Heliyon 9 (2023) e16625

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Gamma-irradiation effect on the chemical composition and antibacterial activity of the moroccan *tanacetum annuum* L. essential oil

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ARTICLE INFO

Keywords:
Tanacetum annuum L.
Essential oil
Gamma irradiation
Chemical composition
Antibacterial activity

ABSTRACT

The primary objective of the present inquiry was to assess the impact of gamma irradiation on the chemical composition and antibacterial potential of the essential oil extracted from the aerial parts of Moroccan *Tanacetum annuum* L. to this end, two distinct irradiation doses of 5 kGy and 10 kGy were administered to the essential oil, and the resultant effects were evaluated via analysis of the oil's chemical composition and antibacterial activity. The study findings have revealed that irradiation technology possesses the remarkable ability to modulate the concentrations of specific chemical constituents in a manner that effectively amplifies the antibacterial activity of the essential oil. Moreover, the technology has evinced the generation of novel compounds while also demonstrating the eradication of certain pre-existing ones upon the oil's exposure to irradiation. These discoveries have emphasized the potential of irradiation technology for augmenting the chemical profile of essential oils, thereby mitigating the risk of contamination via microbiological, physical, or chemical means, ultimately enhancing the therapeutic efficacy of the plant and its essential oil. Furthermore, the results of this study signify the possibility of harnessing irradiation technology in the production of various natural products and essential oils. The present research has thus broadened the horizons for the application of irradiation technology in advancing the potency and safety of essential oils, paving the way for a diverse range of applications in different fields, such as medicine.

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https://doi.org/10.1016/j.heliyon.2023.e16625
Received 15 January 2023; Received in revised form 10 May 2023; Accepted 23 May 2023
Available online 24 May 2023

1. Introduction

The wealth of Morocco in Aromatic and Medicinal Plants (AMPs) has garnered significant attention in recent years due to their potential applications in the food, cosmetics, and pharmaceutical industries. AMPs have been explored for their properties as natural preservatives, flavoring agents, and antimicrobial agents [1]. However, microbial contamination during various stages of processing, transportation, drying, and storage, can cause physical or chemical changes in their properties or metabolites [2]. Gamma irradiation has been shown to be an effective and safe method for the microbiological decontamination of herbs and essential oils (EOs), which are particularly vulnerable to degradation during processing and storage [3]. The Codex Alimentarius Commission (CAC) has given its endorsement for the use of ionizing radiation as a physical means for microbiological decontamination of food, which includes spices and herbs. The consensus among experts in the field is that the use of such radiation does not induce any toxicological changes or provoke the activation of irradiated food products. Literature data suggest that gamma irradiation is effective in improving the microbial quality of medicinal herbs such as ginkgo and guarana. This finding has significant implications for the food industry and underscores the potential for ionizing radiation to be used as a safe and effective method for preserving food quality and safety [4]. As should that γ-irradiation is an ionizing radiation technique that utilizes high-energy gamma rays emitted by radioactive sources, such as cobalt-60 or cesium-137, for various industrial applications, its ability to penetrate deeply into materials and disrupt chemical bonds or kill microorganisms has made it a valuable technique for sterilization, food preservation, and radiation therapy [5]. In addition to its decontamination advantages, γ-irradiation can improve the chemical profile of aromatic plants and their volatile oils, enhancing their biological activity and ensuring safe and effective use [6]. While γ-irradiation has several advantages, including effective microbial elimination and extended shelf life of food products, it also has associated demerits, such as improper handling of γ-irradiation sources can be hazardous to human health, causing radiation sickness, DNA damage, and an increased risk of cancer. Therefore, the use of γ-irradiation is regulated by national and international organizations such as the International Atomic Energy Agency (IAEA) and the U.S. Food and Drug Administration (FDA) to ensure its safe and effective use. Nonetheless, γ-irradiation remains a promising method for the decontamination and valorization of aromatic and medicinal plants (AMPs) [7]. However, the effect of gamma rays on the chemical profile and antibacterial activity of the EO of *Tanacetum annuum* L., (*T.annuum L.*), has not been extensively studied, despite containing over 200 different components, with chemovariation observed within species and subspecies [8,9]. The *T. annuum L.*, is one of the most widespread plants in Morocco and has received the least attention from scientific studies regarding it, and the value of its EO. *T. annuum L.*, is a member of the Compositae (or Asteraceae) family, which is considered the largest and most prolific flowering plant family in the world, consisting of about 1700 genera and 25,000 species [10], it is found all over the world, but it is most common

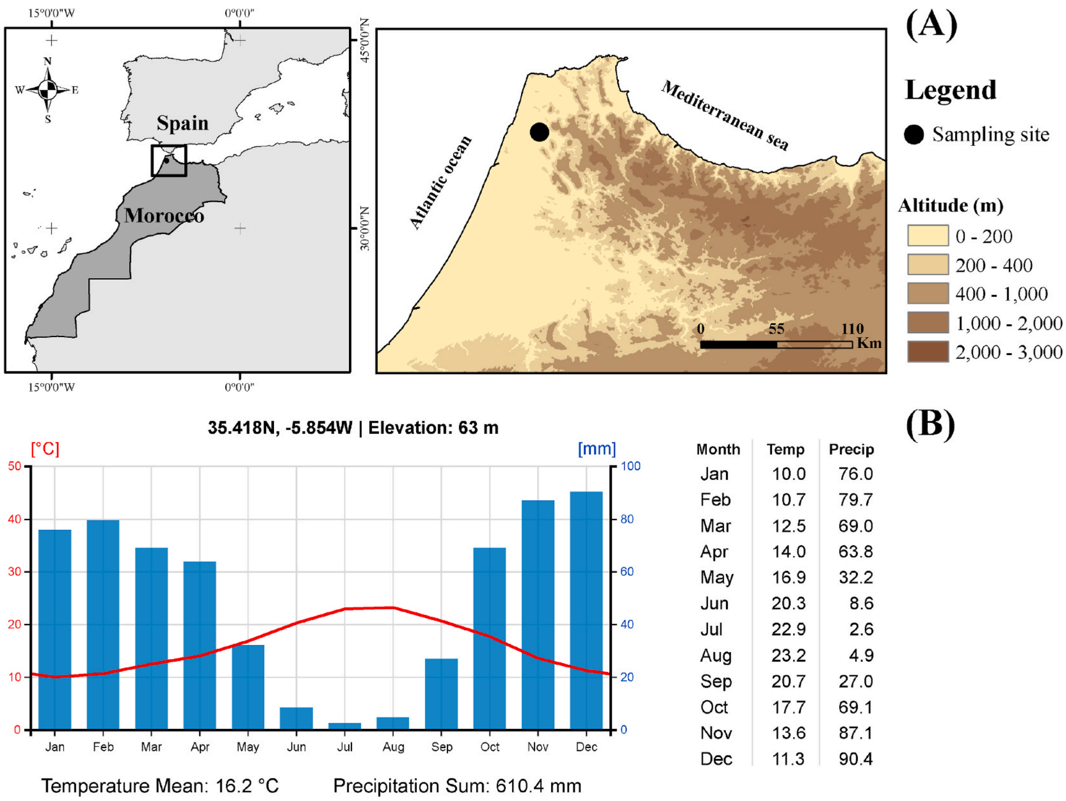

Fig. 1. Climatographic of the sampling site, built over the most recent 30-years period of monthly climatic data from 1990 to 2019 [16], temperature in red line and precipitation in blue bars. (A): the sampling site, (B): the local's climate.

