
**International Journal of**

# Secondary Metabolite

Volume: 5 Number: 1
January 2018

***ISSN-e:*** **2148-6905** **Journal homepage: http://www.ijate.net/** **http://dergipark.gov.tr/ijsm**

## In Vitro Evaluation of Antimicrobial and Antibiofilm Potentials of Essential Oil of *Tanacetum argenteum* (Lam.) Willd. subsp. *canum* (K.Koch) Grierson var. *canum*

**Nazime Mercan Doğan, Arzu Cansaran, Mirac Yılmaz, Naime Nur Bozbeyoğlu**

**To cite this article:** Mercan Doğan, N., Cansaran, A., Yılmaz, M., & Bozbeyoğlu, N. N. (2018). In Vitro Evaluation of Antimicrobial and Antibiofilm Potentials of Essential Oil of *Tanacetum argenteum* (Lam.) Willd. subsp. *canum* (K.Koch) Grierson var. *canum*. TURKEY. *International Journal of Secondary Metabolite*, 5(1), 34-41. DOI: 10.21448/ijsm.356601

**To link to this article:** http://www.ijate.net/index.php/ijsm/issue/archive
http://dergipark.gov.tr/ijsm

IJSM International Journal of Secondary Metabolite

*Int. J. Sec. Metabolite, Vol. 5, Issue 1 (2018) pp. 34-41*

*Research Article*

*ISSN: 2148-6905 online* Journal homepage: http://www.ijate.net/index.php/ijsm

# In Vitro Evaluation of Antimicrobial and Antibiofilm Potentials of Essential Oil of *Tanacetum argenteum* (Lam.) Willd. subsp. *canum* (K.Koch) Grierson var. *canum*

**Nazime Mercan Doğan[1*], Arzu Cansaran[2], Mirac Yılmaz[3], Naime Nur Bozbeyoğlu[4]**

[1]Department of Biology, Faculty of Science and Arts, Pamukkale University, Denizli, Turkey
[2]Department of Mathematics and Science Education Faculty of Education, Amasya University, Amasya, Turkey
[3]Department of Science and Mathematics Fields in Secondary Education, Faculty of Education, Hacettepe University, Ankara, Turkey
[4]Plant and Animal Production Department, Tavas Vocational High School, Pamukkale University, Denizli, Turkey

**Abstract:** This study was performed to determine the antimicrobial and antibiofilm activities of essential oil of *Tanacetum argenteum (Lam.)* Willd. subsp. *canum (K.Koch)* Grierson var. *canum*. The plant was collected from Amasya. The antimicrobial activity was determined by disc diffusion and microdilution broth methods. Essential oil was active against indicator organisms. The maximal inhibition zone diameter were as follows: *E. cloacae* ATCC 28355 (14mm), *P. fluorescens* ATCC 55241 (16mm), *P. aeruginosa* ATCC 27853 (19mm), *S. sonnei* RSKK 8177 (18mm), *E. coli* ATCC 25922 (21mm), *E. coli* O157:H7 (13mm), *Y. enterocolitica* RSKK 1501 (11mm), *C. jejuni* ATCC 33291 (12mm), *K. pneumoniae* ATCC 27736 (13mm), *S. enteritidis* RSKK 171 (12mm), *S. aureus* ATCC 33862 (26mm) and *S. aureus* ATCC 25923 (29mm), *M. luteus* NRLL-B-4375 (20mm), *E. faecalis* ATCC 19433 (19mm), *B. cereus* NRRL-B-3711 (30mm), *B. cereus* RSKK (32mm), *C. albicans* ATCC 10231 (19mm) and *C. tropicalis* (17mm). The minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) were ranged from 62.5-900 µg/ml and 125-1000 µg/ml, respectively. Plant essential oil was the most active against *B. cereus* NRRL-B 3711 and *B. subtilis* RSKK 867. Also, the essential oil has shown a strong anti-candidal activity against two *Candida* species. For determining the antibiofilm effect, various dilutions of oil were studied on *M. luteus, E. cloacae, P. fluorescens, Y. enterocolitica and S. enteritidis* by using microplate biofilm assay. According to results, antibiofilm effect was found as weak. Maximum antibiofilm effect was determined on *E. cloacae* ATCC 28355 with 32.92% biofilm inhibition rate.

