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Obtaining essential oil from clove (Syzygium aromaticum) and non-clinical safety assessment using zebrafish (danio rerio) adult

Obtaining the essential oil from the Indian carnation (Syzygium Aromaticum) and non-clinical safety assessment using adult zebrafish (Danio Rerio)

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Summary

Essential oils are odorous substances found in various parts of plants. Locomotor activity evaluates drugs that cause locomotor impairment or not. The objective of this work was to obtain clove essential oil and carry out a non-clinical safety assessment using zebrafish . The EO was obtained through hydrodistillation . For the open field test, zebrafish received doses of EO (4, 20 and 40 mg/kg); DMSO 3% and DZP 40mg/kg. After 30 min, the animals were added to Petri dishes, and locomotor activity was analyzed by counting the number of CL (5 min). To assess toxicity, the animals received the same treatments and after 96 h of analysis, the values obtained with the number of dead ZFa were submitted to statistical analysis to estimate DL to kill 50%. The EO composition had a high eugenol content and low levels of eugenyl acetate and ß- caryophyllene . In addition, it altered the zebrafish 's locomotor system and is considered safe, as it did not prove to be toxic up to 96 hours of analysis.

Abstract

Essential oils are odorous substances, found by various parts of plants. Locomotor activity evaluates drugs that cause locomotor impairment or not. The objective of this work was to obtain the essential oil of cloves and perform a non-clinical safety assessment using zebrafish. The EO was obtained through hydrodistillation . For the open field test, zebrafish received doses of OE (4, 20 and 40 mg / kg); DMSO 3% and DZP 40mg/kg. After 30 min, the animals were added in Petri dishes, and locomotor activity was analyzed by counting the number of CL (5 min). For toxicity assessment, the animals received the same treatments and after 96 h of analysis, the values obtained with the number of ZFa killed were subjected to statistical analysis to estimate DL to kill 50%. The EO had a high eugenol composition and low eugenyl acetate and ß- karyophylene levels. In addition, it changed the zebrafish locomotor system and is considered safe, as it was not toxic until 96 hours of analysis.

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1. Introduction

Essential oils (EO) are odorous substances, volatile at room temperature, and can be extracted from flowers, roots and fruits. Hydrodistillation is the most widely used method for extracting EO [1]. They are recognized as therapeutic agents since antiquity due to their pharmacological and psychological properties. They are a complex mixture of volatile compounds consisting primarily of benzenoids , phenylpropanoids , monoterpenoids and sesquiterpenoids [2]. Possess some biological activities such as antibacterial, antifungal, antiviral, anti-inflammatory , antioxidant, anticancer and antinociceptive properties [2].

The chemical composition of EO can vary significantly depending on several factors, including the condition of the aromatic part of the plant, the geographic location, the method of extraction and the time of extraction. Various extraction methods are used to produce EO, including steam distillation, dry distillation, hydrodistillation , and high pressure steam distillation [3]. Cold pressing is used in the case of extracting peel oil specifically from citrus fruits. Steam distillation is the most common method widely used for the industrial production of EO. Although the application of solvent extraction methods using various solvents (e.g. hypercritical $CO_{2\ extraction}$ or supercritical fluid extraction) is gaining popularity in the flavor and flavor industry, extracts obtained by these techniques are not considered true EOs . [2].

Studies point to the multiple benefits that EO have on the mental health of humans, including anxiolytic/antidepressant effects, enhancement of cognitive processing, enhancement of attention, psychostimulant effects , and memory enhancement. A survey of these studies was recently carried out by [4]and shows an overview of preclinical and clinical experiments studying the effect of essential oils (EO) on the nervous system and their properties on the Central Nervous System (CNS).

Clove is a dried flower bud of the *Syzygium aromaticum* , a tree-like plant with a characteristic elongated crown that can reach an average of 8-10 meters in height. Its most scientifically known name is Eugenia caryophyllus . The main chemical components of clove oil are: eugenyl acetate , caryophyllene and eugenol [1].

