Review

A systematic review on antimicrobial properties of Mediterranean Wild Edible Plants: we still know a little of the whole, but it is worth to persevere

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Abstract: Introduction. Bacterial resistance to antibiotics is estimated to cause the major number of deaths by 2050 if we do not find strategies to slow down the rise of drug resistance [1].
Reviews on Mediterranean Wild Edible Plants (MWEPs) with antimicrobial properties are scarce in the mean databases (Pubmed, Scopus and WoS).
Hence, we proceeded to a new review of the studies on MWEPs.
Methods. We used Wild Edible Plant and Antimicrobial as keywords. We only included the Mediterranean plants, and studies in non-Mediterranean countries, but for plants growing in Mediterranean basin. Exclusion criteria were the document type, studies not concerning plant, plants not edible, not antimicrobial properties, or totally out of topic.
Results. Finally, the number of studies reviewed, starting from one hundred and ninety-two, was thirty-eight (19,8%), concerning the antimicrobial properties of seventy-four MWEPs species, belonging to twenty-five Families. Fifty-seven (77%) out of seventy-four species, proved to be antimicrobial with a stringent threshold selection.
Conclusions. The studies are still very heterogeneous. We still know too little about MWEPs properties, but what we already know seriously recommends continuing.

Keywords: Wild Edible Plants, antimicrobial effect, Mediterranean plant, Gram+ bacteria, Gram- bacteria, extraction protocols, bioactive compounds, essential oils

Glossary: Wild Edible Plants, **WEPs**; Mediterranean Wild Edible Plants, **MWEPs**; Minimum Inhibitory Concentration, **MIC;** Minimum Bactericidal Concentration, **MBC**; Minimal Fungicidal Concentration, **MFC**; Antibiotic Bacterial Resistance, **ABR**; essential oils, **EOs**; Total Phenolic Compounds, **TPCs**; 50% inhibitory concentration, **IC50**; amount of antioxidant necessary to decrease the initial DPPH absorbance by 50%, **EC50**.

1. Introduction

Bacterial resistance to antimicrobial drugs is an emerging threat [1]. Pathogenic and opportunistic bacteria in nosocomial-acquired infections more and more often cause complications in the postoperative course. This is even more worrying given the modern medicine approaches inducing immune suppression, the misuse of antibiotics in the last fifty years, and the unbalance of healthy nutrients in the western diet. On the other hand, many people rediscover herbal medicine, which blurs the line between foods and medicines - a line that, in many cultures, was never drawn definitely. In this scenario, the Mediterranean Wild Edible Plants (MWEPs) and their antimicrobial properties are known since ancient times and nowadays a growing number of people rediscovers them as natural remedies to common infections [2].
In this respect, one of the problems concerning their use is the heterogeneity of the protocols used to extract and to analyze their active principles properties, which still distinguishes the overall set of scientific studies on MWEPs.
In this picture, we asked whether a comprehensive and updated review on this issue was so far available in the literature, but we have found very few reviews in three main literature databases (n=20 in figure S1).

1.1. Rationale

Before starting, in order to find the number of available reviews in the recent literature on Wild Edible Plants with antimicrobial properties, growing in the Mediterranean basin, we searched through three databases: Pubmed, Web of Science (WoS) and Scopus. By progressively filtering the number of matches with the first (Wild edible plant), the second (antimicrobial) and the third (Mediterranean) keywords, we realized that, without applying other temporal or geographical filters, the matches were just twenty (see figure S1).

We therefore decided to proceed with a new comprehensive analysis of the experimental studies describing this issue to provide an updated review on MWEPs.

1.2. Objectives

To provide an updated survey (last 20 years, see figure S2) of the MWEPs antimicrobial properties and still used in the daily diet by part of the population.

To make a list of the MWEPs species proved to possess anti-bacterial, anti-fungine and anti-viral properties.

To present the overall picture of the diverse protocols employed to extract the active principles from the plants.

To reinforce the belief that they are beneficial for human health and extend their use in the daily diet based on the data collected on their antimicrobial effects.

To convince the scientific community that, although we know too little about MWEPs properties, what we already know seriously recommends continuing

2. Materials and Methods

2.1. Eligibility criteria. The time interval we choose was 2001-2021, twenty years during which the number of publications on WEPs and antimicrobial grew significantly (two LOGs, from 10 to 1000 per year, see figure S2). Only for one out of the three databases (Scopus) was possible to exclude four non-Mediterranean countries such as India, China, USA and Japan, which produce a relevant number of publications on the same issue. The field of search was: Title-abstract-keywords for all the three databases.

2.2. Information sources. Three databases were the selected sources to retrieve the scientific studies: Pubmed (https://pubmed.ncbi.nlm.nih.gov), Web of Science (WoS) (https://apps.webofknowledge.com/WOS_GeneralSearch_input.do?) and Scopus (https://www.scopus.com/search). We performed the last search on the three databases on 14 April 2021.

2.3. Search strategy. The first keyword (Wild Edible Plant) allowed retrieving a very large number of studies (n= 4256). For each of the three databases this group of matches we re-analyzed the presence of the second keyword (antimicrobial, n=292); we then verified the presence of duplicates and took them away (n= 36); finally, we re-analyzed the latter group of matches (n= 256) for containing the third keyword (Mediterranean, n=192), as illustrated in figure 1 and figure S3.

2.4. Selection process.

Exclusion criteria for not eligible studies: review, non-Mediterranean plant, not describing antimicrobial property, not plant, not edible plant, out of topic (see figure 1, Prisma Flow Diagram and figure S3).

Inclusion criteria for eligible studies:

- experiments performed in countries defined Mediterranean, according to the biogeography definition, which includes countries characterized by a Mediterranean climate and ecotype, even if they do not overlook the Mediterranean Sea (such as Portugal and Jordan);

- performed in non-Mediterranean countries but analyzing plants growing in the Mediterranean basin (by checking the species geographical distribution on https://www.gbif.org/, see figure S4).

2.5. Data collection process.

The consistent reduction of the included studies after the filtering process allowed the two reviewers to collect independently the data from half of the thirty-eight reports, which have been divided taking care to balance the number of the same botanical families in the two groups.

2.6. Data items.

We paid a great attention to antimicrobial properties data generated with, at least, one of the most robust assays widely employed (Disk diffusion agar, Minimal Inhibitory Concentration, or MIC, Minimal Bactericidal/Fungicidal Concentration, or MBC/MFC). In this way, we could include thirty-seven out of thirty-eight studies (see Table 1). The only one not employing those assays, either single or in combination, but one protocol similar to MIC (IC50, measuring the 50% growth inhibitory capacity) was reported as a single study.

2.7. Study risk of bias assessment.

We paid particular attention to the assays used to assess the inhibitory or microbicide properties of the MWEPs, as previously reported. The studies performing three antimicrobial assays (Disk Diffusion, MIC and MBC) represent the more reliable group of experimental observations. We decided anyhow to group the results according to the bacterial or fungal species analyzed, to strengthen the principal goal of this review, that is the antimicrobial power of MWEPs.

2.8. Effect measures.

We measured the results of the assays on antimicrobial properties in two ways:

1. The diameter of the growth inhibition zone on the agar plate in mm, for disk diffusion agar test. According to Hudzicki 2009 [3], we adopted the following significance of the nearest whole inhibition zone, when obtained with an extract concentration ≤ 0.5 mg/ml:

Diameter zone, nearest whole mm		
Resistant	**Intermediate**	**Susceptible**
≤10	11-12	**≥13**

Accordingly, we classified as susceptible to a given extract a microorganism with an inhibition zone ≥ 13 mm.

2. The extract concentration of the Minimal Inhibiting Concentration (MIC) and/or MBC/MFC in w/v (mg/ml) or in % (v/v).

For the antibiotics determination of MIC the drug is tested in a wide range of concentrations, from 0,002 to 256 μg/ml. Very often, for different antibiotics, the concentration of 0,5-1 μg/ml represent the threshold to classify as clinically resistant a bacterial pathogen [4]. Given that for medicinal plants the MIC is calculated at a 1000-fold more concentration (mg/ml), we choose to adopt a very stringent threshold with a MIC ≤ 0.5 mg/ml, to perform a rigorous selection of eligible studies. We therefore classified as antimicrobial an extract with a MIC and MBC/MFC below that threshold. For the volume/volume dilutions, we evaluated each single study experimental plant.

3. The combination of 1. and 2. measures end up with the very stringent threshold, which we decided to adopt to classify the antimicrobial features of the MWEPs extracts. This means that, in the diverse studies, we classified as antimicrobial:

i. In the studies with the disk diffusion test and the MIC, the extracts inducing a zone of inhibition ≥13 mm obtained with a MIC ≤ 0.5 mg/ml;

ii. In the studies with MIC only, a MIC value ≤ 0.5 mg/ml;

iii. In the studies with Disk diffusion test only, but reporting the concentration of the extracts, an inhibition zone ≥ 13mm, obtained with a concentration value ≤ 0.5 mg/ml,.

2.9. Synthesis method.

Once having set the threshold of MIC concentration value and inhibition zone diameter to classify a MWEP as antimicrobial, as specified in the Effect measures paragraph above, we synthesized the study's results according to the bacterial (Gram-negative and Gram-positive) and fungi species assayed with the extracts.

A group of MWEPs species described in more than two studies were grouped to compare their antimicrobial effects.

The mean heterogeneity was the unclear specification of the concentration of the efficacious extracts in the group of thirty-eight articles reviewed. Given that our including threshold was exactly based on MIC values (in mg/ml), we had to very carefully look for this data in the materials and methods section of each study. Several of them did not clearly specify the MIC concentration used to obtain a certain inhibition zone in the disk diffusion test, even if, in the majority of cases, we could go up to this data by analysing the protocols adopted to prepare the extracts or to analyse their antioxidant properties**.**

2.9. Certainty assessment.

Beyond the differences between the studies, what gave us the most confidence in the results analyzed was the comparison of the different MWEPs species against the same pathogen.This means that we obtained a consistent picture of reliable data proving either (a) inhibiting effects or (b) not inhibiting ones, by a general evaluation of the number of the studies assaying a single pathogen which, with a certain degree of variation, where reasonably balanced for (a) and (b) type of results.

3. Results

3.1. Study selection.

In figure 1 the Prisma Flow Diagram describes the process of selection applied to the initial 292 studies retrieved by the respective search engines of the three databases inquired: Pubmed (n=61 matches), Web of Science (WoS) (n=61 matches) and Scopus (n=170 matches). Only thirthy-six records resulted duplicated in two or three databases, therefore the number of records screened became two hundreds fifty-six (n=256).

