Int.J.Curr.Microbiol.App.Sci (2017) *6*(10): 3608-3615

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 10 (2017) pp. 3608-3615**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.610.425

Supercritical Fluid Extraction of Oil from Sweet Flag Rhizome (*Acorus calamus* L.) and Its Insecticidal Activity on Pulse Beetles (*Callosobruchus maculatus*)

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Keywords

Supercritical fluid, β-asarone, sweet flag rhizome, insecticides, SC-CO_2

Article Info

Accepted:
xx August 2017
Available Online:
xx October 2017

A B S T R A C T

Sweet flag rhizome (*Acorus calamus* L.) oil was extracted by supercritical fluid extraction method. Different temperature levels (45, 55 and 65 °C) and pressure levels (100, 150 and 200 bar) were selected for the optimization of the parameters for SC-CO_2 extraction process. The highest extraction yield of 3.15 g/100 g at the rate of 90.08% were obtained at 45 °C and pressure of 200 bar. The quality of sweet flag rhizome oil was analysed and determined by using HPLC and the β-asarone content in oil were recorded at the rate of 26.80%. Further, the oil has been tested for its insecticidal activity against pulse beetles with different levels of concentration (10 to 70 µl/ml). The insecticidal activity of oil was found to be increased with increase in oil concentration and exposure time. The highest mortality of pulse beetles were observed in treatment T_7 (70 µl/ml) on 7th day of storage.

Introduction

Sweet flag (*Acorus calamus*) belongs to family Acoraceae comprises about 110 genera and more than 1800 species. The genus name, Acorus is derived from Acoron (coreon = the pupil of the eye) and the species calamus is derived from the Greek word, *Calamus* (a reed). The members of the family are rhizomatous or tuberous herbs. The genus Acorus comprises about 40 species, however, only a few species like *Acorus calamus* L. *Acorus christophii, Acorus tatarinowii (Schott.)* and *Acorus gramineus (Solandin Ait.)* have been investigated for their chemical properties and their applications. *Acorus calamus* is extensively studied due to its medicinal and pharmacological significance (Ganjewala *et al.,* 2011). Calamus oil is a brownish and yellow-coloured essential oil obtained from different parts of *A. calamus*. This oil contains several phytochemical constituent, but the major active constituent is the phenolic ether 'asarone' (upto 96%).

Acorus calamus is a native of Eastern countries and is indigenous to the marshes of the mountains of India. It is cultivated throughout India in the marshy tracts of Kashmir, Himachal Pradesh, Manipur, Nagahills and some parts of Karnataka state in peninsular India (Raja *et al.,* 2009). It is a hardy plant found to grow in tropical and subtropical climates. The rhizomes of *Acorus calamus* possess antibacterial, bio pesticide and antifungal properties (Gaw, *et al.,* 2002; Rani *et al.,* 2003 and Parab *et al.,* 2002).

The essential oil from the rhizomes is also used in the production of beer and alcoholic beverages such as bitters, cordials, vermouths and at lower level in foods such as frozen desserts, yoghurts, cakes and confectionery (Raina *et al.,* 2003).

Normally, only the oil of the diploid variety is used for flavouring aromatic alcoholic beverages. The roots and rhizomes of *Acorus calamus* have been used in the ayurvedic system of medicine for treating a variety of diseases such as epilepsy add hysteria (Madan *et al.,* 1960).The rhizome and leaf produce a light brown to brownish yellow volatile aromatic oil known as calamus oil. The yield of oil from different parts of the plant is upto 1.8% in fresh rhizome, 1.5 to 3.5% in dried rhizome (Chopra *et al.,* 1986).

There are numerous methods for extraction of sweet flag rhizome oil. It can be extracted by solvent extraction using ethyl acetate as a solvent (Devi *et al.,* 2013), steam distillation (Malik *et al.,* 2014), Soxhlet extraction (Muthulakshmi *et al.,* 2015) and also by super critical fluid extraction (Bruno *et al.,* 2005). The oil contains many different compounds, in which β-asarone is the major compound.

