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Original Article

Reversed-phase Liquid Chromatographic Quantification of Pyrethrins in the Extract of Wild *Tanacetum parthenium* (Feverfew) from Northern Khorasan Province (Iran)

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Abstract

Chemical insecticides application for pest control pose serious impacts on human health and environment. Nowadays, intensified efforts to find safer and environmentally friendly alternatives have resulted in identification and production of some plant-derived natural ingredients that can use against insect pests. Amongst these plants, feverfew, *Tanacetum parthenium* (L.) Sch.Bip., from Asteraceae family is reputed to have insecticidal properties in addition to its excellent medicinal values. In this study, we quantitatively evaluated the extract of *T. parthenium* collected from Northern Khorasan province (Northeast of Iran) for its pyrethrin content using RP-HPLC chromatography.

Flowers and leaves of *T. parthenium* harvested at flowering stage were dried at cool and dark place and subjected to 3 steps maceration with (30ml) chloroform and shaking for 1 hr. followed by filtration. Pyrethrin contents were then read by chromatographic method at 230 nm wavelength against the background of calibration regression equations. Our results indicated that dry flowers contain 0.46% total pyrethrin (I+ II), whereas leaves and stems include 0.06% pyrethrum. Pyrethrin was more concentrated in flower than stem. The wild population of *T. parthenium* of Northern Khorasan province demonstrates high potentiality to be commercially cultivated if it undergoes a plant-breeding program to manipulate phenotypic variation in the concentration of bioactive compounds present at harvest.

Keywords: *Tanacetum parthenium*, Pyrethrin, RP-HPLC, Feverfew, Botanical insecticides

Introduction

Insecticides residues have serious negative effects on human health and environment [1]. In the recent years, many plant-derived natural products have been tested for their insecticidal properties against insect pests as adulticides, larvicides, ovicides, growth regulators, or repellents [2-7]. Some of the products derived from *Chrysanthemum roseum* Web. and Mohr. [Compositae], *Nicotiana tabaccum* L. [Solanaceae], *Derris elliptica* Benth (Fabaceae), *Azadirachta indica* A. Juss (Meliaceae), *Melia azaderach* L. (Meliaceae), and *Xanthium strumarium* L. (Solanaceae) have been marketed as natural insecticides [8,9]. Being a member of Asteraceae family, *Tanacetum parthenium* (Feverfew) is reputed to have medicinal and insecticidal properties [10,11]. Taxonomically, feverfew has been placed in 5 different genera;, hence, controversy exists over its botanical classification [12]. Former botanical names include *Chrysanthemum parthenium* (L.) Bernh., *Leucanthemum parthenium* (L.) Gren and Gordon, *Pyrethrum parthenium* (L.) Bernh and

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Matricaria parthenium (L). [13,14]. This plant has been used for medicinal purposes to treat psoriasis, dermatitis, asthma, tinnitus, headache, inflammation, fever, vomiting and worms [15]. In addition to its numerous many medicinal traits, feverfew extract has strong potential to be applied as an organic insecticide [16-20]. The plant extracts and essential oils have exhibited effective insecticidal activity against several species. Essential oils are complex mixtures of various active ingredients, which may exert their biological effects as standalone molecules or via synergism of all molecules [21]. Two main constituents of feverfew essential oil with insecticidal activity are pyrethrin I and pyrethrin II (Fig. 1).

In this study, we quantitatively evaluated the essential oil of wild populations of *Tanacetum parthenium* [feverfew] collected from high lands of Goloul Dam of Shirvan as natural habitats in Northern Khorasan province (Northeast of Iran) for its pyrethrum content in order to validate its potential as an indigenous bio-insecticide.

Material and Methods

Materials

Pyrethrum extract containing 25% pyrethrin I + pyrethrin II (82670-25g) was purchased from Sigma-Aldrich (Germany). Acetonitrile (99.9%) and chloroform 99.4% HPLC grade were obtained from Merck (Germany).

Plant Materials

The plant, *Tanacetum parthenium*, was harvested at flowering stage from high lands of Goloul Dam of Shirvan as natural habitats in Northern Khorasan province (Northeast of Iran) during September 2015. The plant identification was confirmed by Herbarium of Research Center of Plant Sciences at Ferdowsi University (Mashhad, Iran). The flowers, leaves and stems were dried under cool and dark conditions for extraction purposes.

