Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 15, Issue 1, 2026, 35
https://doi.org/10.33263/LIANBS151.035

Ethnobotanical, Phytochemical Study and Evaluation of the Antibacterial Activity of *Calendula arvensis* L. Against Fire Blight (*Erwinia amylovora*)

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Received: 30.08.2024; Accepted: 24.02.2026; Published: 30.03.2026

Abstract: *Calendula arvensis* L. "field marigold, " is a medicinal plant commonly used in phytotherapy for its anti-inflammatory, anti-cancer, and anti-oxidant properties. The present study is part of the valorization approach of medicinal natural resources through an ethnobotanical, phytochemical, and biological study of *Calendula arvensis* L. from the Meknes region, Morocco, using the flowers and leaves of the plants. An ethnobotanical survey was conducted among herbalists and villagers, and we detected and studied the species of the Calendula genus present in this region, as well as the influence of altitude on their density. Phytochemical screening was performed to identify the main chemical classes present in the extracts. Moreover, the effect of *Calendula arvensis* L. extracts on the causal agent of fire blight, "*Erwinia amylovora*." The results showed that the most common species was *C. arvensis*, which grew in abundance between 499 and 689 m. Phytochemical screening showed that field marigold is rich in secondary compounds, particularly polyphenols, which differ according to the extract type and plant part; the flowers are richer in carotenoids, terpenes, and condensed tannins, while the leaves are rich in saponosides. GC-MS analysis showed that 2,4-di-tert-butylphenol was the dominant compound in the ethanolic (74.92%) and methanolic (19.60%) extracts. The extracts had low *in vitro* antibacterial activity (<8 mm) against Erwinia amylovora, the causal agent of fire blight. Future studies should focus on bioassay-guided fractionation and purification to concentrate the active constituents and potentially enhance their antibacterial efficacy.

Keywords: antibacterial activity; *Calendula arvensis* L.; ethnobotany survey, fire blight; phytochemical screening.

1. Introduction

Morocco has appropriate climatic and edaphic conditions for a rich and diverse flora with a high endemism rate; therefore, the country's historical tradition in the field of exploitation of aromatic and medicinal plants remains one of the most important assets of the

phytotherapy sector [1,2]. Indeed, the Moroccan population has a rich and ancient tradition in phytotherapy [3]. It is an Arab-Berber heritage, largely influenced by Islam, and the use of several medicinal plants to treat various diseases is an integral part of Moroccan culture [4]. Thus, at the regional level, Morocco is a true phyto-genetic reservoir and occupies a privileged place among Mediterranean countries that have a long medical tradition and traditional know-how based on medicinal plants [5]. According to the latest assessment, the national vascular flora has 3913 species and 1298 subspecies, with 16% and 32% endemism, respectively [6]. However, their development as natural sources of therapeutic molecules requires a thorough understanding of their chemical profiles, which inevitably involves the extraction of their secondary metabolites [7,8].

In this context, the present study is part of valorizing Moroccan natural resources, particularly interested in the field marigold (*Calendula arvensis* L.), a plant commonly used in herbal medicine, known under the name of "Aljemrah," *C. arvensis* is abundant in the spring, found as gregarious in open areas, wastelands, roadside, cemetery, and field margins [9]. It's traditionally used as a food color, spice, and tea as well as dye, ointment, or cosmetic cream [10]. Several phytochemical studies have shown that *C. arvensis* contains various families of compounds, such as phenolic acids and flavonoids [9,11]. In addition, the aerial parts of this plant are rich in sesquiterpenic glycosides, and some Triterpenoids and Saponins (e.g., arvenside A and B) have been isolated [12]. *C. arvensis* is known mainly for its anticancer and antibacterial properties [12,13] as well as its antioxidant and antifungal activities [14].

The purpose of this study was to investigate the effect of altitude on the density of calendula species in the Meknes region of Morocco. This objective also includes the contribution to the ethnobotanical and phytochemical studies of *Calendula arvensis* L., as well as GC-MS analysis and the evaluation of its antibacterial activity against the fire blight disease "*Erwinia amylovora*."

2. Materials and Methods

2.1. Study area.

Observations were made on Calendula samples collected at the flowering stage from 14 sites in the province of Meknes, Morocco (Table 1). The coordinates (longitude, latitude, and altitude) of the visited sites were recorded using GPS (Global Positioning System). Plants were randomly selected from the samples collected at each site.

Table 1. Geographical coordinates of sampling sites.