in arid and semi-arid subtropical and lower temperate zones, so more than 200 species of the *Tanacetum* genus can be found from northern Africa to western Asia [11], and according to a review of the literature, Tanacetum species have been utilized as treatments in traditional medicine all across the world from ancient times [11]. Given the limited research on the EO of *T. annuum* L.*,* the rationale for selecting *Tanacetum annuum* L., as an oil source was predicated upon its insufficiently characterized yield and chemical composition, despite its potential utility, the high market value of this plant's oil, estimated to be approximately 3000 DH per kilogram, has precluded extensive commercial exportation. As a result, the utilization of *T. annuum* L., as an oil source remains constrained [12]. This study aims to examine the effect of gamma irradiation on its chemical profile and antibacterial activity, by improving our understanding of the potential applications of this EO, which will be obtained through hydro-distillation, and its chemical composition will be determined using gas chromatography-mass spectrometry (GC-MS). The EO will be divided into two groups, one exposed to γ-irradiation at two different doses and the other serving as a control. This study will evaluate the changes in the chemical composition and antibacterial properties of the EO following γ-irradiation. This research can contribute to the further development of the *Tanacetum* plant, and is a promising avenue for further exploration of the potential applications of this EO in various industries.

2. Materials and methods

2.1. Materials

2.1.1. Plant material

In July 2021, the aerial components of *T. annuum L.,* encompassing the leaves, stems, and flowers, were procured from a natural populace situated in the Beni Aerousse region of the Tanger-Tétouan-Al Hoceima province in northern Morocco. The plant material was air-dried at ambient temperature (25 °C) in a shaded area until its mass stabilized. The locale's climate is classified as a Mediterranean warm subhumid climate, characterized by wet autumns and winters and arid summers [13], with an annual mean temperature and rainfall of 16.2 °C and 610.4 mm, respectively (Fig. 1). The Scientific Institute of Rabat authenticated the plant species under the barcode RAB113321, employing The Practical Flora of Morocco [14] and the Catalog of Vascular plants of northern Morocco [15] for plant identification. Subsequently, the samples were purified and air-dried for seven days in the shade, following which nearly 2 kg of biomass was utilized in the hydrodistillation process, in accordance with the parameters outlined in Table 1.

2.1.2. Microbiological material

The investigation utilized cultures of *Staphylococcus aureus ATCC 29213* and *Escherichia coli ATCC 25922*, which were procured from the Epidemiological Laboratory of Fez. These microorganisms were activated by transferring them from slanted nutrient agar cultures (Fluka, Seelze, Germany) onto a nutrient agar medium (Fluka, Seelze, Germany) to stimulate growth, followed by incubation at 35 ± 2 °C for 24 h (Table .2).

2.2. Methods

2.2.1. Essential oil extraction

According to the (British pharmacopoeia 1993) [17] and the (European pharmacopoeia 2005) [18], a hydro-distillation procedure employing a Clevenger type apparatus was approved for the extraction of essential oils (EOs) [19]., based on this technique and following extraction, the EO samples were subjected to drying over anhydrous sodium sulfate and stored in opaque glass vials at a refrigerated temperature of 4 °C until further use. The chemical composition of EOs is same across all samples thanks to the standardization of the extraction procedure. For every sample, the extraction procedure was meticulously recorded and continuously followed. The EO of *T. annuum L.,* was obtained and divided into different samples, one of which served as the control*,* the other samples were exposed to two different doses of gamma irradiation (5 and 10 kGy).

2.2.2. Analysis of non-irradiated and irradiated essential oils by GC-MS

For the species *T. annuum L.,* GC-MS analysis was carried out in (LIMOME) Laboratory. Below is the protocol applied for the analysis of the irradiated and un-irradiated samples of the specie *T. annuum L.*.

- **Gas Chromatography Type:** GCMS-TQ8040 NX (Shimadzu brand)
- **Type of detector:** TRIPLE quadrupole
- **Column:** Apolar capillary RTxi- 5 Sil MS - 30 m × 0.25 mm ID x 0.25 μm
- **Gas used:** Helium
- **injection volume:** 1 Microlitre (μl)
- **source temperature:** 200 °C

Table 1
Parameters concerning *Tanacetum annuum* L. plant.

Species	Parts used	State	Mass in grams	Mass in EO	Harvest date	Drying time
T. annuum L.	Leaves stems and flowers	Dried	2164.48	5.06 g	13-07-2021	7 days

Table 2
Characteristics of the tested microbial strains.

A bacterial strain	n° ATCC	Type	Family	Main infections caused	Culture Media
Escherichia coli	25,922	Gram-negative bacteria	Enterobacteriaceae	- Diarrhea - Dysenteriformes - Gastroentérites	Mueller-Hinton
Staphylococcus aureus	29,213	Gram-positive bacteria	Micrococcaceae	- Gastroenteritis; - Urinary tractinfections; - Osteomyelitis and arthritis.	Mueller-Hinton

- **Interface temp:** 280 °C
- **Injection mode:** splitless
- **opening of the split at** 3.5 min
- **Injection temperature:** 250 °C
- **Pressure:** 37.1 kPa
- **Temperature program:** Ti = 50 °C for 2 min
- **Ramp 1:** 5 °C/min up to 160 °C for 2 min
- **Ramp 2:** 5 °C/min up to 280 °C for 2 min
- **Analysis time:** 50min
- **Diluting solvent:** Hexane

The GC/MS analyzes were carried out within the NIAR Institute in Tangier using a gas chromatograph coupled with a mass spectrophotometer (GC-MS) operating in electron impact mode (70 eV, *m/z* 40–450). The capillary column used: 1MS (30 m × 0.25 mm x 0.25 μm film thickness).

The analytical conditions were:

Injector and transfer line temperatures 250 and 300 °C, respectively, oven temperature programmed 50–200 °C at 10 °C/min and 200–290 °C at 35 °C/min, Helium carrier gas, at 1 mL/min, injection of 1 μL (10% hexane solution), the split ratio was 1h20.

2.2.3. Antimicrobial activity

2.2.3.1. Agar disk diffusion method. The antibacterial activity of EOs was carried out against bacterial strains by using the diffusion method on an agar disc [20]. A fresh bacterial suspension prepared in sterile saline solution and adjusted to 0.5 McFarland was served for inoculation of Mueller Hinton Agar plates (Biokar diagnostics, Beauvais, France). Then, 10 μL of EO was impregnated in sterile Whatman paper discs (6 mm in diameter) and applied to the surface of each plate. Incubation of the plates was at 37 °C for 18–24 h. Control test discs were soaked in 5 μL of sterile water. The measurement of the diameters of the zones of inhibition was in millimeters. However, all the experiments were performed in triplicate.