Plant Extraction

Dry flowers, leaves and stems of *Tanacetum parthenium* were separately pulverized (with an electric mixer) and the powders were passed through a sieve of 250 µm mesh size. The dry powder (0.6 g) was then macerated with (30ml) chloroform by 1 hr. shaking before being filtrated through filter papers. The remnant powder was re-extracted twice by adding [30ml] fresh chloroform and shaking for extra 30 minutes followed by filtration. The filtrates were then combined and the solvent was evaporated at room temperature under an airflow cabinet.

Plant Chromatographic Conditions (RP-HPLC)

To determine pyrethrin contents, the crude extracts were subjected to HPLC analysis. The analytical HPLC system consisted of an lc-6ap pump [Shimadzu, Japan], an SPD-M20 AUV-detector (Shimadzu, Japan), an Shimadzu injection value with a 20µl sample fixed loop, and an vp-C18 analytical column (4.6 mm*250 mm ,Shimadzu, Japan). The mobile phase components used were acetonitrile and water mode (gradient 35% H2O in 65% CH3CN). The flow rate was 1 ml/ min. The pyrethrins were detected at wavelength of 230 nm. Spectra for each ester were obtained over a wavelength range from 200 to 400 nm using the same gradient program.

Pyrethrin Method Evaluation

Quantitative analysis was performed using a range of concentrations including 1, 2, 4, 5, 10, 50, 100, 157, 250, 315, and 500 ppm of standard pyrethrum. A volume of 20 µl of each concentration was injected into the HPLC to produce calibration curve for pyrethrin I and II (Fig. 2). Based on retention times of peak1 and peak 2 and the calibration data, the concentration of compounds in the extract was determined (Fig. 2). Identification of compounds was confirmed by comparing the retention time of the flower and leave plus stem extracts with that of an authentic standard. (Fig. 4 and 5)

Result and Discussion

*Tanacetum parthenium (*Feverfew) and its synonymous species such as *Chrysanthemum parthenium* are well known for their bioactive ingredients of excellent medicinal and insecticidal application [22]. *T. parthenium* has a broad spectrum of biological activities and traditionally been used in the manufacturing of cosmetics, insecticides and herbal medicines [22, 23]. Mojab *et al* found similarity between the essential oil compositions of the root and the aerial part of *T. parthenium*, of which terpenoids constitute major components. [24]. This was not the case in the present study as we found significant difference between the insecticidal pyrethrins (monoterpenes) contents of flowers and other parts. Pavela *et al*

showed that insect mortality correlates with terpenoid content of *Tanacetum parthenium* extracts [25]. Undertaking bioassays on housefly *Musca domestica* using flowers extracts, we observed dose dependent mortality and paralysis both at larval and adult stages (data not shown). In fact, pyrethrins kill insects by disrupting their nervous systems, but are safe to mammals due to their enzymatic capabilities to detoxify pyrethrins into harmless compounds [26, 27]. Pyrethrins exist in plant extracts as a combination of six esters; pyrethrin I, cinerin I, jasmolin I, and pyrethrin II, cinerin II and jasmolin II, of which pyrethrins I and II are the major components [28,29]. While, pyrethrin I is especially effective in killing insects, pyrethrin II produces rapid knockdown but both molecules degrade rapidly under harsh environmental conditions [30,31]. Given the thermal instability of pyrethrins, the RP-HPLC method, used in this study, showed to be suitable for analyzing pyrethrin content of *T. parthenium* [27]. After several trials, the chromatographic method was set to yield the best result using a liquid phase solvent composition of 35% water to 65% acetonitrile and the flow rate of 1 mL/min. Also, the detector wavelength at 230 nm allowed the least chance of interference at the other components' retention times. Among the three solvents namely acetone, acetonitrile and chloroform used for dry powder maceration, the later was more effective, yielding 4.91% oil soluble extract.

Linearity was tested by injecting 2 groups of 11 standard solutions of pyrethrin I and II over the range of 1 to 500 µg/mL (ppm) of total pyrethrins. Linear regressions were established between the elution peak areas and the concentrations for each of the esters. Correlation coefficients were then obtained to be higher than 0.99 as in Figure 1. This confirmed the linear relationships for the two esters within the used concentration range. The retention times were fluctuated around 9.2 min for Pyrethrin II and 20.6 min for Pyrethrin I as in Figure 2. However, as shown in Figure 3 and 4, no interference by other chemical components was observed at the retention times of the two pyrethrins.

