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Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2017.01.013

Natural variability of essential oil and antioxidants in the medicinal plant *Turnera diffusa*

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ARTICLE INFO

Article history:
Received 23 Nov 2016
Received in revised form 24 Dec 2016
Accepted 9 Jan 2017
Available online 20 Jan 2017

Keywords:
Essential oil
Antioxidants
Phytochemicals
Chemical variation

ABSTRACT

Objective: To evaluate differences in yield and composition of the essential oil and antioxidant contents in *Turnera diffusa* plants from localities in central region of Tamaulipas.

Methods: Samples were collected in Tamaulipas, Mexico in the arid zone. Essential oil was obtained through steam distillation and analyzed using GC–MS. Polyphenol contents, antioxidant activities using ABTS and ferric reducing antioxidant power (FRAP) methods also were evaluated.

Results: A total of 21 compounds were identified in the essential oils; nevertheless, only Eucalyptol, 1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1à,4à,4aà,10aà) y Ethanone, 1-(1,3-dimethyl-3-cyclohexen-1-yl) were detected in the three sites. Highest contents were registered in the sample from Padrón y Juárez with phenolic content of 33.85 mg GAE/g of dry material and antioxidant activities with ABTS 72.32% and with FRAP 21.33 mg GAE/g of dry material. Statistical differences were observed in essential oil, phenolics and antioxidants contents between populations.

Conclusions: Results suggest that climatic differences and origin influence the phytochemicals in the medicinal plant *Turnera diffusa*, and thus, it is worth to consider such effects for industrial and medicinal purposes.

1. Introduction

Consumers' demands for safer and more natural products are increasing, mainly in food, cosmetic and medicine industries. One of the most important opportunity to deal with this kind of demands is the use of phytochemicals, which have been demonstrated to be useful as condiments, food, source of colors and flavors, and they have been associated with medicinal properties [1]. Due to natural origin, their consumption through plant material consumption by several methods directly from the wild or from the market is recommended. Nevertheless, it should consider the fact that phytochemical composition of plants could present variations depending of environmental factors, which could interfere with benefits, especially with medicinal properties.

Under natural conditions, plants respond in several ways to environmental pressures, including alterations in synthesis and accumulation of phytochemicals, which can differ through the space and time. Likewise, they can vary chemical diversity and pattern distribution in tissues and organs, depending from local adaptations and genetic variability in heterogeneous habitats [2]. These responses impact directly on all produced metabolites, even on those compounds potentially useful for medicine, food or industrial purposes. Therefore, such variations could affect the quality of products and beneficial effects attributed to plants [2–5] and this is particularly true for *Turnera diffusa*.

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Peer review under responsibility of Hainan Medical University.

Foundation project: This research was financially supported by the Universidad Autónoma de Tamaulipas with project UAT PFI2015-14.

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T. diffusa (Turneraceae) commonly known as damiana [6,7] is a small-branched shrub 60 cm to 1 m height; nevertheless, plants from 30 cm to 2 m also have been reported [8]. It presents lance-shaped leaves that range from 10 to 25 mm length, with fragrant, small-rounded fruits and yellow conspicuous flowers that appear on the summer [7]. This plant is used as raw material in the industry as flavor material to prepare infusions and liquors. In the Mexican herbalism, several medicinal effects are attributed to *T. diffusa*, including stimulation of the nervous system, aphrodisiac, diuretic, hypoglycemic effects and antimicrobial activities [9–11]. The main bioactive components of *T. diffusa* include the phenolics (flavonoids, phenolic acids and derivatives), cyanogenic glycosides, fatty acids, alkaloids, sugars conjugates and its essential oil, which are obtained mainly from leaves and stems. Some authors have associated the essential oil and the antioxidant effects with the medicinal properties of *T. diffusa* [1,11,12]. Therefore, it is imperative to know the accumulative patterns of such compounds in all harvested plants, to ensure the benefits to consumers.