Station	Longitude (°)	Latitude (°)	Altitude (m)
Majjate	33.83	-5.49	636
Boufekrane	33.78	-5.49	689
Toulal	33.87	-5.59	499
Moulay Driss Zerhoun	34.08	-5.53	444
Ouislane	33.9	-5.49	540
Oued jdida	33.92	-5.36	549
Sidi Slimane Moul lkifane	33.87	-5.46	591
Sebaa Ayoun	33.9	-5.37	590
Aïn Taoujdate	33.93	-5.21	914
El Hajeb	33.68	-5.36	810
Agouraï	33.64	-5.58	792
Azrou	33.43	-5.23	1200
Ifrane	33.52	-5.11	1000
Dait Aoua	33.48	-5.15	960

2.2. Altitude effect on the density of the Calendula genus.

The plots method was adopted to study the altitude effect on the density of the Calendula genus by placing 30 squares with a 1m x 1m surface, spaced 5m apart at each station, with an accurate altitude measurement. The density of the genus Calendula was estimated for each square.

2.3. Ethnobotanical survey.

Diverse survey sites were chosen in Meknes city, namely, Lahdim, El Borj, and two villages around Meknes (Haj Keddour and M'haya). The number of respondents was 30, equally divided between the two sexes, selected through a non-probabilistic convenience sampling method that selects people arbitrarily and intuitively within a specified age range. As part of this preliminary ethnobotanical inquiry, no personal or sociodemographic data beyond gender were collected, and the interactions were informal, voluntary, and anonymous. Therefore, no formal ethical approval or written informed consent was required for this initial phase. Future extended ethnobotanical investigations will follow institutional ethical guidelines and include appropriate consent procedures where applicable.

2.4. Sample collection.

The present study focused on Calendula species, which were identified based on plant size, stem color, inflorescence type, and flower description. Two different samples were considered: leaves with full vegetative growth and flowers with full bloom. These samples were collected from the botanical garden of the National School of Agriculture, Meknes, whose geographical coordinates correspond to latitude 33° 49'54"N, Longitude 5° 26'40 " W, and Altitude 631 m.

2.5. Sample preparation and extraction.

The essential oil of *C. arvensis* was extracted using the hydrodistillation Clevenger apparatus system. However, organic, ethanolic, and methanolic extracts were obtained by Soxhlet extraction of 200 g of *C. arvensis* for 6h in approximately 200 mL of each solvent. The filtered extracts were evaporated in vacuo until dry using a rotary evaporator, concentrated to dryness, and the residue was stored at 48°C.

The aqueous extracts were produced using 50 g of the defatted plant material was placed in 1 L of distilled water heated beforehand at 100°C, then stirred for 24 h (using a magnetic stirrer), and a few hours later, the solution was centrifuged at 3600 rpm for 30 min, and the supernatant was recovered and filtered using filter paper, followed by evaporation on a rotary evaporator at 50°C. The extract was placed in an oven at 45°C to recover the water and obtain a dry aqueous extract.

2.6. Phytochemical screening.

Phytochemical screening was used to prepare extracts from each organ (flowers and leaves). Two extraction methods were used: infusion and decoction extraction. In the first method, an infusion of 2.5% was obtained by pouring 200 ml of boiling distilled water onto 5 g of vegetable powder, which was left to infuse for 24 h. For the decoction method, a 10% extract was obtained by pouring 100 mL of distilled water over 10 g of vegetable powder, then

boiling for 15 min. Then, to remove debris, the extracts obtained were filtered through cotton wool and Whatman No. 1 filter paper. Phytochemical characterization tests were carried out according to the methods described by Guessan *et al*. [15] to search for quinones, saponosides, mucilages, terpenoids and steroids, organic acids, starch, carotenoids, and phenolic compounds, including condensed tannins.

2.7. Dosage of total polyphenols.

The determination of the total polyphenols in the various extracts was carried out according to the method adopted by Abudunia *et al*. [12]. A dilution range was prepared in the appropriate solvent of the extract to be assayed to obtain a concentration within the range of gallic acid; in fact, 200 µl of each extract was placed in a test tube and 1 ml of the reagent of folin ciocaleu was added to the latter, the mixture was stirred vigorously, and after 1-4 minutes, 0.8 ml of 7.5% sodium carbonate solution (prepared in distilled water) was added, and the mixture was incubated at room temperature for 2 h. Absorbance was measured at 765 nm using a UV-visible spectrophotometer (T60).

2.8. GC/MS analysis.

Samples of both extracts (methanolic and ethanolic) were subjected to GC/MS analysis using a Shimadzu GC/MS series system (TQ8040) (Tokyo, Japan) with an AOC-20i Plus auto-injector. The analysis was conducted on an Rxi-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm). The oven temperature was initially set at 50°C for 5 min, then increased to 290°C at a rate of 10°C/min and maintained for 10 min. The injector and detector temperatures were set at 200°C. The ionization energy was 70 eV, covering a mass range of 40–650 atomic mass units. The GC/MS system management, parameter settings for both the GC and MS, and data reception and processing were performed using Shimadzu GC/MS Solution Software ver. 4 (Tokyo, Japan). Compounds were identified by comparing their mass spectra with data from the Wiley Registry 11th Edition/NIST 2017 Mass Spectral Library (Wiley, National Institute of Standards and Technology).

