SHORT REPORT

A C G publications

Rec. Nat. Prod. 11:4 (2017) 401-405

records of natural products

Fatty Acid Composition and Biological Activities of *Tanacetum zahlbruckneri* (Náb.) Grierson Growing in Turkey

Pinar Caglar Eyol[*1], Nazli Boke Sarikahya[2], Omer C. Karakoc[3], Ayhan Gokce[4], Fatih Demirci[5], Suheyla Kirmizigul[2] and Nezhun Goren[1]

[1] *Department of Molecular Biology and Genetic, Faculty of Arts & Science, Yildiz Technical University, 34210, Istanbul, Türkiye*
[2] *Department of Chemistry, Faculty of Science, Ege University, 35100, Bornova-Izmir, Türkiye*
[3] *Department of Biology, Faculty of Science & Arts, Karatekin University, 18100, Cankiri, Türkiye*
[4] *Department of Plant Protection, Faculty of Agriculture, Gaziosmanpasa University, 60150, Tokat, Türkiye*
[5] *Faculty of Health Sciences Anadolu University, Eskişehir, Türkiye*

(Received September 02, 2016; Revised March 13, 2017; Accepted March 24, 2017)

Abstract: *n*-Hexane extracts of the roots, stems and flowers of *Tanacetum zahlbruckneri* (Náb.) Grierson (Asteraceae) were analysed for their fatty acid contents by Gas Chromatography–Mass Spectroscopy (GC-MS) method and their antimicrobial and insecticidal activities were evaluated as well. Total FAMEs percentages of the *n*-hexane extracts were detected as 69.94, 73.72 and 87.29 in the roots, in the stems and in the flowers, respectively. While palmitic acid was found as a major component for roots (26.12 %) and stems (21.28 %), flowers contain 33.78 % of linoleic acid as a major component. Roots and stems also contain considerable amount of *α*-linoleic acid as 15.30 % and 18.09 %, respectively. Additionally, *α*-linolenic acid percentage was found as 17.17 in the stems. Single dose screening insecticidal activity tests with *Sitophilus granarius* (L.) showed that *n*-hexane root extract has a considerably high bioactivity (83 % mortality at 100 g/L dose after 48 h). While antibacterial activity results ranged between 0.625-1.25 mg/mL for all three extracts, the antifungal activity was observed of the flowers as 0.156 mg/mL, against *C. parapsilosis* and *C. albicans*.

Keywords: Fatty acids; *Tanacetum zahlbruckneri*; antibacterial activity; insecticidal activity.

1. Plant Source

Plant material was collected from Bahçesaray, Van region in 2006 and identified by Dr. Kerim Alpinar from Istanbul University. The voucher specimen was deposited at the Herbarium of the Faculty of Science, Istanbul University (Voucher no. ISTE 79273a), Turkey.

2. Previous Studies

Tanacetum, belongs to the Compositae (Asteraceae) family, is a genus includes 45 species, 18 of them being endemic to Turkey. The genus is known to exhibit exceptional range of activities against different types of microorganisms [1]. These activities involve cytotoxic, antibacterial [2,3], antioxidant [4,5], anti-inflammatory[6] tests.

T. parthenium (feverfew) oil is generally used to treat migraine headaches, arthritis and bronchial complaints [7]. It's reported that *T. corymbosum* and *T. zawadskii* seed oils are a rich source

* Corresponding author: E-Mail: fcaglar@yildiz.edu.tr ; Phone: +90 212 383 4473
The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 05/17/2017 EISSN:1307-6167

for unsaturated fatty acids [8]. According to the literature data *n*-hexane extracts of *T. balsamita* flowers had shown remarkable antimicrobial activity against a wide spectrum of microorganisms. In addition to these literature data, we determined the essential oil content of this plant in our previous study [9]; To the best of our knowledge, no study was carried out concerning the fatty acid profile, antimicrobial and insecticidal activities of *T. zahlbruckneri*.

3. Present Study

The specimen *Tanacetum zahlbruckneri* (Náb.) Grierson (Asteraceae) is endemic to Irano-Turanian region. Fatty acid composition of *n*-hexane extracts of the roots, stems and flowers of *Tanacetum zahlbruckneri* was investigated using GC-MS technique for the first time. The antimicrobial and insecticidal activities of these extracts were evaluated by MIC method. The air dried material was separated into three parts as stems, flowers and roots. These parts were powdered with a Retsch SM 100 grinder and extracted with *n*-hexane (3X3.0 L). The extracts were concentrated under vacuum at 40 ºC to dryness and the residue was stored at +4 ºC before using for GC-MS analysis.