Previously, differences in contents of essential oil, antioxidant and minerals were reported as responses to environmental influence and genetic background of plants [12,13]. Several studies have focused on chemical variations of the essential oil obtained from *T. diffusa* grown as crop and from wild conditions, indicating that chemical diversity and contents will depend on the environment [13–15].

By the other hand, antioxidants are related with beneficial effects on several illnesses and there is an interest for their incorporation in diet through natural products or supplements [16]; nevertheless, antioxidants from *T. diffusa* also present variations in their accumulation depending on growing conditions of plants [12]. These evidences suggest that accumulation of metabolites in *T. diffusa* is influenced in a multifactorial way.

In the semiarid zone of Mexico, the collect of *T. diffusa* is a common practice in rural communities, and consequently this plant material is sold in local markets for consumption as infusions, and for medicinal purposes. However, there are no controls either in collecting practices neither in certifying the geographical origin of the materials, which could affect bioactive properties of the derivatives. Therefore, the goal of this work was the chemically characterization of three natural populations of *T. diffusa*, geographically different to thus reported in literature.

2. Materials and methods

2.1. Area of study

Collection of samples was done on three sites in the central region of Tamaulipas, México: Site 1 (Padrón y Juárez) (S1) was located in municipality of Jaumave, 1 km at south part of the Padrón y Juárez town (23° 20′ 33″ N and 99° 25′ 43″ W) at 930 masl. This site has a hot semiarid climate, with average annual temperature of 20 °C and average annual precipitation of 500 mm. The predominant soil is haplic xerosol and submontane scrub as the major plant community. Site 2 (S2) was located in Nogales town at the same municipality (23° 16′ 32″ N and 99° 24′ 01″ W) at 1 100 masl within a warm subhumid climate with rains in summer, including an average annual temperature of 18 °C and average annual precipitation of 900 mm. The predominant soil was the lithosol and the oak forest and secondary forest as the major plant communities. Site 3 (S3) was located in the municipality of Victoria near the main town (23° 44′ 06″ N and 99° 07′ 51″ W), at 321 masl with warm climate with rains in summer, with average annual temperature of 25 °C and average annual precipitation of 900 mm, lithosol as the main soil and grassland and submontane scrub as the major plant communities.

2.2. Plant material

Plant material was collected by selective sampling, which considered collection of plants around 25 cm height. Four compound samples of 500 g were randomly taken from the aerial parts (leaves and stems) in each site.

2.3. Moisture content determination

Samples were transported to the laboratory, individually weighted and were dried in a convection oven at 45 °C during 72 h. Then samples were weighted and differences between first and second weights were used to calculate the moisture content as percentage of total weight.

2.4. Extraction and yielding of essential oil

Dried samples were crushed and submitted to water steam distillation [17,18] followed by extraction with CH_2Cl_2 (Sigma–Aldrich, St. Louis, MO, USA) to separate water and oil phases. Essential oil samples were recovered after evaporation of CH_2Cl_2 using nitrogen injection. Recovered essential oil was kept at 4 °C in sealed containers covered with aluminum foil until use. Yield of recovered essential oil was calculated by P = M1/M2 × 100, where M1 = final weight of essential oil, M2 = weight of plant material and 100 = mathematical factor.

2.5. Gas chromatography–MS

Identification of compounds in the essential oil was done by GC–MS using a gas chromatographer Clarus 680 (PerkinElmer, MA, USA) with a Carbowax 20 M® column 0.25 mm id 0.25 μm (Agilent Technologies, CA, USA) and a db-i Rxi-iht® column (Restek, PA, USA) 30 m 0.25 mm id 0.25 μm. The Kovats retention index and comparison of the NIST database were used for identification.

2.6. Antioxidants extraction

Samples of 2 g were grounded and then stirred in 45 mL of distilled water at 60 h during 1 h. Each extract was filtered and purified using Amberlite XAD16 (Sigma–Aldrich, St. Louis, MO, USA); after this, the solvent was evaporated and polyphenols were obtained.

