DOI: 10.46793/SBT28.509M

CHEMICAL COMPOSITION OF LEMON GRASS EXTRACTS

Jelena Mladenović[1], Đorđe Jovanović[1], Nenad Pavlović[1] , Milena Đurić[1] , Ljiljana Bošković-Rakočević[1],Jasmina Zdravković[2]

Abstract: The plant lemon grass (*Aloisia citrodora*) was used as material in this work. The percentage of dry matter, organic acids and cellulose, was determined from the fresh plant material. Extracts are obtained from chopped dry lemongrass leaves. Extraction was done by maceration, Soxhlet and ultrasound. The content of extracted matter in the obtained extracts was determined, as was the content of vitamin C.

Keywords: lemon grass, extraction, organic acids, vitamin C.

Introduction

The most important group of herbal preparations is represented by extracts, which are obtained by applying different extraction methods, starting from simpler technologies to advanced techniques. Extraction is the separation and concentration of certain constituents from plant and animal tissues using selective solvents using standard procedures, (Lampe,1999). Depending on the consistency, extracts are divided into liquid, semi-solid and solid. Plant extracts are obtained by bringing chopped, mostly dry material into contact with an extraction solvent in a suitable device, an extracto, (Damjanović, 2007).

Lemongrass is a perennial evergreen plant from the verbena family (*Verbenaceae*). It has a bushy growth, well branched, up to 3 m tall. The leaves are opposite or clustered in groups of three, up to 8 cm long, green and shiny, when crushed between the fingers they release a pronounced lemon scent. The flowers are small, white or purple, fragrant gathered in oblong, branched spikes about 10 cm long, (Lakušić, 1990).

The leaves are used as tea or as a spice for flavoring drinks, marinades, creams, ice cream, cakes. They can be used fresh or dried, and by drying they

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (jelenamala@kg.ac.rs)
[2]Institute for forage crops, 37000 Kruševac, Serbia (jasna.zdravkovic@gmail.com)

retain their aroma for many years. It is used for stress, depression and in the production of perfumes and some hygiene products.

Materials and methods

A plant, lemon grass (*Aloysia citrodora*) was used as a material in this final work. Plant material for analysis was collected in October 2017 in Crete. The percentage of dry matter was determined from the fresh plant material, i.e. content of water, organic acids and cellulose. The plant was dried in the shade and stored in a dry place. Extracts are obtained from chopped dry leaves of lemon grass.

In laboratories, the determination of water content by drying is most often applied. Three measurements of the crushed sample were performed. The samples were dried to a constant mass in a dryer under atmospheric pressure at a temperature of 105 ^{0}C. Before the samples were placed for drying, the Vegeglas was dried with a lid in an oven to a constant mass (1 hour) at the prescribed temperature (105 ^{0}C), (Piletić i Miletić, 1989).

The examined sample was placed in a dry Vegeglas and measured. Then it was put to dry in a dryer. During drying, the Vegeglas with the sample must be open, i.e. the lid is next to it during drying. After drying, vegeglas is cooled in a desiccator, and then measured.

The process of drying, cooling and measuring was repeated until a constant mass. Since three measurements were made, the mean value was recalculated. The moisture content is obtained from the difference in mass before and after drying the tested sample. The mean value is recalculated for three measurements.

Cellulose content was determined by the Scharrer-Kürschner method, which involves destroying the sample with a mixture consisting of nitric, acetic and trichloroacetic acids. Nitric acid oxidizes and nitrates all substances, except cellulose, and the decomposed products are dissolved in acetic acid (Šiler-Marinković, 2009).

In an Erlenmeyer with a ground neck, 1.00 g of the cause is transferred and then poured with 25 cm^3 of the reagent for cellulose, connected to the return condenser and heated for 30 minutes on an electric heater, over an asbestos mesh. It is necessary to periodically stir the contents of the Erlenmeyer flask in order to remove particles from the vessel walls. After cooking, the content is filtered through previously dried and weighed filter paper. The precipitate is washed with hot water and ethanol, then dried at 105 °C to a constant mass, cooled in a

desiccator and measured. From the difference in mass of filter paper with cellulose and filter paper, the amount of cellulose (including mineral substances) is obtained.

The determination of organic acids was carried out by the volumetric neutralization method, where the solution is titrated using a base solution (NaOH) of known concentration, in the presence of the phenolphthalein indicator, (Milić i sar., 2012).

