Variation bioactive metabolites of *Tanacetum parthenium* L. in two regions in North of Iran.

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Abstract

Tanacetum parthenium L. is one of the most important medicinal herbs, has been used by the rural people of Golestan province for sedative pain, fever, headache, arthritis, asthma, toothache and menstrual disorders.Variation in bioactive components of *Tanacetum parthenium* L. were studied and compared for their essential oil, flavonoids and alkaloids of different parts of plant in two geographical regions of this province. Results showed *T. parthenium* which originated from 2200m produced the highest essential oils, flavonoids and alkaloids, especially in leaves but with low quality and quantities and reduced in flowers. Further more stem have not any bioactive components in two regions.

Key words: *Tanacetum parthenium* L, essential oil, flavonoid, alkaloid, Golestan province

Introduction

Tanacetum species, belongs to Asteraceae family totaling over 200 and distributed over Europe and West Asia and growing up to altitudes of 2000 meters contains several strongly scented annual and perennial species, and now grows in North and South America, Europe, North Africa, China, Japan and Australia (Jaime and Teixeira., 2004).

The aromatic herb fever few (*Tanacetum parthenium* L.) has long been used as a folk remedy for fever, migrain and arthritis (Thersa et al., 2001). It has been historically used for the treatment of headache, menstrual irregularities, stomachache and fevers by Greek and European herbalist (Tiuman et al., 2005 and Tyler, 1993). How ever, this aromatic plant was somewhat forgotten by pharmacists until late 1970 when claims of its efficacy in prevention 0f migraine were publicized in Britain (Groenewegen, Knight, & Heptinstall., 1992).

The terpenes in the essential oil are thought to associate with the biological activity of *Tanacetum*. Some earlier works have been reported on the essential oils of various *Tanacetum* species (Nori-Shargh et al., 1999, Baser et al., 2001, Goren et al., 2001 and Beauchamp et al., 2001). The volatile compounds from *T. vulgare* have been examined in detail (Keskitalo et al., 2001). In the case of *T. argyrophyllum*, α-thujone is reported to predominate in its essential oil (Goren et al., 2001). With respect to *T. parthenium*, many studies have been found that relate to the sesquiterpene lactone parthenolide of essential oil and flavonoids, which exhibit strong biological activity (Changquing et al., 2006, Awang, 1998, Jain and Kulkarni, 1999, Williams et al., 2003 and Long et al., 2003). Camphor and chrysanthenyl acetate were the main components of the essential oil of *T. parthenium* originated from England and Netherlands, it is aromatic perennial plants, wich wide distributed in the northern hemisphere (Hulten, 1968; Hussey, 1974; and Heywood, 1976).

Fever few is now among the 50-top-selling supplements in USA, which is commercially available like other common herbs in capsules or tea bags for migraine prevention (Susrurluk et al., 2007). Many researchers have studied that the bioactive component corresponding for the pharmacological functionality in essential oil and flavonoids (Mittra et al., 2000).

Further studies also showed that parthenolide of essential oils were effective in inhibition of some tumor cells (Wen et al., 2002) and inflammatory (Tiuman et al., 2005).

In addition fever few contains inherent constituents such as aromatic components (e.g.camphor) and flavonoids (e.g.luteolin and apigenin) was reported to significantly inhibit UV-induced mouse skin tumor genesis (McVean et al., 2000) and other tumors (Pedrialla & skibsted, 2002).

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As well known, many flavonoid in leaves and flowers of this plant possess anti-inflammatory and antioxidant activities such as metal chelating and free radical scavenging capacities (Pedrialla and skibsted, 2002) with privation of chronic diseases such as cardio vascular disease and cancer (Sultana & Saleem., 2004).

However due to wild dispersal of this plant in North of Iran (Golestan province) and has been long used in Golestanian traditional medicine, the objectives of our study were to investigate and compare of variation bioactive components of plant different parts in two natural geographical origins in south of Golestan province (Deraznoo mountain in 2200m and Ziarat in 500m).

Material and methods

Plant material

Aerial parts in blooming (leaves and flowers) of plant were collected in late of August of 2006 from two natural different regions (Deraznoo in 2200m and Ziarat in 500m) in Golestan province in North of Iran. They were dried in the shade for one week and powdered. Their botanical name identified in the plant systematic laboratory, college of science, Islamic Azad University of Gorgan Branch, Iran where voucher specimens were deposited.

Essential oil

200 gr of the dried powders of separate leaves and flowers were separately subjected to hydro distillation for 2h, in full glass apparatus. The oil was isolated using a Clevenger type apparatus and stored frozen in dark glass bottles until they were used. (Orav et al., 2006)

Oil analysis

The oils were analyzed by GC (9-A-shimadzu) and GC-MS (Varian - 3400) column (DB - 1, 60 mm 0.25 mm fused silica capillary column film thickness 0.25μm using a temperature program of 50-250°C at a rate of 4°C/min, injector temperature 260°C, carrier gas: Helium, the constituents were identified by comparison of their mass spectra with those in the computer liberary and with authentic compounds. The identifications were confirmed by comparison of their retention indices with those of authentic compounds or with literature data. The components of the oils were identified by matching their mass spectra and retention indices with those of the Wiley 275 library in the computer library and literature. The yield of each component was calculated per kg of the plant material, while its percentage composition was determined from the peak areas of the total oil composition.