**ARTICLE HISTORY**

*Received: 17 July 2017*
*Revised: 30 October 2017*
*Accepted: 11 November 2017*

**KEYWORDS**

*Tanacetum argenteum* subsp. *canum* var. *canum*, essential oil, antimicrobial, antibiofilm effect

## 1. Introduction

The incidence of infectious agents such as human, animal and plant pathogens has increased dramatically over the past few decades. The most common of these pathogens are developed multi-drug resistance and there is a reduction in the number of effective drugs.

*Corresponding Author E-mail: nmercan@pau.edu.tr

ISSN: 2148-6905 online /© 2018 DOI: 10.21448/ijsm.356601

Because of this, the new and effective natural compounds against pathogens are searched by scientists. Aromatic plants and their essential oils are commonly used for the treatment or prevention of disease in traditional medicine as antimicrobial agents. Moreover, their pharmacological effects such as antimicrobial, antioxidant and anticholinesterase are scientifically proven [1, 2]. The chemical analysis of *Tanacetum* species has been well documented [3-11]. Also, the essential oils and sesquiterpenes isolated from some *Tanacetum* species have various biological properties, such as antifeedant, insecticidal, antimicrobial, larvicidal, cytotoxic, anticoagulant and antifibrinolytic, etc. [12-19]. Plant oils in traditional medicine are used to treatment of infectious diseases. Particularly, searching of new drugs against to resistant microorganisms and/or biofilm forming microorganisms has received much attention. Because, treatment of chronic infections related with biofilm are very difficult as biofilms are exceptionally resistant to antibiotics and host immune response. Therefore, natural products have also been screened for their antimicrobial or antibiofilm activity.

*T. argenteum* (Lam.) Willd. subsp. *Canum* (K.Koch) Grierson var. *canum* used in our study has distributed in south Anatolian regions of Turkey [20]. Its local name is Bodur Pireotu. In the literature, there are many works about the composition essential oil and sesquiterpenoids of *T. argenteum* subsp. *canum* [4, 21]. But, we have not found any published report on the antimicrobial and antibiofilm effects of essential oil of this subspecies. Therefore, present study will be the first scientific research to provide data that the antimicrobial and antibiofilm activity of this essential oil. Also we expect that results obtained from this study will become a good reference for detailed screening of other biological and pharmacological properties.

## 2. Material and Methods

***Plant materials:*** *T. argenteum* (Lam.) Willd. subsp. *canum* (K.Koch) Grierson var. *canum* plant was collected during the flowering period and natural populations in A5 Amasya (between Direkli village and Yassıçal town, rocky areas and steppes, at 1300 m, 20.06.2010, Cansaran 5402) which is a city in the Black Sea Region of Turkey. The identification of plant was confirmed by Assoc. Prof. Dr. Arzu Cansaran according to the description given by Davis [20] and Cansaran et al. [22].

***Test microorganisms:*** *Enterobacter cloacae* ATCC 28355, *Pseudomonas fluorescens* ATCC 55241, *Pseudomonas aeruginosa* ATCC 27853, *Shigella sonnei* RSKK 8177, *Escherichia coli* ATCC 25922, *Escherichia coli* O157:H7, *Yersinia enterocolitica* RSKK 1501, *Campylobacter jejuni* ATCC 33291, *Klebsiella pneumoniae* ATCC 27736, *Salmonella enteritidis* RSKK 171, *Staphylococcus aureus* ATCC 33862, *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NRLL-B-4375, *Enterococcus faecalis* ATCC 19433, *Bacillus cereus* NRRL-B-3711, *Bacillus cereus* RSKK 867, *Candida albicans* ATCC 10231 and *Candida tropicalis* were used as test organisms in this study. Microorganisms tested in present study were obtained from Bacteriology Laboratory, Department of Biology, Pamukkale University (Denizli) and were regenerated twice before use in the activity studies. Tryptic Soy Broth (Merck), Tryptic Soy Agar (Merck), Sabouraud Dextrose Broth (Oxoid) and Sabouraud Dextrose Agar (Oxoid) were used for bacteria and yeasts, respectively. The culture was aerobically incubated and adjusted by comparing with 0.5 McFarland Standard Dilutions in all manipulations.

