yield of extractions from different processing of these plant materials (whole and crushed).

MATERIAL AND METHODS

Piper nigrum COLLECTION

The collection of fresh green leaves and seeds of *P. nigrum* was performed on plants grown in an urban backyard in Tangará da Serra/MT (coordinates 14º36'23" S 57º29'04" W, alt: 380 m) (Figure 1). The collections were carried out in the afternoon, between 14:30 and 18:30.

Figure 1. Leaves and spikes/peppers with green seeds of black pepper (*Piper nigrum*) collected in Tangará da Serra/MT. Source: the authors.

DETERMINATION OF MOISTURE CONTENT (MC%)

To determine the moisture content (MC%), after the collection of leaves and green seeds from *Piper nigrum*, samples were separated into triplicates of 20 g for drying in an oven at 50 °C, until constant weight (approximately 15 days). The moisture content was calculated using the equation:

$$MC\% = (wm - dm) / (wm) * 100$$

Where:
MC%= Moisture Content;
wm= wet mass (g);
dm= dry mass (g); and
100= conversion factor to percentage.

The determination of CM% was used in the EO yield calculations, more specifically the values of fresh plant mass (leaves and green seeds) in relation to the wet basis (FPM WB) and dry basis (FPM DB) of the plant material. The dry-based vegetable mass (FPM DB) was corrected using the equation:

$$FPM\ DB = ((100 - MC) * FPM\ WB) / 100$$

ESSENTIAL OIL EXTRACTION OF LEAVES AND GREEN SEEDS FROM *Piper nigrum*

Green leaves and seeds whole and crushed of *P. nigrum* were submitted to hydrodistillation for EO extraction, in a modified Clevenger-type apparatus, for 4 hours. In this method, the distilled oil is retained in a glass tube and the aqueous phase automatically returns to the distillation flask, being reused (SARTOR, 2009). The extractions were performed in triplicate of 100 g. The content of essential oil extracted from plant biomass was calculated based on dry matter or moisture free base (MFB), through the equation:

$$OC = (meo / dm) * 100$$

Where:

OC= Oil content (%);

meo= total mass of extracted essential oil (mg);

dm= dry mass of 100 g of plant material (g); and

100= conversion factor to percentage.

This equation was applied to determine the essential oil content in MFB, and the calculated value is expressed as a percentage, which corresponds to the weight (mg of essential oil per dry biomass obtained from 100 g of fresh plant material) and indicates the correct value of oil content contained in the dry biomass. For the EO yield, the total volume of EO obtained from the extraction of 100 g of fresh plant material was considered. To calculate the EO volume, we use the following equation:

$$VO = meo / de$$

Where:

VO= total volume of essential oil obtained from 100 g of plant material;

meo= Total mass of extracted essential oil (mg); and

de= density of the extracted essential oil.

The essential oil density was calculated using a 2-20 μL LabMATE® micropipette and a Shimadzu® AY220 precision balance, using the equation:

$$DE = m / v$$

Where:

DE = essential oil density;

m= calibrated mass in the micropipette (mg);

v= volume obtained after weighing on the scale (μL).

STATISTICAL ANALYSIS

The content and yield data were submitted to the assumptions of normality and homogeneity of variances. To analyze the results obtained, analysis of variance (ANOVA) performed by the F test was used with subsequent application of the Scott-Knott test to compare means with the aid of the statistical software Assistet version 7.7 beta (SILVA; AZEVEDO, 2016).

RESULTS AND DISCUSSION

Our results showed that there was a significant difference both in the content and in the yield of the EO obtained in extractions between different plant parts and between the processing or not of the green leaves and seeds of *P. nigrum* (Table 1). When we compared the fragmentation result in each plant part separately (leaves or seeds), we noticed that there was no difference, in the EO content and yield, in the extractions made with fresh green and crushed leaves (Figure 2). What was not observed in the extractions with the green seeds, which showed that the crushing process increases considerably obtaining the OEs of this plant part, both in terms of content and yield (Figure 2).

Source of variation	D.f.	F values	
		Content (%)	Yield (mm³)
Treatments	3	1384.1908**	1394.9813**
Residue	8	-	-
p-valor		<.0001	<.0001
C.V. (%)	-	5.63	5.28

D.f.= Degree of freedom; C.V.= Coefficient of variation; ***significant at 1%.*

Table 1 - Analysis of variance for content (%) and yield (mm^3) of essential oil from leaves and green seeds of whole and crushed from black pepper, *Piper nigrum*. Tangará da Serra/ MT, 2022.