2.9. Antibacterial activity.

The antibacterial activity was measured in the same way as in an antibiogram, with antibiotics replaced by previously selected and recognized aromatic essences. The aromatogram or diffusion method in a solid medium, or the disc method, is a technique for determining the sensitivity of microorganisms towards a tested antimicrobial substance. This method is based on the migratory power of plant extracts inside a petri dish. This sensitivity test was performed according to the method described by Konan *et al*. [16], with slight modifications. The extracts of *Calendula arvensis* L. were individually tested against *Erwinia amylovora*.

2.9.1. Preparation of media and cultures.

The conserved *E. amylovora* samples were transplanted into Levane's medium. The cultures were incubated at room temperature (28°C), and colonies were visible after 24h to 72h of incubation [17]. The bacteria on Levane medium are generally mucous, smooth, and circular with a whitish color [18].

2.9.2. Inoculum preparation.

The inoculum was prepared from a fresh culture (24 hours) and homogenized in sterile distilled water. The optical density was measured at 620 nm using a spectrophotometer to obtain an optical density of> 1, corresponding to a concentration of > 10^7 CFU/ml.

2.9.3. Extracts preparation.

A 200 mg/ml solution was prepared in sterile distilled water for the various dry extracts of *C. arvensis*. This solution underwent a series of double dilutions in a geometric progression of half (½), which made it possible to obtain concentrations ranging from 200 to 3.12 mg/ml.

2.9.4. Antibacterial test.

For the antibacterial test, the paper disks (6 mm in diameter) were separated and impregnated with 15 µl of the *C. arvensis* extracts at different dilutions and placed on the inoculated medium at a rate of 3 disks/petri dish [19]. According to the previously defined concentrations, the negative control was prepared using sterile distilled water, whereas the positive control was prepared using a 20 mg/mL streptomycin solution.

2.10. Statistical analysis.

All manipulations, namely extractions, colorimetric assays, phytochemical screening, and antibacterial tests, were repeated three times. One-way analysis (One-way ANOVA) was used to investigate the effect of altitude on the density of *C. arvensis*, as well as extract yields and dosage of total polyphenols. However, statistical analysis of the antibacterial test was performed using two-way ANOVA (extract and concentration), and then the SNK test was used for multiple comparisons of the averages. Statistical analysis was performed using the IBM SPSS Statistics software version 20.

3. Results and Discussion

3.1. Altitude effect on the density of the Calendula genus.

In general, the *C. arvensis* species were abundant at low and medium altitudes, but density decreased with increasing altitude; they were abundant in Toulal, Mejjate, Boufekrane, Oued Jdida, Ouislane, Sebaa Ayoun, and Sidi Slimane Moul El Kifane (Figure 1). However, the density significantly decreased at high altitudes recorded in El Hajeb, Agourai, Ain Taoujdate, Moulay Driss Zerhoun, Azrou, Ifrane, and Dait Aoua. Indeed, *C. arvensis* species tolerate altitudes ranging from 499 m to 689 m, exceeding this interval; it becomes scarce and scanty.

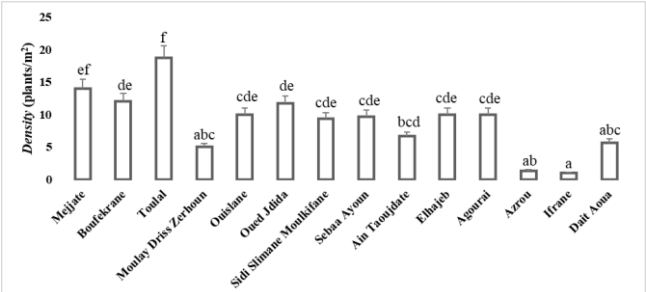

Figure 1. Density of *Calendula arvensis* L. from the prospected stations. Different letters indicate the significant differences in means according to the SNK test, where p≤0.05.

3.2. Ethnobotanical survey.

Among the herbalists and villagers of the Meknes region, C. arvensis is known for its effect on dental pain and gum inflammation, obtained by grinding the leaves and flowers into a mixture, then taking a spoonful and placing it on the tooth or gum. The infused *C. arvensis* species (10 g of powder in ½ liter of heated water) was used to treat several common dermatological pathologies, namely, Herpes, Eczema, and burns. In addition, some villagers extended their use to digestive illnesses as well as headaches.

The results are supported by the clinical study by Abutaha et al. [13] on the preventive effects of the ointment against 2nd-grade acute dermatitis during postoperative irradiation for breast cancer. The anti-inflammatory effect reported by the herbalists is also supported by Abudunia *et al*. [20], who have demonstrated the anti-inflammatory activity of *C. arvensis* on the skin and the mucous membranes, intimate or not? According to this study, the care of the field marigold has soothing and antiseptic virtues for superficial cutaneous lesions. In addition, the traditional use of *C. arvensis* for dental pain and gingival conditions has been reported by Shahane *et al*. [21].