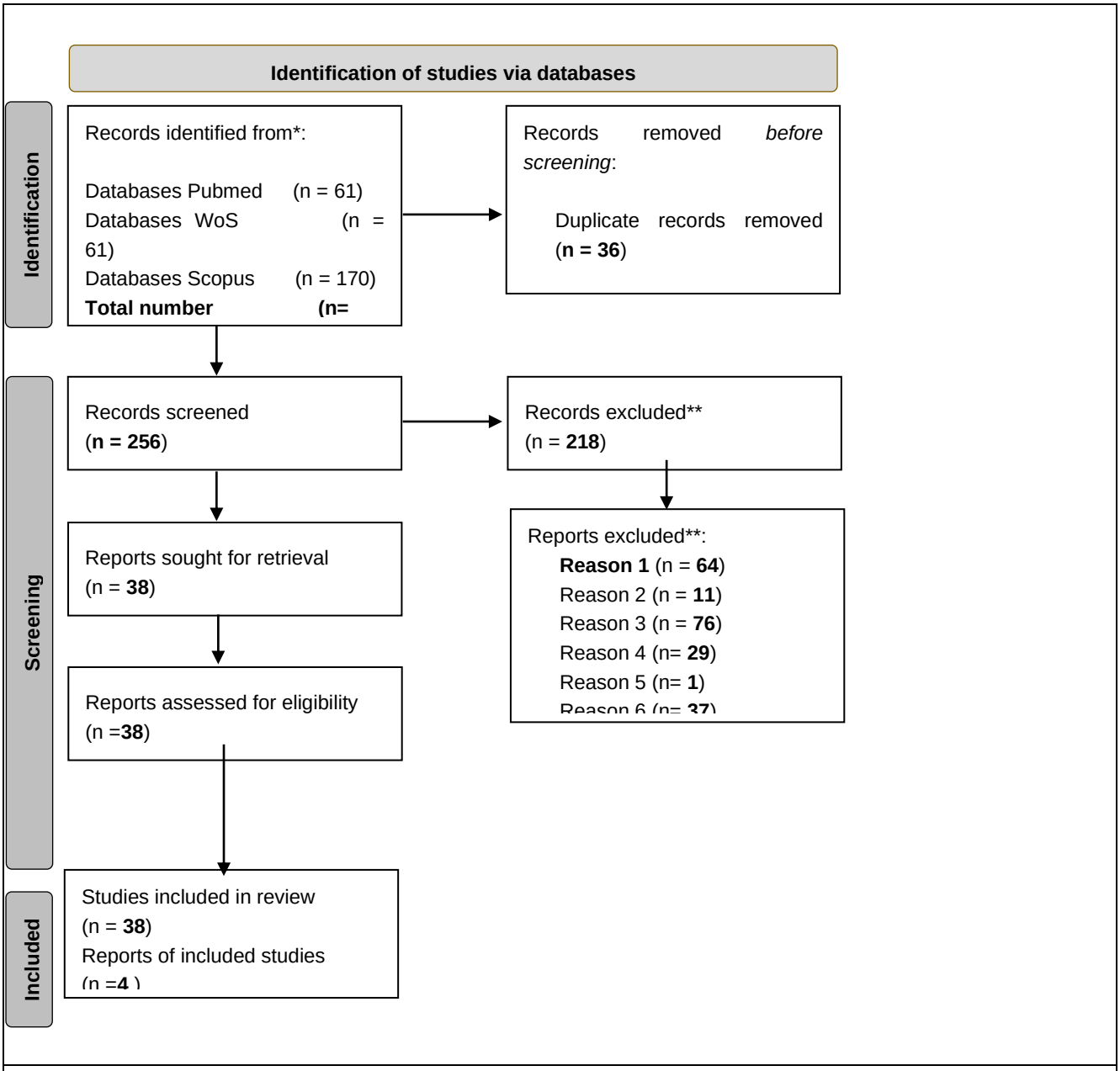


Figure 1. Prisma 2020 flow diagram.
*Number of records identified from each database.
** **Exclusion criteria**: **Reason 1, non-Mediterranean plant (n=64, ending up in a new initial number of 256-64=191),** Reason 2, review (n=11), Reason 3, not antimicrobial property[§] (n=76), Reason 4, not plant (n=29), Reason 5, not edible (n=1), Reason 6, out of topic (n=37).
[§] Mostly studies describing the ethnobotanical use of the described species, against a wide range of non-transmissible disease (diabetes, hypertension, chronic pain, tumours, etc.)
From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021; 372: n71. doi:10.1136/bmj.n71

By analyzing the abstract of each manuscript retrieved, we realized that many of them did not satisfied the inclusion criteria adopted. Sixty-four (n=64) studies were not performed either in Mediterranean countries or with plants growing also in the Mediterranean basin, though the three databases search engines retrieved them (final resulting set of 192 studies). Seventy-four (n=74) studies did not described the antimicrobial properties of the plants, but instead their anti-inflammatory, anti-proliferative or nutraceutical properties. Twenty-nine (n=29) studies performed experiments with algae and mushrooms and not with plants. Thirty-seven (n=37) were totally out of the topic we choose (for example the wild edible plant was shown to be able to prevent adverse effects of chemotherapeutic drug). One (n=1) of them described a Wild plant not edible (fern) and eleven (n=11) of them were reviews. At the end of this careful analysis, out of two hun-

dreds ninety-two studies retrieved, two hundreds eighteen (n=218) of them could not be included in the further analysis, and we proceeded with the remaining thirthy-eight studies (n=38, 19,8% of 192 Mediterranean), actually possessing all the characteristics chosen at the beginning.

It is remarkable that the selection process, though performed with a rather stringent threshold to classify antimicrobial activity, brought to review less than 1% (0,89%) of the initial number of studies on WEPs (articles selection flow numbers in figure S3).

3.2. Study characteristics.

In figure S4 we show some examples of the geographical distribution of the WEPs species in the Mediterranean basin, starting with those present mostly in the northern Mediterranean countries (S4.a.), those diffused also in the Middle East countries (S4.b.) and those widely distributed also in the North African cost (S4.c.). To define a species non-Mediterranean we checked whether there was no single accession in the entire Mediterranean basin (as in S4.d. for *Thymus daenesis*).

Table 1 contain the complete list of the studies analyzed in the present review (thirty-eight). For each study is indicated the botanical Family, the species name, and whether the plant extracts were tested against bacteria or fungi. Surprisingly, no one of them presented MWEPs properties against viruses.

Family	**Species**	**Assayed *vs***		**Ref.**
		Bacteria	**Fungi**	
Asteraceae	***Sonchus oleraceus* ***	yes	no	[5]
	Sonchus arvensis *	yes	no	“
	***Sonchus asper* ***	yes	no	“
	Sonchus uliginosus *	yes	no	“
Asteraceae	*Reicardia picroides* *	yes	yes	[6]
	Picris echioides *	yes	yes	“
	Urospermum picroides	yes	yes	“
	***Taraxacum officinale* ***	yes	yes	“
	Hymenonema graecum	yes	yes	“
	***Sonchus oleraceus* ***	yes	yes	“
	Hedypnois cretica *	yes	yes	“
	Taraxacum spp *	yes	yes	“
Fabaceae	*Ononis natrix* *	yes	yes	[7]
Brassicaceae	***Raphanus raphanistrum***	yes	no	[8]
Asteraceae	*Bidens pilosa* *	yes	no	[9]
Amaranthaceae	***Chenopodium album* ***	yes	no	“
Apiaceae	*Heracleum pyrenaicum subsp. orsinii* *	yes	yes	[10]
Asteraceae	***Sonchus oleraceus* ***	no	yes	[11]
	Cichorium pumilum	no	yes	“
Portulacaceae	*Portulaca oleracea* *	no	yes	“
Myrtaceae	*Psidium cattleianum*	yes	no	[12]
	Psidium guajava	yes	no	“
Apiaceae	*Scandix pecten-veneris* *	yes	yes	[13]
Asteraceae	***Centaurea raphanina* ***	yes	yes	[14]
Asteraceae	***Centaurea raphanina***	yes	yes	[15]
Asphodelaceae	***Eremurus spectabilis* ***	yes	no	[16]
Boraginaceae	*Borago officinalis* *	no	yes	[17]
Orobanchaceae	*Orobanche crenata* *	no	yes	“
Plantagineceae	*Plantago coronopus* *	no	yes	“
Plantagineceae	*Plantago lanceolate* *	no	yes	“
Rosaceae	*Sanguisorba minor* *	no	yes	“
Caryophyllaceae	***Silene vulgaris***	no	yes	“

Asteraceae	***Sonchus asper* ***	no	yes	“
	***Sonchus oleraceus* ***	no	yes	“
	Taraxacum officinale	no	yes	“
Asteraceae	***Centaurea raphanina* ***	no	yes	[18]
Amaryllidaceae	***Allium roseum* ***	yes	yes	[19]
Asphodelaceae	***Eremurus spectabilis***	yes	yes	[20]
Rutaceae	*Ruta angustifolia*	yes	yes	[21]
Apiaceae	***Foeniculum vulgare* ***	yes	no	[22]
Lamiaceae	*Salvia palaestina fruticose* *	yes	no	“
Lamiaceae	*Micromeria fruticose* *	yes	no	“
Fabaceae	*Trigonella foenum-graecum* *	yes	no	“
Asteraceae	***Cichorium pumilum*** jacq *	yes	no	“
Lamiaceae	*Salvia hierosolymitana* boiss *	yes	no	“
Rutaceae	*Ruta chalepensis* *	yes	no	“
Asteraceae	***Chrysanthemum coronarium* ***	yes	no	“
Lamiaceae	***Ziziphora clinopodioides* ***	yes	no	[23]
Crassulaceae	*Umbilicus rupestris*	yes	no	[24]
Amaryllidaceae	***Allium roseum* ***	yes	no	[25]
Lamiaceae	*Origanum syriacum* *	yes	no	[26]
Euphorbiaceae	*Mercurialis annua*	yes	yes	[27]
Papaveraceae	***Papaver rhoeas***	yes	yes	“
Apiaceae	***Foeniculum vulgare***	yes	yes	“
Amaranthaceae	***Chenopodium murale***	yes	yes	“
Asteraceae	*Scolymus hispanicus*	yes	yes	“
Brassicaceae	*Sinapis arvensis* *	yes	no	[28]
Polygonaceae	***Polygonum aviculare* ***	yes	no	“
Asteraceae	***Tragopogon aureus* ***	yes	no	“
Apiaceae	***Foeniculum vulgare* ***	yes	yes	[29]
Amaryllidaceae	***Allium roseum* ***	yes	yes	[30]
Amaryllidaceae	***Allium roseum* ***	yes	yes	[31]
Oleaceae	*Olea europeae*	yes	no	[32]
Oleaceae	*Olea ferrugineae*	yes	no	“
Asteraceae	***Chrysanthemum coronarium* ***	yes	yes	[33]
Amaryllidaceae	*Allium macrochaetum* *	yes	yes	[34]
Asteraceae	***Centaurea raphanina* ***	yes	yes	[35]
Polygonaceae	*Polygonum hydropiper*	yes	no	[36]
Caryophyllaceae	*Silene alba* *	yes	yes	[37]
Caryophyllaceae	*Silene conoidea* *	yes	yes	“
Caryophyllaceae	*Silene dichotoma* *	yes	yes	“
Caryophyllaceae	*Silene italica* *	yes	yes	“
Caryophyllaceae	*Silene supine* *	yes	yes	“
Caryophyllaceae	***Silene vulgaris* ***	yes	yes	“
Lamiaceae	***Ziziphora clinopodioides* ***	yes	yes	[38]
Amaranthaceae	***Chenopodium murale* ***	yes	no	[39]
Brassicaceae	*Eruca sativa* *	yes	no	“
Brassicaceae	*Malcolmia africana* *	yes	no	“
Malvaceae	*Malva neglecta* *	yes	no	“
Fabaceae	*Medicago polymorpha* *	yes	no	“
Fabaceae	*Melilotus officinalis* *	yes	no	“
Brassicaceae	***Nasturtium officinale* ***	yes	no	“
Apocynaceae	*Carissa macrocarpa*	yes	no	[40]
Apiaceae	*Smyrnium olusatrum*	yes	yes	[41]
Apiaceae	*Smyrnium perfoliatum*	yes	yes	“
Apiaceae	*Smyrnium rotundifolium* Miller	yes	yes	“

Apiaceae	*Smyrnium cordifolium* Boiss	yes	yes	“
Apiaceae	*Smyrnium connatum* Boiss and Kotschy	yes	yes	“
Apiaceae	*Smyrnium creticum* Miller *	yes	yes	“
Araceae	*Arum dioscoridis* *	yes	yes	[42]
Amaranthaceae	***Chenopodium album* ***	yes	yes	“
Malvaceae	*Malva sylvestris* *	yes	yes	“
Lamiaceae	*Mentha longifolia* *	yes	yes	“
Brassicaceae	***Nasturtium officinale* ***	yes	yes	“
Papaveraceae	***Papaver rhoeas* ***	yes	yes	“
Polygonaceae	***Polygonum aviculare* ***	yes	yes	“
Polygonaceae	*Rumex acetosella* *	yes	yes	“
Brassicaceae	*Sinapis alba* *	yes	yes	“
Urticaceae	*Urtica dioica* *	yes	yes	“

Table 1. List of the thirty-eight studies analyzed in this review, with a total number of 74 MWEPs species. In bold are indicated the species studied in more than one article. The species classified as antimicrobial, by passing our stringent threshold, are 57 (77%), and are marked with an asterisk (*) when associated to the reference study in which the antimicrobial properties were demonstrated.

We grouped the MWEPs by their botanical Families (Table S1), and it’s worth noting that eight Families account for 69% of the species, namely Asteraceae, Apiaceae, Brassiceae, Caryophyllaceae, Lamiaceae, Fabaceae, Polygonaceae and Rutaceae.

The MWEPs species which are analyzed in more than one study are indicated in Table S2, being *Allium roseum* (Amaryllidaceae), *Centaurea raphanina* and *Sonchus oleraceus* (Asteraceae) described in four studies each, and *Foeniculum vulgare* (Apiaceae) in three studies, all together accounting for 39.5 % of the experimental studies retrieved.