The regression equations of the standard solutions of pyrethrin I (Y=3.2867X+30.5184) and pyrethrin II (Y=2.2063X+12.8162) were used to calculate concentration of pyrethrin content of the plant extracts. Given the dry weight of extracts obtained from 100 gr dried *Tanacetum parthenium* flowers or leaves, the total pyrethrum contents were calculated. The total pyrethrin (I+ II) content of feverfew flowers was found to be 0.46%, whereas that of its leaves and stems was 0.06% per dry weight. Pyrethrins were more concentrated in feverfew flower than its stems and leaves. Other investigators reported that dried pyrethrum flowers have been shown to contain about 0.5-2% of natural pyrethrins [32,33]. Also, Végh *et al* revealed that the essential oil of Feverfew leaves comprises between 0.59 to 0.69 g/100 g drug [15]. On the other hand, Grdiša *et al*, [34] reported that Croatian populations of the same species contain higher quantity of pyrethrin approximately 0.60 to 0.79%. In conclusion, the wild population of *Tanacetum parthenium* of northern Khorasan province demonstrates high potentiality to be commercially cultivated if it undergoes a plant-breeding program to manipulate phenotypic variation in the concentration of bioactive compounds present at harvest.

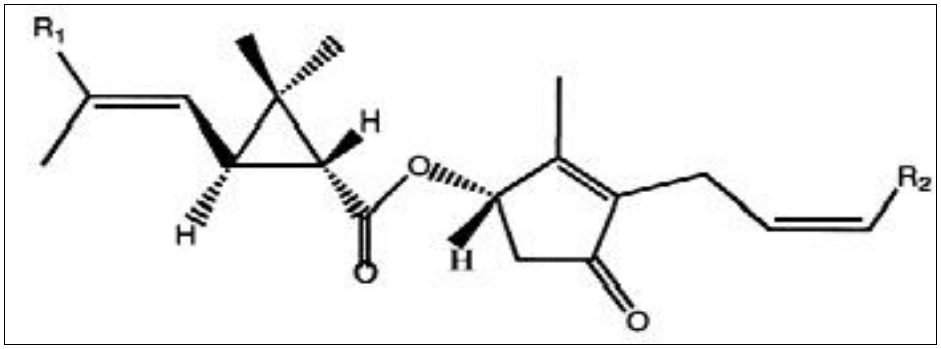

Fig. 1 Structures of main pyrethrin esters (pyrethrin I: $R_1 = CH_3$, R2 = $CH{=}CH_2$; pyrethrin II: $R_1 = CH_3O_2C$, $R_2 = CH{=}CH_2$)

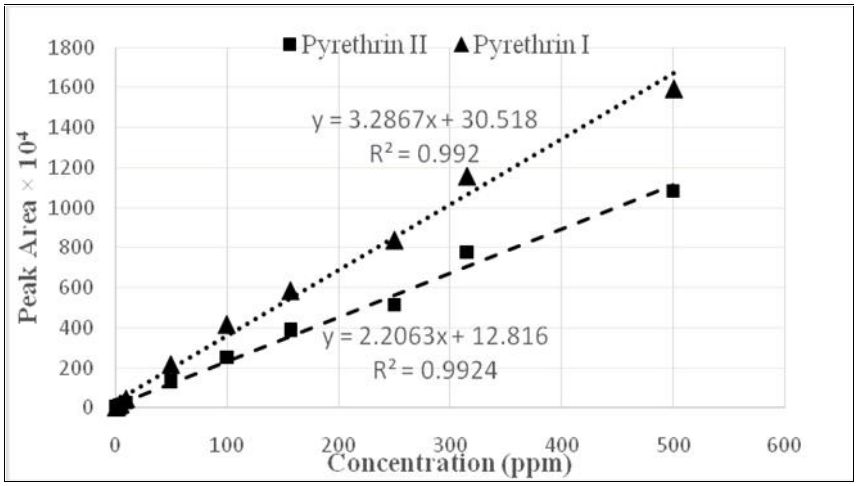

Fig. 2 linear regression results of standard calibration concentrations of pyrethrins I and II obtained from HPLC chromatography at 230 nm