***Extraction of the essential oil:*** Air-dried aerial parts of plants were subjected to hydro-distillation for 3-5 h using a Clevenger-type apparatus to obtain essential oil in a yield of 1.905% (v/w) for *T. argenteum* subsp. *canum* var. *canum* based on the dry weight of the samples. The essential oil was stored in a sealed dark vial at 4 °C.

***Determination of inhibitory effect by the disc diffusion method:*** The antimicrobial activity of essential oil was assigned by disc diffusion method (23). 100 µl suspensions of

microorganisms were spread on the solid medium plates. Empty sterilized disks of 6 mm (Schleicher and Schuell, No. 2668, Germany) were each impregnated with 50 µl of essential oils. The discs injected with essential oils were placed on the inoculated agar. Petri dishes were sealed with sterile laboratory parafilm to avoid eventual evaporation of the essential oils. The dishes were left for 2 h at 4 °C to allow the diffusion of oil, and then were incubated at 37°C for 24 h (at 30°C for *M. luteus* NRRL-B-4375, at 42°C for *C. jejuni* ATCC 33291 and at 28°C for yeasts). The diameters of the inhibition zones formed on the medium were evaluated in millimetres. All the tests were performed in duplicate. The inhibition zones were compared with those of Meropenem and Nystatin as antibiotic and antifungal.

***Analysis of the minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC):*** MIC value of essential oils was determined by the broth microdilution method in 96-well microplates [24]. 95 µl of TSB, 100 µl of the essential oil and 5 µl of the culture were added into each wells. Our controls (with no cells and no essential oils) were also included on each microplate. The MIC was defined as the lowest concentration of the essential oils where no growth was visually observed. The growth of the microorganism was indicated by turbidity. After determination of the MIC, the microplates were mixed and 25 µl from each well (MIC and higher concentrations) was plated on solid medium. After 24 hours for bacteria and 48 h for yeasts, the lowest concentration of essential oil which no growth of colonies (98%) was considered as MLC. Standard powder of Meropenem and Nystatin were dissolved in distilled water and DMSO, respectively.

***Antibiofilm activity:*** Crystal violet method was applied using 96-well polystren plate for antibiofilm activity [25]. The culture suspensions was adjusted at 0.5 McFarland standard tube and 100 µl was dispensed into each well of 96-well plates in the presence of 100 µl essential oil (1/2 MIC, ¼ MIC and 1/8 MIC) or 100 µl TSB (control). The plates were incubated for 48 h at 37 °C and 30 °C. Following incubation, crystal violet staining assay was performed. Measure the optical density (OD) of each of these samples at a wavelength of 550 nm. Each experiment was performed in duplicate. And the biofilm inhibition percentage was calculated by using the following formula:

$$[(OD_{\text{growth control}} - OD_{\text{sample}}) / OD_{\text{growth control}}] \text{ x}100$$

## 3. Results and Discussion

Undoubtedly, infectious disease agents cause major problems in the worldwide and antimicrobial resistance is a critical public health issue. Therefore, the scientific community has been focused on various fields to combat pathogens. Biological and pharmacological properties of aromatic plants and their seconder metabolites are scientifically screened to control the colonization and growing of pathogens in environment. It is the known fact that essential oils obtained from aromatic plants are very rich in complex compounds and the biological and pharmacological properties of some of them are confirmed scientifically. Moreover, it was reported their potential applications as natural and safe food preservatives or an antibacterial agent for both pharmaceutical and pesticide industries [26, 27]. According previous reports, camphor, borneol and 1,8-cineole (eucalyptol) were common compounds of the essential oils of *Tanacetum* species and antimicrobial potentials of these compounds were well determined [28-31]. The antimicrobial and cytotoxic activities of caryophyllene oxide and thujone, the main constituents of the oil of *T. argenteum* subsp. *canum* var. *canum* [21], were also verified by some researchers [32, 33].