Methylation of the Extracts: Fatty acids were transformed to their methyl-esters derivatives by trans-esterification reaction, in order to be analysed by means of GC-MS. Derivation process was carried out as described at the International Olive Oil Council [10] and IUPAC [11] reports. Dried hexane extracts (0.5 g) were dissolved in 5 mL GC-MS grade *n*-hexane. Afterwards they were extracted with 2M methanolic KOH at room temperature for 30 sec. Clear hexane phases were analysed by GC-MS [12].

GC-MS Conditions: The system used for the analyses was an Agilent 5975 GC-MSD with Innowax FSC (60m x 0.25 mm, 25 μm) column. Inlet temperature was set to 250 °C. Helium was used as carrier gas with the flow of 1 mL/min, temperature program was carried out by raising the temperature by 2 °C/min. from 170-210 °C and was kept at 210 °C for 30 min. Mass spectra were recorded at 70 eV and the mass range was *m/z* 35–450. Fatty acids were identified by comparing their mass peaks with Wiley GC-MS Library data. Retention times were compared to Supelco® 37 Component FAME Mix (Sigma-Aldrich Chemie GmbH, Germany) [13]. According to the results, totally thirteen different fatty acid components were identified ranging their carbon numbers from C12 to C22. The identified total fatty acid percentages of the extracts were varied from 69.94 (root) to 87.29% (flower) (Table 1).

Antimicrobial Assay: The microdilution method was carried out for the antimicrobial activities. Microorganisms were stored at -85 °C until used and inoculated on Mueller-Hinton agar (MHA, Merck, Germany) in Petri dishes for purity evaluations prior to use in the bioassay. Dimethyl sulfoxide (DMSO), (Carlo Erba, Italy) was used as the solvent for stock solutions for plant extracts and the antimicrobial agents. The sources and codes of each microorganism used for antimicrobial assay are given in brackets as follows: *Bacillus subtilis* (NRRL B-4378 obtained from American Type Culture Collection (ATCC), *Escherichia coli* (NRRL B-3008), *Staphylococcus aureus* (ATCC 6538, obtained from Northern Regional Research Laboratory (NRRL, currently National Centre for Agricultural Utilization Research, US-Department of Agriculture, Agricultural Research Service), *Staphylococcus epidermidis* (ATCC 12228), *Proteus vulgaris* (NRRL B-123), *Salmonella typhimurium*(ATCC 13311), MRSA (Clinical Isolates, obtained from Clinical isolates from Osmangazi University (OGU, Eskisehir, Turkey), *Candida parapsilosis* (NRRL Y-12696), *Candida albicans* (NRRL Y-12983) and *Aspergillus niger* (ATCC 10549). Microdilution activity results were given in the supporting information S17.

Insecticidal Assay: For the development of Insect Cultures; *Sitophilus granarius* (Coleoptera: Curculioniade) adults were obtained from the insect stock culture at the Department of Plant Protection, Faculty of Agriculture, University of Gaziosmanpasa. The single aged individuals were reared by means of the method described in previous studies [14]. Method for the contact toxicity assay was conducted as described in our previous studies [15]. Extracts were diluted to the concentration of 100 g/L with acetone. One μL of the extract suspension was applied to each insect dorsal site with micro applicators (Burkard Manufacturing Company Ltd., England). In the control group, 1 μL pure acetone was applied to each insect. After treatments, ten insects were transferred to a petri dish (60 mm diameter) and 5 gr. of sterilised wheat was provided to the insects. The insects were incubated at 27±2 ºC and %60±10 relative humidity (RH) for three days. Mortality rates were observed at 24h intervals for three days. Dose-mortality studies were carried out as described above.

The hexane root extracts were diluted with acetone to give concentration 10 g/L, 20 g/L, 40 g/L, 60 g/L, 80 g/L and 100 g/L. One μL of the extract suspension was applied to each insect while 1 μL pure acetone was applied in the control group.

Table 1. Retention times and % contents of fatty acids for root, stem and flower parts with standard derivations and oil yields (in g/100g of dry plant)