2.2.3.2. Statistical analysis. As the average of the three tests and their standard deviation, the statistical analysis of the obtained results was conducted using the “ANOVA" application incorporated in the STATISTICA 5.5 software. The level of significance for the analysis was set at $P < 0.05$, indicating a high degree of statistical significance.

Fig. 2. Distribution of samples exposed to gamma rays.

2.2.4. Treatment by irradiation

For gamma irradiation, *T. annuum L.* EO was packaged in 5 mL glass vials for a total of 32 vials as shown in (Fig. 2), and then exposed to 5 and 10 kGy gamma irradiation at room temperature (20 °C) at the Boukhalef ionization station (SIBO) of Tangier-Morocco, by using the radioactive sources of cobalt-60. To ensure that the vials receive the desired doses, dosimetry work was performed in which similar vials (of the same composition, position, and packaging) were irradiated to determine dose rate and also dose uniformity. The EPR/alanine dosimetry system manufactured by ADANI was used. This system is considered as a reference system for the dose measurement in the interval (50 Gy-100 kGy), due to its advantageous metrological properties ("ISO/ASTM 51607:2013 (en), Practice for using the alanine-EPR dosimetry system," n. d.). Because such systems allow for very reproducible and stable measurements [21], the uncertainties in the experimental dose were rather low and amounted to 3.5% [22]. Alanine is an amino acid, and alanine dosimeters are in the granules form with a nominal diameter of 5.0 mm and a thickness of about 2.3 mm, their irradiation produces free radicals, the concentration of these is proportional to the absorbed dose, and measured by instrumentation is called electron paramagnetic resonance spectrometer (EPR) [23]. Immediately after irradiation, the EOs were extracted and stored at (−18 °C) for later use.

3. Results and discussion

3.1. Extraction yield

Based on the results of this study, the yield of essential oil (EO) obtained from the aerial parts of *T. annuum* L. after 7 days of drying was found to be 0.23%. This yield was lower than that reported in previous studies on the same species with the same drying time. Specifically [24], reported a yield of 1.0% (v/w), while [25] reported a yield of 1.2% (v/w). However, the yield obtained in this study was higher than the yield reported for T. aucheranum (0.15%) and T. chiliophyllum (0.22%) from Turkey [26]. It is important to note that the EO yield can vary significantly depending on various factors, such as the plant part used, the harvesting time, the drying conditions, and the extraction method [27]. reported that the EO yield of harvested and cultivated tansy plants varied between 0.35% and 1.90% (v/w) with an average value of 0.81%, which is higher than the yield obtained in this study. Similarly Ulukanli et al. [28] reported a yield of 0.4% (v/w) for the same parts of *Tanacetum cilicicum*, which is also higher than the yield obtained for *T. annuum* L., in this study. In conclusion, the present study provides insight into the yield of EO obtained from *T. annuum* L., after 7 days of drying. While the yield obtained was relatively low compared to previous studies on the same species with the same drying time, it was higher than the yield reported for some other species of the same genus. The EO yield is influenced by various factors, and further studies are needed to optimize the yield and quality of EO from *T. annuum* L., in order to fully understand its potential for various applications.

Table 3
Chemical Composition of Essential Oil of *T. annuum* L., non-irradiated.

N°	Compound[a]	Area%
1	**α**-Pinene	2.12
2	Camphene	0.94
3	**Sabinene**	**14.39**
4	**β-pinene**	**5.27**
5	**β**-Myrcene	2.49
6	**α-phellandrene**	**5.10**
7	α-terpinene	0.76
8	***p*-Cymene**	**8.87**
9	D-Limonene	3.21
10	1,8-cineole	1.07
11	γ-Terpinene	1.30
12	**Camphor**	**14.21**
13	Borneol	2.67
14	4-terpineol	**2.06**
15	Thymol	1.43
16	**β**-Caryophyllene	2.22
17	β-Sesquiphellandrene	1.79
18	Germacrene D	1.30
19	**3,6-Dihydrochamazulene**	**6.95**
20	5,6-Dihydrochazulene	0.72
21	β-eudesmol	1.58
22	**Chamazulene**	**17.74**
23	Geranyl-α-terpinene	1.82
	Monoterpene Hydrocarbons	**44.45**
	Oxygenated monoterpenes	**21.41**
	Non-aromatic sesquiterpenes	**06.89**
	Aromatic sesquiterpenes	**25.41**
	Diterpene	**01.82**
	Total	**99.98%**

[a] Identificated by CG-MS. Spectra Library: W11N17M1. lib.

3.2. *Chemical composition of the essential oil of Tanacetum annuum L.*

3.2.1. *Chemical composition of the essential oil before irradiation*

The current study is centered on the aerial components of *T. annuum* L. sourced from Morocco, with the aim of scrutinizing the constitution of the hydrodistilled EOs both prior to and post-exposure to 5 kGy and 10 kGy irradiations, utilizing gas chromatography-mass spectrometry (GC-MS). For comparative purposes, the composition of the non-irradiated EOs has been presented in Table 3.

The results of the GC-MS analysis revealed that the non-irradiated EO of *T. annuum* L. was mainly composed of Monoterpene Hydrocarbons, which accounted for 44.45% of the EO (Fig. 3). This finding is consistent with previous studies which reported that Monoterpene Hydrocarbons are the major constituents of the EO derived from *T. annuum* L. [29]. Additionally, the Oxygenated monoterpenes and Aromatic sesquiterpenes constituted 21.41% and 25.41% of the EO, respectively. Among the identified compounds, Chamazulene, Camphor, and Sabinene were the most abundant constituents, with Chamazulene being the major component, accounting for 17.74% of the EO. Chamazulene is known to have anti-inflammatory and antioxidant properties [30], which could be beneficial for various health conditions. The presence of Camphor, which is widely used in the cosmetic and pharmaceutical industries due to its antimicrobial and anti-inflammatory effects [31], suggests that the EO derived from *T. annuum* L. has potential applications in these industries. Overall, the results of this study provide valuable information about the composition of the non-irradiated EO of *T. annuum* L., and the abundance of its individual components, which can be used as a basis for further investigations into the potential applications of this EO in various industries.

The EO studied was analyzed using GC-MS, which identified 23 constituents, accounting for almost all of the total oil composition. Monoterpenes were the dominant constituents, comprising 65.86% of the EO profile, with approximately 21.41% of them being oxygenated. Sabinene, camphor, *P*-cymene, β-pinene, and α-phellandrene were the major oxygenated monoterpenes. Sesquiterpenes accounted for 31.58% of the EO composition, with the majority being aromatic hydrocarbons of the Chamazulene family. Chamazulene, along with its derivatives 3,6-dihydrochazulene and 5,6-dihydrochazulene, were found to be responsible for the blue color of the EO. The presence of a significant amount of monoterpenes, particularly oxygenated ones, in the EO suggests that it may possess therapeutic properties such as antimicrobial, anti-inflammatory, and antiviral effects. The sesquiterpenes, particularly the Chamazulene family, are known for their anti-inflammatory and antioxidant properties, indicating that the EO may have potential in treating inflammatory conditions and oxidative stress-related diseases. The identification of chamazulene and its derivatives in the EO is particularly noteworthy since chamazulene is known to possess anti-inflammatory and analgesic properties. Its derivatives, 3,6-dihydrochazulene and 5,6-dihydrochazulene, are believed to enhance its anti-inflammatory effects. The presence of these compounds in the EO may make it a potential candidate for developing natural remedies for inflammatory conditions such as arthritis and asthma [32]. Fig. 4 suggests the chemical structures of main compounds in *T. annuum* L. EO.