The β-asarone (cis-2, 4, 5-trimethoxy-1-propenylbenzene) is a sesquiterpenoid, is a major active principle found in oil of the rhizomes, along with a few fatty acids, terpenoids and flavonoids. The component β-asarone obtained from sweet flag rhizome has antimicrobial, insecticidal and pesticidal properties (Balakumbahan *et al.,* 2010).

In view of this applications the present study is designed for the extraction of oil from the rhizome by supercritical fluid extraction method, β-asarone content in the extracted oil and its impact on the pulse beetles (*Callosobruchus maculatus*).

Materials and Methods

Plant material

The rhizome of the *Acorus calamus* L. was procured from the local market of Raichur, Karnataka (India). The dried rhizome was made powder by using water cooled pulverizer and sieved with 500 μ mesh (0.5 mm). The chemicals, reagents (analytical and HPLC grade) and pure standard of β-asarone were obtained from M/s. Himedia Chemical Co., Bengaluru.

Supercritical fluid/ supercritical carbon dioxide (SC-CO_2) extraction

Sweet flag rhizome oil is extracted and carried out by supercritical fluid extraction method at different pressure as describes by (100, 150 and 200 bar) and temperature (45, 55 and 65 °C) at constant dynamic extraction time of 120 min.

Extraction yield

The extraction yield of rhizome oil was calculated as per the method described by Marongiu *et al.,* (2005). The extract from the SC-CO_2 was collected and the residue of the co-solvent from the extract was removed by using a rotary flash vacuum evaporator. The extract was then dried in the oven at 40 °C for 30 min. The extraction yield was computed by using the following expression.

$$\text{Extraction yield (\%)} = \frac{M_{extract}}{m_{feed}} \times 100$$

Where,

$M_{extract}$ = Mass crude extract, g
m_{feed} = Feed mass, g

Extraction efficiency

The extraction efficiency was calculated as per the method described by Olawale *et al.*, (2012). Extraction efficiency (oil recovery) is the quantity (%) of oil present in extract residue per 100 g of sweet flag rhizome powder. The extraction efficiency was calculated by using the following expression

$$\text{Extraction efficiency (\%)} = \frac{\text{Quantity of oil recovered after extraction}}{\text{Known quantity of oil in sweet flag rhizome powder}} \times 100$$

Quantitative analysis of β-asarone by HPLC method

β-asarone analysis of sweet flag rhizome oil is carried out by using HPLC (Shimadzu Series LC-10A system Kyoto, Japan) consisting of a liquid chromatography connected to a UV-VIS detector (10 A), binary pump and controlled by Shimadzu class VP workstation software. The C_{18} column has been (15 × 4.6 mm, 5.0 µm) and UV detector (210 nm). The flow rate was 1 ml/min and mobile phase consisted of water with 0.1% trifluoro-acetic acid (TFA) (solvent A) and methanol (solvent B). The instrument was run in isocratic mode (A: 35; B: 65).

The detection was monitored at 210 nm. The β-asarone compound from the SC-CO_2 extracted sweet flag rhizome oil was identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library to relative retention indices (Shailajan *et al.*, 2015; Avadhani *et al.*, 2013).

Effect of sweet flag rhizome oil against pulse beetles (*Callosobruchus maculatus*)

The pulse beetles were collected from Department of Entomology, University of Agricultural Sciences, Raichur. Acetone was used as a solvent to disperse the sweet flag rhizome oil.

Plastic jars of 1000 ml capacity were used as exposure chamber. Red gram grains (150 g) were taken for each treatment.

Twenty pulse beetles adults were placed in each glass jar. These adults were exposed to different dosage of sweet flag rhizome oil.

The doses of sweet flag rhizome oil were 10, 20, 30, 40, 50, 60 and 70 µl. All treatments were maintained triplicate for greater accuracy with there was one untreated control.

Application of sweet flag rhizome oil was carried out by releasing the required volume of appropriate oil solution from an automatic pipette to (8 cm) filter paper disk (Whatman No.2) attached to inner surface of the lid of the glass jars. Exposure periods for each treatment were 3, 5 and 7 days. At the end of exposure period, jars were opened and beetles were separated from the grains and mortality was assessed (Mansoor *et al.*, 2006).