FAMEs	**Symbols**	**M^+**	**Roots (%±SD)**	**Stems (%±SD)**	**Flowers (%±SD)**
Decanoic acid ME	10:0	172	-	0.38±0.046	-
Tetradecanoic acid ME	14:0	242	1.71±0.137	1.54±0.107	0.57±0.087
Hexadecanoic acid ME	16:0	270	26.12±0.880	21.28±1.198	7.99±0.707
(*cis*) 9-Hexadecenoic acid ME	16:1 ω7	268	0.91±0.041	1.01±0.073	-
Heptadecanoic acid ME	17:0	284	0.88±0.004	-	-
(*cis*) 2-Hexadecenoic acid ME	16:1 ω14	268	-	-	1.28±0.103
Octadecanoic acid ME	18:0	298	2.66±0.068	2.60±0.121	4.21±0.340
(*cis*) 9-Octadecenoic acid ME	18:1 ω9	296	9.72±0.309	6.65±0.342	15.44±0.118
(*cis*) 11-Octadecenoic acid ME	18:1 ω7	296	1.32±0.045	-	1.6±0.139
(*cis*) 9,12-Octadecadienoic acid ME	18:2 ω6	294	15.30±0.736	18.09±1.191	33.78±2.409
(*cis*) 9,12,15-Octadecatrienoic acid ME	18:3 ω3	292	9.03±0.474	17.17±0.855	-
Eicosanoic acid ME	20:0	326	2.29±0.120	5.00±0.246	8.10±0.701
Docosanoic acid ME	22:0	354	-	-	14.31±0.445
Phytol	-	296	10.24±0.042	18.14±0.752	-
Other hydrocarbons			19.82	8.14	12.71
Total Saturated FAME (%)			33.66	30.8	35.18
Total Unsaturated FAME (%)			36.28	42.92	52.11
Total FAME Percentages			69.94	73.72	87.29
Unsaturated/Saturated FAME			1.08	1.39	1.48
LA (ω-6) / ALA (ω-3)			1.69	1.05	-
Oil Yield (%)			0.20	0.51	1.29

ME: methyl ester

The study was conducted with randomised block design and each block consisted all concentrations and control group. In each replicated three groups of ten insects were used and the whole study was repeated three different occasion. For the execution of statistical analyses; the first, single dose screening test results were converted into mortality % values, and then they were subjected to the arcsine transformation for normalization [16]. Variance analysis was carried out with the transformed data. Tukey's range test ($P \leq 0.05$) was used for comparison of differences between treatments. MINITAB Release 14 packet program was used for all of the statistical analysis [17]. Dose-mortality bioassay's lethal concentrations (LD_{50}, LD_{90}), slopes and intercepts were calculated with Polo-PC probit packet program [18]. Results of single dose screening with *T. zahlbruckneri*

showed that the greatest insecticidal activity with the root extract that was significantly different from the other extracts and the control group. Stem and flower extracts showed moderate toxicity against *S. granarius* with 63 % and 49 % mortality rates after 24h and they were significantly different from the control ($F=147.26$; d.f.: 3.8; $P<0.05$). As the incubation time was extended, mortalities observed in the treatment increased, whereas the activity order of the extracts did not change. There were significant differences between tested extracts' treatments ($F=100.77$; d.f.: 3.8; $P<0.05$) (Table 2).

Table 2. Results (% Mortality ±SEM) of *T. zahlbruckneri* single dose screening at 100 g/L dose on *Sitophilus granarius* after 24 h and 48h.

Sample	24h	48 h
Control	0.00±0.00 c	0.00±0.00 c
Flower	48.89±0.04 b	52.24±0.49 b
Root	82.99±1.85 a	84.48±2.59 a
Stem	63.35±0.12 b	66.67±0.00 b

a-c indicate the statistical differences according to the Tukey test ($P < 0.05$)

The dose-response study was carried out with the root extract, produced the greatest contact toxicity in the single dose screening test. The calculated LD_{50} and LD_{90} values for *S. granarius* after 24h were detected as 4.95 and 9.52 g/L respectively (Table 3).

Table 3. Dose-Mortality Study Results with *T. zahlbruckneri* root extract on *S. granarius* after 48 h.

Sample	LD_{50} (g/L) Fiducial Limits	LD_{90} (g/L) Fiducial Limits	Slope±SE	Intercept±SE	
T. zahlbruckneri (Root)	49.53 (45.10±54.53)	95.20 (83.03±114.52)	4.52±0.43	-3.14±0.31	7.45

X^2: Pearson's chi-squared test

These results indicate that *T. zahlbruckneri* root extract has a potential in controlling *S. granarius* with 85% mortality at 100g/L dose and 50 g/L LD50 value. Further purification studied of this extract would increase its biological activity, which enable to show its potential as a contact insecticide.

It is known that EFAs undertake important roles for health. Prevention of many diseases is possible with a healthy and balanced diet including EFAs. Especially flower part of this plants have high amount of LA. Generally, many plants have parallel results for LA. During the dietary intake, increase amount of LA may decrease the ALA owing to some oxidation reactions in the body. So, it is essential to increase the ALA and decrease the LA intake, in order to balance the ALA and LA ratio [20, 21]. However, when assessed together with the results provided from insecticidal activity, the potential use of the plant as food supplement should be supported by further research.

The obtained fatty acids are common among many plants, so they cannot be used for the chemotaxonomic evaluation of the plant.