To compare this EO of *T. annuum* L. from Morocco with other studies (Table 4), we need to look at studies that have analyzed the composition of *T. annuum* L. EOs from different regions. Grech et al. have discovered over 27 components in our essential oil, and most of them are present in varying proportions. Table 4 reveals that Camphor is present in the oil with a proportion of 8.87%, which is less than expected. However, the oil has a higher amount of Sabinene and *p*-Cymene and a lower concentration of chamazulene. This suggests that the composition of the essential oil studied is distinct from what was expected based on previous research, the findings of Grech et al. regarding the constituents present in EO. The analysis reveals a different composition than anticipated, with some components present in lower amounts than expected and others in higher amounts. These findings can help expand our understanding of the chemical makeup of EOs and may have implications for their use in various applications [24]. Zaim et al. found that the oil they analyzed had a distinct chemical composition, characterized by higher levels of monoterpene hydrocarbons and lower levels of aromatic sesquiterpenes, particularly chamazulene and its derivatives. The primary compounds identified in the oil studied by Zaim et al. were myrcene (13.67%), camphor (12.67%), sabinene (9.49%), β-pinene (7.70%), α-phellandrene (6.95%), and chamazulene (5.87%), with borneol (4.79%), limonene (3.78%), terpinene-4-ol (2.89%), 3,6-dihydrochamazulene (2.18%), α-pinene (2.16%), and

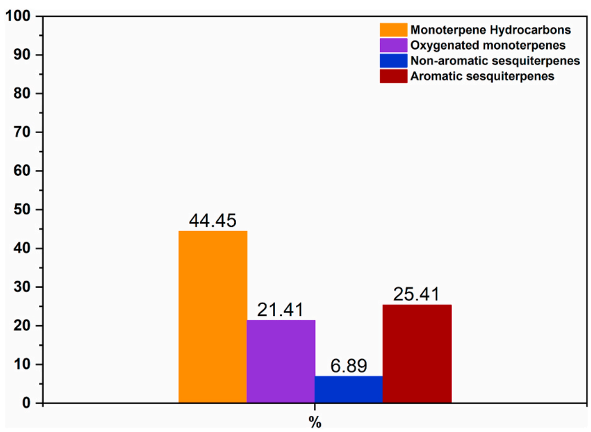

Fig. 3. Graphic representation of the percentages of the chemical groups of the essential oil of *Tanacetum annuum* L., before irradiation.

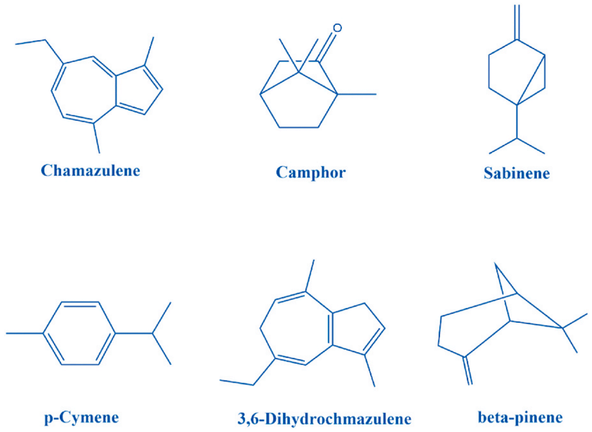

Fig. 4. The chemical structures of main compounds in *Tanacetum annuum* L. essential oil such as Chamazulene, Camphor, Sabinene, *p*-Cymene, 3,6-Dihydrochamazulene, and beta-pinene.

β-caryophyllene (2.13%) also present at rates between 2% and 3%. The results of the study by Zaim et al. demonstrate the importance of analyzing the chemical composition of EOs from various sources, as even oils extracted from the same species and harvested from the same region can have significantly different chemical makeups. These differences can have important implications for the therapeutic and aromatic properties of EOs, as well as their use in various applications. The findings of this study suggest that environmental factors, such as soil type, climate, and extraction methods, may play a crucial role in determining the chemical composition of EOs. By providing a detailed analysis of the chemical constituents of EOs, studies such as that conducted by Zaim et al. can inform the development of new products and applications for these valuable natural resources [25]. The EO extracted from the species found in the Larache area, as studied by a separate research team, contains significantly lower percentages of myrcene (6%) and chamazulene (2.8%), but higher levels of sabinene (22.3%), β-pinene (10.1%), and *p*-cymene (8.9%). It is worth noting that the remaining percentages of other constituents in this oil are also less than 2% [33]**.** The finding that the chemical profile of the EO studied is significantly different from that of the EO extracted from the same species highlights the importance of considering geographic and environmental factors in the analysis of natural products. Indeed, variations in soil composition, temperature, and altitude can have a substantial impact on the chemical makeup of essential oils. By identifying these differences, researchers can develop a deeper understanding of the complex interactions between natural compounds and their therapeutic and aromatic properties [8]. The present study provides valuable insights into the chemical composition of EO derived from *T. annuum* L., and how it differs from other species within the *Tanacetum* genus. Notably, the study sheds light on the high concentration of chamazulene and its derivatives in the EO of *T. annuum* L., which are responsible for its blue coloration. These findings are significant as chamazulene has been shown to possess a wide range of biological activities, including anti-inflammatory, antimicrobial, and antioxidant effects, which suggest that the EO of *T. annuum* L. may have therapeutic potential. In addition, the study reveals that *T. annuum* L. EO is rich in oxygenated monoterpenes, which have been shown to exhibit various biological activities, including antifungal, antibacterial, and antiviral effects. This suggests that the essential oil of *T. annuum* L. may have a broad spectrum of biological activity due to the presence of multiple active compounds. Overall, the unique chemical composition of the essential oil of *T. annuum* L. suggests that it may have potential as a source of novel therapeutic agents [26].

3.2.2. Chemical composition of the essential oil after irradiation

The outcomes of analyzing EOs using GC-MS technique, which were exposed to gamma radiation with doses of 5 kGy and 10 kGy, have been compiled in Table 5.