In the present research, the antimicrobial activity of essential of *T. argenteum* subsp. *canum* var. *canum* collected from Amasya (between Direkli village and Yassıçal town) was examined against some pathogens on the basis of disc-diffusion and microdilution assay. The activity results, quantitatively estimated by the presence or absence of inhibition zones and also

MIC and MLC values, were given in Table 1 and 2. According to the initial antimicrobial screening test, it was shown that the activity of essential oil was good against all the used organisms. But, *E. coli* 0157:H7, *Y. enterocolitica* RSKK 1501, *Campylobacter jejuni* ATCC 33291, *Klebsiella pneumoniae* ATCC 27736 and *Salmonella enteritidis* RSKK 171 were more resistant than other bacteria. The inhibition zones of disc, MIC and MLC values for the microorganisms were in the range of 11-32 mm, 62.5-900 µg/ml and 125-1000 µg/ml, respectively. The essential oil was more active against gram-positive bacteria than gram negative bacteria.

**Table 1.** Growth inhibition zones of pathogens in millimetres (±standard deviation) of *Tanacetum argenteum* subsp. *canum* var. *canum*

| Tested bacteria | Inhibition zone diameter (mm; 50 µl) | Antibiotics | |
|---|---|---|---|
| | | Meropenem (10 µg) | Nystatin (100 U) |
| *Enterobacter cloacae* ATCC 28355 | 14±0 | 22±0 | NT |
| *Pseudomonas fluorescens* ATCC 55241 | 16±0 | 24±0 | NT |
| *Pseudomonas aeruginosa* ATCC 27853 | 19±0 | 23±0 | NT |
| *Shigella sonnei* RSKK 8177 | 18±0 | 22±0 | NT |
| *Escherichia coli* ATCC 25922 | 21±2 | 27±0 | NT |
| *E. coli* O157H7 | 13±0 | 25±0 | NT |
| *Yersinia enterocolitica* RSKK 1501 | 11±1 | 25±0 | NT |
| *Campylobacter jejuni* ATCC 33291 | 12±0 | 18±0 | NT |
| *Klebsiella pneumoniae* ATCC 27736 | 13±0 | 19±0 | NT |
| *Salmonella enteritidis* RSKK 171 | 12±2 | 24±0 | NT |
| *Staphylococcus aureus* ATCC 33862 | 26±0 | 12±0 | NT |
| *Staphylococcus aureus* ATCC 25923 | 29±0 | 10±0 | NT |
| *Micrococcus luteus* NRLL, B-4375 | 20±2 | 5±0 | NT |
| *Enterococcus faecalis* ATCC 19433 | 19±0 | 9±0 | NT |
| *Bacillus cereus* NRRL,B-3711 | 30±2 | 10±0 | NT |
| *Bacillus cereus* RSKK 867 | 32±0 | 8±0 | NT |
| *Candida albicans* ATCC 10231 | 19±0 | NT | 18±0 |
| *Candida tropicalis* (clinic isolate) | 17±0 | NT | 16±0 |

NT: Not tested; ATCC: American Type Culture Collection; NRRL: a culture collection of the Agricultural Research Service (ARS); RSKK: Refik Saydam National Type Culture Collection

As known, spore forming bacteria are critical issue in food industry. For example, *Bacillus cereus* is an important bacterium for the safety of prepared foods. Because, spores of *B. cereus* are found widely in environment such as soil, water, dust, etc. and they produce toxins in foods [34]. In our study, *Bacillus cereus* NRRL-B-3711 (30 mm) and *Bacillus cereus* RSKK 867 (32 mm) were the most sensitive bacteria among all tested organisms. The MIC and MLC values of essential oil against these bacteria were 62.5 µg/ml and 125 µg/ml, respectively. On the other hand, it was noted that the bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, known to be multi-resistant to antibiotics and concern to public health, were moderate sensitive to the essential oil. For example, *Escherichia coli* O157:H7 is an enterohemorrhagic foodborne pathogen and causes human disease [35]. *Campylobacter jejuni* is the most important source of gastrointestinal illness. Moreover, its infection happens more frequently than do infections caused by *Salmonella* and *Shigella* species, or *Escherichia coli* O157:H7 [36]. It is well known that such infections are fatal in children and elderly. Therefore,