Acknowledgments

The authors acknowledge YTU BAPK (2011-01-07-YULAP06) for the financial support.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] N. Gören, N. Arda and Z. Çaliskan (2002). Chemical characterization and biological activities of the genus *Tanacetum* (Compositae), In: Studies in Natural Products Chemistry, ed: Atta-ur Rahman, Elsevier Science, Amsterdam,Netherlands, 547-658.

[2] N. Gören, H.J. Woerdenbag and C. Bozok-Johansson (1996). Cytotoxic and antibacterial activities of sesquiterpene lactones isolated from *Tanacetum praeteritum* subsp. *praeteritum*, *Planta Med*. **62,** 419-422.

[3] H. Shamkhani, N. Nasiri, A.Aliahmadi and A. Sonboli (2016). The essential oil composition and antibacterial activity of *Tanacetum hololeucum* from Iran, *Rec. Nat. Prod*. **10 (6),** 818-823.

[4] M. Firozy, Z. Talebpour and A. Sonboli (2012). Essential oil composition and antioxidant activities of the various extracts of *Tanacetum sonbolii* Mozaff.(Asteraceae) from Iran, *Nat. Prod. Res*. **26,** 2204-2207.

[5] I.H. Gecibesler, A. Kocak and I. Demirtas (2016). Biological activities, phenolic profiles and essential oil components of *Tanacetum cilicicum* (Boiss.) Grierson, *Nat. Prod. Res*. **6**, 1-6.

[6] M. Abad, P. Bermejo and A. Villar (1991). Antiinflammatory and anti-ulcerogenic activities of the organic extracts of *Tanacetum microphyllum* DC. in rats, *Phytother Res*. **5,** 179-181.

[7] E.S. Johnson, P.J. Hylands and D.M.H. Jessup (1988). Oil extract of *Tanacetum parthenium* for treating migraine. US 4,758,433.

[8] N. Tsevegsuren, W.W. Christie and D. Lösel (1998). *Tanacetum* (*Chrysanthemum*) *corymbosum* seed oil—a rich source of a novel conjugated acetylenic acid, *Lipids* **33,** 723-727.

[9] K. Polatoğlu, B. Demirci, N. Goren and K.H.C Baser (2011). Essential oil composition of endemic *Tanacetum zahlbruckneri* (Náb.) and *Tanacetum tabrisianum* (Boiss.) Sosn. and Takht. from Turkey, *Nat. Prod. Res*. **25,** 576-584.

[10] International Olive Oil Council (2001). Preparation of the fatty acid methyl esters from olive oil and olive-pomace oil. Spain; Madrid.

[11] C. Paquot (1979). Standard methods for the analysis of oils, fats and derivatives the commission on oils, Fats and Derivatives of the IUPAC: France; Thiais.

[12] S. Kırmızıgül, S. N. Boke, H. Sumbul, R.S. Gokturk and N. Arda (2007). Essential fatty acid components and antioxidant activities of eight *Cephalaria* species from southwestern Anatolia, *Pure Appl. Chem*. **79**, 2297-2304.

[13] F. David, P. Sandra and P.L. Wylie (2002). Improving the analysis of fatty acid methyl esters using retention time locked methods and retention time databases. Application Agilent Technologies: CA; Palo Alto.

[14] Ö.C. Karakoç, A. Gökçe and I. Telci (2006). Fumigant activity of some plant essential oils against *Sitophilus oryzae* L., *Sitophilus granarius* L. (Col.: Curculionidae) and *Acanthoscelides obtectus* Say. (Col.: Bruchidae), *Turk Entomol Derg-TU*. **30,**123-136.

[15] K. Polatoğlu, Ö.C. Karakoç, A. Gökçe and N. Gören (2011). Insecticidal activity of *Tanacetum chiliophyllum* (Fisch. & Mey.) var. *monocephalum* grierson extracts and a new sesquiterpene lactone, *Phytochem Lett*. **4,** 432-435.

[16] J.H. Zar (1999). Biostatistical Analysis. Prentice Hall, New Jersey.

[17] J. McKenzie (2005). The student guide to MINITAB release 14 manual. Pearson Education, Boston.

[18] LeOra Software (1987). Polo-PC a user's guide to probit or logit analysis. California; Berkeley.

[19] A.S. Pereira, D.S. Siqueira, V.O. Elias, B.R. Simoneit, J.A. Cabral and F.R. Aquino Neto (2002). Three series of high molecular weight alkanoates found in Amazonian plants, *Phytochemistry* **61,** 711-719.

[20] A.P. Simopoulos (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids, *Biomed. Pharmacother*. **56,** 365-379.

[21] W. E. Connor (2000). Importance of n−3 fatty acids in health and disease, *Am. J. Clin. Nutr*. **71**, 171S-175S.