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Antioxidant capacity, insecticidal ability and heat-oxidation stability of *Tagetes lemmonii* leaf extract

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ABSTRACT

The aim of this study was to examine the effect of process factors such as ethanol concentration, extraction time and temperature on the extraction yield and the bioactive contents of *Tagetes lemmonii* leaf extracts using response surface methodology (RSM). ANOVA results showed that the response variables were affected by the ethanol concentration to a very significant degree and by extraction temperature to a lesser degree. GC/MS characterization showed that the extract is rich in bioactive compounds and those present exhibited important biological activities such as antioxidant, insect repellence and insecticidal activities. The results from the toxicity assay demonstrate that the extract obtained from the leaves of *Tagetes lemmonii* was an effective insect toxin against *Tribolium castaneum*. The radical scavenging activity and *p*-anisidine test results of olive oil spiked with different concentrations of leaf extract showed that the phenolic compounds can retard lipid oxidation.

1. Introduction

The environmental influences such as UV radiation, toxins, pollutants, and diet are major causes of biomolecular oxidation and the oxidative stress that can cause diseases or disorders and accelerate the human aging process (Chew et al., 2008; Gliszczynska-Swiglo, 2006). Compounds with antioxidant properties not only protect the body from some diseases associated with aging but also reduce the effects of oxidative stress (Chyau et al., 2015; Gutteridge and Halliwell, 2010; Vattakandya and Chaudharib, 2013). Plants have been investigated due to their concentrations of potential of bioactive compounds. Phenolic compounds exhibit effective antioxidant properties and are known to be the most promising functional groups for the relief of oxidative stress (Sharma et al., 2015). More and more interest is being directed toward the use of natural antioxidants that can offer more health benefits than synthetic antioxidants and reduce the use of synthetic antioxidants (Dominguez-Perles et al., 2014). Apart from the medicinal applications of bioactive extracts, natural bioactive compounds can act as alternatives to synthetic pesticides in the protection of plants against pests and can be used to improve the oxidative stability of edible oils (Kyarimpa et al., 2014; Vaisali et al., 2016). The presence of a wide variety of bioactive compounds in the extracts serves as an important defensive strategy for plants against insects, and assists in preventing autoxidation or oxidative rancidity in edible oils (Sayyari and Farahmandfar, 2016; Tholl, 2006). Although synthetic antioxidants and insecticides are effective for preventing oxidative rancidity of edible oils and controlling insect pests, these synthetic compounds were not well accepted by customers due to undesirable effects on human and animal health (Ilaiyaraja et al., 2015). Currently, bioactive compounds found in plants are considered important alternatives for applications in food, pharmaceuticals, environmental pest management and cosmetics.

Tagetes lemmonii (Lemmon's marigold) is a perennial herbaceous plant that belongs to the Asteraceaea family, which is native to southern Arizona in the United States as well as the states of Sonora and Sinaloa in northwestern Mexico. *Tagetes lemmonii* is a rich source of antioxidants and therefore has great potential for applications in food chemistry (Trucker and Maciarello, 1996). The solvent extraction process is relatively efficient and is usually applied to extract bioactive compounds from plants. This study prefers to use ethanol as the solvent to extract bioactive compounds from the *Tagetes lemmonii* leaf because of its environmentally friendly nature, high extraction efficiency, lower toxicity, and lower cost. Response surface methodology (RSM) was applied to obtain the optimum extraction conditions for the extraction of *Tagetes lemmonii* leaves. Phenolic compounds include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and other plant metabolites; they can produce antioxidant, anticarcinogenic, antimutagenic, and anti-inflammatory effects and can be harmful to insects (Huang and Cai, 2010; Dieng et al., 2016). Therefore, extraction yield (EY) and total phenolic content (TPC) were considered response values, while ethanol concentration, extraction

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time and temperature were considered the independent variables for maximum TPC extraction. Optimum extraction conditions were used to investigate the insecticide activity of *Tagetes lemmonii* leaf extract against *Tribolium castaneum*. Finally, the extract was used as an additive to improve the oxidative stability of the olive oil, and the oil oxidative rancidity was monitored by the DPPH scavenging activity and the *p*-anisidine value.

2. Materials and methods

2.1. Materials

Tagetes lemmonii leaves were obtained in a local market in Taipei, Taiwan. Olive oil was purchased from the local market. Acetic acid (> 99%), ethanol (99.8%), sodium carbonate (Na_2CO_3) (99.5%) and Folin-Ciocalteu's reagent (2.0 N) were purchased from Fisher Chemical, USA. Gallic acid (98%) and n-hexane (99%) were supplied by Acros, USA. DPPH (2,2-diphenyl-1-picrylhydrazyl) (95%) and *p*-anisidine (99%) were purchased from Alfa Aesar, USA. Permethrin (95%) was provided by AK Scientific, USA.

2.2. Extraction and GC-MS analysis

Tagetes lemmonii leaves were collected and put into an oven at 40 °C. After 210 min, the sample was ground to a fine powder for further experimentation. A sample of approximately 5 g was mixed with 150 ml solvent at the desired ethanol concentration, and held for the desired extraction time and temperature in an incubated shaker and maintained at 100 rpm. After maceration, the extract was filtered through Whatman No. 1 and separated by centrifugation at 6000 rpm (Hsiang Tai centrifuge, CN-2060, Taiwan) for 10 min. Finally, the extract was evaporated to a constant mass at 40 °C under vacuum in a rotary evaporator (Rotavapor R-3, Buchi, Switzerland) and stored at 4 °C until further use. Each experiment was carried out in triplicate, and the EY (%) was determined on a dry weight basis.