Statistical design

Statistical analyses were carried out to study the effect of different parameters on all the dependent variables by Design-Expert software version 7.7.0 (Statease Inc., Minneapolis, USA). Factorial Completely Randomized Design (FCRD) using the statistical software AGRESS and the insecticidal activity of extracted oil was analysed by Completely Randomized Design (CRD) using statistical package.

Results and Discussion

Extraction yield and extraction efficiency

The extraction yield of sweet flag rhizome oil obtained at different SC-CO_2 temperature and pressure combinations. The extraction yield and extraction efficiency varied in the range of 0.73 to 3.15 g/100 g and 20.79 to 90.08%. The interaction effect between treatment combinations are significant ($p < 0.01$) at one percent level (Fig. 1).

From this results it is clear that as pressure increased from 100 to 200 bar, the extraction yield increased. This might be due to the fact that the increase in pressure increased the density of the CO_2 there by increasing the solvent strength and solubility of the oil in CO_2 (Yao *et al.*, 2012). The highest extraction yield of 3.15 g/100 g was recorded at SC-CO_2 pressure of 200 bar, temperature of 45 °C which was considered as the optimum and the best SC-CO_2 extraction condition for obtaining the highest oil yield from sweet flag rhizome powder. Results are in agreement with the earlier findings of (Yao *et al.*, 2012), who reported optimal oil yield of 4.12 g/100 g at a pressure of 35 MPa and temperature of 55 °C.

Similarly the variation of temperature during the supercritical fluid extraction (SFE) affects the density and the volatility of the analytes from the matrix. By increasing the temperature, the volatilities of the analytes increases but the supercritical CO_2 density decreases (Ghasemi *et al.*, 2006).

From figure 2, the pressure increased from 100 to 200 bar, the extraction efficiency increased. The highest extraction efficiency of 90.08% was recorded at SC-CO_2 pressure of 200 bar, and temperature of 45 °C which was considered as the optimum and best SC-CO_2 extraction condition for obtaining the highest oil efficiency from sweet flag rhizome powder.

Fig.1 Effect of pressure and temperature on extraction yield of sweet flag rhizome oil

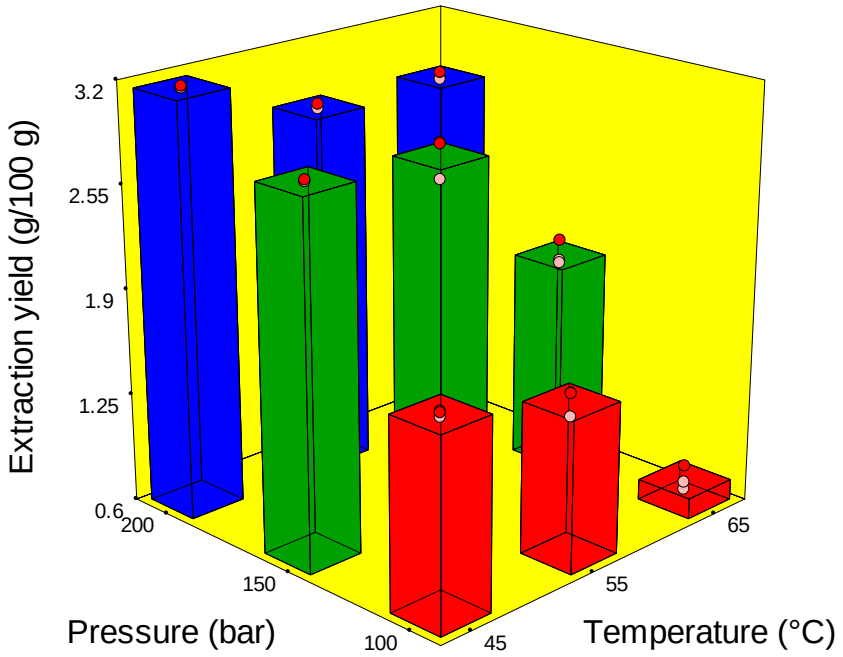


Fig.2 Effect of pressure and temperature on extraction efficiency of sweet flag rhizome oil