2.7. Quantitation of polyphenols

Total phenolic content was determined using the Folin-Ciocalteu method according to Singleton and coworkers [19] with modifications [20]. A standard curve was made with gallic acid (Sigma–Aldrich, St. Louis, MO, USA) in a range of 100–500 mg/L. Samples, standards were incubated in presence of Folin-Ciocalteu reagent (Sigma–Aldrich, St. Louis, MO, USA)

and 2 M Na_2CO_3(Sigma–Aldrich, St. Louis, MO, USA) during 2 h, and then absorbance was registered at 765 nm. Results were calculated as gallic acid equivalents (GAE mg/g of sample).

2.8. Total antioxidant activity using ABTS

The antioxidant capacity using ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] (Sigma–Aldrich, St. Louis, MO, USA) was carried out according to the method reported by Re and coworkers [21]. A stock solution of 7 mM ABTS and 2.45 mM potassium persulfate (Sigma–Aldrich, St. Louis, MO, USA) were mixed at 2:1 ratio and was incubated during 12–16 h. Its absorbance was adjusted to 0.7 at 734 nm. A 10-μL aliquot of samples or standard reacted with 2.9 ml of the ABTS working solution during 10 min at room temperature. Then, absorbance was measured at 734 nm and results were indicated as percentage of inhibition of the radical ABTS.

2.9. Ferric reducing antioxidant power

Reduction of ferric ion was carried out according to method proposed by Çelik and coworkers [22] with minor modifications. Fifty microliters of sample were mixed with 120 μL of PBS buffer. Then 220 μL of 1% potassium ferricyanide (Sigma–Aldrich, St. Louis, MO, USA) were added and mixture was incubated at 50 °C during 20 min. After this, 120 μL of 10% trichloroacetic acid were added (Sigma–Aldrich, St. Louis, MO, USA). Finally 450 μl of distilled water and 100 μL of 0.1% $FeCl_3$ were incorporated. Then absorbance of each sample was registered at 700 nm and results were expressed as GAE according to comparison with a standard curve of gallic acid.

2.10. Statistical analysis

To determine differences in moisture content and yields of essential oils between samples, the analysis of variance was used. The analysis and graphs were done in Statistica® version 8.0.

3. Results

The studied populations grew under natural conditions and without a remarked management with the exception of S1, which comes from a natural site affected by frequent extraction, which implies removal of foliar tissue periodically. Samples from S2 were collected close to foothill of mountains in a place with high humidity and dense vegetation cover, while S1 and S3 were collected from sites with apparent less environmental humidity. Since plants were collected, clear morphological differences were observed under field conditions; and despite of their geographical location S1 were similar to S3, with average height of 24 cm, less branched (three branches by plant) and smaller foliar area. By the other side, plants from S2 had an average height of 60 cm, but some reached up to 1 m height; they were widely branched with average of 18 branches by plant; also with wider and greener leaves. Morphological differences between S1 and S2 were evident, probably associated to environmental humidity differences where they grew. Also, variations were observed in moisture contents of S1, S2 and S3 were 57.47%, 41.00% and 54.06%, respectively, indicating differences between populations [$F_{(1,2)} = 39.716$, $P < 0.01$]. Morphological differences also corresponded with statistical differences in moisture contents, where S1 and S3 were closer and both were different to S2, which grew with higher humidity in its collecting area.

To avoid variations in oil contents due to phenological stage, all collected plants were collected fully established plants with no flowering activity and no visual damage. Yields registered were between 0.02 and 0.2%.

Essential oil content of S1, S2 and S3 were 158.15, 616.90 and 336.00 mg/kg, respectively. Yields of essential oil showed statistical differences between populations [$F_{(1,2)} = 16.3347$, $P < 0.01$]. The S2 had higher content of essential oil (around 600 mg/extracted kg) and was statistically different to S1 and S3, indicating a correspondence with results of moisture content.