The analysis showed that there is a variation in the percentages of the majority of these constituents accompanied by the appearance and disappearance of certain minor constituents. In particular, depending on the increasing dose of irradiation, there is a significant decrease in the percentages of non-aromatic monoterpene hydrocarbons, in particular; the α-Pinene, Camphene, Sabinene, β-Pinene, β-Myrcene, α-terpinene, D-Limonene and the γ-terpinene. Thus, an increase in *p*-Cymene and oxygenated monoterpen, as, 1,8-Cineole, Borneol, Camphor, 4-terpineol, and Thymol. As well as for the sesquiterpene hydrocarbons next to the oxygenated sesquiterpene β-eudesmol and the diterpene geranyl-α-terpinene. However, the percentage of aromatic sesquiterpenes of the Chamazulene family has undergone a significant decrease such as chamazulene and 3,6-Dihydrochazulene (6.95%) on the other hand note the disappearance of 5,6-Dihydrochazulene which was in the non-irradiated oil with a very low percentage (0.72%), (Fig. 5). Based on the analysis, it appears that exposure to irradiation leads to significant changes in the chemical composition of the oil. Specifically, there is a decrease in non-aromatic monoterpene hydrocarbons and an increase in oxygenated monoterpenes, sesquiterpene hydrocarbons, and diterpenes. The changes observed included a reduction in non-aromatic monoterpene hydrocarbons, accompanied by an increase in oxygenated monoterpenes, sesquiterpene hydrocarbons, and diterpenes. These alterations in chemical composition could be attributed to the effects of irradiation on the molecular structure of the EO constituents. The observed changes may have implications for the potential uses and applications of the irradiated EOs.The decrease in aromatic sesquiterpenes in the Chamazulene

Table 4
Chemical Composition of the Essential Oil of *Tanacetum annuum* L. from the Tangier region (Essential oil studied: EO_1, Essential oil studied by Ref. [34]: EO_2, Essential oil studied by Ref. [25]: EO_3.

N°	Compound	% (EO_1)	% (EO_2)	% (EO_3)
1	α-Thujene	–	–	0.25
2	**α**-Pinene	2.12	1.3	2.16
3	Camphene	0.94	0.5	1.01
4	**Sabinene**	**14.39**	**8.1**	**9.49**
5	**β-pinene**	**5.27**	**2.7**	**7.70**
6	**β**-Myrcene	2.49	3.1	**13.67**
7	**α-phellandrene**	**5.10**	**2.0**	**6.95**
8	**δ−3−Carène**	**-**	**-**	**0.87**
9	α-terpinene	0.76	0.5	0.79
10	***p*-Cymene**	**8.87**	**1.6**	**-**
11	***O*-Cymene**	**-**	**-**	**0.42**
12	D-Limonene	3.21	1.1	3.78
13	Camphenilone	–	–	0.70
14	**1,8-cineole**	**1.07**	**0.8**	**-**
15	γ-Terpinene	1.30	–	1.65
16	Terpinoline	–	–	0.28
17	Linalool	–	–	0.57
18	Hydrate de *trans*-Sabinene	–	–	0.34
19	Trans Pinan-2-ol	–	–	0.13
20	All-Ocimene	–	–	0.49
21	*Cis*-β-dihydro Terpineol	–	–	0.16
22	Camphre	–	10.6	**12.67**
23	**Camphor**	**14.21**	**-**	**-**
24	**β-Terpineol**	**-**	**-**	**0.17**
25	**Borneol**	**2.67**	**1.7**	**4.79**
26	4-terpineol	**2.06**	**2.1**	**2.89**
27	α-Terpineol	**-**	**0.2**	**0.24**
28	Polygone	**-**	**-**	**0.19**
29	γ-Terpinen-7-al	**-**	**-**	**1.04**
30	Iso-Longifollene	**-**	**-**	**0.17**
31	**Thymol**	**1.43**	**1.0**	**-**
32	**β-Elemene**	**-**	**0.5**	**-**
33	**β**-Caryophyllene	2.22	1.0	2.13
34	**β**-Farnesene	–	0.1	1.60
35	α-Humulene	–	–	0.23
36	Valencene	–	0.6	0.50
37	α-Farnesene	–	–	0.34
38	β-Sesquiphellandrene	1.79	–	–
39	Germacrene D	1.30	–	1.38
40	**3,6-Dihydrochamazulene**	**6.95**	**7.2**	**2.18**
41	**δ-Cadinene**	**-**	**-**	**0.23**
42	**Elemol**	**-**	**-**	**0.46**
43	**Oxyde de Caryophylene**	**-**	**0.5**	**-**
44	**5,6-Dihydrochazulene**	**0.72**	**3.1**	**0.25**
45	7,12-deshydro-5,6,7,8-tetrahydro Chamazulene	**-**	**2.5**	**-**
46	**β-eudesmol**	**1.58**	**5.5**	**0.45**
47	**Chamazulene**	**17.74**	**24.2**	**5.87**
48	9-(15,16-dihydro-15-méthylène)-α-Terpinene	**-**	**0.3**	**-**
49	9-(15,16-dihydro-15-methylene)-*p*-Cymene	**-**	**1.5**	**-**
50	Geranyl-α-terpinene	1.82	–	–
AS	Monoterpene Hydrocarbons	44.45	31.50	61.09
	Oxygenated monoterpenes	21.41	05.80	11.22
	Non-aromatic sesquiterpenes	06.89	09.90	07.49
	Aromatic sesquiterpenes	25.41	37.00	08.30
	Diterpene	01.82	–	–
	Total	**99.98%**	**84.20%**	**88.10%**

family is particularly notable, as these compounds are known for their anti-inflammatory and anti-allergic properties. The disappearance of 5,6-Dihydrochazulene from the non-irradiated oil may suggest that this constituent is particularly sensitive to irradiation. Overall, the findings of this analysis suggest that the chemical composition of the oil is significantly altered by exposure to irradiation. Further research may be needed to determine the implications of these changes for the potential uses and benefits of the oil.

Fig. 6 provides an overview of the effects of gamma irradiation on specific chemical compounds and also offers a visual representation of these effects. The findings suggest that gamma irradiation possesses sufficient energy to cause ionization of the material being exposed to radiation, a process that involves the removal or addition of electrons from atoms or molecules, leading to the formation of ions and free radicals. As a result of the ionization process enhanced by gamma irradiation, chemical compounds undergo oxygenation, which is demonstrated by a decrease in the percentages of monoterpene hydrocarbons and aromatic sesquiterpenes and

Table 5
Variation in the chemical composition of irradiated and non-irradiated EOs of *Tanacetum annuum* L. as a function of irradiation doses.

