

Medicinal and Pharmaceutical Properties of *Vanilla planifolia*

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Received November 09, 2019; Accepted December 23, 2019; Online Published January 01, 2020

Abstract

Introduction: Spices are an ingredient that today is considered indispensable in the kitchen not only for the important role of flavoring and coloring food, but also for the antibacterial, antioxidant, eupeptic, antidiabetic and anticancer properties, which have been highlighted through chemical and pharmacological analysis.

Methods: In order to use spices in the food sector, it is necessary to have a product with characteristics of authenticity and genuineness such as to protect the consumers from possible health risks. The aim of the present study was to find the best extractive conditions for vanilla capsules with the use of different solvents such as ethyl ether, methyl alcohol and dichloromethane and subjecting the extracts obtained to qualitative and quantitative analysis.

Results: From the analysis carried out it was possible to highlight that the main component of the vanilla pod is vanillin from which the particular aroma derives. Other important compounds are: vanillic acid, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, anisic alcohol; and vanillin degradation products which are also available in small concentrations. In addition, the following flavonoids have been identified: rutin, morine, quercetin, myricetin, kaempferol, apigenin, which are considered to be responsible for pharmacological activities.

Conclusions: Knowledge of chemical composition of a spice is necessary to guarantee its authenticity. In the case of vanilla, possible sophistications can be highlighted considering the relationship between vanillin and the other main components which in the natural extract is well defined and, in addition to being an index of good quality, allows to identify the origin of the plant and to discover any adulterations, as by adding synthetic vanillin or other spices.

Keywords: Spice, Vanillin, Analytical Methods, Pharmacology

Introduction

For several years, much attention has been given to the cultivation and conservation of food products in order to maintain their natural and healthy characteristics. In particular, substances such as aromas, natural or artificial colorings, technological adjuvants, etc., which are added to foods in order to prolong their conservation or characteristics, have been the subject of many research. Among these substances, vanilla is the most common natural flavoring and has been used for a long time both in the food, pharmaceutical and cosmetic fields.

In ancient times, vanilla was widely used by the Mexican people. The Aztecs consumed it in large quantities as a drink by mixing vanilla with cocoa, which was even offered by the emperor Montezuma to the Spanish Hernando Cortéz, conqueror of Mexico.¹ Later on, Cortéz introduced cocoa beans and vanilla to Europe, and in the European courts the vanilla-flavored hot chocolate was well-known. It was Hugh Morgan, apothecary of Queen Elizabeth I, who suggested other uses of vanilla, especially as a flavoring; its diffusion, however, occurred only in the mid-1800s, when it was possible to obtain the production of Vanilla in areas different from the native ones, thanks to the discovery of the manual pollination method by Edmond Albius, a slave who lived on

the Reunion Island.^{2,3} This island later became a world producer of vanilla. After the eighteenth century, vanilla was used in alcoholic beverages, tobacco and perfumes.

The geographical origin influences the physical properties, the organoleptic characteristics and the chemical composition of the vanilla fruits. The major producers of vanilla is considered to be Madagascar, the Comoros and Reunion islands, Indonesia, Mexico, the West Indies and South America. There are various qualities of vanilla, but currently only three species are cultivated for their economic importance: *Vanilla planifolia* Andrews (*V. fragrans*), *Vanilla tahitensis* More, and *Vanilla pompona* Schledde.⁴⁻⁶

Vanilla planifolia Andrews or *Vanilla fragrans* is an herbaceous liana from the Orchidaceae family native to Central America. It lives in the undergrowth of humid tropical forests, is robust, climbing, with a dark green, cylindrical, fleshy, and branched stem, which can reach a length of 10 -15 meters. In the nodes of the branches, on the opposite side to the leaf, there is an adventitious root that absorbs water and supports the plant allowing it to adhere strongly to the available supports.