3.3. Risk of bias in studies.

We paid much attention to the presence of original experiments performed with plant extracts on bacteria, fungi and viruses. Surprisingly we could not find a single study reporting anti-viral properties of WEPs. Given the limited number of studies to analyze, we reported all the experimental data and protocols adopted to produce them. The significant heterogeneity of the experimental procedures was taken into account and the results were grouped according to the bacterial or fungal pathogen, which resulted sensible to the plant’ microbicide effect. A graphical summary describes the different starting material (wild *vs* cultivated, fresh *vs* frozen-lyophilized or air-dried plants), the mean solvents employed to prepare the extracts (alcoholic *vs* aqueous extract, see Figure 2) and the antimicrobial assays performed (Disk Diffusion test *vs* MIC and/or MBC, see Figure 3)

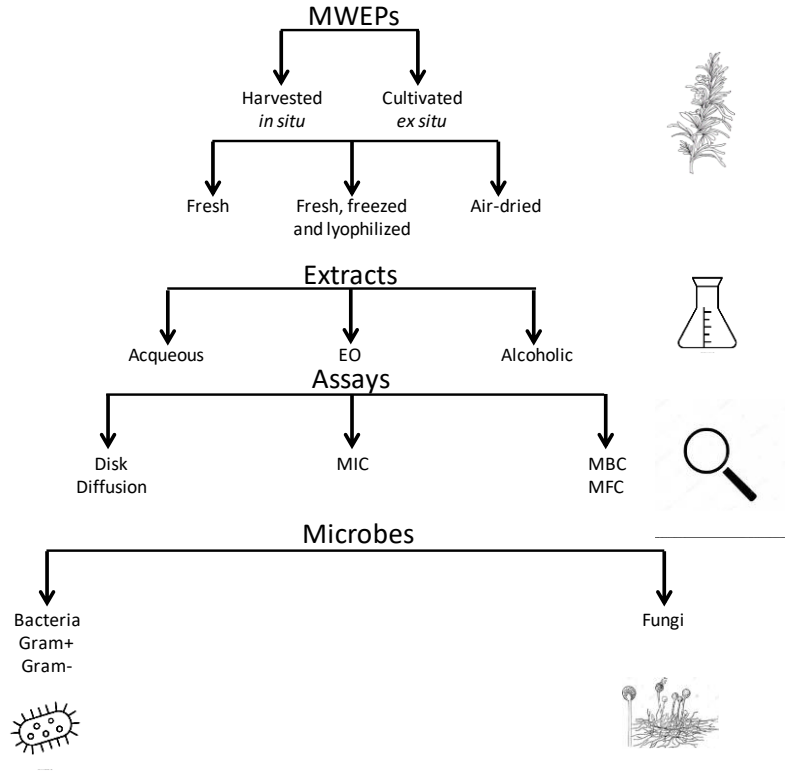


Figure 2. Graphical picture of the different types of plant material, solvents for extracts, assays for antimicrobial properties and pathogenic microbes (bacteria and fungi) tested.

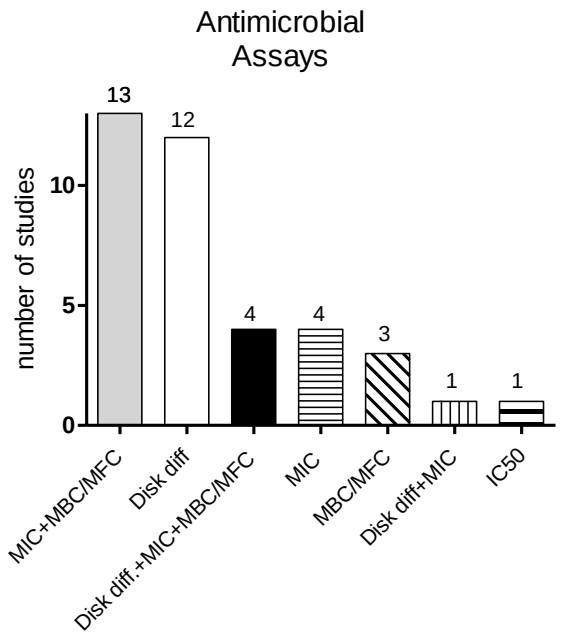


Figure 3. Graphical picture of the distribution of antimicrobial assays in the thirty-eight studies analyzed. The major part of them (n=36, constituting the 65,7%) employed either MIC, or MBC, or they combination also with Disk diffusion agar, while 31,6% was based only on Disk diffusion agar test, and 2,6% (one single study) only on IC50 assay.

3.4. Results of individual studies.

We report those few studies showing no antimicrobial effects at all (8), in which *Raphanus raphanistrum* did not meet our threshold criteria for being classified as antimicrobial (see Materials and Methods). In addition, *Eremurus spectabilis* extracts [20], have not passed the threshold of values established for classification of antimicrobial activity.

There was only one study using exclusively IC50 assay, in which eight MWEPs showed a very high antibacterial capacity measured as less than 20 ppm [22].

There is only one in which the authors isolated the unique active compound cnicin, [18] in *Centaurea raphanina*, which was the only isolated compound displaying antifungal activity.

There are only two studies analyzing *Allium roseum* proteic extract [30-31], both of which did not display any antibacterial activity.

There is only one study performed with Olive oils, extracted from the ripe and unripe fruit by mechanical pressing [32], but they did not show any antibacterial activity by our criteria.

3.5. Results of syntheses.

We have grouped the studies according to:

3.5.1. The antimicrobial effects reported for the species most studies.

The sixteen MWEPs species analyzed in more than one study are listed in Table S2. Fourteen out of sixteen (87, 5%) displayed antimicrobial properties, as classified with our stringent threshold (see Materials and Methods).

Three species were analyzed in four studies: *Allium roseum*, *Centaurea raphanina* and *Sonchus oleraceus.*

For *Allium roseum,* out of four studies, only one was performed with alcoholic and aqueous extracts [19], while the other three were protein extracts [25,30-31]. Interestingly, only the alcoholic extracts (containing Polyphenols) displayed a significant antimicrobial effect *vs* many Gram-positive and Gram-negative bacteria, while the three protein extracts MIC was above the threshold we set up in this review.

For *Centaurea raphanina,* four studies compared the plant grown *in situ*, or wild, with the *ex situ*, or cultivated one [15], by a further analysis of the soil enrichment effects on the plant antimicrobial properties [14,18,35]. The wild *C.raphanina* displayed the highest capacity to inhibit bacterial growth.

Sonchus oleraceus, with two studies on pathogenic bacteria [5-6], and other two on pathogenic fungi [8,14], displayed relevant antimicrobial properties in all the four independent studies.

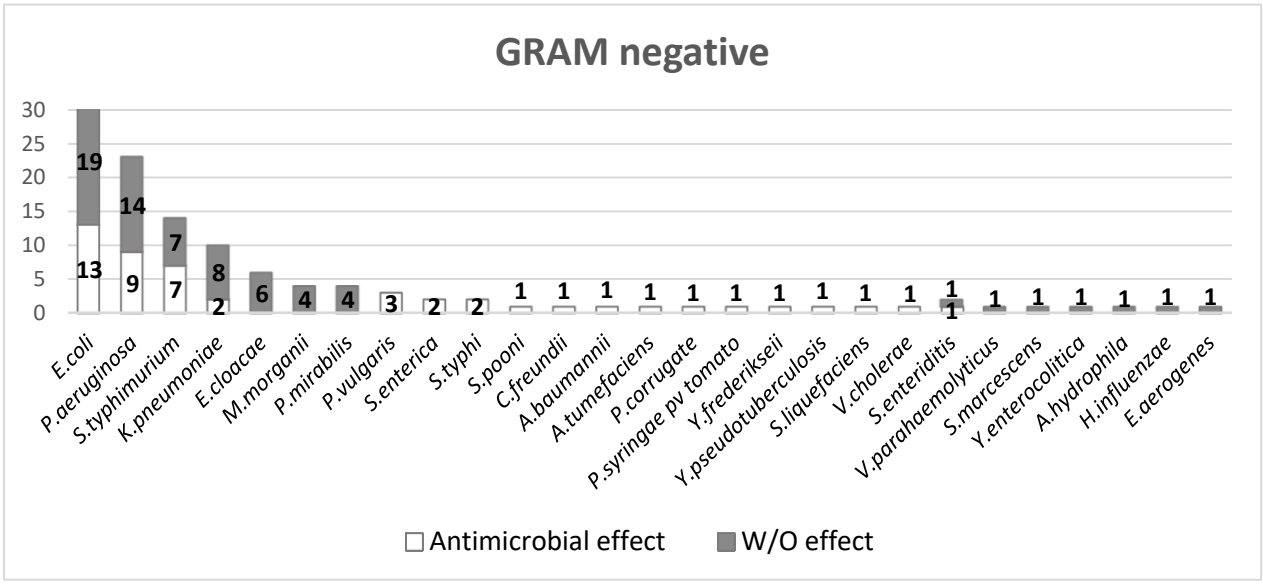
Foeniculum vulgare was analyzed in three studies, and displayed antibacterial IC50 values of less than 20 ppm [22] *vs Staphylococcus mutans,* while it didn't show relevant antimicrobial activities *vs* other bacteria nor fungi [27,29]. It's interesting that the only study showing antimicrobial properties was performed with plants collected from the mountains of the Nablus region and Kabul mountain (north Galilee),while in the other two plants were collected in the Sidi Bennour region (central Morocco, altitude 185 meters

above sea level, 27) and in sisxteen location in Tunisia [29], only two with an altitude > 590 meters above sea level.

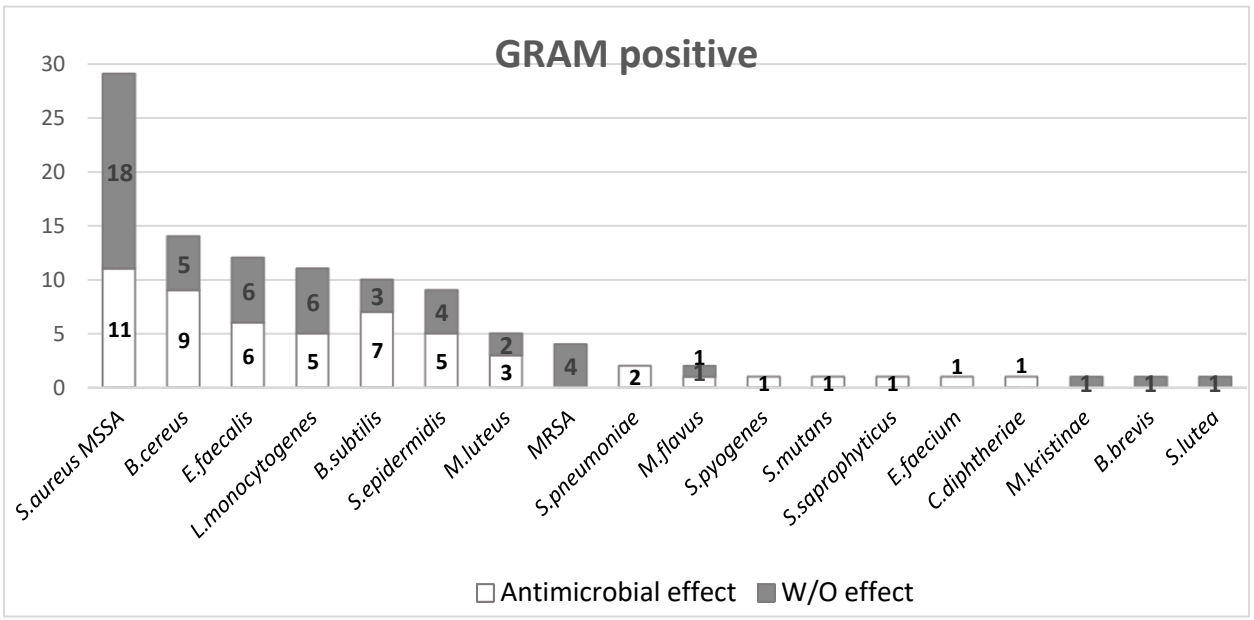
In figure S5 we show the Venn Diagram grouping the Gram-negative and Gram-positive common bacterial species assayed with this group of MWEPs species.

3.5.2. The overall picture of MWEPs antimicrobial effects on Gram positive bacteria, Gram negative bacteria, Fungi.

In Figure 4 we report the number of studies on GRAM-negative (n=27 species), GRAM-positive (n=18 species) bacteria and Fungi (n=25 species) treated with MWEPs extracts.



a.