3.3. Extracts yield.

The essential oil extraction of *C. arvensis* using the hydrodistillation extraction method recovered only certain oily traces of dichloromethane (extraction solvent); the yield of these traces did not exceed 0.03 %. This result is consistent with the results of Paolini *et al*. [22], which were 0.02% up to 0.06%, revealing a meager essential oil yield. However, the *C. arvensis* yield obtained by methanolic, ethanolic, and aqueous extracts was between 8.87 % and 28.88 %; the lowest yield was found in the methanolic extract (8.88%), while the best result was recorded in the aqueous extract (Figure 2). The yield of the methanolic extract was lower than that obtained by Ercetin *et al*. [23], which was 21.70 %, which may be due to the plant locality, period of collection, and/or the extraction technique that can influence the yield of the plant extract and its chemical composition.

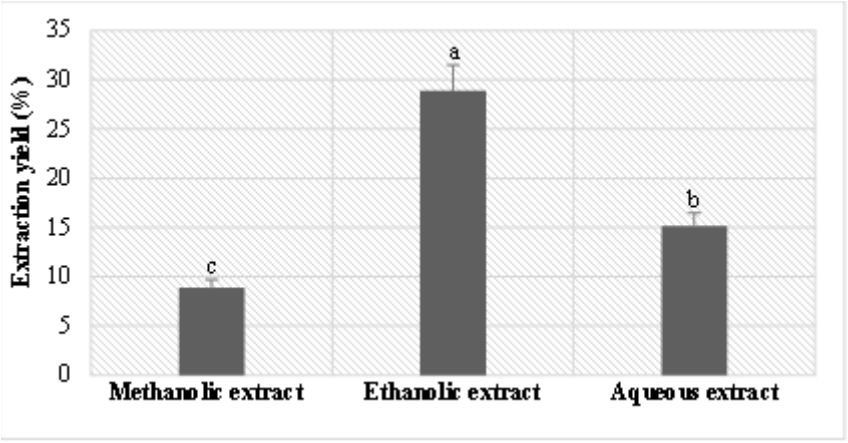

Figure 2. Percentage extraction yield obtained from *C. arvensis* using different solvents. Different letters indicate the significant differences in means according to the SNK test, where p≤0.05.

However, the *C. arvensis* yield obtained with ethanol is consistent with that reported by Abudunia et al. [12] (16.29%). The yield obtained with the aqueous extract was also close to that in this study (30.12%). This allowed us to classify ethanol as the best extraction solvent for this species, followed by distilled water. This classification can vary from one species to another and is strongly correlated with the phytochemical composition of the plant studied [24].

The degreasing of the *C. arvensis* plant made it possible to extract lipids from the flower and leaf parts and to calculate their yield (Figure 3). The results showed that the lipid content of *C. arvensis* was low, and was greater in the leaves (1.25%) than in the flowers (0.53%). The study by Dulf *et al*. [25] on the lipid content of *C. officinalis* seeds revealed much higher values, which are 9 times larger than our result (13.6 to 21.7%). This difference in lipid quantities between C. arvensis and C. officinalis is certainly attributed to the species factor, the plant part, and the geographical origin, which have undergone degreasing [26].

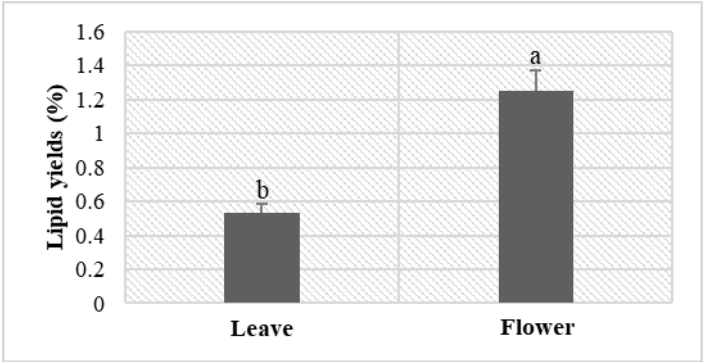

Figure 3. Lipid yield (%) obtained from flowers and leaves of *C. arvensis*. Different letters indicate the significant differences in means according to the SNK test, where p≤0.05.