The leaves are alternate, smooth and fleshy with an oval shape and a pointed tip. Both the leaves and the stem contain an irritating juice which causes persistent burns and itching

on the skin. The flowers, from the classical trumpet shape of orchids, are odorless, have white, pale yellow or greenish color and are joined in racemes to the axil of the leaves. The fruit with a balsamic odor has a cylindrical shape, up to 30 cm long, with a diameter of about 10 mm, of fleshy consistency, and is gathered in clusters of eight or ten and contains numerous small seeds of brilliant black color. The fruit is improperly called "pod" and even more improperly "vanilla stick". It matures after about six/eight months after its fertilization and after harvesting, it undergoes fermentation and drying, obtaining brown, flexible sticks, which are covered with a white patina consisting of vanillin microcrystals.

When cultivated outside its natural habitat, the plant requires artificial pollination, because the shape of its flowers is only suitable for pollination by hummingbirds and Euglossine bees. The fruits are harvested when they are still unripe and are brought to maturity away from the plant by subjecting them to a long fermentation process, during which they are kept in the sun, covered during the day, while they are placed in well insulated wooden crates during the night. This treatment is necessary to extract the vanillin (4-hydroxy-3-methoxybenzaldehyde) which, in the untreated fruit, is present in the form of glucoside.⁷ Vanillin is an aromatic aldehyde with a characteristic aroma and taste and is responsible for the commercially prized value of the fruit.^{8,9}

Traditional methods of pods treatment

The pods undergo various treatments to obtain the formation of vanillin. Its characteristic aroma is released by making the immature pods "sweat" in the sun several times for 10-20 days and then is slowly dried for several months.

Extraction in water

The vanilla sticks, still gathered immature, are grouped by length and immersed in water at a temperature of 80-85°C, taking care to avoid the temperature reaching the boiling point. Each batten is preliminarily immersed in water only once for 15-20 seconds or two or three times for 5-7 seconds. This operation aims to eliminate the development of microorganisms.

After immersion in hot water, the vanilla is drained and then covered with a dark woolen cloth for about a quarter of an hour. Subsequently it is exposed to the sun on a table placed about one meter above the ground to avoid contact with the damp earth. Before nightfall, the woolen cloth enclosing the vanilla is rolled up and placed in a hermetically sealed woolen box. Sun exposure is repeated for 2-3 days for smaller sticks and for 5-6 days for larger ones. Eventually the pods become flexible, dark and wrinkled longitudinally. The last operation is drying. The slats are placed on wooden boards in a well-ventilated and sunny room for a period of

time that can vary between 10 days and 2 months until the woody part does not contain only traces of humidity and in this way the volume is considerably reduced.

Extraction in a Stove

This method is used to prepare large amounts of vanilla. Open wooden barrels filled with water at a temperature of 80°C are used and coated with a cloth cone with the tip pointing downwards and which does not touch the water. In this container, 50 kg of green vanilla sticks are placed and covered with a woolen cloth. After about 12 hours, drying is carried out in an oven at a temperature of 50°C.

Extraction in a Oven

This procedure commonly used in Mexico consists of introducing iron containers each containing 10 kg of vanilla in an oven heated to 65°C. After about 15 hours, the containers are opened to check the progress of drying, which ends when the slats acquire a greyish color. At this point, the containers are taken from the oven and wrapped in a woolen cloth to avoid abrupt cooling. The vanilla sticks are then removed from the containers and exposed to the sun for final drying.

Following these treatments, vanilla acquires its aroma, taste and flavor due to the enzymatic transformations of β -glucosidases and peroxidases. The hydrolytic action of β -glucosidase promotes not only the liberation of vanillin from glucovanillin, but also frees many other compounds such as p-hydroxybenzaldehyde and vanillic acid from their glycosidic forms. The activity of peroxidase enzymes are responsible for the degradation of carbohydrates and the oxidation of vanillin with the formation of quinone compounds that contribute to the overall vanilla aroma.

Currently, vanillin is chemically obtained by synthesis, given that the traditional extraction processes of vanillin from the pods are expensive, time consuming and provide a yield in the finished products of around 20%.