Gas Chromatography

GC analyses were performed using a shimadzu – 9A gas chromatograph equipped with a flame ionization detector and quantitation was carried out on Euro Chrom 2000 from KNAUER by area normalization method and neglecting response factors. The analysis was carried out using a DB – 1 fused-silica column (60m x 0.25mm, film thickness 0.25μm, J & W Scientific Inc., Rancho Cordova, CA). The operating conditions were as follows: injector and detector temperature 250^{o}C and 265^{o}C, respectively; carrier gas, helium. Oven tem-perature programme was 40^{o}C-250^{o}C at a rate of 4^{o}C/min.

Gas Chromatography / Mass Spectrometry

The GC/MS unit consisted of a varian Model 3400 gas chromatograph coupled to a Saturn II ion trap detector was used. The column was same as GC, and the GC conditions were as above. Mass spectrometer conditions were: ionization potential 70eV; electron multiplier energy 2000V.

The identity of the oil components was established from their GC retention indices, relative to C_7–C_{25} n–alkanes, by comparison of their MS spectra with those reported in the literature and by computer matching with the Wiley 5 mass spectra library, whenever possible, by co–injection with standards available in the laboratories.

Ethanolic extraction

Plants parts were dried, ground to find texture and after which it, 70% ethanol were added to 100 gr of dried plants powder in decanter for extended periods and the result ant extracts were obtained in period of 72h. the resultant extracts were concentrated, under reduced pressure finally each samples were diluted with propylene glycol for provide 4 concentration: 200, 100, 50 and 25 mg.ml^{-1}. (Mashhadian, et al., 2005)

Investigate quality of flavonoid and alkaloid

Falvonoids

The alcoholic extract (5ml, corresponding to 1g of plant material) was treated with a few drops of concentrated HCl and magnesium turnings (0.5g). The presence of flavonoids was indicative if pink or magenta-red color developed within 3 min (5).

Alkaloids

The alcoholic extract (corresponding to 2.5g of plant material) was evaporated to dryness and the residue was heated on a boiling water bath with 2N HCl (5ml). After cooling. The mixture was filtered and the filtrate was divided into two equal portions. One portion was treated with a few drops of Mayer's reagent and other with equal amounts of Wagner's reagent (3). The samples were then observed for the presence of turbidity or precipitation. A (+) score was recorded if the reagent produced only a slight opaqueness: a (++) score was recorded if a definite turbidity. But no flocculation was observed and a (+++) score was

recorded if definite heavy precipitate or flocculation was produced (4).

Results and discussion

Tanacetum parthenium L. (Tancy) commonly known as fever few and locally name " Dawoody vahshi "belongs to Asteraceae family is a perennial herb 14-45 cm in height, having a strong smell and greenish yellow feather like leaves. It is widely distributed in hedges and wants places 500 to 2200m in throughout of sunny position the road mountain in south of Golestan province in North of Iran.

The leaves have long been used as infusions or can be chewed in conditions like fever, migraine, headache, arthritis, asthma, toothache and menstrual pains by rural peoples of this region in Golestanian traditional medicine.

In this research we investigated and compared. The quantities of essential oils and quality of the most bioactive metabolites of plant (flavonoids and alkaloids) in different parts (leaves and flowers) in two geographical origins: Deraznoo in 2200m and Ziarat region in 500m, respectively. Results showed in Table 1 and Fig 1, 2.

The essential oil yields (v/w, on dried mass basis) of *T. parthenium* parts grown in Ziarat,were 0.66% in flowers and 0.12% in leaves, but in Deraznoo 0.49% and 0.16%, respectively; close to those reported for *T. argyrophyllum* (0.96–1.03%) and *T. parthenium* (0.30–0.83%) (Hendriks et al., 1996; Goren et al., 2001).

In height region (2200m), the essential oil percentage, quality flavonoids and alkaloids were increased especially in leaves. In two regions, stem have not any bioactive components.

Table1: Essential oil variation in different parts of plant (leaves and flowers).

	Ziarat (900m)		Deraznoo (2200m)	
Tanacetum parthenium	flawers	leaves	flawers	leaves
	0.66%	0.12%	0.49%	0.16%

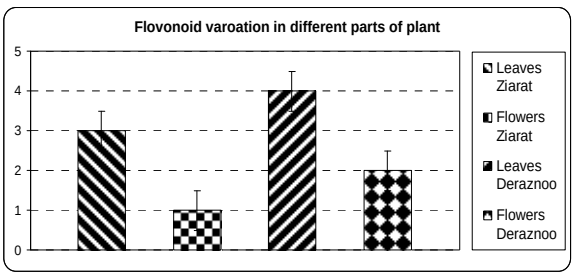

Figure 1: flavonoid variation in different parts of plant (leaves and flowers).