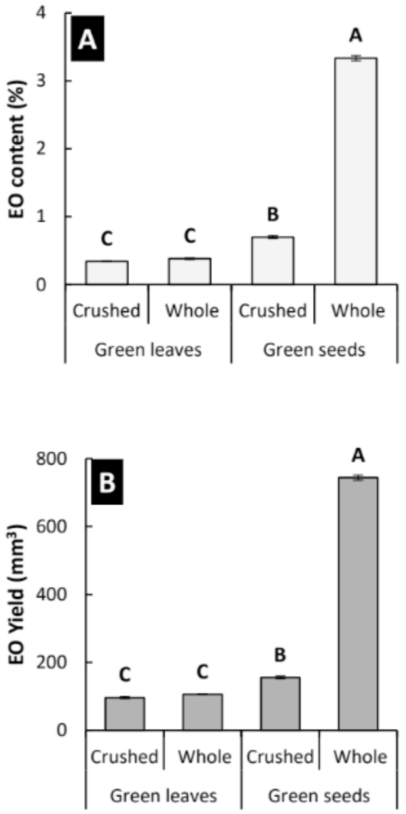

Figure 2. Amount of essential oil (EO) extracted from leaves and green seeds whole and crushed *Piper nigrum*. A) Content (%) of EO found in leaves and green seeds whole and crushed; B) Average yield (± standard error) of EO obtained from leaves and green seeds whole and crushed. Bars followed by different letters show a significant difference by the Scott-Knott test ($p \leq 0.05$).

The average values of content and yields found when the seeds were crushed were almost 500% higher than those extracted from whole seeds. According to May et al. (2007), the mechanical process of grinding this plant part before distillation tends to break some structures of the seeds, which leads to greater release of EO, in addition to which fragmentation also increases the contact area. On the other hand, the leaves showed no significant differences between the extractions performed with whole and crushed leaves (Figure 2).

These results corroborate what was described by Busato et al. (2014), when reporting that many times it is necessary to fragment the plant material to facilitate the removal of the EO. Our data further reinforces that processing can vary according to plant structure, where flowers, leaves and other thin, non-fibrous parts generally do not require shredding, while roots, stems and any woody material need to be cut to short lengths (GÜNTHER, 1948). And when we compared the plant parts of *P. nigrum* (leaves and seeds), we noticed that the seeds presented EO values up to 747% higher than the leaves.

Our work then shows that although the variation in the amount of EO during extraction can be influenced by environmental issues as already reported by Bustamante (1993) and Corrêa-Júnior et al. (1994), that the preparation of the material after its collection, with simple strategies, such as fragmenting the plant parts before carrying out the extraction, should be an issue to be taken into account, because these factors interfere directly on final quantity of the raw material of interest, in this case, the OEs. In addition, the crushing process increases the contact surface of the plant material used, as verified in the seeds of our work, allowing consequently a better distillation yield, as already reported by authors such as Geankoplis (1993) and May et al. (2007).

However, when we compare the different

plant parts, our results are in agreement with data found in research carried out with other species of the same genus of black pepper (*Piper*), comparing the yield between leaves and seeds, as in the studies with *Piper gaudichaudianum*, *Piper aduncum* and *Piper fuligineum*, where the leaves of these species also presented a lower amount of EO than the spikes/infructescences (structure where the seeds are located) (SCHINDLER et al., 2018; NUNES, 2021; OLIVEIRA, 2021).

This type of relationship in the amount of OEs between the different plant parts, such as leaves and seeds, is expected, especially when we consider that seeds are reproductive structures, and possibly the increase in metabolic rates due to the action of phytohormones also stimulate production. of nutrients for the formation of these reproductive organs (SILVA et al., 2015). And since secondary metabolites, such as EOs, are produced and stored, in most cases, in other plant tissues in the vegetative phases (such as leaves, branches and roots), during the reproductive phase of the plant they can be reallocated to structures such as fruits and seeds (AMARAL et al., 2015).

Thus, it is evident that there are several factors that affect the EO yield, whether environmental, edaphic and climatic, atmospheric pollution, as well as those inherent to the plant itself, such as the vegetative cycle, age and plant organ (LIMA et al., 2003; GOBBO-NETO; LOPES, 2007; FIGUEIREDO et al., 2008). And this type of study shows the importance of more research being carried out with plants of the same species, either by comparing different plant parts or different processes that can optimize obtaining a greater amount of OEs for their later use.

Furthermore, new studies with *P. nigrum*, such as those carried out with other *Piper* species, can also be carried out, testing, for example, post-harvest drying in order to further optimize the EO extraction process and thus reduce the time to obtain a greater amount of EO from this species (OLIVEIRA et al., 2021; OLIVEIRA, 2021; SANTOS et al., 2020; NISHIDA et al., 2020; RODRIGUES et al., 2020; and VIEIRA et al., 2020).

Research like these generate important information that improves the processes/protocols for obtaining EO from plants in general, and which provide that the products, when obtained in larger quantities, can be used in various scientific projects, as has already been done with other families and species of plants of economic interest (KRINSKI ; MASSAROLI, 2014; KRINSKI et al., 2014; KRINSKI; FOERSTER, 2016; SANINI et al., 2017; KRINSKI et al., 2018b).

CONCLUSION

Through this work we can conclude that: 1) when comparing the yield of different plant parts, the green seeds of *Piper nigrum* have a higher amount of EO than the green leaves, whether whole or crushed; 2) crushing the green seeds optimizes the obtaining of EO, increasing its quantity in relation to extractions with whole green seeds; and 3) the fragmentation of green leaves does not increase the amount of EO obtained from this plant part.

ACKNOWLEDGMENTS

The authors thank the Universidade do Estado de Mato Grosso (UNEMAT), University Campus Professor Eugênio Carlos Stieler, Tangará da Serra, for the logistical structure made possible to carry out this work.

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