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Phytochemistry Of *Piper Hispidinervum* Cultivated Under The Edafoclimatic Conditions Of Lavras, MG, Brazil

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ABSTRACT

An essential oil with a high safrole content produced by *piper hispidinervum* is among the secondary metabolites of economic importance and commercial interest. The present study evaluated the influence of time of harvest and the steam distillation process on the quantity and quality of this oil. Fresh leaves were collected at 8:00 a.m., 10:00 a.m., 1:00 p.m., 3:00 p.m. and 5:00 p.m. The material was dried in a forced-air oven at 30°C for a period of six days and submitted to steam distillation for periods of 1, 2, 3 and 4 hours. Gas chromatographic analysis demonstrated that the collection should be performed at the times of 8:00 a.m., 10:00 a.m. 3:00 p.m. and 5:00 p.m., with a preference for the morning period, but this parameter did not have a significant influence on the major component (safrole). The steam distillation should be performed during a one to two-hour period, depending on the requirements of the consumer market with respect to the safrole content of the oil. These preliminary tests should be performed to determine the operational procedure for the industrial scale extraction.

KEYWORDS

Piper hispidinervum;
pimenta longa;
essential oil;
safrole

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INTRODUCTION

The diversity of Brazilian flora represents a great potential for the production of primary and secondary compounds. These compounds have been continually demanded by industry in the last decades because of the increased utilization of natural products. The research for active substances derived from plants in Brazil is still very incipient. Of the 500,000 plant species that are estimated to exist in the world, about 16% are found in the Amazon forest[1]. Plant organisms represent a rich and complex resource for organic compounds with many stereogenic centers that actively participate in its growth and development. Such compounds, called secondary metabolites, originate via alternative biosynthetic routes and may be characterized as elements for cellular differentiation and specialization, performing a defense function for perpetuation of the plants in the ecosystem[2].

Among the secondary metabolites of major biological activity are the essential oils, which have great economic importance, especially for the food, pharmaceutical, perfume and pesticide sectors. For this reason, the number of studies on the constitution and the biological properties of these essences, as well as taxononic, environmental, and cultivation factors that lead to the variation in the quantity and quality of these oils, has slowly been growing[3]. An example of this type of study is the identification of the *piper hispidinervium* (pimenta longa) in the state of Acre via the Programa de Triagem de Plantas Aromáticas da Amazônia, carried out in the 1970's by INPA (Instituto Nacional de Pesquisas da Amazônia). In recent years, this plant has been stimulating a great interest on the part of national and international producers, processors of essential oils rich in safrole. This interest is a result of the extinction of the only plant in Brazil that furnished safrole, which is sassafras (Lauraceae family) from the natural habitats of the south of Brazil. Its exploitation was prohibited by IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) by decree No. 1557/91[4]. Safrole is an aromatic component that occurs naturally, having been used by the chemical industry as a starting material in the manufacture of heliotropine or piperonal, an important fixer of fragrancies, and the piperonyl butoxide (PBO), used as a synergistic agent in natural insecticides such as pyrethium (Figure 1).

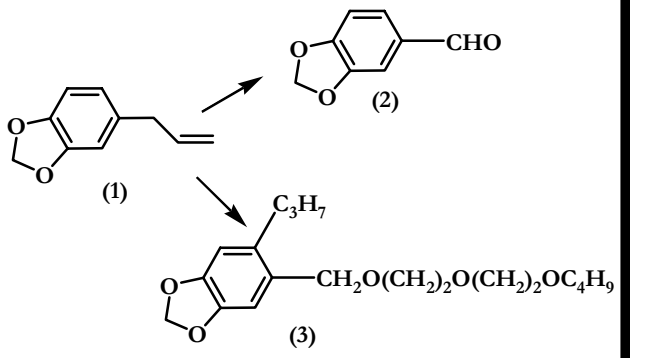

Figure 1: Chemical structures of safrole and its derivatives[4]. 1,Safole (4-Allyl-1,2-methy lenedioxybenzene); 2, Piperonal (Fixer of aromas); 3, Piperonyl butoxide(5-{[2-(2-butoxyethoxy) ethoxy] methyl}-6-propyl-1,3-benzodioxole; synergistic insecticide)

Although *piper hispidinervium* has stimulated commercial interest, its cultivation and the extraction of the essential oil require technical information on its production system, directed principally toward the quantity and quality of this secondary metabolite[5]. Since the existence and distribution of this essence and its chemical composition are quite variable in the plant species and depend on hereditary, ontogenic (developmental stages), and environmental (soil, climate, microorganisms, etc) factors[1,5,6-12], as well as the extraction process (distillation, extraction with CO_2, etc)[3], the present study attempted to evaluate the influence of the harvest time, as well as the steam distillation process on the quantity and quality of the essential oil produced by this piperaceae.