N°	RT	Compound name	un-irradiated EO	EO irradiated at 5 kGy	EO irradiated at 10 kGy
1	7.936	α-Pinene	2.12	2.06	1.17
2	8.411	Camphene	0.94	0.93	0.54
3	**9.067**	**Sabinene**	**14.39**	**13.12**	**9.20**
4	**9.221**	**β-pinene**	**5.27**	**4.91**	**3.43**
5	9.529	β-Myrcene	2.49	2.10	1.95
6	**10.055**	**α-phellandrene**	**5.10**	**4.33**	**2.75**
7	10.378	α-terpinene	0.76	0.64	–
8	**10.599**	***p*-Cymene**	**8.87**	**8.44**	**9.75**
9	10.752	D-Limonene	3.21	2.71	2.48
10	10.850	**1,8-cineole**	**1.07**	**1.97**	**4.97**
11	11.625	γ-Terpinene	1.30	1.21	1.20
12	**14.295**	**Camphor**	**14.21**	**14.62**	**18.69**
13	**15.036**	**Borneol**	**2.67**	**2.77**	**3.70**
14	15.285	4-terpineol	**2.06**	**2.04**	**2.81**
16	**18.335**	**Thymol**	**1.43**	**1.48**	**1.93**
17	21.930	Caryophyllene	2.22	2.21	2.99
18	22.395	β-Sesquiphellandrene	1.79	1.79	2.51
19	23.465	Germacrene D	1.30	1.43	1.64
21	**24.156**	**3,6-Dihydrochamazulene**	**6.95**	**6.59**	**4.19**
22	**24.467**	β-Sesquiphellandrene	**-**	**0.61**	**1.07**
23	**27.085**	**5,6-Dihydrochamazulene**	**0.72**	**-**	**-**
24	**28.262**	**β-eudesmol**	**1.58**	**1.83**	**2.69**
27	**30.250**	**Chamazulene**	**17.74**	**17.67**	**15.08**
28	36.520	geranyl-α-terpinene	1.82	1.94	2.33
		Monoterpene Hydrocarbons	44.45	40.45	32.47
		Oxygenated monoterpenes	21.41	22.88	32.1
		Non-aromatic sesquiterpenes	6.89	7.87	10.09
		aromatic sesquiterpenes	25.41	24.26	19.27
		Diterpene	1.82	1.94	2.33
		Total	**99.98%**	**97.40%**	**96.26%**

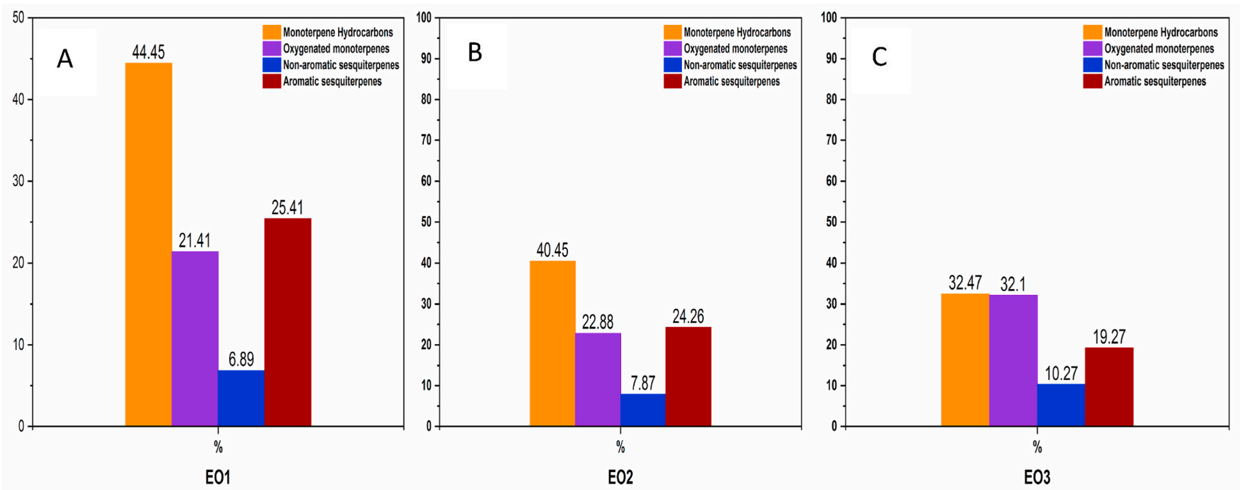

Fig. 5. Graphic representation of the percentages of the chemical groups of the essential oil of *Tanacetum annuum* L., (A: un-irradiated EO, B: EO irradiated at 5 kG y and C: EO irradiated at 10 kG y).

an increase in oxidized monoterpenes. These results suggest that gamma irradiation may have potential applications in altering the chemical composition of certain materials.

According to Fig. 6, the chromatogram (Fig. 7) provides valuable insights into the chemical composition and structure of the EOs before and after exposure to irradiation. The chromatogram of the un-irradiated EO (Fig. 7A) serves as a baseline for comparison with the chromatograms of the irradiated samples. The chromatogram of the EO irradiated at 5 kGy (Fig. 7B) show a decrease in the peak areas corresponding to non-aromatic monoterpene hydrocarbons, while an increase in the peak areas of oxygenated monoterpenes, sesquiterpene hydrocarbons, and diterpenes may be observed. This is consistent with the results obtained from the GC-MS analysis indicating the significant changes in the chemical composition of the EO after exposure to irradiation. Similarly, the chromatogram of

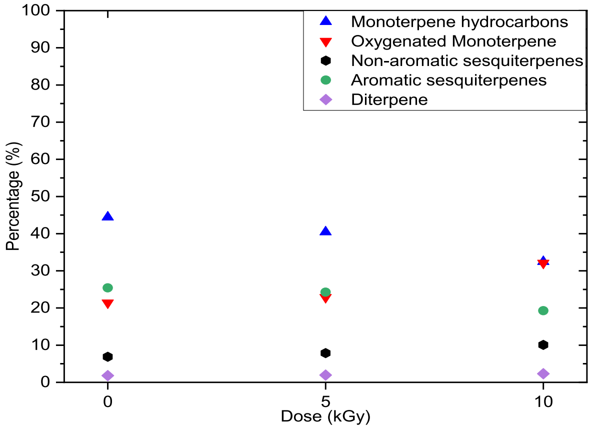

Fig. 6. Variation of the percentages of the classes of organic substances of the EO of *Tanacetum anuum* L. according to the dose of irradiation.