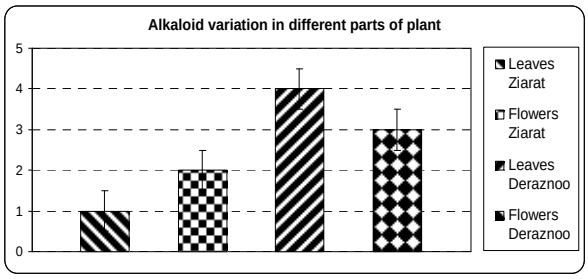

Figure 2: Alkaloids variation in different parts of plant (leaves and flowers).

According similar researches, we reported monoterpens of essential oils and flavonoids were considered as the most important biologically active principal of fever few (Shiravastara et al., 2006). The composition variation of sesquiterpen lactones, flavonoids of *T. parthenium* varies markedly and several chemo types from different geographical origins have already been classified. In similar reported, Tancy have different flavonoids from Tier Adel fuego, Argentina (Gorene, 2001), England and USA, tansy oil terpenoids and flavonoids obtained from two Canadian grown plants contained different quality and quantities (Keskitalo et al., 2001).

T. parthenium L. which originated from south west of Finland produced the highest bioactive compounds in flowers and reduced in leaves but in high concentration of flavonoids from South Finland in leaves (Susruluk et al., 2007).

Several authors have studied the variation of chemo type of terpenoides and flavonoids. Sorsa & et al found that camphor was the main components of flower in northern, but thujon in southern Finland (Susruluk et al., 2007), Sabinene, bornyl acetate and germacrene-D (Holopainen, 1989), essential oil quantities and their chemo type were detected from fever few grown in different regions of Finland.

Fever few also contains other variation secondary products such as less sesquiterpenes (Hethelyi et al., 1999) and several flavonoids compounds, but the other groups that grown different parts of Ognyanov and Todorova consist monoterpene glycocosides, sesquiterpene lactone (Schearer, 1984) such as parthenolide (Hendrisk and Bos, 1990) triterpens (Williams et al., 2003) and flavonoids (Christensen, 1999).

Variation fever few essential oil and chemo types in different origins have been investigated by the other authors (Keskitalo et al., 2001), Many factors are known to influence secondary metabolites (leaf/flower), ontogeny of plant sampling (Hendrisk et al., 1990) seasonal changes and even the extraction method (Gorene et al., 2001) many cause variation in the composition of secondary metabolites.

Jain and Kulkarni were reported that fever few extract exerted anti nociceptive and anti inflammatory effects (Jain and Kulkarni, 1999).

Williams et al. reported f *T. parthenium* showing activity of mitotic blocker, due to rich various flavonoids in leaves and disc-floret useful anti-inflammatory properties (Williams et al., 2003).

Conclusions

The goals of this study to compare the variation quantities and quality bioactive composition different parts of plant in two geographical origins in North of Iran. Assessment variation chemical of *T. parthenium* and its most used by the rural healers for migraine disorders allow us to identify unique chemo types with potential industrial value and bioassay the activity of the secondary bioactive metabolites. With such information the most desirable chemotypes can be selected for plant drugs product.

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مقایسه متابولیت‌های ثانوی اندام‌های مختلف گیاه دارویی *Tanacetum parthenium* L. در دو رویشگاه شمال ایران

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چکیده

گیاه دارویی داوودی وحشی با نام علمی *Tanacetum parthenium* L. یکی از گونه‌های مهم و ارزشمند دارویی استان گلستان است که در طب سنتی مردم آن منطقه از ارزش خاص دارویی مخصوصاً به عنوان تب‌بر قوی، مسکن سردرد، کمردرد، دندان درد، دردهای قاعدگی، آسم و ضدالتهاب مورد استفاده قرار می‌گیرد. در این تحقیق به بررسی و مقایسه مهمترین مواد موثره ثانوی اندام‌های گیاه موردنظر در دو رویشگاه متفاوت و طبیعی جنوب شرق استان گلستان پرداختیم. نتایج نشان داد با افزایش ارتفاع بر میزان روغن اسانسی، فلاونوئید و آلکالوییید مخصوصاً در برگ‌های گیاه افزوده می شود و تقریبا در هر دو رویشگاه ساقه گیاه فاقد متابولیتهای مورد مطالعه می‌باشد.

کلمات کلیدی: *Tanacetum parthenium* L.، روغن اسانسی، فلاونوئید، آلکالوئید، اندام‌ها، رویشگاه‌های متفاوت، استان گلستان