the use of natural antimicrobial agents to combat pathogens is important issue. Because, there are no toxic effects of the plant extracts. MIC values of the essential oil against *E. coli* 0157:H7 and *C. jejuni* ATCC 33291 were 850 µg/ml and 875 µg/ml, respectively. MIC and MLC values of essential oil against *P. aeruginosa* were also determined as 150 µg/ml and 300 µg/ml. Also, *S. aureus* was another pathogen species for public health in our study and it was found that the essential oil has strong antibacterial activity against *Staphylococcus aureus* ATCC 33862 (26 mm) and *S. aureus* ATCC 25923 (29 mm). Essential oil was active against *Candida tropicalis* and *C. albicans*. It was clear from Table 1 and 2 that essential oil of *T. argenteum* subsp. *canum* was not only active against bacteria but it was also effective against yeast species tested in present study. It is considered from this result that this oil is a broad-spectrum.

**Table 2.** Values of MIC and MLC of *Tanacetum argenteum* subsp. *canum* var. *canum* essential oil

| Tested bacteria | MIC (µg/ml) | MLC (µg/ml) | Antibiotics | |
|---|---|---|---|---|
| | | | Meropenem (µg/ml) | Nystatin (µg/ml) |
| *Enterobacter cloacae* ATCC 28355 | 350 | 875≥ | 62.5 | NT |
| *Pseudomonas fluorescens* ATCC 55241 | 350 | 875≥ | 40.0 | NT |
| *Pseudomonas aeruginosa* ATCC 27853 | 150 | 300≥ | 40.0 | NT |
| *Shigella sonnei* RSKK 8177 | 250 | 750≥ | 40.0 | NT |
| *Escherichia coli* ATCC 25922 | 150 | 325≥ | 62.5 | NT |
| *E. coli* O157:H7 | 850 | 1000> | 62.5 | NT |
| *Yersinia enterocolitica* RSKK 1501 | 850 | 1000> | 62.5 | NT |
| *Campylobacter jejuni* ATCC 33291 | 875 | 1000> | 40.0 | NT |
| *Klebsiella pneumoniae* ATCC 27736 | 850 | 1000> | 40.0 | NT |
| *Salmonella enteritidis* RSKK 171 | 900 | 1000> | 62.5 | NT |
| *Staphylococcus aureus* ATCC 33862 | 175 | 300≥ | 125.0 | NT |
| *Staphylococcus aureus* ATCC 25923 | 175 | 300≥ | 125.0 | NT |
| *Micrococcus luteus* NRLL-B-4375 | 150 | 300≥ | 175.0 | NT |
| *Enterococcus faecalis* ATCC 19433 | 150 | 300≥ | 125.0 | NT |
| *Bacillus cereus* NRRL-B-3711 | 62.5 | 125≥ | 125.0 | NT |
| *Bacillus cereus* RSKK 867 | 62.5 | 125≥ | 125.0 | NT |
| *Candida albicans* ATCC 10231 | 200 | 450≥ | NT | 100 |
| *Candida tropicalis* (clinic isolate) | 225 | 425≥ | NT | 125 |

MLC: minimal lethal concentration; MIC: minimal inhibition concentration; NT: Not tested

**Table 3.** Antibiofilm effect of *Tanacetum* essential oil (%)

| Bacteria | % Inhibition | | |
|---|---|---|---|
| | ½ MIC | ¼ MIC | 1/8 MIC |
| *Enterobacter cloacae* TCC 28355 | 32.92 | 29.30 | 18.40 |
| *Micrococcus luteus* NRRL-B 4375 | 24.10 | 18.65 | 12.00 |
| *Pseudomonas fluorescens* ATCC 55241 | 15.62 | 8.16 | - |
| *Yersinia enterocolitica* RSKK 1501 | 12.54 | 8.12 | 6.02 |
| *Salmonella enteritidis* RSKK 171 | 10.26 | - | - |