The test sample, 100g, is crushed in a porcelain mortar. With the addition of water, the sample is quantitatively transferred into a measuring vessel of 250 mL, and all this is diluted with distilled water to 150 mL. After that, the extraction is carried out, which is accelerated by heating in a water bath at a temperature of 70-80°C with occasional stirring for 30 minutes. The obtained extract is then cooled, the measuring vessel is filled with distilled water up to the line and then filtered through pleated filter paper. After filtration, 50 mL of the filtrate with dissolved acids is transferred with a pipette into a 250 mL Erlenmeyer flask, 2-3 drops of 1% phenolphthalein are added and titrated with a 0.1 mol/L NaOH solution.

Free acidity is expressed in g/100g of fresh sample through citric acid, which is dominant in the sample.

Extraction by maceration

Chopped and homogenized plant material (5 g) was poured with solvent (250 mL 96% ethanol) and left in well-closed Erlenmeyer flasks, protected from light. After 5 days, the plant material was separated from the macerate by straining through cheesecloth, and then through filter paper, a black strip. The solvent was removed by evaporation on a water bath, and the obtained extract was dried to a constant mass, (Lajšić i Grujić-Injac, 1998).

Soxlet extraction – y

A measured mass of chopped and homogenized plant material (5 g) was placed in a sleeve. The sample sleeve was then inserted into the middle part of the extractor which was connected to the cooler and balloon. Erlenmeyer was previously dried for 1 hour at 105 ^{0}C and weighed on an analytical balance. Using a small funnel from the top of the condenser, enough solvent was poured into the apparatus to fill the extractor and pour into the Erlenmeyer flask. Then a little more solvent (96% ethanol) was added, making sure that the total amount of solvent did not occupy more than ¾ of the Erlenmeyer volume, (Milić i sar., 2012).

The apparatus was placed on a rack and the solvent was gradually heated in an Erlenmeyer so that the condensed drops of solvent falling on the hilzne could be counted rather than leaking in a continuous stream. The extraction was performed at the boiling temperature of the solvent for 6 hours. After the extraction was completed, the extracted substance was dissolved in the solvent in an Erlenmeyer

flask. The solvent was removed by evaporation and then the extract was dried in an oven to a constant mass.

Ultrasonic extraction

Ultrasonic extraction was performed in an ultrasonic water bath (EUP540A, Euinstruments, France) [12]. The sample (5g) was placed in a flask and poured with 250 mL of 96% ethanol. The mixture was extracted for 30 minutes at a frequency of 40 kHz and an ultrasound power of 90% (216 W), (Milić i sar., 2012).

Determination of vitamin C content

Quantitative determination of total vitamin C is based on the reversible ability of the oxidation-reduction system ascorbic-dehydroascorbic acid, (Aćamović-Đoković i Cvijović, 2009).

The Tillmans method was used for the quantitative determination of vitamin C, which is based on oxidometric titration during which L-ascorbic acid is oxidized to dehydroascorbic acid, with simultaneous reduction of the applied reagent. Titration with 2,6-dichlorophenolindophenol. Tillmans reagent (TR) is performed in an acidic medium at pH 4–6. The oxidized form of the Tillmans reagent solution (which also acts as an indicator) has a dark blue color (at pH 5.2), while in the presence of ascorbic acid, TR changes to its reduced, leuco form. At pH 4.2, TR has a red color (acidic environment), and when all the amount of L-ascorbic acid is oxidized, the next drop of TR colors the tested solution pink because the reaction environment is still acidic, (Džamić, 1984).

For the extraction of ascorbic acid from the plant extract, 10% acetic acid or 5% metaphosphoric acid or their mixture is used. These acids favor protein precipitation and at the same time slow down the reaction of other reducing substances with Tillmans reagent; also, they keep the environment acidic.

Results and discussion

The content of dry matter obtained on the basis of three measurements was calculated as their mean value and is 27.354%, and the moisture content obtained by subtracting the content of dry matter per 100 g of the sample, for each measurement also expressed as their mean value, is 72.645%.

The content of organic acids, as well as the content of cellulose, was determined in fresh plant material calculated on dry matter. Analyzes were performed in three trials and converted to the mean value. The mean value of organic acid content is 3.19%, while the mean value of cellulose content is 35.51%.