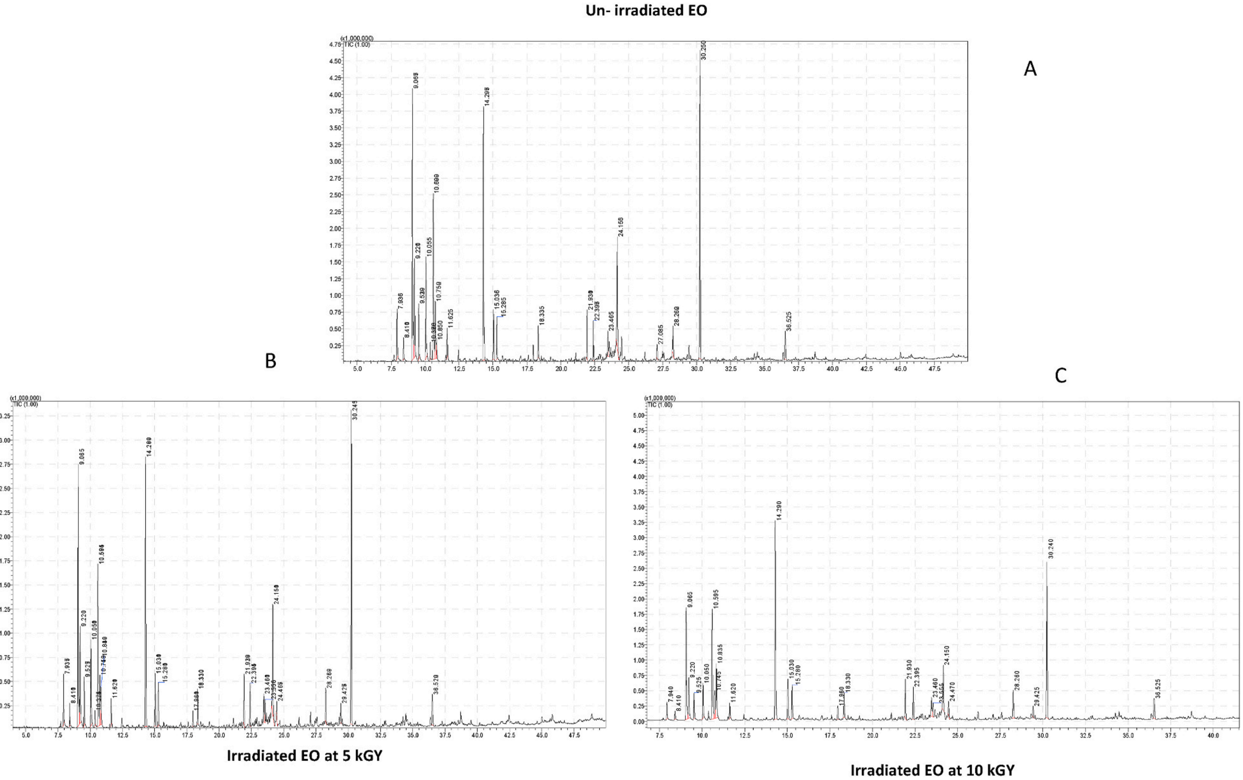

Fig. 7. Chromatogram of: A: un-irradiated EO, B: irradiated EO at 5 kGY and C: irradiated EO at 10 kGY

the EO irradiated at 10 kGy (Fig. 7C**)** exhibit a further decrease in non-aromatic monoterpene hydrocarbons, accompanied by a more pronounced increase in the peak areas of oxygenated monoterpenes, sesquiterpene hydrocarbons, and diterpenes. This indicates a more significant effect of higher doses of irradiation on the molecular structure of the EO constituents. Overall, the interpretation of the chromatograms can help to confirm the results obtained from the GC-MS analysis and provide a visual representation of the changes in the chemical composition of the EO induced by irradiation.

Based on prior investigations conducted on the oil under examination, and taking into account the distinct variations that occur in the chemical profile of the *T. annuum* L. species, it can be inferred that the analyzed oil contains a notable proportion of its chemical constituents. Notably, H. Greche et al. identified more than 130 compounds, accounting for approximately 90% of the oil. The chief constituent of the oil was chamazulene (28%), while among the 16 monoterpene hydrocarbons identified (23.1%), sabinene (6.4%), β-pinene (3.1%), myrcene (4.4%), and 3,6-dihydrochamazulene (3.7%) were the most prominent. These findings provide valuable insights into the chemical composition of the oil and may have implications for its potential applications in various domains.

Nonetheless, further investigations may be required to fully comprehend the implications of these chemical components and their interactions in the oil [34]. In a subsequent study conducted by the same author, it was observed that the chemical profile of *T. annuum L*. oil is marked by the prevalence of sabinene (22.3%), camphor (13.2%), β-pinene (10.1%), and *P*-cymene (8.9%) as the primary compounds [24]. In a study conducted by S. El Haddar et al. it was demonstrated that the EO derived from the same *T. annuum* L., species harvested in the Larache region (located in the north-west of Morocco) exhibited a lower concentration of myrcene (6%) and chamazulene (2.8%), but a higher proportion of sabinene (22.3%), β-pinene (10.1%), and *p*-cymene (8.9%) [33] A study conducted by A. Zaïm et al. on the Moroccan variant of *T. annuum* L., revealed that the predominant chemical components were myrcene (13.67%), camphor (12.67%), sabinene (9.49%), β-pinene (7.70%), α-phellandrene (6.95%), and chamazulene (5.87%) [25]. The GC-MS analysis of the EO that underwent 5 kGy of irradiation showed a chemical composition that was similar to the non-irradiated oil with minor differences in the proportion of some compounds. Notably, chamazulene decreased slightly from 17.74% to 17.67%, while there was a significant decrease in sabinene from 14.39% to 13.12%. On the other hand, camphor showed an increase from 14.21% to 14.62%, and a decrease was observed in *p*-cymene from 8.87% to 8.44%, as well as in 3,6-dihydrochazulene from 6.95% to 6.59%. Moreover, β-pinene decreased from 5.27% to 4.91%, α-phellandrene from 5.10% to 4.33%, and D-limonene from 3.21% to 2.71%. However, borneol increased slightly from 2.67% to 2.77%. The observed changes in the percentage of chemical compounds in the irradiated oil compared to the non-irradiated oil can be attributed to the effect of gamma irradiation on the oil. The decrease in certain compounds such as Sabinene, *p*-cymene, 3,6-Dihydrochazulene, β-pinene, α-phellandrene, and D-limonene could be due to the degradation or transformation of these compounds under the influence of gamma irradiation. On the other hand, the increase in Camphor and borneol could be due to the formation of new compounds or the increase in the concentration of existing compounds. The chemical composition of the EO irradiated at 10 kGy underwent significant changes compared to the non-irradiated EO and that irradiated at 5 kGy. Camphor became the major compound with a percentage of (18.69%), followed by chamazulene with a percentage of (15.08%) in the EO irradiated at 10 kGy. *P*-cymene also became a major compound with a percentage of (9.75%). Sabinene also saw an increase towards (9.20%). The percentage of 1,8-cineole increased from (1.07%) in the non-irradiated oil to (4.97%) in the EO irradiated at 10 kGy. On the other hand, 3,6-Dihydrochazulene decreased from (6.95%) for non-irradiated EO to (4.19%) for the EO irradiated at 10 kGy. Borneol had a high percentage of (3.70%) compared to the non-irradiated EO which had (2.67%), and there was a continuous decrease in β-pinene percentage from (5.27%) before irradiation to (4.91%) at 5 kGy of irradiation and (3.43%) after irradiation at 10 kGy. Caryophyllene and 4-terpineol both experienced successive increases in their percentages. However, the α-phellandrene percentage successively decreased from (5.10%) to (4.33%) and to (2.75%). The results indicate that irradiation at 10 kGy significantly modified the chemical composition of the *T. annuum* L., EO compared to the non-irradiated oil and the oil irradiated at 5 kGy. Camphor became the major compound in the irradiated oil at 10 kGy, followed by chamazulene and *p*-cymene. The percentage of 1,8-cineole increased with increasing irradiation dose. However, 3,6-Dihydrochazulene and β-pinene decreased with increasing irradiation dose. Additionally, borneol increased compared to the non-irradiated oil, while α-phellandrene continuously decreased with increasing irradiation dose. These changes in chemical composition may affect the therapeutic properties of the essential oil and should be considered when evaluating the effects of irradiation on the quality of the oil [35].

4. Conclusion

In order to analyze the limitations of a study that investigated the influence of gamma irradiation on the chemical composition and antibacterial properties of EOs derived from *T. anuum* L., it is imperative to ensure transparency and lucidity with regard to the study's inability to establish causality. The study is restricted in its scope as it exclusively examined one species of plant from a singular family, thus impeding its generalizability to other plant species from disparate families. Moreover, the study utilized a single methodology for essential oil extraction, while there are multiple techniques that may yield varying degrees of chemical composition, alongside other uncontrollable factors, such as soil composition, growth conditions, and climate, that may impact the findings.