-: no inhibition;

Particularly, searching of new drugs against to resistant microorganisms and/or biofilm forming microorganisms has received much attention. Because, treatment of chronic infections related with biofilm are very difficult as biofilms are exceptionally resistant to antibiotics and host immune response [37]. Therefore, natural products have also been screened for their antibiofilm activity. In this study, *T. argenteum* subsp. *canum* oil was also studied for its antibiofilm activity. The antibiofilm effect of the oil was tested at the ratio of 1/2, 1/4 and 1/8 MICs. It was found that, the oil exhibited moderate antibiofilm activity on *E. cloacae* ATCC 28355 (32.92, 29.30 and 18.40% for ½ MIC, ¼ MIC and 1/8 MIC, respectively) and less antibiofilm activity on *S. enteritidis* RSKK 171 (maximum 10.26%), *P. fluorescens* ATCC 55241 (maximum 15.62%) and *Y. enterocolitica* RSKK 1501 (maximum 12.54%). Moreover, any antibiofilm activity could not be determined on other bacteria and candida species (Table 3).

## 4. Conclusion

In conclusion, essential oil of *T. argenteum* subsp. *canum* var. *canum* showed antimicrobial and antibiofilm activity various pathogens. In the literature, there are many works about the biologic and pharmacologic activity and content of essential oils *Tanacetum* species. But, there was no published report about the antimicrobial or antibiofilm effect of *T. argenteum* subsp. *canum* var. *canum*. Since, to the best of our knowledge, no or limited data is available about antimicrobial and antibiofilm activity of this essential oil, our aim was to investigate its effect on some pathogen bacteria and yeasts. The results of the present study have confirmed our hypothesis that this essential oil has the antimicrobial and antibiofilm effect on viability of tested pathogens. Last of all, present study will be the first scientific research to provide data that the antimicrobial and antibiofilm activity of this essential oil possesses on some pathogen organisms.

## 5. References

[1] Lopes-Lutz, D., Alviano, D.S., Alviano, C.S., Kolodziejczyk, P.P. (2008). Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils. *Phytochemistry,* 1732-1738.

[2] Kivrak, I., Duru, M.E., Öztürk, M., Mercan, N., Harmandar, M., Topçu, G. (2009). Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of *Salvia potentillifolia*. *Food Chem*., 116, 470–479.

[3] Goren, N. (1995). Sesquiterpene lactones from *Tanacetum praeteritum*. *Phytochemistry,* 38(5), 1261 1264.

[4] Goren, N., Tahtasakal, E. (1997). Sesquiterpenoids from *Tanacetum argenteum* subsp. *canum* var. *canum*. *Phytochemistry,* 45(1), 107- 109.

[5] Çaliskan, Z., Goren, N., Watson, W.H. (2004). Isolation and structures of eudesmanolides from *Tanacetum cadmeum* ssp. *Cadmium*. *Journal of Chemical Crystallography,* 34(5), 307-310.

[6] Akpulat, H.A., Tepe, B., Sokmen, A., Daferera, D., Polissiou, M. (2005). Compositon of the essential oils of *Tanacetum argyrophyllum* (C.Koch) Tvzel. var. *argyrophyllum* and *Tanacetum parthenium* (L.) Schultz Bip. (Asteraceae) from Turkey. *Biochemical Systematics and Ecology,* 33, 511–516.

[7] Gogus, F., Celik, A., Lewis, A.C., Ozel, M. Z. (2009). Analysis of Volatile Components From *Tanacetum cadmium* by Hydrodistillation and Direct Thermal Desorption Methods Using GC×GC-TOF/MS. *Chemistry of Natural Compounds,* 45(4), 557-559.

[8] Bagcı, E., Kocak, A. (2010). Essential oil composition of two endemic *Tanacetum* (*T. nitens* (Boiss&Noe) Grierson and *T. argenteum* (Lam.) Willd. Subsp. *argenteum*) (Asteraceae) taxa, growing wild in Turkey. *Industrial Crops and Products*, 31, 542-545.