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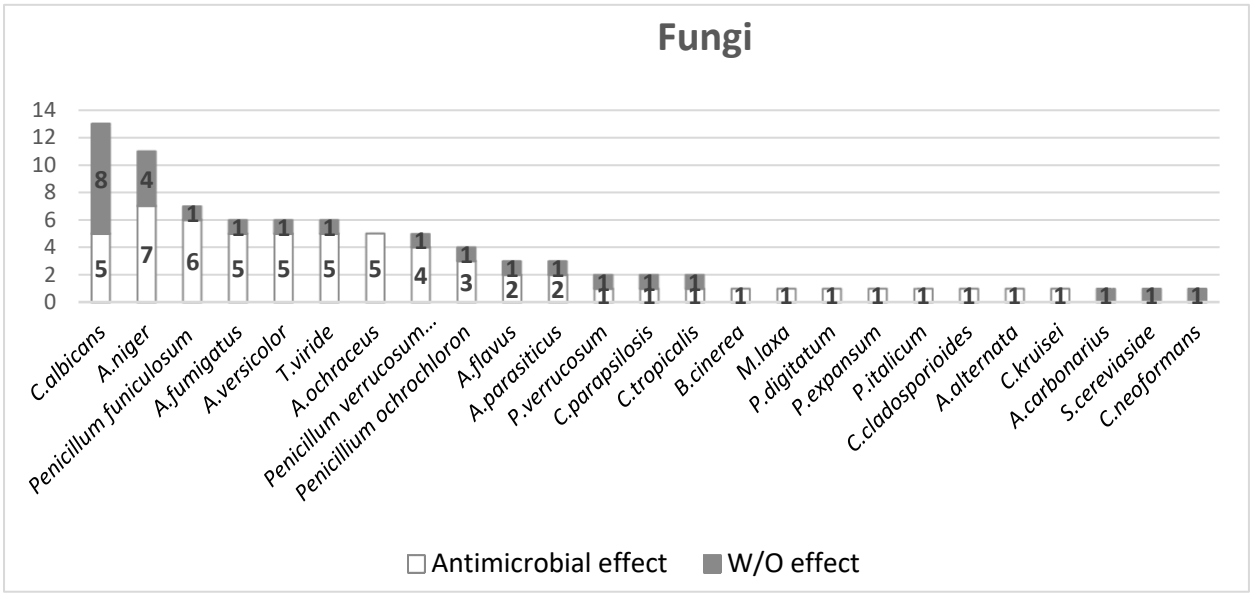


c.

Figure 4. Number of studies (ordinate axis) of antimicrobial properties of MWEPs on GRAM-negative (27 species), GRAM-positive (18 species) bacteria and Fungi (25 species). In different colors, those displaying an antibacterial effect (in white) and those in which no antibacterial effect (in grey) could be included by our thresholds for inhibition zone diameter (≥ 13 mm) where obtained with a MIC value ≤ 0.5 mg/ml. A. GRAM-positive bacteria; B. GRAM-negative bacteria; C. fungi.

1. How many Gram-negative bacteria are susceptible to MWEPs extracts?

In figure 4.a are indicated all the Gram-negative species *vs* which the MWEPs are efficacious or not. Overall, we found that 19 species, out of 27 of Gram-negative bacteria, were sensible to the MWEPs extracts, representing the 70 % of the species analyzed in the thirty-eight studies of this review.

For the mean bacteria of this group, composed by *E.coli*, *P.aeruginosa*, *S.typhimurium* and *K.pneumoniae*, the studies with MIC ≤0.5 mg/ml are slightly less to those over this threshold. It is important to note, though, that these bacteria do display a high incidence of nosocomial-associated ABR strains (being *P.aeruginosa* and *K.pneumoniae* included in the ESKAPE group (composed by six nosocomial pathogens that exhibit multidrug resistance and virulence: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.,) [43].

In this scenario the availability of several MWEPs species able to inhibit, and eventually kill, these dangerous bacterial species, might represent a strategic reservoir of natural products for therapeutic and disinfectants interventions (Table 2.).

It is also worth noting the very limited number of studies on the Gram-negative species not sensible to the MWEPs extracts, very often one or maximum four articles. This is anyhow a suggestion for future research on these species, four of which are nosocomial-associated bacteria, as *E.cloacae*, *M.morganii*, *S.marcescens* and *P.mirabilis*.

Species	Ref	Extract	Disk diff.	MIC mg/ml	MBC mg/ml	IC_{50}	Bacteria	MFC	Fungi
Sonchus oleraceus ***Sonchus arvensis*** ***Sonchus asper*** ***Sonchus uliginosus***	5	Methanolic of aerial parts air-dried, pulverized and stored at -48°C.	√	√			Gram-: ***Escherichia coli*** ***Salmonella enterica*** *Vibrio parahaemolyticus* Gram+: ***Staphylococcus aureus***		
Reicardia picroides ***Picris echioides*** *Urospermum picroides* ***Taraxacum officinale*** *Hymenonema graecum* ***Sonchus oleraceus*** ***Hedypnois cretica*** ***Taraxacum sp***.	6	Hydromethanolic using a 30 g/L solid/liquid ratio with methanol/water (at 25 °C at 150 rpm). Plants cultivated from wild seeds, fresh parts frozen and lyophilized.		√	√		Gram-: ***Salmonella typhimurium*** ***E.coli*** *Enterobacter cloacae* Gram+: ***Bacillus cereus*** ***S.aureus*** ***Listeria monocytogenes***	√	***Penicillium ochrochloron*** ***Penicillum funiculosum*** ***Penicillum verrucosum* var. *cyclopium*** *Aspergillus fumigatus* ***Aspergillus ochraceus*** *Aspergillus niger*
Ononis natrix	7	Methanolic extract of dried leaves.	√				Gram-: *S.typhimurium* *E.coli* *Pseudomonas aeruginosa* Gram+: *S.aureus* ***Enterococcus faecalis***	√	*Candida albicans*
Raphanus raphanistrum	8	Fresh and lyophilized leaves decocted in boiling water and extracted with 80% ethanol/water.		√	√		Gram-: *E.coli* *Klebsiella pneumoniae* *Morganela morganii*		

							Proteus mirabilis *P.aeruginosa* Gram+: *E.faecalis* *L.monocytogenes* Meth-resistant *S.aureus* (MRSA)		
Bidens pilosa, ***Chenopodium album***	9	Acetone, Methanol, Water extracts of fresh and air-dried leaves.		√			Gram-: ***E.coli,*** *P.aeruginosa* *Salmonella pooni* *Serratia marcescens* *K.pneumoniae* Gram+: ***B.cereus*** *S.epidermidis* *S.aureus* *Micrococcus kristinae* ***Streptococcus pyogenes***		
Heracleum pyrenaicum **subsp.** ***orsinii***	10	Air-dried material powdered or crashed and hydrodistilled. Collecting solvent was n-hexane. The oils dried over anhydrous sodium sulfate and kept at 4 °C.		√	√		Gram-: ***E.coli*** ***P.aeruginosa*** ***S.typhimurium*** *E.cloacae* Gram+: ***B.cereus,*** ***S.aureus*** *L.monocytogenes* *Micrococcus flavus*	√	*A.niger* ***A.fumigatus*** ***Aspergillus versicolor*** ***A.ochraceus*** ***Trichoderma viride*** ***P.funiculosum*** *P. ochrochloron* *P. verrucosum* *P. verrucosum* var. *cyclopium*

Sonchus oleraceus *Cichorium pumilum* ***Portulaca oleracea***	11	Fresh leaves dried in an oven, ground to a fine powder. Powder plants extracted by distilled water at room temperature for 24 h, then centrifuged and evaporated to near dryness. The resulting viscous powder dissolved to obtain stock solution.	√					√	***Aspergillus flavus*** ***A.ochraceus*** ***Aspergillus parasiticus***
Psidium cattleianum *Psidium guajava*	12	Fresh, fully ripe fruits lyophilized to dried material. A portion extracted successively with hexane, ethyl acetate and methanol.	√				Gram-: *E.coli,* *P.aeruginosa* Gram+: *S.aureus* *Bacillus subtilis*		
Scandix pecten-veneris	13	Fresh and healthy leaves dried in the shade for at least two weeks, then crushed and stored in the dark. Extraction by soaking dry powdered leaves in methanol.	√				Gram-: ***E.coli*** *P.aeruginosa* ***S.typhi,*** ***Proteus vulgaris*** Gram+: ***S.aureus*** ***B.subtilis*** ***Streptococcus pneumoniae***	√	***A.flavus*** ***A.niger*** *A.parasiticus,* ***C.albicans***
Centaurea raphanina	14	Fresh leaves put in plastic food bags and stored in freezing conditions. Then, frozen leaves lyophilized, ground to powder, and stored in deep-freezing conditions. Solvent employed for antimicrobial assays not specified		√	√		Gram-: ***E.coli*** *S.typhimurium* *E.cloacae* Gram+: *S.aureus* ***B.cereus***	√	***A.niger*** ***A.fumigatus*** ***A.versicolor*** ***Trichoderma viride*** ***P.funiculosum*** ***P.verrucosum var. cyclopium***

Centaurea raphanina	15	Fresh leaves put at −80 °C until lyophilization, ground to powder, and stored again at −80 °C. Hydroethanolic extracts of the samples by stirring the dried plant material with etha-nol-water for 60 min.		√	√		Gram-: ***E.coli*** *S.typhimurium* *E.cloacae* Gram+: ***S.aureus*** ***B.cereus***	√	***A.niger*** ***A.fumigatus*** ***A.versicolor*** ***T.viride*** ***P.funiculosum*** ***P.verrucosum var. cyclopium***
Eremurus spectabilis	16	Leaves and roots air dried at room temperature used for extracts. Acetone used in an amber flask. All extraction solvents evaporated under reduced pressure. Stored in dark at 4°C.	√				Gram-: *Aeromonas hydrophila* ***E.coli*** ***K.pneumoniae*** ***P.aeruginosa*** *S.typhimurium* *Yersinia enterocolitica* Gram+: ***S.aureus*** *Bacillus brevis* *B.cereus* *B.subtilis* ***L.monocytogenes***		
Borago officinalis, ***Orobanche crenata,*** ***Plantago coronopus,*** ***Plantago lanceolata,*** ***Sanguisorba minor,*** *Silene vulgaris,* ***Sonchus asper,*** ***Sonchus oleraceus***,	17	Species dried in a ventilated oven, finely ground in a grinder to obtain a dry powder, and stored under vacuum in a cool room. An amount of dry powder extracted twice with refluxing aqueous methanol. After extraction, the methanolic extracts filtered and evaporated to dryness under reduced pressure at 35 ◦C.						√	***Botrytis cinerea*** ***Monilinia laxa*** ***Penicillium digitatum*** ***P.expansum*** ***P.italicum*** *Aspergillus carbonarius* ***A.niger***

Taraxacum officinale									
Centaurea raphanina	18	Fresh plant material finely ground and extracted at room temperature with cyclohexane-Et2OMeOH. The extract washed with brine, the aqueous layer re-extracted with EtOAc, and the organic layer dried with Na2SO4 and concentrated under reduced pressure.						√	***A.niger*** *A.flavus* ***A.versicolor*** ***A.ochraceus*** *T.viride* *P.funiculosum* ***P.ochrochloron*** ***Cladosporium cladosporioides*** ***Alternaria alternata***
Allium roseum	19	Separated stems, bulbs and flowers cleaned, washed, cut into small pieces, and lyophilized. Each sample extracted separately with either cold acetone/water, methanol/water, or distilled water. Extracts centrifuged and supernatants concentrated using a rotary evaporation under vacuum. Extracts immediately used or stored at −20◦C.	√	√	√		Gram-: ***E.coli*** ***P.aeruginosa*** ***S.typhimurium*** Gram+: ***S.aureus*** ***S.epidermidis*** ***B.subtilis*** *B.cereus* ***Micrococcus luteus*** ***E.faecalis***	√	***C.albicans***
Eremurus spectabilis	20	Leaves and roots air dried at room temperature. Acetone used in an amber flask. Extract mixed by a magnetic stirrer. Mixture left at room temperature for 24 h. Extract filtered to obtain particle free extract. Residue re-extracted twice with acetone and filtered. All extraction solvents evaporated under reduced pressure. Stored in dark at 4°C.	√				Gram-: *E.coli* Gram+: *S.aureus* *B.subtilis* *L.monocytogenes*	√	*Saccharomyces cerevisiae*
Ruta angustifolia	21	Plant material reduced to a fine powder and		√	√		Gram-:	√	*C. albicans* *A.niger*

		extracted with ethanol or methanol by percolation. Ethanolic and methanolic extracts of wild growing plants obtained after evaporation to the dryness under reduced pressure below 40 ºC.					*E.coli,* *P.aeruginosa* *S.enteritidis* *Enterobacter aerogenes* Gram+: *S.aureus* *B.cereus* *L.monocytogenes*		
Foeniculum vulgare ***Salvia palaestina*** ***Micromeria fruticose*** ***Trigonella foenum-graecum*** ***Cichorium pumilum*** ***Salvia hierosolymitana*** ***Ruta chalepensis*** ***Chrysanthemum coronarium***	22	Specimens dried in the shade for one month. Ground up and packed in the tubes of Soxhlet. A mixture of solvents (water, ethanol, ethyl acetate, and hexane) introduced. The solvents from each extract evaporated in an oven under reduced pressure. The polar extracts dissolved in water, while the non-polar extracts dissolved in dimethyl sulfoxide (DMSO).				√	Gram+: ***Staphylococcus mutans***		
Ziziphora clinopodioides	23	Leaves dried in shadow and crushed by mill. Each sample soaked in methanol and 48 hours later smoothed by filter paper. Extracts obtained using rotary machine, concentrated and dried at the same temperature for 2 days and gradually dried. For production of oil, water distillation method applied.	√	√	√		Gram-: ***E.coli*** ***K.pneumoniae*** *P.aeruginosa* ***Proteus vulgaris*** ***Citrobacter freundii*** Gram+: ***S.aureus*** ***L.monocytogenes*** ***S.epidermidis* (coag. Neg)**		