MATERIAL AND METHODS

The present study was conducted in the Laboratório de Química Orgânica of the Departamento de Química da Universidade Federal de Lavras-MG (UFLA). Dried *piper hispidinervum* leaves, cultivated in the medicinal plants garden of the university, located at 21°14' south latitude and 45°00' west longitude at an altitude of 920 m. The

local climate is of the Cwb type according to the Koppen international climatic classification, characterized by a rainy summer and a dry winter with an average annual precipitation of 1,411 mm and an average annual temperature of 19.3°C. The studies were performed during the autumn in the period from May to June. At this location, the autumn season has an average annual pluvial precipitation of 17.5 mm, an average temperature of 27.0 °C, 53.03% relative humidity and 6.9 hours of light per day[13].

Collection

The fresh leaves of *piper hispidinervium*, unattacked by pests or diseases, were collected at 8:00 a.m., 10:00 a.m., 1:00 p.m., 3:00 p.m. and 5:00 p.m. This procedure was conducted in three repetitions with 15-day intervals.

Climatic conditions

Temperature, relative humidity and sunlight data were collected at the site. The Hygrotherm, Ref. 7429, Marca MERSE apparatus was used for measurement of the first two types of data. The sunlight data were obtained from the university Climatological Station employing a heliograph (direct sunlight without clouds).

Drying

The fresh leaves were dried at 30°C in a forced-air oven for a period of six days[10].

Biomass humidity

Humidity was determined using 5.0 g of chopped leaves immersed in 125 mL of cyclohexane. The starting material was suspended in the solvent in a 500 mL volumetric flask coupled to a modified Clevenger apparatus. The mixture was heated at 100 ± 5°C. The distillate was collected in graduated tubes, the volume of water was measured and the content of water in 100 grams of sample was calculated[11].

Extraction of the essential oil

The extraction of the essential oil was achieved by steam distillation from 50 g of dried leaves using a modified Clevenger apparatus. The extractions were performed during one-, two-, three- and four-hour periods[7].

Qualitative and quantitative analyses

The distillates obtained above were centrifuged to eliminate the aqueous phase and to separate the essential oil. The volume and density of the essential oil were measured, and the yield was calculated based on the dry mass of the starting material.

The chromatographic analyses of the essential oil of *piper hispidinervum* were performed on a Shimadzu CG – 17A gas chromatograph equipped with a DB5 capillary column containing 35% phenylmethylpolysiloxane, at an injector temperature of 200°C; FID detector temperature of 210°C; temperature program with an initial temperature of 130°C for 4 min; increase in the column temperature to 150°C at 5°C/min, and a plateau at 150°C for 4 min. Nitrogen was used as the carrier gas with a split of 1:100. A 1-μL volume of the sample, diluted 1:40 (v/v) in dichloromethane, was injected. An internal standard of safrole (furnished by Embrapa-CTAA), diluted 1:50 (v/v) in dichloromethane was used for identification. Analyses were performed in triplicate.

Experimental procedure

The experiment was conducted in a random delineation with three repetitions, and the treatments were arranged in a 4 x 5 factorial scheme constituted by the factors: distillation times (1, 2, 3 and 4 hours) and collection times (8:00a.m., 10:00a.m., 1:00p.m., 3:00p.m. and 5:00p.m.). The yield of essential oil and the safrole content were the variables determined.

RESULTS AND DISCUSSION

Of the five times at which the *piper hispidinervum* was collected, only that collected at 1:00 pm furnished a lower yield of the essential oil in its biomass (2.05 %). There was no significant difference in the yields (2.22 and 2.64%) obtained at the other four times (8:00 a.m. 10:00 a.m., 3:00 p.m. and 5:00 p.m.). The highest yields of oil were obtained in the morning, from 8:00 am to 10:00 am, followed by the yields obtained in the afternoon, principally the biomasses collected from 3:00 pm to 5:00 pm. A rela-

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TABLE 1: Verage yields of the essential oil obained from the dried leaves of *piper hispidinervum* as a function of the time of collection

Collection time	Yield of essential oil* (%)
8:00 a.m.	2.64[a]
10:00 a.m.	2.34[ab]
1:00 a.m.	2.05[b]
3:00 a.m.	2.22[ab]
5:00 a.m.	2.27[ab]

***Means followed by the same letter do not differ at the level of 5% of probability according to the Tukey test. CV (%) = 10.27. Overall average = 2.31%.**

tionship between environmental factors (temperature and relative humidity) and the yield of this secondary metabolite was also observed. The highest yields were obtained at these same collection times at low temperatures and high relative humidity. The highest yield was obtained at 8:00 am. The density of the oil was 1.05 g/cm^3 (TABLES 1 and 2).