[9] Polatoğlu, K., Demirci, B., Gören, N., Başer, K.H.C. (2011). Essential oil composition of endemic *Tanacetum zahlbruckneri* (Náb.) and *Tanacetum tabrisianum* (Boiss.) Sosn. and Takht. from Turkey. *Natural Product Research,* 25(6), 576–584.

[10] Polatoglu, K., Karakoc, O.C., Yücel, Y.Y., Demirci, B., Gören, N., Başer, K.H.C. (2015). Composition, insecticidal activity and other biological activities of *Tanacetum abrotanifolium* Druce. essential oil. *Industrial Crops and Products,* 71, 7–14.

[11] Mahdavi, M., Jouri, M.H., Mahmoudi, J., Rezazadeh, F., Mahzooni-Kachapi, S.S. (2013). Investigating the altitude effect on the quantity and quality of the essential oil in *Tanacetum polycephalum* Sch.-Bip. *polycephalum* in the Baladeh region of Nour, Iran. *Chinese Journal of Natural Medicines,* 11(5), 0553−0559.

[12] Thomas, O.O. (1989). Anticoagulant and antifibrinolytic properties of *Tanacetum macrophyllum*. *Fitoterapia,* 15(4), 329–330.

[13] Susurluk, H., Çalışkan, Z., Gürkan, O., Kırmızıgül, S., Gören, N. (2007). Antifeedant activity of some *Tanacetum* species and bioassay guided isolation of the secondary metabolites of *Tanacetum cadmeum* ssp. *cadmeum* (Compositae). *Industrial Crops and Products,* 26, 220-228.

[14] Tabanca, N., Demirci, F., Demirci, B., Wedge, Başer K.H.C. (2007). Composition, enantiomeric distribution, and antimicrobial activity of *Tanacetum argenteum* subsp. *flabellifolium* essential oil. *Journal of Pharmaceutical and Biomedical Analysis,* 45, 714-719.

[15] Polatoğlu, K., Demirci, F., Demirci, B., Gören, N., Başer, K.H.C. (2010). Essential oil composition and antibacterial activity of *Tanacetum argenteum* (Lam) Willd. ssp. *argenteum* and *T. densum* (Lab.) Schultz Bip. ssp. *amani* Heywood from Turkey. *Journal of Oleo Science,* 59(7), 361-367.

[16] Polatoğlu, K., Demirci, F., Demirci, B., Gören, N., Başer, K.H.C. (2010). Antimicrobial activity and Essential Oil Composition of a New *T. argyrophyllum* (C. Koch) Tvzel var. argyrophyllum Chemotype. *Journal of Oleo Science,* 59(6), 307-313.

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

[18] Triana, J., Eiroa, J.L., Morales, M., Pérez, F.J., Brouard, I., Marrero, M.T., Estévez, S., Quintana, J., Estévez, F., Castillo, Q.A., León, F. (2013). A chemotaxonomic study of endemic species of genus *Tanacetum* from the Canary Islands. *Phytochemistry,* 92, 87–104.

[19] Ali, A., Tabanca, N., Kurkcuoglu, M., Duran, A., Blythe, E.K., Khan, I.A., Baser, K.H.C. (2014). Chemical Composition, Larvicidal, and Biting Deterrent Activity of Essential Oils of Two Subspecies of *Tanacetum argenteum* (Asterales: Asteraceae) and Individual Constituents Against Aedes aegypti (Diptera: Culicidae). *Journal of Medical Entomology,* 51(4), 824-830.

[20] Davis, P.H. 1965-1985. *Flora of Turkey and the East Aegean Islands*. vol. 1-9. Edinburgh: Univ. Press.

[21] Goren, N., Demirci, B., Baser, K.H.C. (2001). Composition of the essential oils of *Tanacetum* spp. from Turkey. *Flavour Fragr*. J., 16, 191–194.