							***S.saprophyticus* (coag. Neg)**		
Umbilicus rupestris	24	Leaves lyophilized and reduced to a fine powder. Hydroethanolic extracts prepared by extracting freeze-dried sample with an ethanol:water solution. After filtration, the plant residue re-extracted and the combined filtrates evaporated under pressure at 40 °C and lyophilized. Decoctions prepared using freeze-dried samples and heated distilled water. The mixture boiled using a heating plate and then filtrated. The obtained decoctions frozen and lyophilized to obtain a dried extract.		√	√		Gram-: *E.coli,* *K.pneumoniae* *M.morganii* *Proteus mirabilis* *P.aeruginosa* Gram+: *E.faecalis,* *L. monocytogenes* MRSA		
Allium roseum	25	Leaves air-dried in shade, blended into fine powder and stored in a dry place and at dark. **Aqueous Extracts.** Macerate: plant powder macerated in water. Filtered then the total filtrate lyophilized. Digestion. Dried powdered leaves extracted with water at 50°C then filtered and lyophilized. Decoction. Prepared by boiling the powdered leaves in water. After filtration, the extract lyophilized. Infusion. Powdered leaves held in boiling water. After filtration, the liquid phase frozen and lyophilized. **Organic Extracts**. Powdered leaves homog-	√				Gram-: *E.coli* (ATCC 25922) *P.aeruginosa* (ATCC 27853) Gram+: *S.aureus* (ATCC25923) *S.epidermidis* (CIP106510) *M.luteus* (NCIMB 8166**)**		

		enized in petroleum ether. The final residue extracted again with three solvents: dichloro-methane, methanol and then ethanol 80%. Then evaporated to dryness. The total organic residue dried and then further extracted with distilled water to obtain an exhausted organic extract. Finally, the exhausted residue de-cocted in water.							
Origanum syriacum	26	Leaves separated from the stems, washed and dried in the shade at room temperature. The EOs of the four samples of plant extracted by using a microwave-ultrasonic method. The obtained EO was collected into a clean beaker, dried over anhydrous sodium sulfate (Na2SO4) and stored in the refrigerator at 2–8 ° C.		√			Gram-: *E.coli* (ATCC 25922) *P.aeruginosa* (ATCC 27853) Gram+: ***S.aureus* (ATCC 25923)** MRSA ***Enterococcus faecium* (ATCC 700221)**		
Mercurialis annua *Papaver rhoeas* *Foeniculum vulgare* *Chenopodium murale* *Scolymus hispanicus*	27	Fresh plant material dried in an oven, and the powder material weighed. Powder is soaked for 48 hours in a mixture of two solvents, a polar solvent (ethanol) and an apolar solvent (dichloromethane). The mixture filtered, and the filtrate concentrated under reduced pres-sure using a rotary evaporator and the crude extract dried. The extracts stored at 4°C.	√				Gram-: *E.coli* (CIP54127) *P.aeruginosa* Gram+: *S.aureus* (CIP 209 (ATCC 25923) *E.faecalis* (ATCC19433)	√	*Cryptococcus neoformans* CIP 960, *C.albicans*
Sinapis arvensis, *Poly-gonum aviculare* *Tragopogon aureus*	28	Leaves separated from plants and dried at 50ºC in an oven, then dried leaves ground to a fine powder with a mortar and pestle and kept at room temperature prior to extraction for anti-	√				Gram-: *Agrobacterium tumefaciens* ***P.aeruginosa*** *Pseudomonas corrugate*		

		oxidant activity and total phenolics analysis.					*Pseudomonas syringae pv. tomato* *Yersinia frederiksenii* *Yersinia pseudotuberculosis* *Yersinia enterocolita* *S. typhimurium* *Serratia liquefaciens* *Vibrio cholerae* Gram+: *B.cereus,* *Corynebacterium diphtheriae* Proteobacteria: *Xanthomonas compestris*		
Foeniculum vulgare	29	Dried seeds were hydrodistilled using a Clevenger-type apparatus. Essential oil dried over anhydrous sodium sulfate and stored in dark vials at 48°C.	√	√	√		Gram-: *P.aeruginosa* CIP 82118 *S.enterica* CIP 8039 *E. coli* CIP 53126 Gram+: *S.aureus* CIP 53156 *B. subtilis* CIP 5262 *M. luteus* CIP 5345	√	*A. niger ATCC 16404* *C.albicans ATCC 10231*
Allium roseum	30	Plant extracted by three methods. Method 1: powdering each sample with a mortar and pestle, stirring the powder in 0.05 M sulfuric acid, neutralizing the suspension with NaOH and removing the insoluble material by centrifugation and microfiltration. Method 2: material homogenized with 1 M Tris HCl buffer (pH 8.8) filtered and centri-	√	√	√		Gram-: *E.coli* *P.aeruginosa* *S.typhimurium* Gram+: *S.aureus* *S.epidermidis* *B.cereus*	√	*C.albicans*

		fuged. Method 3: each sample homogenized in 0.02 M phosphate buffer (pH 7.2) containing 0.1 M NaCl, stirred overnight, filtered, adjusted to pH 4.0 with acetic acid (50%, v/v), stirred and centrifuged. All extraction steps done at 4°C. Plant extracts stored at -20°C.					*B.subtilis* *M.luteus*		
Allium roseum	31	Plant extracted separately by three methods. Method 1: powdering each sample with a mortar and pestle, stirring the powder in 0.05 M sulfuric acid, neutralizing the suspension with NaOH and removing the insoluble material by centrifugation and microfiltration. Method 2: material homogenized with 1 M Tris HCl buffer (pH 8.8) filtered and centrifuged. Method 3: each sample homogenized in 0.02 M phosphate buffer (pH 7.2) containing 0.1 M NaCl, stirred overnight, filtered, adjusted to pH 4.0 with acetic acid (50%, v/v), stirred and then centrifuged. All extraction steps done at 4°C. Plant extracts stored at -20°C.	√	√	√		Gram-: ***S.typhimurium* NRRLB 4420** ***E.coli* ATCC 25922** ***P.aeruginosa* ATCC 27853** Gram+: ***S.aureus* ATCC 25923** *S.epidermidis* CIP 106510 *M.luteus* NCIMB 8166 ***B.cereus* ATCC 11778** ***B.subtilis* ATCC 168** ***E.faecalis* ATCC 29212**	√	***C. albicans ATCC 1405***
Olea europeae (cultivated) *Olea ferrugineae* (wild)	32	Leaves crude extraction: fine powder of leaves dissolved separately using different organic solvents. Rotary flash evaporator used to concentrate all the extracts. Concentrated extract weighed and preserved in airtight bottles at 4°. For antibacterial assay, 15 mg of each	√				Gram-: *E.coli* (ATCC 25922) *P.aeruginosa* (ATCC 27853) *K.pneumoniae* (ATCC 15380) *S.typhimurium* (ATCC 14028) Gram+:		

		extract dissolved in 1ml of DMSO as a solvent. Crude oils extraction: Olive oils extracted from the ripe and unripe fruit by mechanical pressing and using n-hexane as a solvent.					*S. aureus* (ATCC 6538) *L.monocytogenes* (ATCC 13932) *B.cereus* (ATCC 11778) *B.subtilis* (ATCC 19659) *E.faecalis* (ATCC 49452)		
Chrysanthemum coronarium (or *Glebionis coronaria*)	33	Samples for microbiological studies collected during their blossom stage. The extract of the above-ground part obtained by infusing air-dry material in 40% ethanol. Essential Oil by Clevenger method.		√	√		Gram-: *E.coli* UCMB-906 *P.aeruginosa* UCMB-900 Gram+: ***S.aureus* UCMB-904**	√	***C.albicans* UCMY-1918**
Allium macrochaetum	34	Essential oil: Clevenger apparatus used for obtaining essential oil of bulb with water. EO dried over anhydrous sodium sulfate and diluted by dichloromethane. Petroleum ether: The oil of bulbs obtained using Soxhlet apparatus with petroleum ether. Water and ethanol extracts: samples homogenized separately in ethanol and water using a waring blender at high speed. The extracts filtered through cheesecloth, and the residue re-extracted under the same condition with same solvents. The combined filtrate concentrated under vacuum at 35°C to dryness and lyophilized at −80°C. The extracts storaged at −20°C. Dry extracts diluted and filtrated.		√			Gram-: *E. coli* ATCC 25922 *K. pneumoniae* ATCC 4352 *P. mirabilis* ATCC 14153 *P. aeruginosa* ATCC 27853 Gram+: *S. aureus* ATCC 29213 ***S. epidermidis* ATCC 12228** ***E. faecalis* ATCC 29212**	√	*C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019 *C. tropicalis* ATCC 750
Centaurea raphanina	35	Frozen leaves lyophilized, ground to powder, and stored in deep-freezing conditions.		√	√		Gram-: *E. coli* (ATCC 25922)	√	***A. fumigatus* (ATCC 9197)** *A.niger* (ATCC 6275) *A.versicolor* (ATCC

		The lyophilized plant material used to prepare hydroethanolic extracts, obtained by stirring the powder with of ethanol/water and filtered. The residue then re-extracted with additional hydroalcoholic mixture. The combined extracts concentrated at 40 °C under reduced pressure and further lyophilized.					*S. typhimurium* (ATCC 13311) *E.cloacae* (ATCC 35030) Gram+: *S. aureus* (ATCC 11632) *B. cereus* (food isolate)		11730) ***P.funiculosum*** **(ATCC** 36839) ***P.verrucosum var. cyclopium*** **(food isolate)** ***T.viride*** **(IAM 5061)**
Polygonum hydropiper	36	Fresh leaves and stem separated, washed and air-dried at 25 ° C for 30 days in an air flux drying oven. The plant parts crushed to fine powder. Powdered samples placed in sterile sealed bags each and kept at 4 °C for further analyses. Extracts made with Ethanol, Acetone, Methanol, n-hexane, Chloroform and water.	√				Gram-: *E. coli* *K. pneumoniae* *M. morganii* *Haemophilus influenzae* Gram+: *S. aureus*		
S. alba ***S. conoidea*** ***S. dichotoma*** ***S. italica*** ***S. supina*** ***S. vulgaris***	37	Aerial parts allowed to air dry for 10 days at room temperature. Dried plant samples ground to a fine powder. Powdered plant material extracted with methanol. The extracts filtered and concentrated under vacuum at 40 °C. The extracts stored at +4 °C in dark.		√	√		Gram-: ***E. coli*** **(ATCC 35210),** ***P.aeruginosa*** **(ATCC 27853),** ***S. typhimurium*** **(ATCC 13311)** *E.cloacae* Gram+: ***L. monocytogenes*** **(NCTC 7973)** ***E. faecalis*** **(hu- isolate)** ***B. cereus*** **(clinical isolate)** ***M. flavus*** **(ATCC 10240)** ***S. aureus*** **(ATCC 6538)**	√	***A. versicolor*** **(ATCC 11730)** ***A. fumigatus*** **(plant isolate)** ***A. ochraceus*** **(ATCC 12066)** ***A. niger*** **(ATCC 6275)** ***P.ochrochloron*** **(ATCC 9112)** ***P. funiculosum*** **(ATCC 36839)** ***P. verrucosum*** **(food isolate)** ***T.viride*** **(IAM 5061)**
Ziziphora clinopodioides	38	Samples macerated in ethanol for 24 hours at room temperature. The extract filtered and the		√	√		Gram-: *P.aeruginosa* ATCC 27853	√	*C. albicans ATCC 10231* ***C. parapsilosis ATCC 22019*** ***C. tropica-***

		residue re-macerated under the same condition with ethanol two more times. The combined filtrate concentrated in a vacuum at 35ºC to remove the organic solvent. The extract stored at -20ºC.					*E. coli* ATCC 25922 *K. pneumoniae* ATCC 4352 *P. mirabilis* ATCC 14153 Gram+: *S. aureus* ATCC 29213 ***S. epidermidis* ATCC 12228** *E. faecalis* ATCC 29212		***lis ATCC 750***
Chenopodium murale ***Eruca sativa*** ***Malcolmia africana*** ***Malva neglecta*** ***Medicago polymorpha*** ***Melilotus officinalis*** ***Nasturtium officinale***	39	Non-plant materials and any visible dirt and insect parts removed from the plant samples. Plant leaves dried in shade, pulverized and stored in paper bags and analyzed. The plant material extracted with methanol by maceration and fractionated. The extract evaporated to dryness under vacuum and stored at 4°C.	√				Gram-: ***E. coli*** ***S. typhi*** ***P.vulgaris*** Gram+: ***S.pneumoniae***		
Carissa macrocarpa	40	Samples dried until at a constant weight in an incubator at 35°C. Then, plant material ground and the homogeneous samples stored in a desiccator protected from light. The hydroalcoholic extract obtained by maceration using aqueous ethanolic solution as the extraction solvent. After filtration, the solvent first evaporated at 40°C, under reduced pressure, in a rotary evaporator and the residual solvent removed in a freeze drier.		√	√		Gram-: *E.coli* ESBL *K.pneumoniae* *K.pneumoniae* ESBL *M.morganii* *P.aeruginosa* Gram+: *S.aureus* MSSA MRSA *S.epidermidis* *L.monocytogenes* *E.faecalis*		