When oil samples of *T. diffusa* were analyzed, chemical differences were found and 21 compounds were identified in total for the three populations, several of these compounds were common in the three populations, some only were detected in two and others were exclusive for only one population, each sample was heterogeneous despite they shared some common compounds (Table 1). Samples S2 and S3 showed higher number of identified compounds, including the Eucalyptol, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1à,4à,4aà,10aà) (Diphenhydramine) and the Ethanone, 1-(1,3-dimethyl-3-cyclohexen-1-yl) which were shared in all populations. These analyses only indicated presence or absence, but no quantitative differences; nevertheless, it was clear that oil composition varied in each site. S1 presented eight identified compounds of which three compounds were only detected in this site; in S2, 12 compounds were identified and five compounds were exclusive

Table 1
Comparison of *T. diffusa* essential oil of three populations from Tamaulipas.

Compounds identified	S1	S2	S3
Eucalyptol	x	x	x
Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene	x	x	
Bicyclo[3.1.1]hept-2-en-6-one, 2,7,7-trimethyl	x	x	
1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1à,4à,4aà,10aà)	x	x	x
Ethanone, 1-(1,3-dimethyl-3-cyclohexen-1-yl)	x	x	x
Veridiflorol	x		
5,6-Decadien-3-yne, 5,7-diethyl	x		
Diisooctyl adipate	x		
Bicyclo[2.2.1]heptane, 2,2,3-trimethyl-, endo		x	
1-Methyl-3-(1′-methylcyclopropyl)cyclopentene		x	
à-Thujenal		x	
(Z,Z)-à-Farnesene		x	
1-Hydroxy-1,7-dimethyl-4-isopropyl-2, 7-cyclodecadiene		x	
Selina-6-en-4-ol		x	x
2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-4a, 5-dimethyl-3-(1-methylethylidene)-, (4ar-cis)		x	x
Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)			x
1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene			x
1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)			x
Cycloisolongifolene			x
1-Methylene-2b-hydroxymethyl-3, 3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane			x
1-Oxaspiro[2.5]oct-5-ene, 8,8-dimethyl-4-methylene			x

Table 2
Polyphenols and antioxidant content in leaf samples of *T. diffusa* from three contrasting sites.

Site	Total polyphenols (mg/g)	Total antioxidant capacity*	FRAP (mg gallic acid/g)
S1	33.85	72.32	21.33
S2	27.65	60.847	18.67
S3	23.95	68.995	18.51

*: % inhibition of the radical ABTS; FRAP: ferric reducing antioxidant power.

to this site and S3 showed 11 identified compounds with six compounds characteristic to this site (Table 1).

In relation to the content of phenolic compounds and antioxidant activities, the plants also showed variations between the collecting sites (Table 2).

Phenolic content in S1 was higher than S2 and S3; in the case of antioxidant activity using the ABTS method, S1 were higher than S2 and S3 thus coincides with amounts between phenolic contents and antioxidant contents; however, results of the ferric reduction power shown that all samples presented closer values. This indicates that phenolic content presented a correlation with the radical scavenging but also the plants in all sites have a similar antioxidant properties according to FRAP method. Behavior in both kind of metabolites was different when compare the yielding and composition of essential oils in each site with phenolics and antioxidants, which showed less variation in all sites.

4. Discussion

Essential oil yields could be associated with high environmental humidity; since higher oil content was registered in S2, when plants grown in a humid place and lower content of essential oil was observed in plants from S1 and S3, which corresponded to plants from semiarid climate with less precipitation records. However, the influence of humidity on essential oil yields remains additional experiments to confirmation.