[22] Cansaran, A., Peker, S., Yıldırım, C. (2007). Floristic Characters of the Area between the Direkli (Göndes) Village, Yassıçal (Ebemi) Town and Abacı Village (A5/6 Amasya–TURKEY). *International Journal of Botany*, 3(3), 240-250.

[23] CLSI. (2012). *Performance Standarts for Antimicrobial Disk Susceptibility Test; Approved standard-Eleventh Edition*. (M02-A11), Vol. 32, No. 1, Replaces M02-A10, Vol. 29, No. 1.

[24] Koneman, E.W., Allen, S.D., Janda, W.M., Scherckenberger, P.C., Winn, W.C. (1997). *Color Atlas and Textbook of Diagnostic Microbiology*. Philadelphia: Lippincott-Raven.

[25] Merritt, J.H., Kadouri, D.E., O'Toole, G.A. (2005). *Growing and analyzing static biofilms, Curr Proto Microbiol*. John Wiley and Sons, Inc, pp. 1B.1.1-1B.1.17.

[26] Chen, Z., He, B., Zhou, J., He, D., Deng, J., Zeng, R. (2016). Chemical compositions and antibacterial activities of essential oils extracted from *Alpinia guilinensis* against selected foodborne. *Industrial Crops and Products*, 83, 607-613.

[27] Bokhari, N., Perveen, K., Al Khulaifi, M., Kumar, A., Siddiqui, I. (2016). In Vitro Antibacterial Activity and Chemical Composition of Essential Oil of *Mentha arvensis* Linn. Leaves. *Journal of Essential Oil Bearing Plants*, 19(4), 907–915.

[28] Pattnaik, S., Subramanyam, V.R., Bapaji, M., Kole, C.R. (1997). Antibacterial and antifungal activity of aromatic constituents of essential oils. *Microbios*, 89, 39–46.

[29] Tzakou, O., Pitarokili, D., Chinou, I.B., Harvala, C. (2001). Composition and antimicrobial activity of the essential oil of *Salvia ringens*. *Planta Medica*, 67, 81–83.

[30] Tabanca, N., Kirimer, N., Demirci, B., Demirci, F., Baser, K.H.C. (2001). Composition and antimicrobial activity of the essential oils of *Micromeria cristata* subsp. *phyrgia* and the Enantiomeric Distribution of Borneol. *Journal of Agricultural and Food Chemistry*, 49, 4300–4303.

[31] Vardar-Unlu, G., Candan, F., Sokmen, A., Daferera, D., Polissiou, M., Sokmen, M., Donmez, E., Tepe, B. (2003). Antibacterial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. et Mey var. *pectinatus* (Lamiaceae). *Journal of Agricultural and Food Chemistry*, 51, 63–67.

[32] Sabulal, B., Dan, M., John, J.A., Kurup, R., Pradeep, N.S., Valsamma, R.K., George, V. (2006). Caryophyllene-rich rhizome oil of Zingiber nimmonii from South India: Chemical characterization and antimicrobial activity. *Phytochemistry*, 67, 2469-2473.

[33] Nikolic, B., Vasilijevic, B., Mitic-Culafic, D., Vukovic-Gacic, B. (2015). Comparative study of genotoxic, antigenotoxic and cytotoxic activities of monoterpenes camphor, eucalyptol and thujone in bacteria and mammalian cells. *Chemico-Biological Interactions*, 242, 263-271.

[34] Andersson, A., Ronner, U., Granum, P.E. (1995). What problems does the food industry have with the spore-forming pathogens *Bacillus cereus* and *Clostridium perfringens*? *International Journal of Food Microbiology*, 28(2), 145-155.

[35] Lim, J.Y., Yoon, J.W., Hovde, C.J. (2010). A Brief Overview of *Escherichia coli* O157:H7 and Its Plasmid O157. *J. Microbiol Biotechnol*., 20(1), 5–14.

[36] Allos, B.M. (2001). *Campylobacter jejuni* Infections: Update on Emerging Issues and Trends, *Food Safety*, 32, 1201-1206.

[37] Otto, M. (2008). Staphylococcal biofilms. *Curr. Top. Microbiol. Immunol.*, 322, 207-228.