3.4. Phytochemical screening.

From the results of the phytochemical tests carried out on the two organs of *C. arvensis*, different families of chemical compounds were revealed by the simple reactions of coloring and precipitation. The flower extract of *C. arvensis* showed a positive correlation with condensed tannins, terpenes, sterols, and organic acids. In contrast, leaf extracts showed a positive effect on saponosides, carotenoids, and organic acids. However, tests for quinones, mucilage, and starch produced negative results in various organs of the plant studied. The results showed that *C. arvensis* is rich in phenolic compounds, such as tannins, which are known for their antioxidant properties. Tannins are also known for their protein-binding properties and antiseptic effects; thus, their tissue-renewal properties may explain the traditional use of C. arvensis petals for burns and joint inflammation, as well as for the treatment of colon healing and cancer [27]. To our knowledge, no phytochemical study has been carried out on the organs of the *C. arvensis* plant separately; this study detected the presence of tannins, alkaloids, coumarins, and steroids, and the absence of starch. As for carotenoids, the study by Jiménez-Medina *et al*. [28] investigated the species *C. officinalis* where 19 carotenoids were detected in its yellow petals with different proportions, namely: Flavoxanthine (28%), Luteoxanthin (11%), Lycopene (20%), Carotene (alpha, beta, gamma, theta: 12%), Lutein (8%), Rubixanthines (7%), Auroxanthine (7%), but so far, no preliminary or chromatographic analysis has been carried out on the carotenoids of the *C. arvensis* extracts.

3.5. Dosage of total polyphenols.

Figure 4 shows that the various extracts of *C. arvensis* are rich in phenolic compounds; their contents were between 45.86 and 150.23 µg gallic acid equivalent/mg of extract. Regarding the total polyphenols, the statistical test of SNK did not reveal any significant difference between methanolic and ethanolic extracts of the flowers; however, for the leaves,

a significant difference was detected between the extracts used, which can be attributed to the organ factor and/or the type of extract considered.

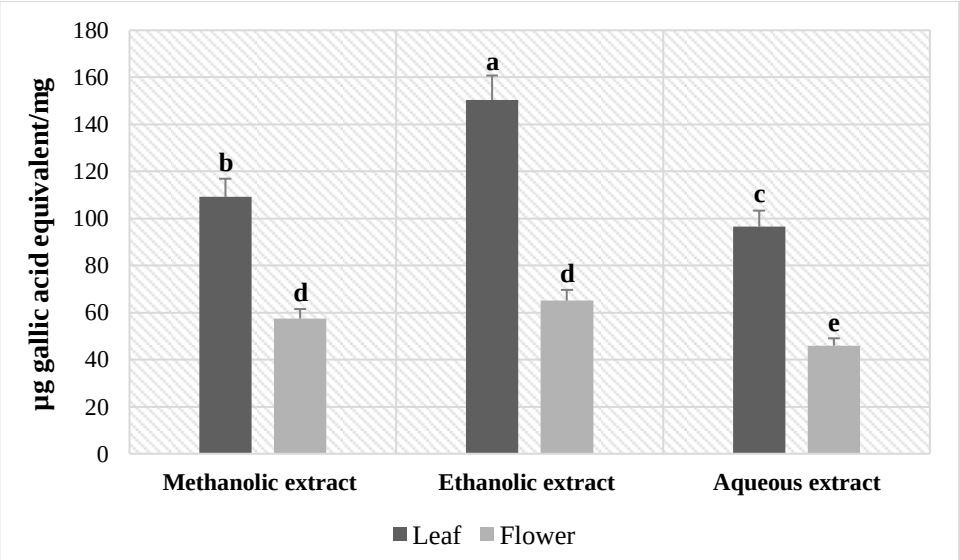

Figure 4. Polyphenol content of various extracts of leaves and flowers of *Calendula arvensis L.* Different letters indicate the significant differences in means according to the SNK test, where p≤0.05.

The highest content of polyphenols was recorded by spectrophotometric colorimetric analysis in the ethanol extract (150.23 µg EqAG/mg), followed by the methanolic (109.28 µg EqAG/mg) and aqueous extract (96.61 µg EqAG/mg extract) of the leaves. However, low contents were noted in the various extracts of *C. arvensis* flowers, and they were classified in decreasing order as follows: ethanolic> methanolic> aqueous. The unequal distribution of polyphenols in different plant organs has been reported previously [29,30]. Phenolic compounds are involved in a large number of physiological processes in plants and their interactions with the environment, and the structure of the plant can confer specific functions [31]. Our results corroborate those of Abudunia *et al*. [12] with a value of 50.26 g EqAG/mg of extract detected in the methanolic extract and 47.89 µg EqAG/mg of extract in the aqueous extract of *C. arvensis* flowers.

3.6. GC/MS analysis.

GC/MS analysis of the methanolic extract of Calendula revealed 17 chemical compounds (Table 2, Figure 5).