Important variations in yields of essential oils have been reported both cultivated and wild plants, with intra- and inter-populational differences, mostly attributed to environmental and genetic influences. For example, in *Lippia javanica* (Houst.), five chemotypes were named according the predominant component in the essential oil; even when these plants were obtained from the same region. Each population presented variation in proportions of main components; this is relevant for studies focused on exploration of potentially useful bioactivities or in the development of new products, because it is imperative to consider this natural variation [23]. Yield variation of the essential oil could range from some thousandths up to 1%–3% of the vegetal weight [24]. In addition, variation depends on the extracted organ and even on the developing state when such organ is processed. In *Lavandula stoechas* L., the yield in the vegetative tissues was between 2.1% and 2.4% (ranging from 0.8% to 2.4%); in contrast, yield obtained from flowers was between 2.7% and 3.1%, which decreased as the floral senescence progressed, which allowed wider yield variation in these organs (0.2%–3.1%) [25].

The yields of essential oils obtained in this work coincides with previously reported [17], where it is indicated that yields in *T. diffusa* ranged from 0.01% to 2.00%. Additionally, variations in essential oil contents have been reported between genotypes of *T. diffusa*, leading to consider the genetic effect as a keystone for the crop management for essential oil production [13]. In this work, we used only one extraction procedure, but it is worth to remark that yield will also depend on the used technique and time of processing since the recollection until extraction, and that should be consider for the design of management strategies [1,26].

In relation to chemical composition of the oil, the eucalyptol has been previously reported in some *T. diffusa* genotypes [13]; while the others components have been reported in other plants but not in *T. diffusa*. This implies a high diversity and variation between populations, according to the particular distribution of some compounds in oils from each site. In addition, it indicates that main components obtained from wild plants grown in the central part of Tamaulipas are different to reports in literature. This is an argument to consider that bioactivities of medicinal and industrial interests could be affected when plant material comes from several locations. And that, composition will be influenced by predominant environmental conditions, season, time before processing, geographical origin and genetic background [13–15,23].

Phenolic contents shown slight differences together with the radical scavenging but when antioxidant activity is compared with FRAP, values between sites seems to be closer, probably associated to synergistic or antagonistic effect of the antioxidant compounds present in the samples. In the case of these metabolites, the differences in general levels were not marked as the case of essential oils, but identification of the phenolic and antioxidant compounds are necessary to be certain.

All evaluated parameters varied between populations collected in Tamaulipas, where accumulation of metabolites seems to be dependent to the growing site conditions; which is similar to variations reported in *T. diffusa* when was submitted to agronomic management in comparison to plants grown in wild [12].

It is important to highlight that knowledge about variations in metabolite contents not only has implications in quality and bioactivity of plants. It also constitutes the base for plants management in field, conservation in germplasm banks, in vitro propagation, because such variations are associated with structural and functional genetic diversity interacting with the environment [27]. According to reports, most of variation is produced by phenotypic plasticity in response to a multifactorial environment, and in an ecological context represent adaptive characters [2] that potentially would influence the uses of the plant.

The use of medicinal plants is recommended as common practice in many regions around the world, but many times, there are many factors that are not considered about the plant material, including the contents of phytochemicals. There is a necessity to know the nature of the plant material, to ensure the beneficial effects to consumers. As found in this work, antioxidant activity and essential oil in *T. diffusa* presented variation in relation to other reports from different geographical origin; so it is imperative to know the determinant factors that affect the yields and homogeneity to ensure the quality of preparations and products [13]. Because of this, it must be ensure the origin, genetic background, the collection season, age, organs and other factors as keystone to ensure homogeneity of the raw material and beneficial effects of this plant to consumers.

The samples from the sites were chemically and morphologically different; this could be attributed to climatic differences; and thus, it is worth to consider the effects of the environment in level and diversity of phytochemicals in this species for industrial and medicinal uses.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgment

Authors would like to thank CONACYT for the Doctorate scholarship for the first author (252383) and to Universidad Autonoma de Tamaulipas for financial support to the project UAT PFI2015-14.

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