No significant relationship of the safrole concentration to the collection times was observed. However, there was a small increase in the safrole content during the cooler periods of the day (TABLES 2 and 3). No relationship between periods of sunlight (average duration was 7.0 hours/day) and the yield of essential oil or safrole concentration.

Studies relating the influence of collection times on the production of essential oil are rare in the scientific literature. However, the responses of different cultures that produce the essential oil to this aspect are quite diversified. Freitas et al.[8], studying the harvesting of japonese mint (*Mentha arvensis* L.) at 7:00 a.m., 9:00 a.m., 11:00 a.m., 1:00 p.m. and 3:00 p.m., obtained a higher production of essential oil and its constituent of commercial interest, menthol, at 1:00 am, contrary to the results observed in the present study. Leal et al.[9], evaluating the production of essential oil in capim-limão (*Cymbopogon citratus* DC. Starf) leaves at 12:00 midnight; 4:00 am; 8:00 am; 12:00 noon; 4:00 pm and 8:00 pm, did not identify significant variations in the production of the oil. Results similar to those obtained with *piper hispidinervium* were observed with basil (*Ocimum basilicum* L.). According to Silva et al.[14], neither the season (January and August), nor the time of collection (8:00 a.m. and 4:00 p.m.) had any effect on the

TABLE 2: Average temperatures and relative humidities of the air at each time of collection

Collection time	Temperature (°C)	Relative humidity (%)
8:00 a.m.	23.07	58.67
10:00 a.m.	25.10	56.67
1:00 a.m.	30.03	48.67
3:00 a.m.	29.47	49.10
5:00 a.m.	25.07	52.00

TABLE 3: Average safrole contents of the essential oil extracted from the dried leaves of *piper hispidinervum* as a function of the time of collection

Collection time	Safrole content* (%)
8:00 a.m.	86.71
10:00 a.m.	84.79
1:00 a.m.	82.47
3:00 a.m.	86.03
5:00 a.m.	86.87

***There was no difference among the average safrole contents at the level of 5% of probability according to the Tukey test. CV (%) = 4.54. Overall average = 85.37.**

composition of the essential oil, although there was a difference between the seasons with respect to the essential oil content. The results demonstrated the dynamism of the *piper hispidinervum* species in relation to environmental conditions as far as the yield of the essential oil and it major constituent (safrole) is concerned.

There was no significant difference in the yields of essential oil obtained during the four periods of steam distillation (TABLE 4). On the other hand, there was a significant difference in the safrole contents of the essential oil according to the distillation times. The highest concentrations were obtained

TABLE 4: Average yields of the essential oil extracted from the dried leaves of *piper hispidinervum* as a function of the duration of the steam distillation

Duration of the distillation (Hours)	Yield of essential oil* (%)
1	2.20
2	2.31
3	2.36
4	2.37

***There was no difference among the average yields of essential oil at the level of 5% of probability according to the Tukey test. CV (%) = 10.27. Overall average = 2.31.**

TABLE 5 : Average yields of the safrole contents of the essential oil extracted from the dried leaves of *piper hispidinervum* as a function of the duration of the steam distillation.

Duration of the distillation (Hours)	Safrole content* (%)
1	86.57[a]
2	88.56[a]
3	84.78[ab]
4	81.58[b]

***Means followed by the same letter do not differ at the level of 5% of probability according to the Tukey test. CV (%) = 4.54. Overall average = 85.37**

between one and three hours of distillation with a maximum observed after distilling for two hours (88.56%). There was a linear increase in safrole concentration until the second hour of distillation, followed by a decrease to values of approximately 82% (TABLE 5). This decrease may be caused by the increased carryover of components with longer chains. This fact is confirmed by the chromatograms, where there was an increased intensity of peaks corresponding to components with retention times greater than that of safrole. The identification of these constituents was not the objective of this study. From the data presented in TABLES 4 and 5, it can be seen that the distillations performed during one to three hours did not present significant differences with respect to the essential oil and safrole contents.

According to Simões et al.[3], the time necessary for extraction varies as a function of the rigidity of the plant material and its degree of division, the nature of the substances to be extracted, the solvent and the use of heating. The choice of an extraction method should take into account the efficiency, quantity and stability of the substances to be extracted. The present study demonstrated that the yield and quality of the essential oil extracted from the dried *piper hispidinervum* leaves depended on the time required for steam distillation.

CONCLUSION

The highest yield of essential oil was obtained when the leaves were collected at 8:00 am, 10:00 am, 3:00 pm and 5:00 pm with the best result being obtained in the morning. The concentration of safrole in the essential oil was not influenced by the time of collection. It is recommended that the steam distillation be performed during one hour, or, at most, two hours, depending on the market requirements with regard to the safrole content. For an industrial scale process, preliminary tests should be performed to determine the operational procedure. The recommendations furnish a basis for the agro-industrial production of a high-quality product with a view to improving the cost/benefit ratio.

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