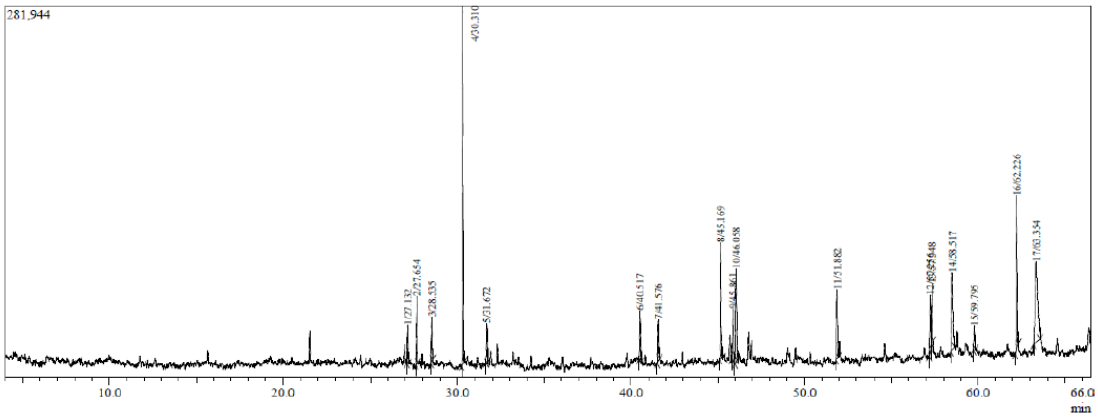

Figure 5. GC/MS chromatogram of methanolic extract.

The most important compounds identified in the methanolic extract, which constituted a substantial portion of the overall composition, were 2,4-di-tert-butylphenol, stigmasterol, tetracosane, methyl hydroxylinolenate, and humulane-1,6-dien-3-ol. Hasan and Alnaqqash [32] identified additional compounds using different GC–MS protocols. These varied results underscore the complexity and diversity of Calendula phytochemicals, highlighting the importance of employing diverse separation and extraction methods to identify as many compounds as possible [33].

Table 2. Chemical compounds of *Calendula* identified by GC/MS in the methanolic extract.

	Compounds	Retention time (min)	Area	Peak area %	Height %	Chemical formula	Molecular weight
1	Gamma-Curcumene	27.132	87949	2.01	2.67	$C_{15}H_{24}$	204.35
2	Zingiberene	27.654	154886	3.54	4.77	$C_{15}H_{24}$	204.35
3	Sesquiphellandrene	28.535	95065	2.17	3.09	$C_{15}H_{24}$	204.35
4	2,4-di-tert-butylphenol	30.310	858091	19.60	25.17	$C_{14}H_{22}O$	206,32
5	Trans-Sesquisabinene hydrate	31.672	96009	2.19	2.80	$C_{15}H_{26}O$	222.37
6	Methyl Palmitate	40.517	129479	2.96	3.54	$C_{17}H_{34}O_2$	270.5
7	Hexadecanoic acid	41.576	114483	2.62	2.88	$C_{16}H_{32}O_2$	256.42
8	Methyl hydroxylinolenate	45.169	332572	7.60	8.16	$C_{19}H_{32}O_3$	308.5
9	Linolenic acid methyl ester	45.861	168322	3.85	3.61	$C_{19}H_{32}O_2$	292.5
10	Eicosane	46.058	277353	6.34	6.59	$C_{20}H_{42}$	282.5
11	Dotriacontane	51.882	189719	4.33	4.86	$C_{32}H_{66}$	450.9
12	Hexatriacontane	57.256	164121	3.75	4.00	$C_{36}H_{74}$	507.0
13	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	57.348	163934	3.75	4.32	$C_{30}H_{52}O_2$	444.7
14	Humulane-1,6-dien-3-ol	58.517	312026	7.13	5.44	$C_{15}H_{26}O$	222.37
15	4-Protoadamantanone	59.795	64186	1.47	1.68	$C_{10}H_{14}O$	150.22
16	Tetracosane	62.226	485443	11.09	10.77	$C_{24}H_{50}$	338.7
17	Stigmasterol	63.354	683441	15.61	5.66	$C_{29}H_{48}O$	412.7

GC/MS analysis of the ethanolic extract of Calendula revealed the presence of fifteen chemical compounds. Table 3 lists the compound name, chemical formula, molecular weight, height, peak area, and retention time. Notable components include 2,4-di-tert-butylphenol, which is similar to the methanolic extract, followed by acetyl-tributyl citrate.

2,4-Di-tert-butylphenol, detected as the most abundant compound in both methanolic (19.60%) and ethanolic extracts (74.92%), is a phenolic antioxidant known for its strong free radical scavenging properties and reported antioxidant, antifungal, and antimicrobial activities against various bacterial and fungal strains [34]. Stigmasterol (15.61% in methanolic extracts) is a plant sterol with documented anti-inflammatory, antioxidant, anticancer, and antidiabetic properties, and it also modulates membrane fluidity [35,36]. Tetracosane (11.09% in methanolic extracts) is a long-chain hydrocarbon that exhibits cytotoxic activity against certain cancer cells [37].