Smyrnium olusatrum *Smyrnium perfoliatum,* *Smyrnium rotundifolium* *Smyrnium cordifolium* *Smyrnium connatum* *Smyrnium creticum*	41	Dried and powdered aerial parts reduced to coarse powder. Plant extracted with methanol at room temperature. The methanol evaporated to dryness after extraction progress. Sample solutions prepared by dissolving the extracts in dimethyl sulfoxide (DMSO).	√				Gram-: *E. coli* ATCC 39628 *E.cloacae* ATCC 13047D *S.typhimurium* CCM5445 Gram+: *Sarcina lutea* ATCC 9341NA	√	*C.albicans ATCC 10231*
Arum dioscoridis ***Chenopodium album*** ***Malva sylvestris*** ***Mentha longifolia*** **Huds.** ***Nasturtium officinale*** **Aiton,** ***Papaver rhoeas*** ***Polygonum aviculare*** ***Rumex acetosella*** ***Sinapis alba*** ***Urtica dioica***	42	Plants cleaned, dried at room temperature and powdered for subsequent extraction process. Step 1 (nonpolar extract): Powdered plant parts macerated with n-hexane. After filtration, once more extraction performed on the residue. Then filtrated extracts combined and concentrated in vacuum at 40ºC. Extracts kept in the dark at +4ºC. The residue kept for the further methanol extraction. Step 2 (polar extract): Residue obtained from hexane extraction macerated with 70% methanol by leaving them overnight. After extract filtration, once more extraction performed on the residue. Then filtrated extracts combined and concentrated in vacuo at 40ºC using a Rotary evaporator. Extracts kept in the dark at +4ºC until tested.		√			Gram-: ***P.aeruginosa*** **(ATCC 10145/isolated strain)** ***Acinetobacter baumannii*** **(RSKK 02026/ isolated strain)** ***S. enteriditis*** **(RSKK 538/ isolated strain)** **Gram+:** ***S.aureus*** **(ATCC25923/ isolated strain)** ***E.faecalis*** **(ATCC 29212/ isolated strain)** ***B.subtilis*** **(ATCC 6633 / isolated strain)**	√	***C.albicans*** **ATCC 10231** ***C.kruisei*** **ATCC 6258**

Table 2. Complete list of the botanical species studied, the extracts employed, the antimicrobial assays used, the bacteria and fungi species analyzed. In bold both the MWEPs proved to be most effective and the bacteria and/or fungi documented by the respective study most susceptible to their extracts.

2. How many Gram positive bacteria are susceptible to MWEPs extracts? Overall, we found that 15 species, out of 18 of Gram-positive bacteria, were sensible to the MWEPs extracts, representing the 83 % of the species analyzed in this review.

In figure 4.b are indicated all the Gram-positive species *vs* which the MWEPs are efficacious or not. For the mean bacteria of this group, composed by *S.aureus* Methicillin Sensible (MSSA), *B.cereus*, *E.faecalis*, *L.monocytogenes*, *B.subtilis* and *S.epidermidis*, the studies with MIC ≤0.5 mg/ml are slightly more than those over this threshold. This is in accordance with the higher sensitivity of Gram-positive bacteria to antimicrobial drugs, but the use of natural products might help also to lower the antibiotic doses in human treatments.

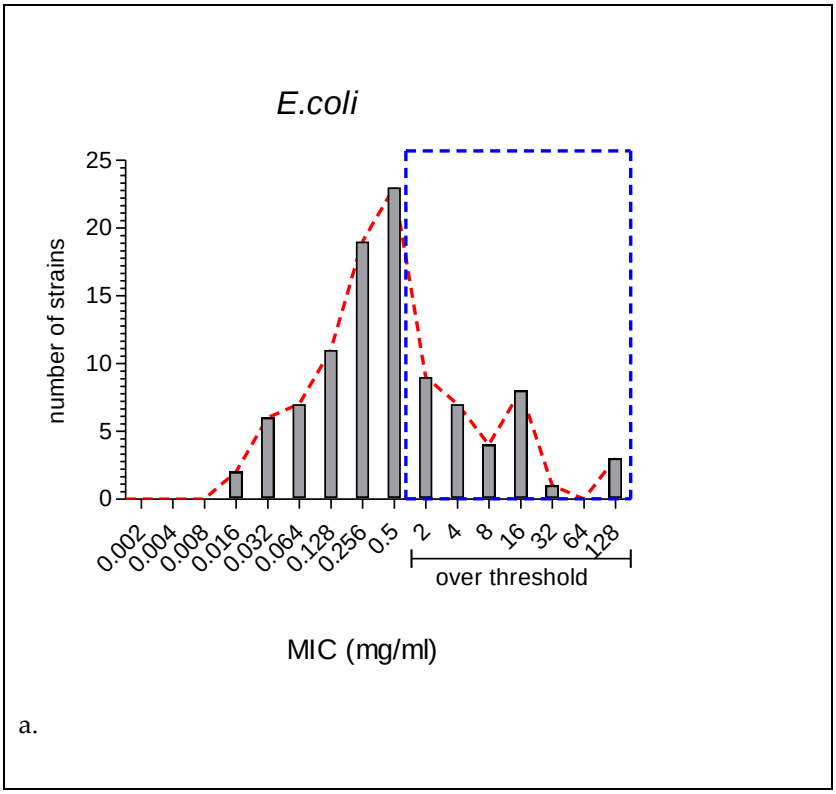
The three species not inhibited by MWEPs extracts are analyzed only in one study for each, and are environmental bacteria, as *B.brevis* and *S.lutea*, or opportunistic bacteria infection immune compromised hosts as *M.kristinae*. Again, the studies concerning these bacteria are very few.

2. How many fungi are susceptible to MWEPs extracts? Overall, we found that 22 species, out of 25 of fungi, were sensible to the MWEPs extracts, representing the 88 % of the species analyzed in the thirty-eight studies.

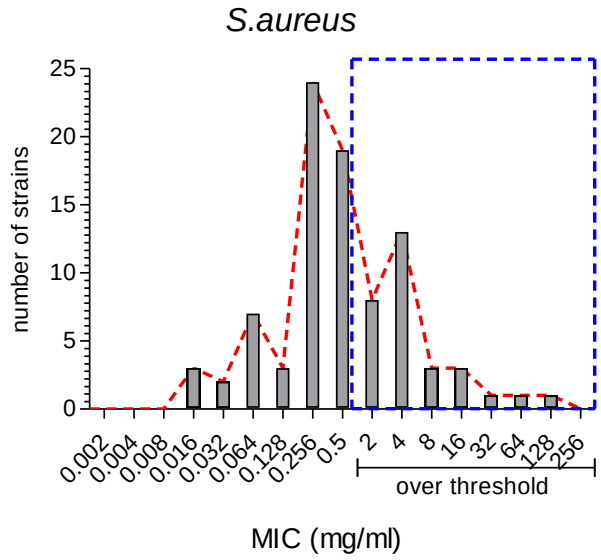
In figure 4.c are indicated all the fungi species *vs* which the MWEPs are efficacious or not. It is quite clear that MWEPs are fairly efficient in inhibiting the fungi growth, being the number of studies demonstrating antimicrobial activity many more than those proving the absence of such effect.

3.5.3. The comparison of MIC values.

The majority of studies analyzed both *E.coli* (Gram-negative) and *S.aureus* (Gram-positive), and therefore we could compare all the MIC values reported for these two mean bacterial species (figure 5).



a.



b.

Figure 5. MIC values (in mg/ml) reported in the thirty-eight studies for the MWEPs extracts *vs* the two mean, and most studied, pathogenic bacteria: a. the Gram negative *E.coli* and b. the Gram positive *S.aureus*.

It is well known that Gram-negative bacteria are more resistant to antibiotic treatments and embody 67% of the ESKAPE group of ABR bacterial species [43]. It is then important to note that the majority of the MWEPs extracts displayed MIC values (figure 5.a) below the very stringent threshold adopted in our selection (see Materials and Methods).

Instead, *S.aureus* showed MIC values distributed at the turn of the threshold value (as shown in figure 5.b).

Only three studies analyzed the antimicrobial properties of the plants against clinically isolated strains [31,37,42]. It is worth noting that, for all the comparison between collection and clinically isolated strains, the MIC values not always are higher for the latter (16 vs 256 µg/ml for the most effective plants, as *Chenopodium album*), but in some cases significantly lower, even than conventional antibiotics ones (as for *Silene conoidea*, 0,01 *vs* 0,1 mg/ml Ampicillin).

Finally, there are only six studies using Essential Oils (EO), being the one distilled from *C.coronarium* [33] the only one in which the MIC values are given in EO dilutions (v/v). Overall the EOs are shown to be highly efficient in inhibiting bacterial and fungal growth [10,23,26,34], displaying in some cases, as compared with routinely employed antibiotics, a comparable (*vs* Streptomycin) or even higher efficiency (*vs* Ampicillin) *vs* Gram-negative bacteria [7]. The only exception was EO of *F.vulgare*, which did not show any antibacterial activity [29].

3.6. Reporting biases.

The thirty-eight studies reviewed were quite heterogeneous in respect with:

i. the protocols to extract the active principles from the plants and

ii. the assays employed to evaluate the antimicrobial properties of the extracts.

We therefore grouped the studies according to the bacteria against which they showed antibacterial properties of some MWEPs species.

We also sub-grouped the studies according to the assay reported (disk diffusion or MIC/MBC) to provide an overall picture of the most employed techniques (figure 2 and 3). This kind of approach could not bring to zero the bias in evaluating the study's results, but tried to present a wide picture of the range of antimicrobial properties of selected WEPs against bacteria and fungi.

3.7. Antioxidant vs antimicrobial properties: direct or inverse association?

Despite the vast majority of studies underpin the role of antioxidant and reducing power of a MWEP in conferring it effective antimicrobial capacity, there are still contradictory results to be evaluated. Overall, we cannot draw yet a conclusion.

For example, *Sonchus* species with the higher reducing power are the very same displaying the higher their antimicrobial properties [5]. *C.raphanina* cultivated plants have less polyphenols in respect with the wild ones, and this correlates with their lower antimicrobial capacity [15].

In *Allium roseum* the authors find a direct association between antimicrobial properties and Total Phenolic Compounds (TPCs) in the extracts made with different parts of the plant (either leaves, flowers or bulbs) [19] . For *Silene* spp, the order of descending antimicrobial properties was compared with the respective order for metal chelating potential [37]. While it is true that the species more antimicrobial (*S.conoidea*) it is also the more powerful in chelating metals, we have to acknowledge that the descending order for both the features, in the six *Silene* species, does not allow to establish a direct association between them. In another example [33], *P.hydropiper*, proved to be a highly antioxidant but a weak antimicrobial: at our threshold, none of the extracts was able to inhibit bacterial growth. In another study of MWEPs, the total antioxidant and free radical-scavenging activities of the plant species showed a linear correlation with the total phenolics. For example, *M. polymorpha* was found to be the most active and also high antioxidant activity was observed for *G. laevigata*. Nonetheless, the antibacterial activity of methanolic extracts of all of the plant species was lower than positive control (streptomycin) against the tested bacterial species [39].