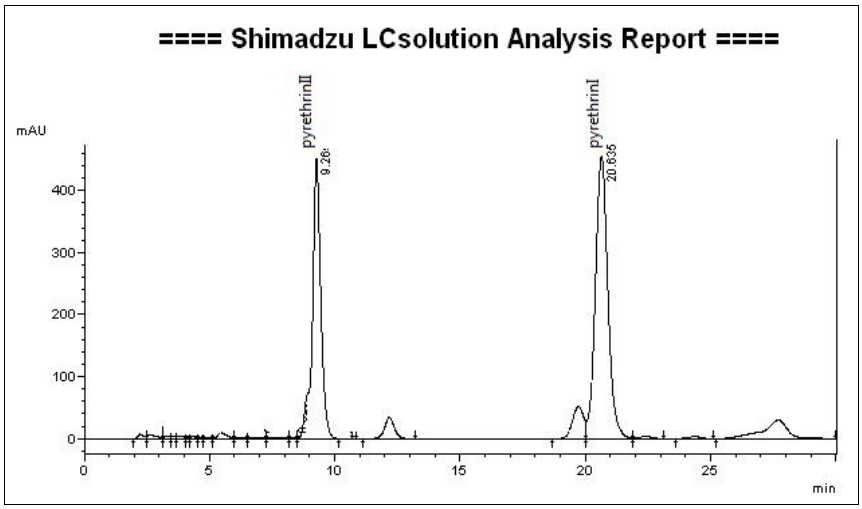

Fig. 3 Reversed-phase HPLC chromatogram of standard pyrethrum. (Mobile phase flow rate 1 mL min-1, injection volume 20 µL, UV detector at 230 nm)

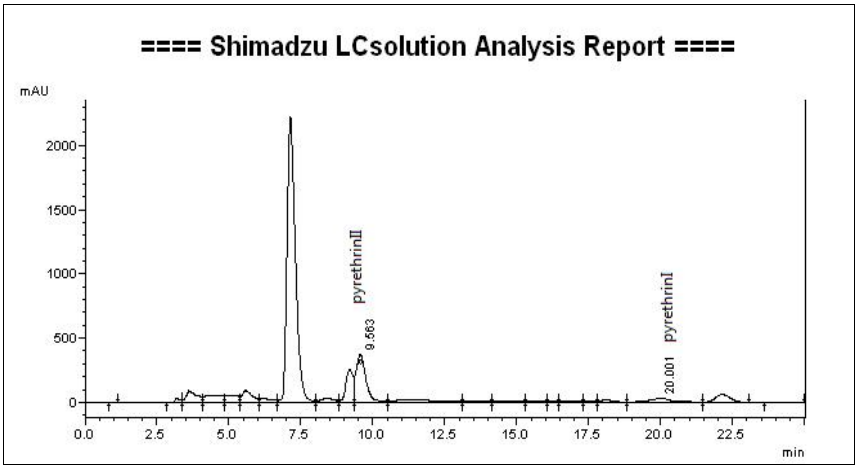

Fig. 4 Reversed-phase HPLC chromatogram of *Tanacetum parthenium* (L.) Sch.Bip.flower extracts. [Mobile phase flow rate 1 mL min-1, injection volume 20 µL, UV detector at 230 nm]

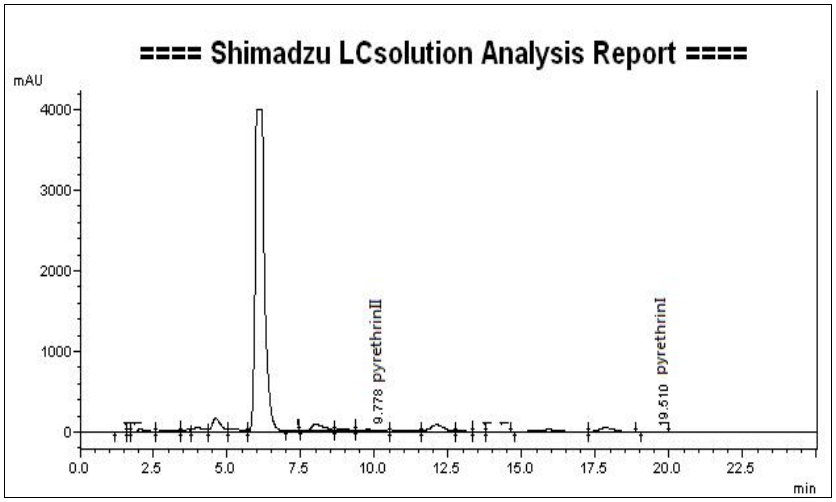

Fig. 5 Reversed-phase HPLC chromatogram of *Tanacetum parthenium* (L.) Sch.Bip. stems and leaves extracts. [Mobile phase flow rate 1 mL min-1, injection volume 20 µL, UV detector at 230 nm]

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