The research aimed to investigate the effects of gamma radiation on both the chemical composition and antibacterial activity of EOs obtained from *T. annuum* L. Nonetheless, the study presents novel avenues for future research, such as broadening the scope of inquiry to various types of EOs derived from diverse families and examining a wider range of bacteria. Additional investigation could examine how various levels of gamma radiation affect the subject, starting with doses ranging from 0 to 5 KGy, then progressing to doses between 5 and 10 KGy, and eventually exceeding 10 KGy.

4.1. Antibacterial power of irradiated and non-irradiated essential oils of Tanacetum annuum L.

The results of the microbial activity inhibition tests are shown in Table .6. The values given are the average of three tests. The statistical analysis of the results is carried out with the "ANOVA" application using STATISTICA 5.5 software and the significance

Table 6
Inhibition diameters.

	Inhibition Diameters in mm	
	Staphylococcus aureus ATCC	*Escherichia coli* ATCC
Un-irradiated EO	22.33 ± 0.44	42,00 ± 0.66
Irradiated EO at 5 kGy	39.33 ± 1.55	40.00 ± 0.00
Irradiated EO at 10 kGy	35.66 ± 2.22	40.66 ± 1.55

threshold is set at $P < 0.05$.

These findings indicate that the EO of non-irradiated *T. annuum L.* exhibited a significant inhibitory effect against *Escherichia coli*, with zones of inhibition ranging from 06.00 mm to 42.00 mm, suggesting a potent bactericidal activity against this strain. For the EOs of *T. annuum* L. subjected to irradiation at 5 kGy and 10 kGy, a slight reduction in the inhibition diameter was observed, implying that the antibacterial activity against *Escherichia coli* remained stable between the non-irradiated oil, with an inhibition diameter of 42.00 mm, and the irradiated oil at 5 kGy, with a diameter of 40.00 mm, and 40.66 mm for oil irradiated at 10 kGy. As for the effectiveness of the essential oil of *T. annuum L.*, these results suggest that irradiation did not significantly affect its antibacterial activity against *E. coli.* When tested on *Staphylococcus aureus*, it was observed that the crude oil of *T. annuum* L. that was not irradiated resulted in inhibition diameters ranging from 06.00 to 22.33 mm. In contrast, the use of the oil that underwent irradiation at a dose of 5 kGy showed a significant increase in antibacterial activity, with the inhibition diameter increasing to 39.33 mm from the initial 22.33 mm before irradiation. However, the inhibition diameter was found to decrease to 35.66 mm at the 10 kGy dose. This indicates that irradiation could enhance the antibacterial effectiveness of the essential oil against *Staphylococcus aureus* at doses below 10 kGy. The findings from the study suggest that irradiation can have a significant impact on the antibacterial activity of the EO of *T. annuum* L. against *Staphylococcus aureus*. The increase in antibacterial activity observed after irradiation at a dose of 5 kGy could be attributed to the alteration of the chemical composition of the oil. This alteration may have caused the major components responsible for the antibacterial activity to become more available or to have undergone structural changes, leading to a more potent antibacterial effect. However, the decrease in the inhibition diameter observed at the 10 kGy dose suggests that there may be a threshold beyond which further irradiation leads to the degradation or alteration of these components, resulting in a reduction in antibacterial activity. The results of this study suggest that gamma-irradiation can be used as a means to enhance the antibacterial activity of *T. annuum* L. EO against *Staphylococcus aureus*, particularly at doses below 10 kGy, Fig. 8. Further studies are required to identify the optimal irradiation conditions that can maximize the antibacterial activity while minimizing any adverse effects on the chemical profile and quality of the oil. Nonetheless, the use of irradiated *T. annuum* L. EO as a natural antimicrobial agent may offer a promising alternative to conventional antibiotics in the future.

This is what reminds of the central objective of this research and its prospects, which is to determine the optimal dose which makes it possible to obtain a concentrated chemical composition with compounds of great effectiveness and thus to increase the biological activity of the oil and why not the anticorrosion activity too. By comparing the susceptibility of the two strains to the oils tested, so that the effectiveness of the oil differs from one bacterium to another as well as to the type of oil, whether it is irradiated or not. *Escherichia coli* is the most resistant of the non-irradiated oil, however, *Staphylococcus aureus* is the most sensitive to the non-irradiated oil tested while the antibacterial efficacy of the irradiated oils is highly convergent against both bacteria. The significant bioactivity observed in the EOs of *T. annuum* L. can be attributed to its chemical composition, as noted in previous research [36]. The efficacy of the *T. annuum* L. oil can be explained by its high levels of Chamazulene, sabinene, and camphor. However, the antibacterial effect of the oil may not be solely due to the presence of these specific compounds, but rather a result of the interaction between different aromatic structures. These molecules may act synergistically, either alone within the essential oil or in combination with each other. It's also important to note that minor compounds in the EOs can significantly contribute to their antibacterial activity [37].

4.2. Conclusion

The study on the gamma-irradiation effect on the chemical composition and antibacterial activity of the Moroccan *T. Annuum* L. essential oil revealed that the irradiation process significantly affected the chemical composition of the essential oil. The major components, chamazulene and α-pinene, decreased while other minor components increased. The irradiation also led to a reduction in the antibacterial activity of the essential oil. However, the irradiated oil still showed some level of antibacterial activity, especially against Gram-positive bacteria. In conclusion, our study highlights the significant impact of gamma-irradiation on the chemical composition and antibacterial activity of the Moroccan *T. Annuum* L. essential oil. The alterations in the chemical composition may have resulted from the degradation and rearrangement of the essential oil molecules during the irradiation process. The reduction in the antibacterial activity may be attributed to the altered chemical structure of the major components responsible for the antimicrobial activity. Despite the decrease in antibacterial activity, the irradiated essential oil still demonstrated some level of efficacy, particularly against Gram-positive bacteria. Our findings suggest that further research is necessary to identify the optimal irradiation dose and conditions to preserve the maximum antibacterial activity while minimizing the changes in the essential oil's chemical composition. In the future, the utilization of gamma-irradiated essential oils as a natural antimicrobial agent may be possible with further investigation and development.

Author contribution statement

Hasna Belcadi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ahmed Ibnmansour, Adil Aknouch Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Soukaina EL Amrani: Performed the experiments.

Mohammed Lachkar, Mohamed Mouhib, Said Zantar, and Anas Chraka: Contributed reagents, materials, analysis tools, or data.

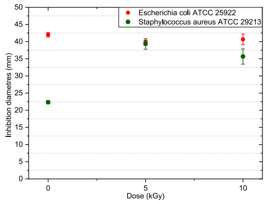

Fig. 8. The graph illustrating how inhibition diameters change with increasing doses of gamma radiation.

Data availability statement

Data included in article/supplementary material/referenced in article.

Funding

The authors did not receive support from any organization for the submitted work.
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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