In most scientific research, some animals are used to better study the causes of human diseases and apply tests for innovative therapies. The Zebrafish was used in scientific research by George Streisinger , a biologist, professor at the University of Oregon. He realized that there is a great advantage in using fish in scientific studies, as the fish are small in size, their maintenance is simple and their breeding is economical compared to rodents [5]. Thus, the present work aimed to obtain the EO of cloves and carry out a non-clinical safety assessment using zebrafish (*Danio rerio*) adult.

Materials and methods

plant material

The flower buds of *S. aromaticum* were purchased at the public market in Sobral - CE and taken to the Natural Products Laboratory of the State University of Vale do Acaraú to obtain clove essential oil (OECRAVO).

Animal acclimatization protocol

Zebrafish (*Danio rerio*) adult, wild, both sexes aged 60-90 days, sizes 3.5 ± 0.5 cm and weight 0.4 ± 0.1 g were obtained from Agroquímica: Comércio de Produtos Veterinários LTDA, a supplier in Fortaleza (Ceará, Brazil). Groups of 60 fish were acclimatized for 24 h in glass aquariums (30 x 15 x 20 cm), containing dechlorinated water (*ProtecPlus* ®) and air pumps with submerged filters, at 25 ºC and pH 7.0, with a circadian cycle of 14: 10 hours light/dark. The fish received feed *ad libitum* 24 h before the experiments. After the experiments , the fish were sacrificed with cold water (5 ºC). All experimental procedures were approved by the Ethics Committee for the Use of Animals of the State University of Ceará (CEUA-UECE), under protocol nº 7210149/2016.

The tests were carried out based on methodologies proposed by [6]and [7]On the day of the experiments, the fish were randomly selected, transferred to a damp sponge, treated with the test or control samples, intraperitoneally (ip .). They were then placed individually in beakers (250 mL) containing 150 mL of aquarium water for resting. For intraperitoneal (*ip) treatments* , an insulin syringe (0.5 mL ; UltraFine ® BD) with a 30G needle was used.

Obtaining the essential oil

The EO of *Syzygium* flower buds *aromaticum* was obtained by hydrodistillation in a Clevenger -type apparatus for 2h. Then, the essential oil was collected, stored in glass vials (Fig. 1) and stored in a freezer.

Figure 1- Flower buds of *S. aromaticum* (**A**), CARAVE (**B**)

Assessment of Locomotor Activity

the zebrafish ZFa (n=6/group) were treated intraperitoneally (ip .) with 20 µL of EO solutions at doses (4, 20 and 40 mg/kg) and vehicle (DMSO 3%) and diazepam (DZP; 40mg /kg). After 30 min of treatments, the animals were added to Petri dishes, marked with quadrants, and locomotor activity was analyzed by counting the number of lines crossing, for 5 min. Animals without treatments (Naive) were considered as 100% and the percentage of locomotor activity (%AL) was calculated.

Acute toxicity 96h

The animals received the same treatments described in 4.1. After 96h of analysis, the number of dead ZFa were submitted to statistical analysis, estimating the Lethal Dose to kill 50% (LD_{50}).

Results and discussion

Chemical analysis

The chemical composition of the EO of floral buds of *S. aromaticum* is described in Table 1. It is observed that it was possible to identify three constituents totaling 99.00% .

Table 1- Chemical composition of the EO of floral buds of *S. aromaticum*

Compound	IK_{lit} [2]	Relative area (%)
eugenol	1359	83.90
β- Caryophyllene	1419	3.57
Eugenyl Acetate	1523	11.53
Total		99.00

[1] IK_{lit} : Kovats index of literature

The analysis of the EO of the flower buds of *S. aromaticum* allowed the identification of phenylpropanoid eugenol (83.90%), in addition to non - oxygenated sesquiterpene β- caryophyllene (3.57%) and eugenyl acetate (11.53%) (Fig. 2). according to the literature where a sample of essential oil of *S. aromaticum* collected in the southern region of Ilhéus, showed high eugenol content and low levels of eugeyl acetate and ß- caryophyllene . The order of retention times (TR) of the major constituents of *S. aromaticum* is eugenol, ß- caryophyllene and eugenyl acetate [1], confirmed in the analysis of the chromatogram of the EO of the flower buds of *S. aromaticum* (Fig.2).