On the other hand, in a study on *C.macrocarpa*, even if the MIC values were over the threshold we set, it is interesting to note the strong correlation values (0.7–0.9 and >0.9) between reducing power and total flavan-3-ols (TF3O), total phenolic compounds (TPC), and inhibiting properties *vs* some Gram positive bacteria tested (*E.faecalis*, *L.monocytogenes*, MRSA and MSSA). Differently, for DPPH scavenging activity, the strong correlation values were associated to total phenolic acids (TPA), total flavonols (TF), and inhibiting properties *vs E.faecalis* and MRSA only [40].

Lastly, another study wondered, in view of the obtained results, whether their results can lead to the conclusion that there is no correlation between antibacterial activity against *S. mutans* and free radical scavenging [22]. However, an in-depth analysis revealed that the extracts of plants that exhibited an EC50 (amount of antioxidant necessary to decrease the initial DPPH absorbance by 50%) ≤ 100 ppm, showed some degree of enrichment (four orders of magnitude) toward more antibacterial activity.

It is also evident from the analysis of the studies' results that geographical location of plant collection (as altitude in the case of *F.vulgare*) and extraction procedures also have substantial effects on the activity of the extracts [32].

3.8. Certainty of evidence.

Studies exhaustively documenting the antimicrobial properties against the very same bacterial and fungal species of pathogens are grouped and the data are summarized.

4. Discussion

4.1. MWEPs Weaknesses

4.1.1. After all, how much do we actually know about antimicrobial MWEPs?

It was rather surprising to find so a big number of studies erroneously included in the bulk of matches retrieved with the keywords Wild Edible Plant AND antimicrobial by the three search engines (see figure 1 and figure S3). This has to be borne in mind if we are convinced that MWEPs' antimicrobial properties are widely studied, just because we find a high number of articles using them as keywords.

In our search, out of one hundred and ninety-two initial matches, only 19,8% of them (thirty-eight) could be actually included in the detailed analysis of experiments, describing the antimicrobial properties of Mediterranean WEPs.

Surprisingly, we could not find a single study reporting anti-viral properties of MWEPs and this poses the problem of increasing this kind of research activities.

4.1.2. How many are the most studied MWEPs?

Ten percent of all vascular plants are used as medicinal plants, and there are estimated to be between 350,000 and almost half a million species of them [44]. Other authors more conservatively estimated 1,300 medicinal plant species in EU of which 90 % are harvested from wild resources [45].

In this picture, this review on seventy-four MWEPs, even if limited to the Mediterranean basin, immediately gives us the idea of how much the study of these plants is to be enlarged.

4.1.3. How much MWEPs are necessary *vs* Antibiotics?

It is worth noting that, for conventional antibiotics, to determine the value of MIC and MBC against different microorganisms, the drugs are analyzed at concentration of μg/ml (or mg/L). In the case of MWEPs, differently, the antimicrobial assays are performed using their extracts at a one thousand fold-more concentration, which is mg/ml (or g/L) [46].

Such a ratio might give us the erroneous impression that these plants are much less efficient than conventional antibiotics, but we have to carefully consider some important differences between MWEPs extracts and drugs of synthesis.

The extracts reported in this review are documented to contain an heterogeneous mixture of compounds, ranging from phenolic, tannin and flavonoids, to minerals, fibers, organic acids, tocopherols, proteins, lipids, carbohydrates, free sugars, macro and micro elements (calcium, potassium , iron, manganese, etc), and antinutrients (alkaloids, saponins, phytate).

The first group (collectively defined Polyphenols) is known to be mainly responsible for the antimicrobial properties of MWEPs; its concentration in the vegetal tissues is highly variable and constitutes a very small percentage of the plant compounds (normally expressed in mg/g dry weight). This means that, to reach a significant antimicrobial effect, we have to increase 1000 fold-times the extract concentration as compared to the antibiotic one.

On the other hand, these extracts, exactly because they are composed by many different active principles, do not allow bacteria or fungi to develop a resistance against their antimicrobial effects. Unfortunately, instead, the growing bacterial and fungal resistance against the conventional antibiotic drugs, composed by a single molecule, has become a public health emergency worldwide for many years now [1].

Nonetheless, in many studies, MWEPs and antibiotics are used at the very same concentration (mg/ml) to directly compare their antimicrobial activities. In several cases MWEPs extracts do work as the conventional antibiotics (as Ampicillin), if not at lower concentrations [6,10,14,15,19,24,28,34-37,42], especially for bacterial species known for displaying ABR.

It is anyhow necessary to state that the future studies on MWEPs will also have to carefully examine the problems posed by various authors on natural products effective bioavailability and efficacy *in vivo* [47].

4.2. MWEPs Opportunities

4.2.1. Antimicrobial Efficacy

1. Gram negative bacteria are sensitive to MWEPs extracts.

70% of the assayed Gram-negative species were inhibited by MWEPs extracts. For *E.coli* the majority of inhibiting MIC values were significantly below our stringent threshold. This effective antimicrobial property might render their possible use, in co-administration with antibiotics, a mean to fight Gram negative bacteria infections.

Some of the Gram-negative, not inhibited by MWEPs, are opportunistic bacteria, responsible for urinary tract infections in immune compromised hosts, also for catheter insertions, and the search for inhibiting natural products derived from MWEPs is definitely recommendable.

2. Gram positive bacteria are sensitive to MWEPs extracts.

83 % of the assayed Gram-positive species were inhibited by MWEPs extracts. For *S.aureus* the inhibiting MIC values were distributed at the turn of the threshold value, being the majority of them below the threshold.

3. Fungi are sensitive to MWEPs extracts.

For Fungi, 88 % of the species analyzed in the thirty-eight studies were sensitive to MWEPs extracts. Approximately 300 fungal species on Earth are known to cause illnesses such as *Candida* spp. and dermatophytes. In the food industry, bacteria and fungi cause problems during product processing and storage [48]. MWEPs could be a new safe and effective antimicrobial agent which could be applied in many fields.

4.2.2. MWEPs *vs* antibiotic-resistant species.

We report some examples of the advantages of widening the use of MWEPs extracts, also co-administered with conventional Antibiotics to decrease the drug amount and contribute to slow down the rise of antibiotic resistance [49].

For example, *Bacillus cereus* is an opportunistic pathogen, which became a serious cause of nosocomial infections [4]. Nine studies (see Table 2) reported in this review provide experimental data of MWEPs efficacious on this pathogen [6,9,10,14-15,28,30-31,37].

Also for *Enterococcus faecalis*, responsible for urinary tract infections, and under surveillance for resistance to aminoglycosides, six studies (see Table 2) reported MWEPs extracts efficient in inhibiting its growth [7,19,31,34,37,42].

Another pathogen belonging to the ESKAPE group of ABR bacteria is *Klebsiella pneumonia*, *vs* which two studies (see Table 2) reported efficient inhibiting activity of MWEPs extracts [16,23].

All together, the studies here reviewed provide natural tools to inhibit the growth of five out six ESKAPE pathogens, and the only one missing is an *Enterobacter*, for which we could include only one study.

4.2.3. Antioxidant vs antimicrobial properties.

There is still some controversy, in the thirty-eight studies reported, concerning the type of association between antioxidant power of MWPs and their corresponding antimicrobial power, and this issue deserves further investigation. What is, instead, clearer so far, is the adjuvant role of natural antioxidant compounds in Immune system good performance [50-51].This might suggest that, even if the MWEPs antioxidant properties are not always those solely responsible for microbes killing, certainly they ameliorate the capacity of the Immune system to orchestrate the microbicide response of the infected host.

4.2.4. Implications of the results for practice, policy and future research.

MWEPs are being more and more rediscovered by consumers, both in Mediterranean and Northern European countries [2]. Their use in the daily diet might provide important micronutrients and healthy active principles. We should start, then, to face the too big heterogeneity characterizing the way in which MWEPs are studies, in order to render their precious properties more systematically documented and consistent.

Trying to figure out a future MWEPs publication checklist for authors, we propose a list of necessary steps to be requested.

The journals' editors might suggest to the authors to perform the very same test to describe the antimicrobial properties of plant extracts. This means that at least the disk diffusion test and the MIC/MBC test should be included in the study, in order to be compared with similar studies.

For what is concerned with the protocols of extraction, the editors might suggest to include at least and alcoholic, hydro-alcoholic and aqueous extract from the very same part of the plant analyzed, in order to be compared with similar studies.

For aromatic plants, the analysis of Essential oils, highly efficient in inhibiting bacteria and fungi, should be included.

Finally, for what is concerned with the pathogens analyzed, the editors might suggest to include at least the more characterized Gram positive (*S.aureus*, MRSA) and Gram negative (*E.coli*, *P.aeruginosa*) bacteria, responsible for the major number of infections in humans, in particular for community-associated infections.

4.2.5. Limitations of the evidence included in the review.

Many studies using the Disk Diffusion agar test do not specify at which extract concentration they perform the experiments, and this does not allow the comparison of the same assays in different studies with the same MWEPs species.

5. Conclusions.

Weaknesses

About MWEPs, we still know very little and it is necessary to standardize the protocols to further study these plants.

The MWEPs cultivation it is advisable to avoid their extinction, but the agronomic practices have to be as closer as possible to the *in situ* growth, to preserve their active principles.

The study of their effect on viruses has to be increased.

Opportunities

MWEPs are able to inhibit both Gram-negative and Gram-positive bacteria, and fungi. Importantly, the effective MIC on Gram-negative is significantly below the stringent threshold we employed, and some extracts do inhibit clinical isolates.

Their common antioxidant and metal chelating properties, despite some controversy, exert a positive effect on human and domestic animals' immune system further helping them to face infections.

Supplementary Materials

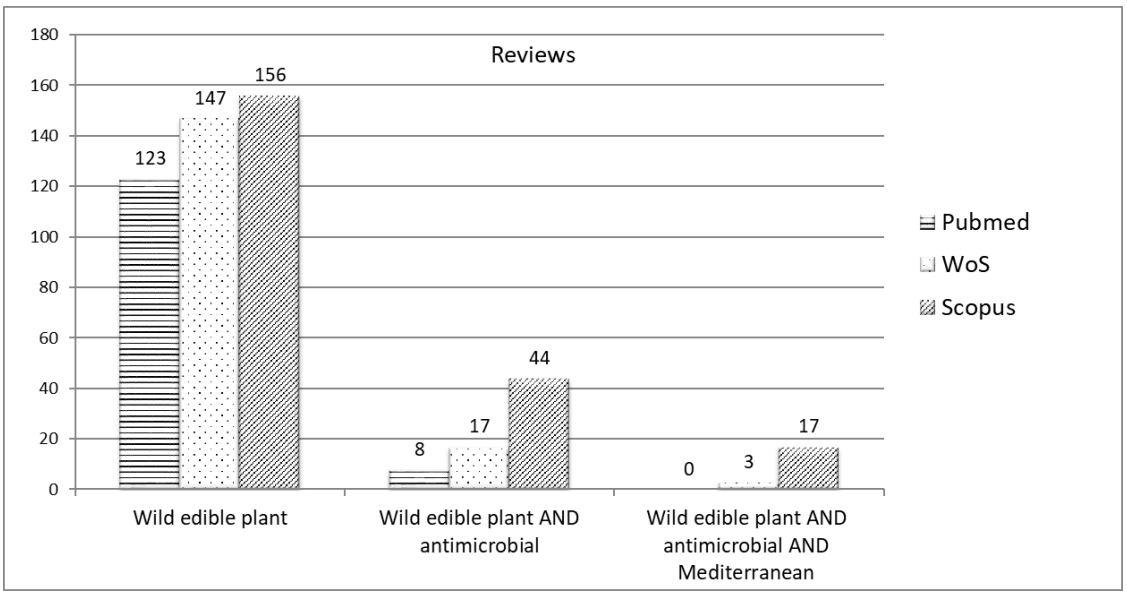


Figure S1. Initial survey on published reviews concerning Mediterranean Wild Edible Plants. Number of reviews retrieved by the three databases engine search, progressively filtered with the three selected keywods, whitout any time range and geographic filters.