Synthetic vanillin was obtained by synthesis in 1874 by two German chemists, Ferdinand Tiemann and Wilhelm Haarmann, who produced it starting from coniferine, a substance present in the pine resin, in the presence of potassium dichromate and hydrogen sulfide. The process, which provided a vanillin yield of 350g / kg. was abandoned because it was considered uneconomical with respect to the processes developed later.^{10,11} Vanillin has also been synthesized from guaiacol obtained from guaiac resin, wood tar or coal tar, as well as from eugenol and derived from carnation oil. All these production methods have the defect of requiring various phases of recrystallization before arriving at having sufficiently pure vanillin to be used in the food sector.

The sachets or vials of vanillin on the market do not contain information on the composition or purity, therefore it is difficult to understand which excipients or other substances have been added. Since the synthetic vanillin was produced at a lower cost than the natural one, the need arose to be able to distinguish the two substances, synthetic and natural vanillin and the derived products in order to avoid fraud by the producers. In order to be able to distinguish a natural vanillin extract from the one to which synthetic vanillin has been added, many identification methods have been developed based on analytical methods, on carbon isotopic ratios, on the ratios of the levels of some substances present. It was noted that the vanillin / potassium, vanillin / nitrogen and vanillin / phosphate ratios differed in the various types of pods but were constant in the extract obtained with pods of the same type. Therefore, if the ratios of an extract are greater than those of an authentic extract, it is likely that the producer has added synthetic vanillin. Furthermore, the vanillin extracted from the pods can be distinguished from those produced synthetically by lignin, eugenol or guaiacol, due to the different ratio between the two carbon isotopes C_{13} and C_{12} , thus allowing to detect possible frauds.^{12,13} In the present study, the analytical composition of the main vanilla constituents obtained by extraction have been compared with different solvents.

Experimental part

Materials and methods

GC-MS HP 5890 Mass 5970 appliance; capillary column ZB-1 of 30 m with I.D. 0.25 mm and F.T 0.25 μ ; HPLC Sunicom Oy device with ERC 3215 degasser; reverse phase column Ultracarb 5 ODS (20) l = 250 mm; i.d. = 4.6 mm (Phenomenex, Torrance, CA, USA); Standards: rutin, morina, quercetin, myricetin, kaempferol, apigenin (Sigma),

Capsules of *Vanilla planifolia* Andr. commercially available, they were coarsely chopped and 2.5 g of the triturate were hot-drawn with the Soxhlet method using dichloromethane, methyl alcohol and diethyl ether respectively as solvent. After removal of the solvent by a rotary evaporator at a temperature of 35 °C, residues of 1.0453, 0.2666 and 0.2149 g were obtained, respectively. The solubilized residues in the three mentioned solvents were analyzed by gas chromatography-mass spectrometry using a HP 5890 Mass 5970 GC-MS apparatus with a 30 m capillary column ZB-1 with I.D. 0.25 mm and F.T 0.25 μ .

The GC-MS analysis of the dichloromethane fraction, which was found to be richer in components, was performed with a temperature gradient method, starting from 100°C with an increase of 5°C / min up to 250°C; using helium as a carrier gas at a flow of 1.4 mL / min. In this analysis the following substances have been identified: vanillin,

glucovanillin, methyl ester of margaric acid, heptacosanoic acid and eicosandioic acid.

The HPLC analysis performed in the isocratic mode using a Sunicom Oy device with ERC 3215 degasser and injecting 10 μ L of each residue, dissolved in methanol. Methanol and 10-3 M phosphoric acid in water (25:75) was used as eluents at a flow rate of 0.7 mL/min. a reverse phase column Ultracarb 5 ODS (20) l=250 mm used; i.d.=4.6 mm (Phenomenex,) with detection in the ultraviolet at a wavelength of 280 nm. The following substances have been identified: p-hydroxybenzoic acid, vanillic acid, p-hydroxybenzaldehyde and anisic alcohol.