Table 1. Content of organic acids and cellulose

Organic acids, (%)	Cellulose,(%)
3.20	35.35
3.15	35.43
3.22	35.75

After the extractions have been completed, the extraction yield is calculated by evaporating the obtained plant extracts to dryness and measuring the obtained dry residues. 0.4215g of dry macerate matter, 0.2545g of Soxhlet extract and 0.695g of ultrasonic extract were obtained from 5g of plant material of lemongrass leaf. The obtained results in percentages are shown in Table 2.

Table 2. Percentage yield of density extraction of obtained extracts

	maceration	Soxhlet extraction	Ultrasonic extraction
extraction yield, %	8.43	5.09	13.09
density of extracts, g/cm^3	0.75	0.68	0.82
Vitamin C, mg / 100 g	9.5	4.5	17.5

Based on the obtained results, it can be concluded that the lowest yield was obtained by Soxhlet extraction, followed by maceration, while the highest yield was obtained by ultrasonic extraction, which proved to be the extraction method with optimal conditions for the lemongrass plant. Given that this plant contains a lot of vitamins and bioactive compounds, we assume that during Soxhlet extraction, the mentioned thermolabile compounds were decomposed, and during maceration, the length of extraction affected their decomposition.

Based on the density measurement, we see that the highest density in the ultrasonic extract is 0.82 g/cm^3, and the lowest in the Soxhlet extract is 0.68 g/cm^3, which correlates with the extraction yield.

When determining the content of vitamin C, we concluded that the highest content of this vitamin was determined, also in the ultrasonic 17.5 mg/100g, then in the macerate 9.5 mg/100g, and the lowest in the Soxhlet extract 4.5 mg/100g. With ultrasonic extraction, we managed to isolate the largest amount of this vitamin because the temperature in the bath was 40 C^0, which is a lower temperature than the decomposition temperature of this vitamin (50-60 C^0), in

Soxhlet extraction the temperature of the solvent was at the boiling point, i.e. 60 C^0, and with maceration even if the solvent was at room temperature, the process lasted 5 days, which probably led to the breakdown of vitamins. We assume that the ultrasonic extraction method proved to be the most optimal with the highest yield because it lasted the shortest (30 min), at the lowest temperature. In this way, the vitamin was quickly extracted and preserved from degradation.

The plant, lemongrass, was not examined chemically, but by comparing the values of the content of organic compounds in the plant as well as the content of water, the obtained values are within the limits of the expected results (MacVicar J.,2006).

Conclusion

When determining the content of vitamin C, we concluded that the highest content of this vitamin was determined, also in the ultrasonic 17.5 mg/100g, then in the macerate 9.5 mg/100g, and the lowest in the Soxhlet extract 4.5 mg/100g. With ultrasonic extraction, we managed to isolate the largest amount of this vitamin because the temperature in the bath was 40 C^0, which is a lower temperature than the decomposition temperature of this vitamin (50-60 C^0), in Soxhlet extraction the temperature of the solvent was at the boiling point, i.e. 60 C^0, and with maceration even if the solvent was at room temperature, the process lasted 5 days, which probably led to the breakdown of vitamins. We assume that the ultrasonic extraction method proved to be the most optimal with the highest yield because it lasted the shortest (30 min), at the lowest temperature. In this way, the vitamin was quickly extracted and preserved from degradation.Please check each item to be certain that the article is in compliance with the requirements.

Acknowledgement

The research was financed by the Ministry of Education, Science an Technological Development Republic of Serbia, project ref, number 451-03-47/2023-01/20008

References

Aćamović-Đoković G., Cvijović M. (2009). Praktikum iz Organske hemije, Agronomski fakultet, Čačak.

Damjanović B. (2007). Ekstrakcija biakativnih komponenti, Metalurško-tehnološki fakultet, Podgorica.

Džamić M. (1984). Biohemija, Beograd. Lajšić S., Grujić-Injac B. (1998). Hemija prirodnih proizvoda, Tehnološki fakultet, Novi Sad.

Lakušić R. (1990). Planinske biljke, Beograd.

Lampe, J.W. (1999). Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies, Am. J. Chin. Nutr., Vol. 70, 475-490.

MacVicar J. (2006). Ljekovito i začinsko bilje , Naklada Uliks, Rijeka.

Milić J., Primorac M., Savić S. (2012). Frarmaceutska tehnologija I, Farmaceutski fakultet, Beograd.

Piletić V. M., Miletić LJ. B. (1989). Organska hemija, Novi Sad, Tehnološki fakultet.

Šiler-Marinković S. (2009). Vitamini, Beograd, Tehnološko-metalurški fakultet.