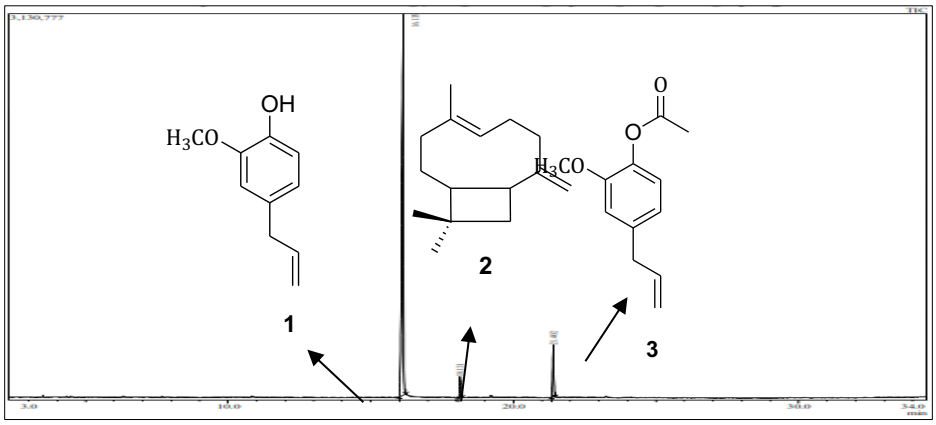

Figure 2- Chromatogram of the EO of flower buds of *S. aromaticum* eugenol (**1**), β - caryophyllene (**2**) and eugenyl acetate (**3**).

Assessment of locomotor activity

Locomotor activity is one of the behavioral analysis parameters that has been used to evaluate the action of drugs that can act on the central nervous system of zebrafish (*Danio rerio*) adult and cause locomotor impairment or not [8–10].

This activity can be explored through the Open Field Test, in an aquarium [11], as well as Petri dishes [12]. As a result, it was observed that (OECRAVO) caused motor impairment in zebrafish , where there was a reduction in the number of line crossings in the petri dish by the animals, a result significantly different from the naïve group ($p<0.01$; $p< 0,1$ *vs.* naive) (Fig.3).

The natural behavior of zebrafish in the open field is characterized by constant swimming activity and manifestations of immobility, which are rarely observed in zebrafish 's natural conditions. [13]. The analysis of locomotor activity explored through an open field can be a model used to evaluate hyperactivity as an indication of anxiety [5]. Treatment of zebrafish with anxiolytic drugs such as benzodiazepines may increase exploratory activity in the open field [13]as well how to cause a sedative effect and decrease locomotor activity [14,15].

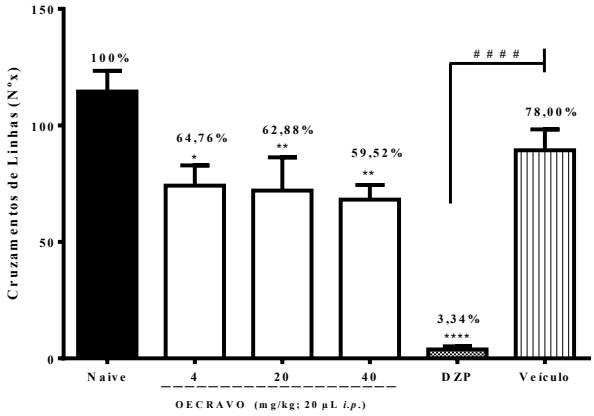

Figure 3 - Effect of clove essential oil on the locomotor activity of zebrafish (*Danio rerio*) adult .

The decrease in locomotor activity in adult zebrafish caused by (OECRAVO) suggests a possible sedative action, such as benzodiazepines (anxiolytic drugs), which decrease locomotor activity (mobility) of zebrafish in the open field, as highlighted. [16, 17]

Toxicity 96

Our results showed that CRAVO was not toxic against FaZ up to 96 h of analysis (LD_{50} > 40 mg/kg).

Conclusion

The data obtained revealed that OECRAVO presented in its composition a high eugenol content and low levels of eugeyl acetate and ß- caryophyllene . The results indicate that the EO alters the zebrafish 's locomotor system and is considered safe, as it did not prove to be toxic up to 96h of analysis.

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