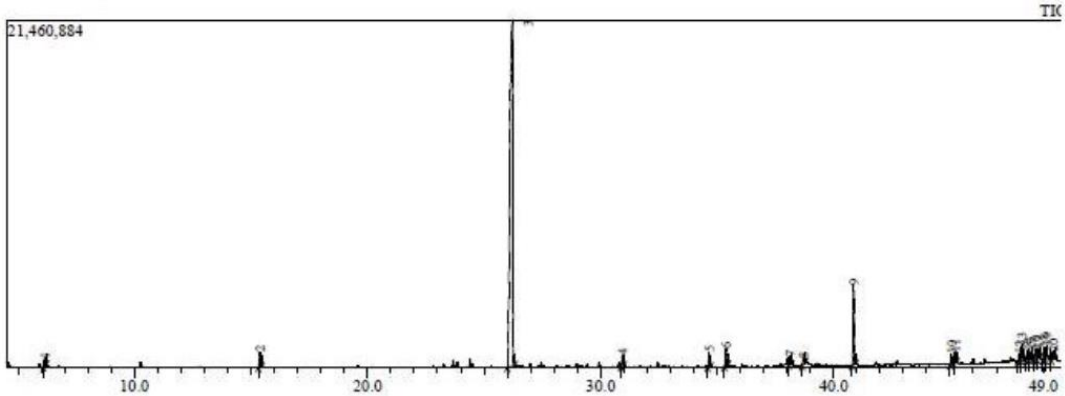

Figure 6. GC/MS chromatogram of ethanolic extract.

Table 3. Chemical composition of the ethanolic extract of *Calendula* identified by GC/MS.

	Compounds	Retention time	Peak area (%)	Height (%)	Molecular formula	Molecular weight (g/mol)
1	O-Xylene	6.148	0.77	1.34	C_8H_{10}	106.16
2	Naphthalene	15.381	1.32	2.48	$C_{10}H_8$	128.17
3	2,4-di-tert-butylphenol	26.219	74.92	56.00	$C_{14}H_{22}O$	206.32
4	Loliolide	30.943	0.97	1.87	$C_{11}H_{16}O_3$	196.24
5	Methyl palmitate	34.659	1.01	2.29	$C_{17}H_{34}O_2$	270.5
6	Hexadecanoic acid	35.391	1.42	2.75	$C_{16}H_{32}O_2$	256.42
7	Linolenic acid, methyl ester	38.141	0.73	1.33	$C_{19}H_{32}O_2$	292.5
8	Methyl heneicosanoate	38.695	0.58	1.01	$C_{22}H_{44}O_2$	340.6
10	Octyl hexadecanoate	45.054	0.92	1.55	$C_{24}H_{48}O_2$	368.6
11	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	45.215	0.85	1.78	$C_{30}H_{52}O_2$	444.7
12	2,4-Dimethylheptane	47.960	0.84	1.09	C_9H_{20}	128.25
13	Eicosyl nonyl ether	48.078	1.59	2.50	$C_{29}H_{60}O$	424.8
14	Dinonyl cyclohexane-1,2-dicarboxylate	48.667	1.11	1.60	$C_{26}H_{48}O_4$	424.7
15	1,2-Cyclohexanedicarboxylic acid, cyclohexylmethyl nonyl ester	49.143	1.79	2.22	$C_{24}H_{42}O_4$	394.6

3.7. Antibacterial activity.

Table 4 shows that the extract types, the concentrations used, and their interactions were very significant ($p<0.003$). The largest inhibition diameter was recorded for the ethanolic extract at 200 mg/ml, with a value of 3.58 mm. On average, the ethanolic extract was the most effective, with an average inhibition zone of 1.26 mm, followed by the aqueous extract (0.69 mm) and the methanolic extract (0.27 mm). The highest concentration (200 mg/ml) was the most effective (2.35 mm), followed by 100 mg/ml (1.39 mm), whereas the other concentrations yielded inhibition zones of less than 1 mm.

The negative control (sterile distilled water) showed no zone of inhibition around the disc. However, *Erwinia amylovora* was sensitive to streptomycin, which inhibited growth by (10.34 ± 0.5) mm. A previous study conducted by Wimmer *et al*. [38] found that *E. amylovora* is sensitive to streptomycin; the difference between the zones obtained is potentially due to the difference in the concentration used in the two studies. According to the indices developed by Ponce *et al*. [39], the bacteria studied were considered insensitive to the various C. arvensis extracts used in this study, as all zones of inhibition were less than 8 mm (Table 4). Other studies have shown the inhibitory effects of natural plant extracts against *E. amylovora*, notably *Peganum harmala* [40] and *Moringa oleifera* [41]. The relatively low antibacterial activity observed in this study may be attributed to several factors. One possible reason is the low concentration of bioactive compounds in the crude extracts, which may not have reached the minimum levels of inhibition required to exert potent antibacterial effects. Furthermore, crude extracts often contain inactive or antagonistic constituents that may dilute or mask the activity of key antimicrobial molecules. Resistance mechanisms inherent to the bacterial strains tested, such as efflux pumps or biofilm formation, may also contribute to reduced susceptibility. It is plausible that the fractionation or purification of extracts could concentrate the active constituents and eliminate interfering compounds, thereby improving antibacterial efficacy. Future studies focusing on bioassay-guided fractionation could provide further information on the most active fractions and their potential applications.