For the determination of flavonoids, 2.5 g of finely ground vanilla capsules were treated in water with sulfuric acid up to pH 3 and the mixture was boiled for 24 hours. The obtained aqueous mixture was filtered and extracted 5 consecutive times with ethyl acetate in a separating funnel. The organic phases were collected in a single portion which was concentrated by means of a rotary evaporator until the complete removal of the solvent was obtained. The obtained residue, equal to 0.2144 g, was solubilized in 1mL of methanol and 5 μ L of the solution were analyzed by High Pressure Liquid Chromatography (HPLC) applying the elution gradient consisting of 0.01M sodium acetate at pH 4.8/ acetonitrile. The used column is the same column mentioned above with UV detection at a wavelength of 350 nm. The following flavonoids have been identified: rutin, morina, quercetin, myricetin, canferol, apigenin, comparing the retention times with those of pure standards eluted under the same experimental conditions.^{14,15}

The analytical results showed a constant presence of the main components in the three organic extracts, although in different concentrations. In the methanolic fraction among the substances of lower concentration there are degradation products of vanillin such as ferulic aldehyde and isoeugenol, identified by TLC. In the dichloromethane extract, which differs from the other two for a gelatinous appearance, there are waxy substances and fatty acids, as evidenced by the GC-MS analysis. Also, the presence of flavonoids identified in the organic phase is separated from the aqueous solution, by extracting with ethyl acetate.

Products of Vanilla

The basic products derived from vanilla used as flavorings or fragrances are: the extract, oleoresin, absolute vanilla and powdered vanilla. These represent different forms of vanilla flavoring that are used in the food industry, dairy industry, beverage production, pharmaceuticals or perfume production. Each form has its own organoleptic, physical and functional characteristics determined by the choice of the used pods and the method by which they were treated.

The vanilla extract is obtained in a solution of water and ethyl alcohol in which the principles responsible for the taste and fragrance of the pods are dissolved by maceration. The ethyl alcohol content is not less than 35% by volume, but the solution may also contain other ingredients such as glycerin, propylene glycol, sugar (also inverted), dextrose and corn syrup. The composition of the medium used for the extraction plays an important role in determining the quality of the extract, as different ratios of alcohol and water dissolve different extractable substances. Depending on the polarity of the solvent, gums, sugars, and soluble proteins, waxes and resins are extracted to different degrees and these non-aromatic ingredients may be able to modify the taste, although they are important for providing body and consistency to the vanilla extract. Even the vanilla extract can be adulterated, in particular using the fava beans Tonka that have a high concentration of coumarins. This adulteration causes toxic effects in the liver and blood and is also very difficult to highlight.¹⁶⁻¹⁸

Vanilla oleoresin is a dark brown viscous liquid obtained by extraction with a solvent composed of water and ethanol at 50% (v/v) at a temperature of 50°C of finely chopped pods and is then proceeded to the removal of the solvent under vacuum.

Absolute vanilla is the most concentrated form of vanilla aroma. In order to be used in cosmetics it must be completely soluble in ethanol and perfumed oils. Absolute vanilla is prepared both with extraction techniques with selective solvents such as benzene, hexane, methylene chloride, alcohol and acetone and with supercritical extraction with liquid carbon dioxide. By varying the combination of polar and apolar solvents, as well as mixing pods of different species or of different geographical origins, it is possible to obtain absolute vanilla products with different physical properties and aromatic characteristics. Absolute vanilla obtained from supercritical extraction with carbon dioxide is different in aromatic characters from that extracted with solvents and is described as having a cleaner aroma which however lacks intensity.

Powdered vanilla is obtained by mixing ground pods, vanilla oleoresin or concentrated vanilla extract, with other suitable support ingredients such as sugar, lactose, dextrose, dehydrated corn syrup, acacia gum and food starch that can be used alone or combined with the addition of up to 2% anti-caking agents to prevent the formation of lumps. The vanilla powder produced in this way is completely soluble in many liquid food products.