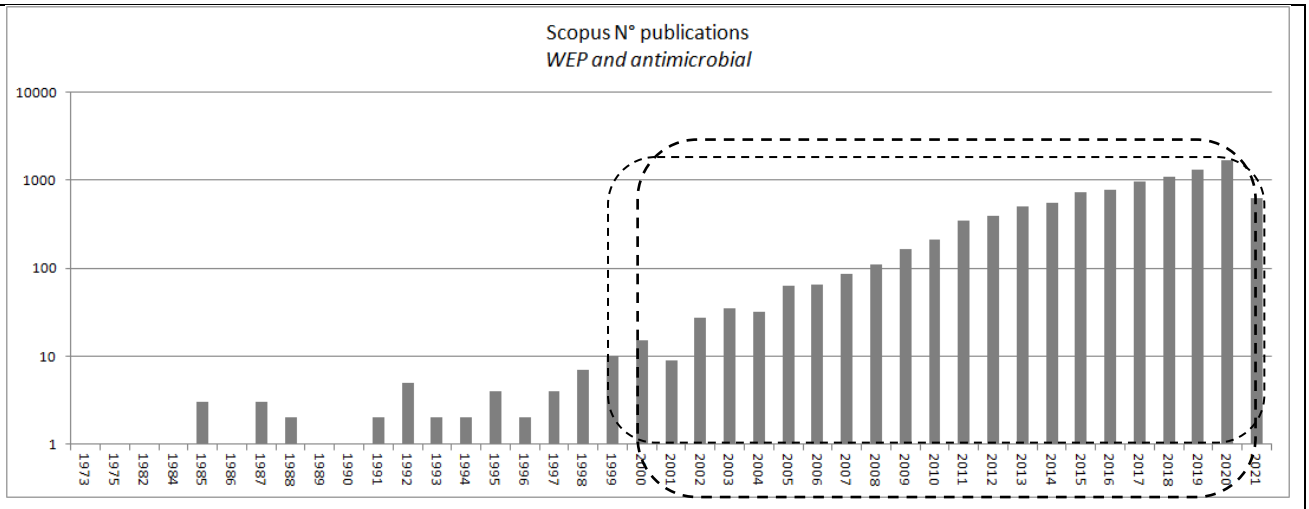


Figure S2. Selection of the time interval, according to the number of publications found with Wild Edible Plant (WEP) and antimicrobial keywords. We selected the last 20 years, in which the publication number grew significantly (from ten to thousand per year). Last search made on 14 April 2021.

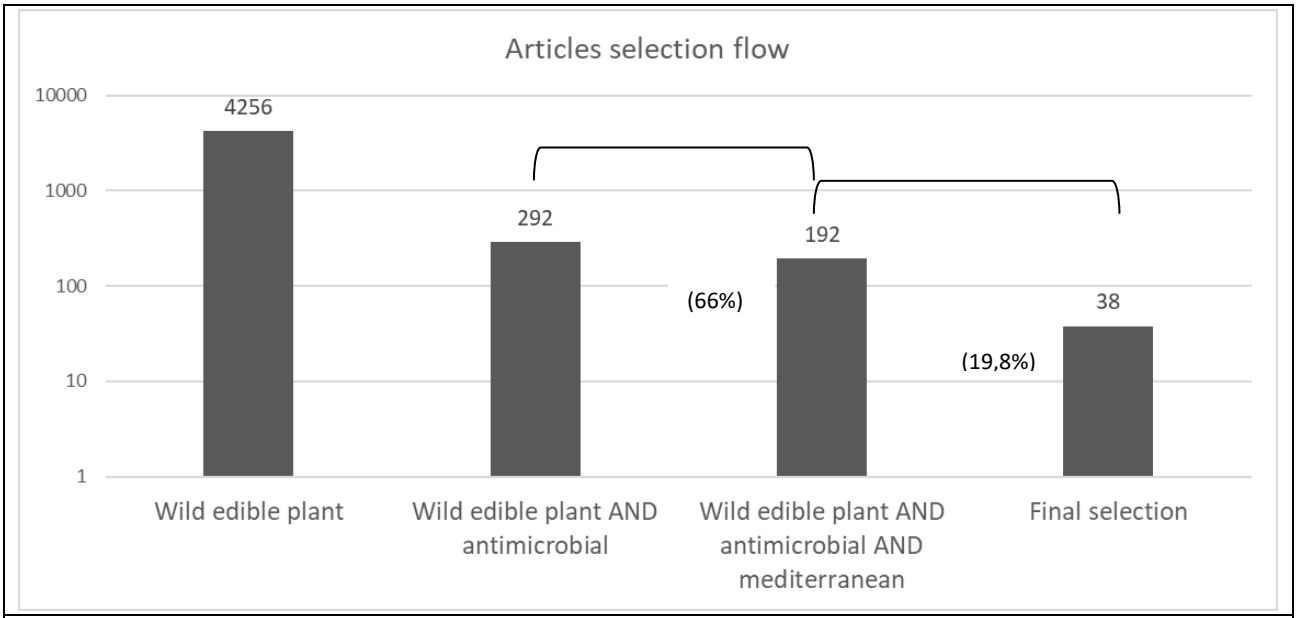
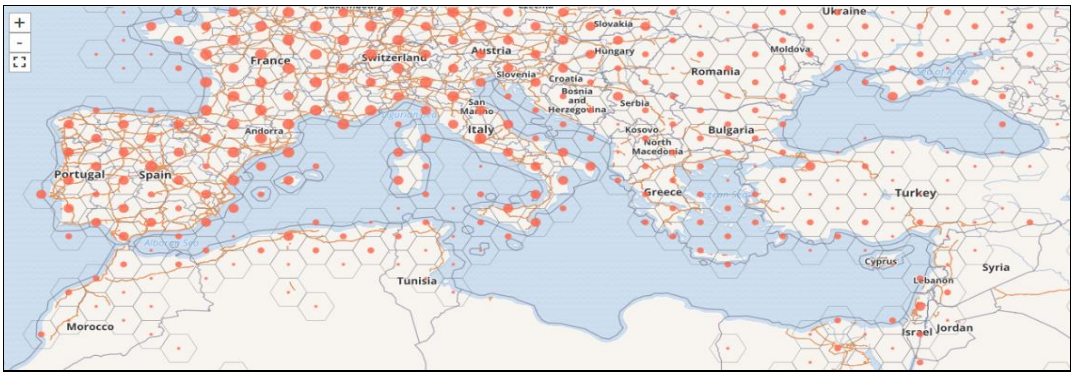


Figure S3. Articles selection flow, starting from the total matches obtained in the three database with the first keyword, Wild Edible Plants (WEPs), in the publication years range 2001-2021, and excluding USA, China, Japan and India countries of authors' affiliation in the Scopus database. The number of articles retrieved on antimicrobial Mediterranean WEPs (MWEPs) represents the 66% of the total articles on antimicrobial WEPs, and the final number of articles included in this review represent the 19,8% of MWEPs ones. Last search made on 14 April 2021.

a.

b.



c.

e.

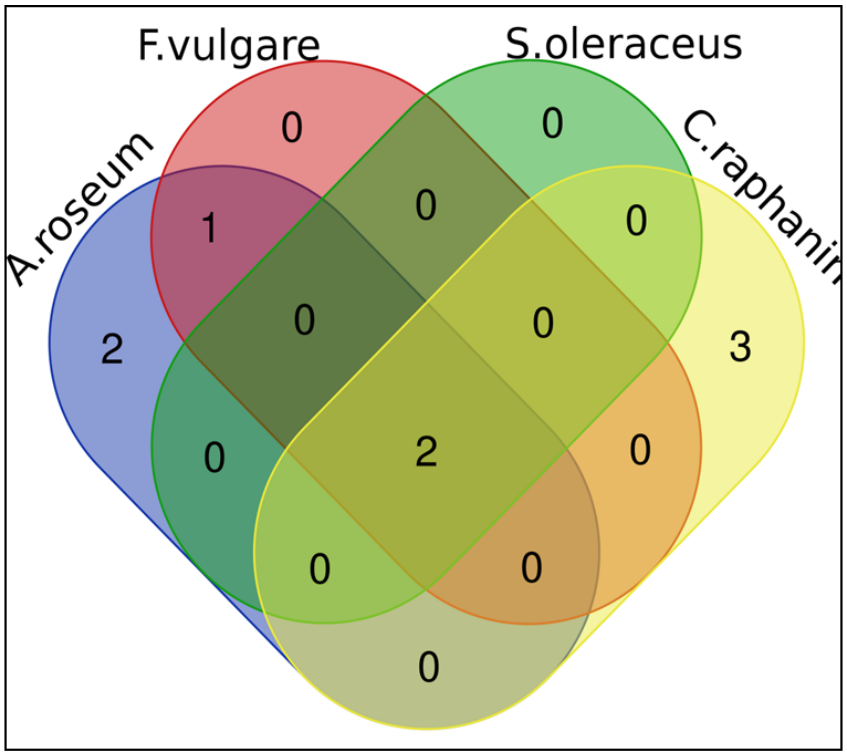
Figure S4. Examples of species geo-localization by search on GBIF | Global Biodiversity Information Facility homepage (https://www.gbif.org/species). Example of a. *Diplotaxis tenuifolia*, a plant species present in the Mediterranean basin; b.Taraxacum Genus, widely distributed in the Mediterranean basin; c. *Portulaca oleracea*, a plant species abundantly distributed in the Mediterranean basin; e. *Thymus daenesis*, a plant species totally absent in Mediterranean basin.

Species	Families	% of species	% of species	Grouped
14	**Asteraceae**	19	**69**	**8 Families 51 Species**
9	**Apiaceae**	12		
6	**Brassicaceae**	8		
6	**Caryophyllaceae**	8		
6	**Lamiaceae**	8		
4	**Fabaceae**	5		
3	**Polygonaceae**	4		
3	**Rutaceae**	4		
2	Amaranthaceae	3	**16**	**6 Families 12 Species**
2	Amaryllidaceae	3		
2	Malvaceae	3		
2	Myrtaceae	3		
2	Oleaceae	3		
2	Plantagineceae	3		
1	Apocynaceae	1	**15**	**11 Families 11 Species**
1	Araceae	1		
1	Asphodelaceae	1		
1	Boraginaceae	1		
1	Crassulaceae	1		
1	Euphorbiaceae	1		
1	Orobanchaceae	1		
1	Papaveraceae	1		
1	Portulacaceae	1		
1	Rosaceae	1		
1	Urticaceae	1		
74	**25**	**100,0**	**100,0**	

Table S1. List of all the Families and Species described in the analyzed studies. It is worth noting that the first 8 families accounted for 69% of the studied species (51 species out of 74).

Families with the most studied species	Species	N° publications	Ref.
Amaranthaceae	*C.album*	2	6-39
Amaranthaceae	*C.murale*	2	24-36
Amaryllidaceae	***A.roseum***	**4**	16-22-27-28
Apiaceae	***F.vulgare***	**3**	19-24-26
Asphodelaceae	*E.spectabilis*	2	13-17
Asteraceae	***C.raphanina***	**4**	11-12-15-32
Asteraceae	*C.coronarium*	2	19-30
Asteraceae	*C.pumilum*	2	8-19
Asteraceae	*S.asper*	2	2-14
Asteraceae	***S.oleraceus***	**4**	2-3-8-14
Asteraceae	*T.officinale*	2	3-14
Brassicaceae	*N.officinale*	2	36-39
Caryophyllaceae	*S.vulgaris*	2	14-34
Lamiaceae	*Z.clinopodioides*	2	20-35
Papaveraceae	*P.rhoeas*	2	24-39
Polygonaceae	*P.aviculare*	2	25-39

Table S2. List of the 16 most studied species (described in more than one publication), belonging to 10 out of the total 25 botanical families. Four species (in bold) are analyzed in more than two studies each, accounting for 39,5% of the whole thirty-eight studies reviewed.



MWEPs Species	total	Bacterial species
Allium roseum ✚ *Centaurea raphanina* ✚ *Foeniculum vulgaris* ▬ *Sonchus oleraceus* ✚	2	*E.coli S.aureus*
Allium roseum ✚ *Foeniculum vulgaris* ▬	1	*P.aeruginosa*
Allium roseum ✚	2	*M.luteus S.epidermidis*
Centaurea raphanina ✚	3	✧ *E.cloacae B.cereus S.typhimurium*

http://bioinformatics.psb.ugent.be/cgi-bin/liste/Venn/calculate_venn.htpl

Figure S5. Venn Diagram of the common bacterial species used to assess their antimicrobial properties by the four MWEPs species analyzed in more than two studies. The capacity to inhibit the bacterial growth is indicated with a plus symbol, the absence of inhibiting effect is indicated with a minus symbol, and a star symbol specify the case of a single bacterium sensible to the extract, as for the case of *C.raphanina* inhibiting only *E.cloacae* and not *B.cereus* and *S.typhimurium*.

In the case of *F.vulgaris,* the only bacterium sensible to its extract was the Gram-positive *Staphylococcus mutans,* not assayed with the other MWEPs species.

Author Contributions: "Conceptualization, F.M.; methodology, F.M.; validation, F.M. and G.C.; formal analysis, F.M. and G.C.; investigation, F.M.; resources, F.M.; data curation, F.M.; writing—original draft preparation, F.M.; writing—review and editing, F.M. and G.C.; visualization, G.C.; supervision, F.M.; project administration, F.M.; funding acquisition, F.M. and G.C. All authors have read and agreed to the published version of the manuscript."

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