The extract was analyzed using GC/MS techniques in an ISQ Series GC/MS system equipped with a Trace™ 1300 gas chromatograph (Thermo, Massachusetts, USA). Each sample (1 µl) was injected in a 30 m Rxi-5 ms fused silica column (0.25 µm) with a carrier gas flow rate of 1 ml min^{-1}. The injector and detector temperature of the GC-MS were set at 300 °C. The oven temperature was programmed as follows: 70 °C for 1 min, raised to 200 °C at 3 °C min^{-1} and held for 5 min, raised again to 300 °C at 20 °C min^{-1}, and held for 10 min. The compounds were identified by comparison of the mass spectra with the mass spectra library (NIST 14 and Wiley Libraries).

2.3. Phenolic content analysis

The TPC of the extracts was determined with the Folin-Ciocalteu reagent according to the method of Singleton and Rossi (1965), with some alterations. A 50 µl aliquot was mixed with 100 µl of Folin-Ciocalteu's reagent and 2 ml of distilled water. After 5 min, 1 ml of sodium carbonate (15%) was added, and the mixture was incubated at room temperature for 2 h. The absorbance was recorded at 760 nm on a UV–vis spectrophotometer (Genesys™ 10S UV–vis, Germany). Total phenolic content is expressed as milligrams of gallic acid equivalent per gram of dried leaf (mg GAE g^{-1} DL) and was determined using the calibration equation obtained using different standards of gallic acid.

2.4. Insect cultures

The insecticide activity of *Tagetes lemmonii* leaf extract against *Tribolium castaneum* was evaluated in this study. The insect rearing conditions used were from Bougherra et al. (2015) and were 70% relative humidity, room temperature in plastic boxes (40 × 31 × 26 cm) containing rice bran with constant air circulation. The adults raised in this way were used for bioassays.

2.5. Toxicity bioassays

The toxicity bioassays were conducted following the method proposed by Peixoto et al. (2015) with modifications. In each experiment, a glass Petri dish (8 cm diameter) containing 20 adult insects was used. The insecticide permethrin (PER; 0.5%) diluted with ethanol (99.5%) was used as the positive control, and ethanol without permethrin was used as the negative control. Different concentrations (0–1250 ppm) of extract were diluted with ethanol and prepared as the test sample for the evaluation of insecticidal activity against *Tribolium castaneum*. A 500 µl volume of each test sample was dropped on a piece of filter paper (Whatman No. 1) which was placed on the bottom of the Petri dish. The Petri dish was sealed with Parafilm to prevent fast leakage of the test sample, and several pinholes were made in the Parafilm to avoid the death of the insects due to suffocation. All the experiments were maintained at 25 ± 1 °C, 60% relative humidity in the dark and three replicates were performed for each bioassay. The number of dead insects was recorded after 3 h for the determination of observed mortality (%). To correct the control mortality (%), Abbott's formula (Abbott, 1987) was applied to obtain the corrected mortality (%):

$$Corrected\quad mortality(\%) = \frac{(observed\quad mortality(\%) - Control\quad mortality(\%))}{100 - Control\quad mortality(\%)} \times 100 \tag{1}$$

The LC_{50} value (the lethal concentration of the sample to kill 50% of the insects) was determined from the plot of test sample concentrations (ppm) versus corrected mortality (%).

2.6. Oxidative stability of olive oil

According to the study by Yim et al. (2013), leaving an oil sample in a forced-draft oven at 65 °C for one day is equivalent to one month of storage at ambient temperature. The oxidative stability test of olive oil was designed based on this concept. First, 150 µl of the dilute extract at various concentrations (200–800 ppm) was added to 50 ml of olive oil in amber bottles; then, the bottles were placed in a sonicator for 5 min to disperse the extract homogeneously. To accelerate the oxidation of the olive oil, the oil samples were stored at 100 °C oven for 3 h (preheated) and then cooled in a 4 °C refrigerator for 21 h; this 24 h process of heating and cooling constitutes one cycle. The oil sample without the extract was subjected to the same procedure and served as the control sample for comparison. The oil oxidative rancidity was monitored by the DPPH scavenging activity and the *p*-anisidine value at regular periods (cycles) for 0–3 days.

2.6.1. Determination of radical scavenging activity

The scavenging activity of the olive oil against DPPH radical was determined according to the method described by Martinez and Maestri (2008), with some modifications. The diluted oil sample (0.1 ml) was mixed with 1.9 ml of 99% n-hexane and 1 ml of DPPH solution (0.1 mM in n-hexane). The solution was then vigorously shaken and allowed to settle in the dark at room temperature for 30 min. The absorbance of the test samples with (A_s) and without (A_b) olive oil was measured at 517 nm using a spectrophotometer. The DPPH radical-scavenging activity was calculated using the following equation:

$$radical\quad scavenging\quad activity(\%) = \frac{(A_b - A_s)}{A_b} \times 100 \tag{2}$$

2.6.2. Measurement of p-anisidine value (AV)

The measurement of the *AV* of the oil sample was conducted following the method described by Chong et al. (2015), with slight modifications. A certain mass of olive oil sample (*W*) was dissolved in 25 ml of n-hexane (99%), and then 5 ml of the mixture was taken to react with 1 ml *p*-anisidine solution in acetic acid (0.25% w/v). The