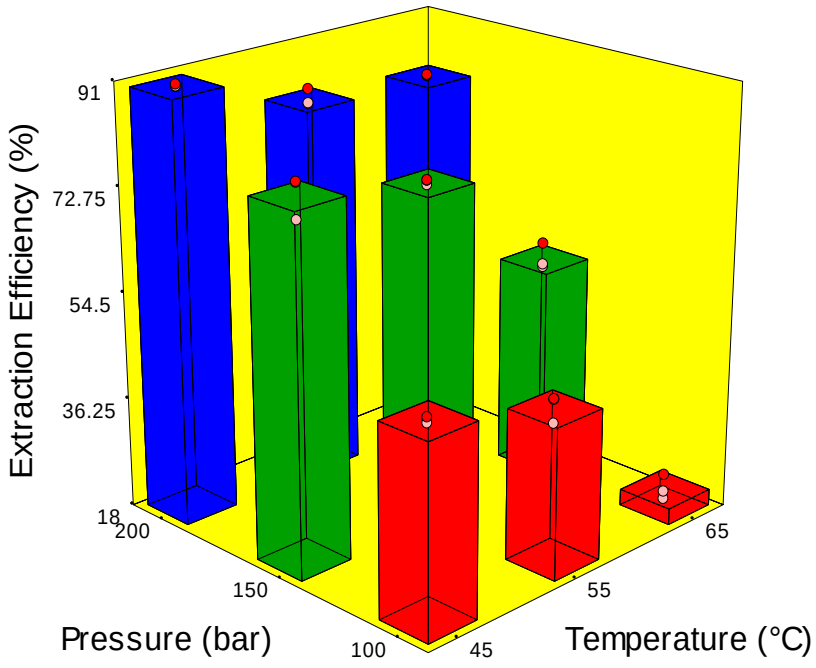


Fig.3 β-asarone content of extracted sweet flag rhizome oil

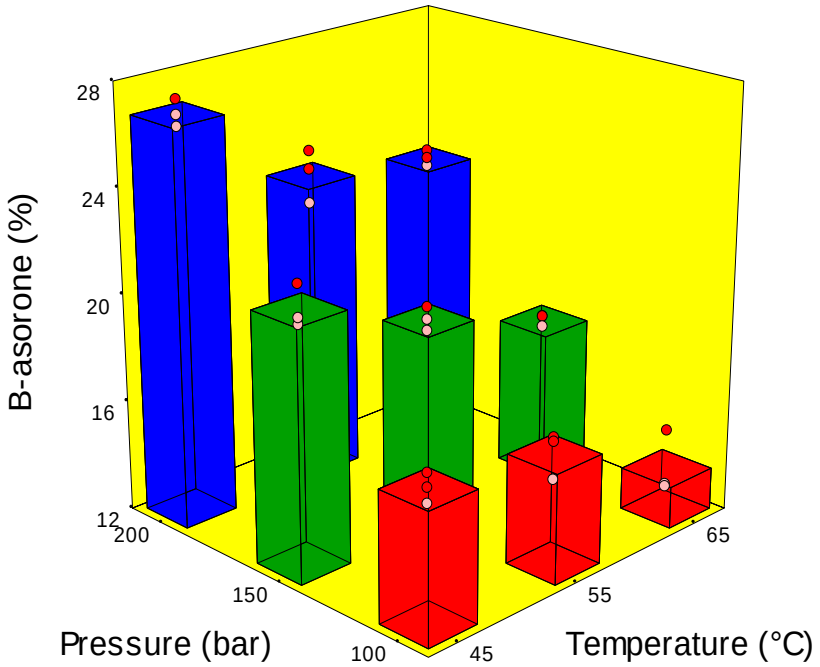
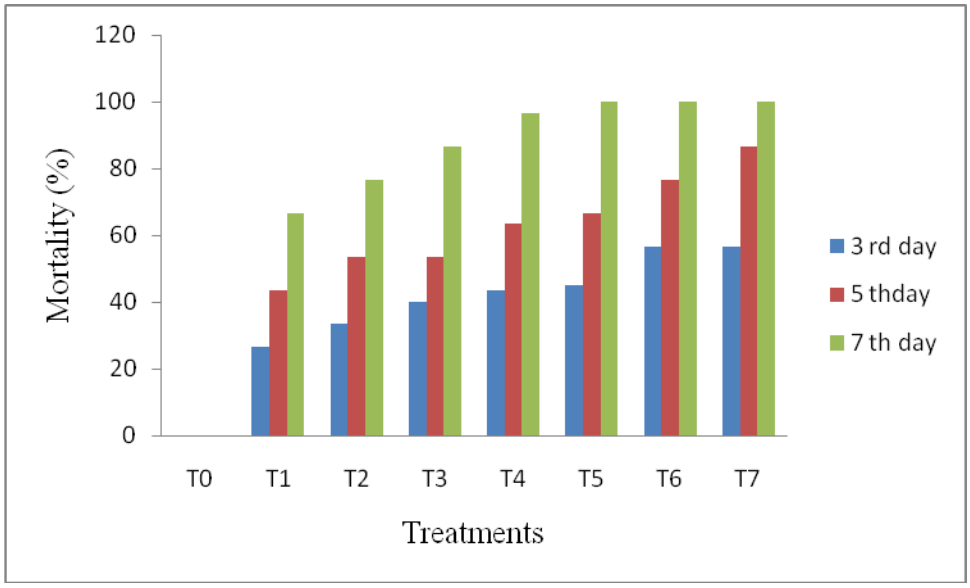