Pharmacological activity

The main use of vanilla is in the food and cosmetic industry, however it is also used in galenic technique for the correction and aromatization of preparations based on bitter or odorless

substances, for the aromatization of paraffin oil and also castor oil.¹⁹ Recent research has highlighted various pharmacological properties. An important choleric action highlighted both for oral and parenteral administration is attributed to vanilla and to some synthetic derivatives such as divanillidene-cyclohexanone, isovanillin and ethylisovanillin. This activity was correlated to the structure of the molecules, manifesting itself higher when the aldehyde group is present in a meta position with respect to the free phenolic group.

By using rat liver mitochondria, the ability of natural vanillin to protect membranes from oxidative damage by photosensitization, at concentrations normally used in food preparations, was examined.²⁰ Vanillin at a concentration of 2.5 mmol/L acts effectively avoiding the natural oxidation of proteins and the peroxidation of lipids induced by exposure to light in the hepatic mitochondria. The inhibitory effect is comparable to ascorbic acid and is manifested by an attack on the oxygen radical, responsible for the damage induced during exposure to light.

Studies carried out on bacteria have also shown antimutagenic activity, so that a possible use is expected in the future to prevent the proliferation of cancer cells in particular types of cancer²¹ liver,²² colon and breast²³. Vanillin acts effectively as an antiseptic. A 1.5% solution prevents the development of microorganisms in food, therefore it is used as a natural antibacterial in cocoa-based products. This property is related to the presence of flavonoids such as quercetin and apigenin.^{24,25}

In the ancient pharmacopeia, vanilla was used to promote hunger and entice the individual to eat in conditions of apathy towards food. The intense aroma of vanillin, moreover, allows to reduce the quantity of sugar in drinks, in particular in milk, with significant health benefits especially for children and the elderly.²⁶

Inflammatory bowel disease is a chronic and recurrent inflammatory disease of the intestine in which the activation of the Nuclear Factor- κ B (NF- κ B) and the production of proinflammatory cytokines play a decisive role. Vanillin has been shown to inhibit the activation of NF- κ B stimulated by lipopolysaccharides and the expression of the cyclooxygenase-2 gene in murine macrophages. To evaluate the beneficial effects on intestinal inflammation, vanillin was administered orally to mice that had been caused by trinitrobenzene sulfonic acid colitis. The obtained results were extremely encouraging and highlighted the possibility of using vanillin for therapeutic purposes for patients who had inflammatory bowel disease which has currently greatly increased in all countries throughout the world.²⁷

Although vanilla is not really harmful, people involved in the processing of vanilla are affected by allergic phenomena which are known as vanillism due to the presence of a volatile

essential oil, with a pungent odor that spreads quickly in the air causing internal and external disturbances. Among the external symptoms, the most common is believed to be a type of urticaria with rashes, swelling of the skin with severe itching especially on the back of the hands and forehead. Internal symptoms include intense headaches, dizziness that last for three to four days and are insensitive to medications. Gastric pain is also frequent, which can be alleviated by drinking milk and taking emollient herbal teas.

The extracts of the stem of the plant contain a large amount of calcium oxalate which easily transforms into oxalic acid whose caustic and rubefacient properties are known, much attention must be paid when collecting vanilla pods.

Conclusion

Vanilla is a flavoring that occupies a prominent place for its multiple use, being used in the food sector, especially pastry and drinks, in the pharmaceutical sector to make certain drugs more pleasant to the taste and also in the cosmetic sector for creams and perfumes. However, only few studies have been carried out in regards to vanilla applications in the therapeutic field, while containing substances of particular interest for the action on the gastrointestinal tract or with antibacterial and anti-tumor activity.

The continuous increase in the cost of vanilla beans has led to an increase in extracts obtained by mixing natural and synthetic vanilla or adulteration with other plant species. To guarantee the quality of vanilla extracts and products containing pure vanilla, it is important to develop techniques to verify their authenticity. Actually, vanillin is the main component of vanilla pods and its determination is fundamental to guarantee natural vanilla extracts.

Conflict of Interests

The authors declared no potential conflict of interests with respect to the research, authorship, and/or publication of this article.

Funding/Support

The authors received no financial funding or support for the research.

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