Table 4. Diameters of the inhibition zones obtained with different concentrations and extracts of *C. arvensis* L.

Extracts	Concentrations	Diameters of the inhibition zones (Mean ± SD) mm
Aqueous extract	200	1.97 ± 0.25^{c}
	100	1.60 ± 0.07^{d}
	50	0.74 ± 0.16^{e}

Extracts	Concentrations	Diameters of the inhibition zones (Mean ± SD) mm
	25	0.46 ± 0.11 ^{f}
	12.5	0.05 ± 0.09 ^{g}
	6.25	-
	3.12	-
Ethanolic extract	200	3.58 ± 0.39 ^{a}
	100	2.28 ± 0.26 ^{b}
	50	1.58 ± 0.06 ^{d}
	25	0.79 ± 0.06 ^{e}
	12.5	0.38 ± 0.05 ^{fg}
	6.25	0.19 ± 0.05 ^{fg}
	3.12	-
Methanolic extract	200	1.52 ± 0.41 ^{d}
	100	0.30 ± 0.09 ^{fg}
	50	0.06 ± 0.06 ^{g}
	25	-
	12.5	-
	6.25	-
	3.12	-
Extraction type		
Aqueous extract		0.69 ± 0.59 ^{b}
Ethanolic extract		1.26 ± 0.63 ^{a}
Methanolic extract		0.27 ± 0.45 ^{c}
Concentration (mg/mL)		
3.12		-
6.25		0.06 ± 0.01 ^{d}
12.5		0.14 ± 0.09 ^{cd}
25		0.42 ± 0.35 ^{cd}
50		0.80 ± 0.67 ^{c}
100		1.39 ± 0.88 ^{b}
200		2.35 ± 0.99 ^{a}
P extract		0.003
P concentration		<0.001
P concentration × extract		<0.001

Means presented within each column with no common letter(s) are significantly different according to the SNK test, where p≤0.05.

4. Conclusions

The primary ethnobotanical survey revealed the use of *C. arvensis* in the Moroccan traditional pharmacopeia, specifically in the Meknes region. Only the leaves and flowers that are exploited are well known for their anti-inflammatory properties, which are used to treat certain dermatological diseases, gingivitis, and headaches. Qualitative analysis of *C. arvensis* extracts revealed the presence of condensed tannins, terpenes, steroids, and carotenoids in the flowers, and saponosides in the leaves, in addition to organic acids revealed at the level of the two organs tested (leaves and flowers). The colorimetric assay revealed that field marigold is a significant source of polyphenols, with uneven distribution across organs and extraction solvents. The highest polyphenol content was observed in the ethanolic and methanolic leaf extracts, with values of 150.23 and 109.28 µg EqAG/mg of extract, respectively. GC-MS analysis revealed that 2,4-di-tert-butylphenol was the predominant compound in both ethanolic (74.92%) and methanolic (19.60%) extracts. The extracts applied at the tested concentrations were not very active against *Erwinia amylovora*, which is responsible for fire blight disease. All extracts led to an inhibition zone of less than 8 mm, suggesting that E. amylovora is a strain resistant to different *C. arvensis* extracts. These findings provide a useful baseline for future

studies. Subsequent studies should focus on bioassay-guided fractionation and purification to isolate and concentrate the active constituents, which may enhance their antibacterial potential. In parallel, exploring other plant species with documented antimicrobial properties, particularly those adapted to similar ecological conditions, could broaden the search for effective plant-based agents against fire blight and contribute to the development of environmentally friendly plant protection strategies.

Author Contributions

Conceptualization, S.B. and A.T.; methodology, C.T. and A.B.; software, A.B.; validation, A.B., A.T., and S.B.; formal analysis, A.B.; investigation, C.T. and A.B.; resources, A.T. and S.B.; data curation, C.T., As.B. and A.B.; writing—original draft preparation, A.B.; writing—review and editing, A.B., A.T., As.B. and S.B.; visualization, A.B.; supervision, A.T. and S.B.; project administration, A.T. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

This research received no external funding.

Acknowledgments

The authors express their gratitude to the Regional Center of Interface at Sidi Mohamed Ben Abdellah University in Fez, Morocco, for facilitating the gas chromatography-mass spectrometry (GC-MS) analysis.

Conflicts of Interest

The authors declare no conflict of interest.

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