Fig.4 Effect of sweet flag rhizome oil on mortality of pulse beetles



Quantitative analysis of β-asarone by HPLC method

The β-asarone content in sweet flag rhizome is mainly dependent on polidity level. The concentration of β-asarone was found to be from 13.58 to 26.80%. The highest percentage of β-asarone was at 200 bar pressure and 45 °C temperature and the lowest percentage was found at 100 bar pressure and 65 °C temperature (Fig. 3).

β-asarone was further identified by comparing retention times with those of standard compounds. Similar results were reported by several other authors with different protocols for sample preparation and quantification of β-asarone in sweet flag rhizome oil (Avadhani *et al.*, 2013).

Effect of sweet flag rhizome oil on mortality of pulse beetles (*Callosobruchus maculatus*)

The sweet flag rhizome oil with 10 to 70 µl/ml concentration was tested. It was observed that highest mortality of pulse beetles were in treatment T_7 (70 µl/ml), which recorded 70.38% mortality followed by T_6 and T_5 and lowest mortality was observed in treatment T_0 and T_1. Mortality was increased with increase in dose of oil and exposure time. The T_7 values were on par with the treatment T_6, T_5, and T_4. T_5 is the optimum dosage for the control of pulse beetles (Fig. 4) to manage the pulse beetles in storage of food grains.

Similar results were reported by Mansoor *et al.*, (2006) who studied the insecticidal activity of different doses of *Acorus calamus* oil against *Trogoderma granarium* (Everts).

SC-CO_2 extracted sweet flag rhizome oil yield (3.15%) and extraction efficiency (90.08%) at 200 bar pressure at 45 °C.

The extraction yield and efficiency was decreased with increase in extraction temperature and increased with increase in the pressure. The β-asarone content was highest at 200 bar pressure at 45 °C.

The insecticidal activity of extracted sweet flag rhizome oil on pulse beetle was increased with dosages and exposure time.

Acknowledgement

All the authors acknowledge their heartfelt gratitude to UAS, Raichur for the financial support extended for conducting the present investigation.

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How to cite this article:

Shreelaxmi, H. Sharanagouda, C.T. Ramachandra, R.S. Roopa and Hanchinal, S.G. 2017. Supercritical Fluid Extraction of Oil from Sweet Flag Rhizome (*Acorus calamus* L.) and Its Insecticidal Activity on Pulse Beetles (*Callosobruchus maculatus*). *Int.J.Curr.Microbiol.App.Sci.* 6(10): 3608-3615. doi: https://doi.org/10.20546/ijcmas.2017.610.425